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Research Article

Scarification and Seed Biomatriconditioning Effect Using Endophytic-Rhizobacteria in Areca Nut (*Areca catechu* L.) Seedling Vigor

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Abstract

Background and Objective: Intensive and commercial development of areca nut requires the provision of high-vigour areca nut. This study aimed to evaluate the effect of scarification and seeds biomatriconditioning using endophytic-rhizobacteria in increasing seedlings vigor of areca nut. **Materials and Methods:** The research was carried out at the Agronomy Unit of Agrotechnology Laboratory, Agriculture Faculty, Halu Oleo University, from November, 2020-March, 2021. The research design was split-plot in a Completely Randomized Design (CRD). The main plot, seed scarification, consisted of 2 treatments, without scarification and scarification. Sub-plots, seeds biomatriconditioning using endophytic-rhizobacteria, consisted of 6 treatments, control, L1-R, M5-R, LA6-R, LA2-E and RJ6-R. **Results:** The results showed that the scarification treatment did not affect the seedlings vigor of the areca nut. Seed biomatriconditioning using endophytic-rhizobacteria was able to increase seedling vigor both without scarification and with scarification. There were 3 isolates of endophytic-rhizobacteria which were more able to increase the vigor of areca nut without scarification, namely L1-R, LA6-R and LA2-E, with an increase of 137, 104 and 102%, respectively compared to the control, while in scarified seeds, L1-R isolate was able to increase the seedlings vigor of areca nut by 194% compared to the control. **Conclusion:** Scarification treatment did not affect the vigor of the areca nut. Seed biomatriconditioning with endophytic-rhizobacteria was able to increase the vigor of areca nut seeds either without scarification or with scarification.

Key words: Areca nut seeds, endophytic-rhizobacteria, scarification, seedlings vigor, biomatriconditioning, dormancy arecoline

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Areca nut (*Areca catechu* L.) is originally from the Philippines but widely grown in many countries in Southeast Asian country¹, including Indonesia. In this region areca nut cultivation with crops in a model of agroforestry. Growing areca nut in association with other agricultural plantation crops is a widespread practice elsewhere². Areca nut has promising prospects for commercial development³ and the demand for dried areca nut for both domestic needs and as an export commodity continues to increase from year to year. The high demand for areca nut is due to its many and varied benefits, including as raw material for bio-polymer⁴, medicine^{5,6}, cosmetics, health and textile industries⁷. The main chemical constituents of areca nut are alkaloids⁸, flavonoids, tannins, triterpenes, fatty acids^{9,10}, which are useful as anti-bacterial, anti-viral, antioxidant¹¹. Arecoline compounds (alkaloid components) was found in areca nut¹², can function as an anthelmintic (anti-worm)¹³. In addition, the ethanol extract of areca nut has antidepressant activity or stress medication^{14,15}.

The increasing demand for areca nut must be followed by an increase in areca nut production, either through intensification or extensification programs. In Southeast Sulawesi areca nut plants have begun to be developed, although not intensively. One of the problems related to the extensification program or the expansion of the areca nut cultivation is seed dormancy. The high composition of coir in areca nut (60-80%) and the content of lignin, cutin and suberin in the seed coat cause physical dormancy due to the hard skin on the areca nut. Several methods have been used to overcome the problem of dormancy due to hard skin, including scarification with sandpaper¹⁶, soaking in coconut water¹⁷, application of growth regulators¹⁸ and scarification¹⁹.

The study of isolation of endophytic bacteria and rhizobacteria (endo-rhizobacteria) from the roots of areca nut plants obtained several potential isolates as plant growth promoters. The results of the initial inoculation of isolates on dormant upland rice seeds showed that these isolates were also able to break the dormancy of upland rice seeds^{20,21}. The ability of endo-rhizobacteria to stimulate seed germination is related to their ability to produce growth hormones, solubilize phosphate and fix N²². Evaluation of endophytic and rhizobacterial isolates using scarification and biopriming techniques on dormant areca nut was able to reduce the intensity of areca seed dormancy and increase germination of areca nut by 136% compared to the control.

The study aimed to evaluate the effectiveness of scarification and seeds biomatriconditioning using endophytic-rhizobacteria in increasing the seedlings vigor of areca nut.

MATERIALS AND METHODS

Place and time: The research was conducted at the Agrotechnology Laboratory of the Agronomy Unit, Faculty of Agriculture, Halu Oleo University, Kendari. This research takes place from November, 2020-March, 2021.

