The effectiveness of endo-rhizo bacterial isolated from areca nut rizosphere (Areca catechu L.) in breaking dormancy and improvement of seed vigor

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Abstract. This study aims to evaluate the effectiveness of endophytic and rhizobacteria (endo-rhizo bacterial) isolates in breaking dormancy while increasing its viability and vigor. The research was carried out at the Agronomy Unit of Agrotechnology Laboratory, Faculty of Agriculture, Halu Oleo University from February to October 2020. This study used a divided plot design in a completely randomized design (CRD). The main plot, namely seed scarification, consisted of 2 treatments, namely without scarification and scarification. Subplots, namely the application of bacterial endo-rhizo isolates using seed biomatric conditioning techniques, consisted of 6 treatments, namely control, L1-R, M5-R, LA6-R, LA2-E, and RJ6-R. The treatment was repeated 3 times so that there were 36 experimental units. The observed data were analyzed using analysis of variance followed by the Duncan Multiple Range Test at α = 0.05. The results showed that The scarification treatment had no effect on the dormancy of areca nut seeds, while the bacterial endo-rhizo treatment had a very significant effect on breaking dormancy and germination of areca nut seeds in all observed variables. There were 2 bacterial endo-rhizo isolates that were better able to break dormancy and increase the germination of areca nuts compared to the control and other isolates, namely isolates L1-R and LA6-R. The 2 isolates were able to reduce dormancy of areca nuts by 36% and 14%, respectively, while the increase in germination reached 136% and 104% respectively compared to the control.

1. Introduction
Areca nut (Areca catechu L.) is a type of plantation that has good prospects to be developed on a commercial scale. Areca nut has many uses, including for consumption, cosmetics, health, and coloring materials in the textile industry. The main product of areca nut is dry seeds, which contain lots of alkaloids, flavonoids, and tannins [1]. Some of the benefits of areca seeds include anti-cancer, anti-microbial and anti-inflammatory drugs. The dominant compounds found in areca seeds are tannins and alkaloids. Tannin content ranges from 15% and alkaloids from 0.3 to 0.6% [2].
The Southeast Sulawesi plantation and horticulture service noted that areca nut production in 2014 reached 261 tons. The total achievement of areca nut production in 2014 has increased by about 40 tonnes or the production achievement in 2013 was only 221 tonnes. In 2015 the production level decreased to 232 tonnes and in 2016 areca nut production decreased again, namely 205 tonnes.

A common obstacle in supplying areca seeds for cultivation purposes is dormancy in areca seeds caused by hard skin. The dormancy characteristic of betel nut seed germination is the length of time it takes to germinate and the number of germinates is only a small amount. This is because fresh betel nuts contain 60–80% coir. An effort which can be done to break the dormancy of hard-shelled seeds is scarification. Scarification is a process which can break dormancy in hard seeds through increased seed imbibition.

Apart from physical treatment, microbes can also break seed dormancy. Various studies on the ability of microbes (endophytic bacteria or rhizobacteria) to stimulate plant growth have been carried out and have been shown to improve growth and increase crop yields. Endophytic bacteria are able to increase the availability of nutrients and produce growth hormones. Endophytic bacteria are also able to increase plant resistance to various pathogenic microbes by inducing plant resistance known as induced systemic resistance (ISR) so that they can withstand plant disease attacks.

Microbes in the soil environment can play a major role in energy flow and nutrient cycling related to primary productivity. The root area of plants is generally called the rhizosphere, while the area around plants is called the phylosphere. Plant roots during biosynthesis will supply oxygen to the rhizosphere or vice versa during respiration will free carbon dioxide in the rhizosphere. Roots in a sustainable manner will free nutrients from exudates, root secretions or lysis of root cells. Various microbes live and thrive in the rhizosphere (rhizosphere bacteria) and benefit from the availability of oxygen and nutrients. The important role of rhizosphere bacteria is also related to phosphate dissolving, nitrogen fixation and growth hormone production. There is very limited information regarding the role of the two groups of bacteria (endophytic-rhizosphere / endo-rhizo bacteria) in breaking dormancy of hard-shelled seeds. Through this study evaluated the ability of endo-rhizo bacteria to break dormancy of areca nuts, integrated with scarification treatment.

2. Materials and methods

2.1. Place and time
This research was conducted in Agronomy unit of Agrotechnology Laboratory of Agriculture Faculty, Halu Oleo University from February to October 2020.

2.2. Preparation of Areca seeds
The areca nut seeds used are local superior seeds of areca nut, obtained from Korong Koto Nagari Sikucur Barat, V Koto Kampung Dalam Subdistrict, Padang Pariaman Regency, West Sumatra Province. Areca seeds are relatively uniform in brownish yellow color.

2.3. Propagation and suspension of endo-rhizo bacterial isolates
Endo-rhizo bacterial isolates used in this study were L1-R, M5-R, LA6-R, LA2-E, RJ6-R isolates. The propagation and suspension of isolates used in this study followed the previous research procedures.

2.4. Seeds treatment
Areca nut seeds with uniform color and size were selected and then according to the treatment, some seeds were scarified by cutting 2 cm across the base of the seeds. After scarification, all seeds were moistened with husk charcoal powder mixed with endo-rhizo bacterial isolate suspension (seed biomatriconditioning technique). Furthermore, the seeds were incubated for 24 hours.
2.5. Preparation of seedling media and planting seeds
The seedling medium used was a mixture of soil and sterile rice husks with a ratio of 2:1. The media for seedlings is put in a nursery box measuring 30 cm x 20 cm x 10 cm. The betel nut seeds that have been treated are then sown in 10 seed boxes per box. During the seeding process, the media for seedlings is kept moist so that the germination process takes place optimally.

2.6. Research design
This study used a divided plot design in a completely randomized design (CRD). The main plot, namely seed scarification, consisted of 2 treatments, namely without scarification and scarification. Subplots, namely the application of bacterial endo-rhizo isolates using seed biomatricconditioning techniques, consisted of 6 treatments, namely control, L1-R, M5-R, LA6-R, LA2-E, and RJ6-R. The treatment was repeated 3 times so that there were 36 experimental units. The variables observed in this experiment were dormancy intensity (percentage of seeds that did not grow until the end of the observation), maximum growth potential, and T50 [17]. The research data were analyzed using analysis of variance and if the treatment had a significant effect, then proceed with the Duncan Multiple Range Test (DMRT) \( \alpha = 0 \).

3. Results and discussion

3.1. Results
The treatment of areca seed planting with biomatricconditioning techniques using endophytic bacteria and rhizobacteria isolated from the roots of areca plants was able to break dormancy of areca seeds. In both the scarified and unscarified seeds, rhizobacteria L1-R isolates had the ability to break the dormancy of betel nuts better than the control and other treatments, except for the LA2-E and RJ6-R endophytic isolates. The increased dormancy breaking in seeds inoculated with L1-R isolates reached 36% in unscarified seeds and 46% in scarified seeds (figure 1).

![Figure 1](image-url)

Figure 1. The dormancy intensity of areca nut seeds on scarification and inoculation of endo-rhizo bacterial treatment. Means on the same bar color (blue or red) suffixed with different letters (a, b, c) are different at 5% levels of significance according to DMRT.

The biomatricconditioning technique of areca seeds using endophytic bacteria and rhizobacteria was also able to reduce the time needed for seeds to germinate by 50% (T50). In both the scarified and unscarified seeds, all tested bacterial endo-rhizo isolates had the same ability to reduce T50. The reduction of T50 in seeds inoculated with endo-rhizo bacterial isolates reached 9% -17% in unscarified seeds and 12% -18% in scarified seeds (figure 2).
Figure 2. The T50 of areca nut seeds on scarification and inoculation of endo-rhizobacterial treatment. Means on the same bar color (blue or red) suffixed with different letters (a, b) are different at 5% levels of significance according to DMRT.

Areca nut seeds which were inoculated with endophytic and rhizobacterial isolates using biomatricconditioning techniques were significantly able to increase their maximum growth potential. In unscarified seeds, rhizobacteria L1-R isolates had the ability to increase the maximum growth potential of betel nuts compared to controls and other treatments. Meanwhile, in the seeds that were clarified, rhizobacteria isolates L1-R, LA6-R and M5-R were more able to increase the maximum growth potential of betel nuts compared to controls and other treatments. The increase in the maximum growth potential of seeds inoculated with rhizobacterial isolates L1-R reached 150% for unscarified seeds, while those with clarified seeds reached a range of 88% -138% (figure 3).

Figure 3. The maximum growth potential of areca nut seeds on scarification and inoculation of endo-rhizobacterial treatment. Means on the same bar color (blue or red) suffixed with different letters (a, b, c) are different at 5% levels of significance according to DMRT.
3.2. Discussion
The results showed that the areca seed scarification by cutting crosswise the hard skin at the base of the seeds did not affect the seeds that did not get bacterial inoculation (control). This can be seen in the three observed variables (dormancy intensity, T50 and maximum growth potential) which give the same value. It is suspected that the scarification technique used was not sufficient to make the seed coat more permeable to water and gas, so the process of breaking the dormancy of both took place naturally. The scarification carried out in this study only removed the seed mesocarp without injuring the endocarp, while the effective scarification process should be by removing the mesocarp and injuring the endocarp until the endosperm of the seeds is visible [18-19].

Inoculation of seeds with endo-rhizo bacteria using biomatricconditioning techniques was significantly able to break dormancy and increase germination of betel nuts. This is relevant to the results of previous studies that the use of endophytic bacteria was able to increase seed germination [20-24]. Likewise, the use of rhizobacterial isolates was able to increase seed germination [9].

Based on the results of previous screening, the endophytic bacteria LA2-E significantly increased the viability and vigor of rice seeds. Therefore, these isolates were further tested on betel nuts to evaluate their effectiveness in increasing betel nut germination [25-26]. The role of endophytic bacteria as plant growth promoters has been widely reported [27-28].

The results showed that the treatment of seeds with endo-rhizo bacteria was significantly able to overcome seed dormancy in betel nuts and increase seed vigor. The increase in seed vigor and the breaking of the betel nut seed dormation are related to the ability of rhizobacteria to produce the IAA growth hormone which plays an important role in seed germination. Indole acetic acid (IAA) is a plant growth hormone that plays an important role in stimulating plant growth. The role of IAA, which is produced exogenously from bacteria, is able to accelerate plant growth in spurring the differentiation process at the roots in forming root hairs [27]. Endophytic bacteria are also reported to be able to produce the IAA hormone [23,28], so did the rhizobacteria group [12,16,26]. Furthermore, it was reported that, PGPR inoculation was able to increase seed vigor, plant height, root length, and seed germination [29]. Relevant studies reported that the rhizobacter consortium was able to increase seed germination 114% and seed biomass 65% [30].

4. Conclusions
The scarification treatment had no effect on the dormancy of areca nut seeds, while the bacterial endo-rhizo treatment had a very significant effect on breaking dormancy and germination of areca nut seeds in all observed variables. There were 2 bacterial endo-rhizo isolates that were better able to break dormancy and increase the germination of areca nuts compared to the control and other isolates, namely isolates L1-R and LA6-R. The 2 isolates were able to reduce dormancy of areca nuts by 36% and 14%, respectively, while the increase in germination reached 136% and 104% respectively compared to the control.

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