Experimental design: The study used a Randomized Block Design (RBD), consisting of 9 treatments of growing media composition as shown in Table 1. Each treatment was repeated 3 times so that there were 27 experimental units.

Research design: The research design used in this experiment was a split-plot design in a Completely Randomized Design (CRD). The main plot was seed scarification, consisting of 2 treatments, namely without scarification and scarification. The subplots were seed biomatriconditioning using endophytic-rhizobacterial isolates consisting of 6 treatments, namely control, L1-R, M5-R, LA6-R, LA2-E and RJ6-R isolates. The treatment was repeated 3 times so that there were a total of 36 experimental units.

Culture of endophytic-rhizobacterial isolates and making suspensions: Before culturing the isolates, 1st a culture medium was made using Tryptic Soy Agar (TSA) medium. Endophytic-rhizobacteria isolates namely L1-R, M5-R, LA6-R, LA2-E, RJ6-R²³ were then cultured in Petri (Ø9 cm) containing TSA which had been sterilized at 121 °C, p 1 atm, t 20 min. The isolated culture was then incubated at 28 °C for 48 hrs. After incubation, the bacterial isolates were suspended in 100 mL of sterile distilled water in a glass bottle and then shaken for 24 hrs at a speed of 150 rpm²⁴.

Seed treatment and planting: Areca nut seeds were obtained from Korong Koto Nagari Sikukur Barat, District V Koto Kampung Dalam, Padang Pariaman Regency, West Sumatra Province. Before planting, some of the seeds were scarified (according to the treatment) by cutting obliquely the skin and coir of the areca nut on the microfilm section of 2 cm long without injuring the microfilm, the other seeds were left intact. After the scarification process, all the seeds were treated with biomatriconditioning, namely moistening the seeds with moist solid media (ground burned-rice husk) which had been

inoculated with endophytic-rhizobacteria isolates (according to the treatment). The treated seeds were incubated for 48 hrs, after incubation, the seeds were dried for 5 hrs in a laminar airflow cabinet, then the seeds were ready to be used for testing. Before being used for seed vigor testing, the seeds were 1st germinated in a germination box measuring 30×15×10 cm with a germination medium in the form of a mixture of burned-rice husk and organic fertilizer at a ratio of 1:1 (v/v) for 60 days. The germinated seeds were then transferred to polybags measuring 20×20 cm. The planting medium used is a mixture of soil, burned-rice husk and organic fertilizer with a ratio of 1:1:1 (v/v/v).

Observation variable: The variables observed in this study were plant height, stem diameter and root length, observed at 30 Days After Transplanting (DAT). Plant height was measured from the base of the stem to the tip, stem diameter was measured at the base of the stem using a calliper and root length was measured at the longest root of the seedling with a ruler.

Data analysis: The research data were analyzed by analysis of variance and if the treatment had a significant effect, then continued with Duncan's Multiple Range Test (DMRT) at $\alpha = 0.05$.

RESULTS

The results showed that scarification and biomatriconditioning of seeds with endophytic-rhizobacteria significantly increased the vigor of areca nut seedlings. In general areca nut seeds without scarification showed lower plant height growth performance compared to scarified seeds. In seeds without scarification, the biomatriconditioning treatment of seeds with rhizobacteria LA6-R showed better seedling height compared to control and other treatments but not significantly different from L1-R and LA2-E. Meanwhile, in the scarified seeds, L1-R rhizobacteria showed better seedling height and were significantly different from the control and other treatments (Table 1).

Scarification treatment and seed biomatriconditioning using endophytic-rhizobacteria significantly affected the stem diameter of areca nut seedlings. In seeds without scarification, seed biomatriconditioning treatment with LA6-R rhizobacteria showed better stem diameter compared to control and other treatments but not significantly different from L1-R and RJ6-R. Meanwhile, in scarified seeds, biomatriconditioning treatment with L1-R, M5-R and LA2-E rhizobacteria showed better stem diameter of areca nut and was significantly different from the control and other treatments (Table 2).

Table 1: Seedlings height of areca nut on scarification and seeds biomatriconditioning using endophytic-rhizobacteria

Endophytic-rhizobacteria	Treatments	
	Without scarification	Scarification
Control	4.90±0.96 ^c	4.77±1.66 ^c
Biomatriconditioning L1-R	6.87±0.03 ^{ab}	14.75±0.43 ^a
Biomatriconditioning M5-R	5.65±0.52 ^{bc}	10.50±0.95 ^b
Biomatriconditioning LA6-R	7.62±1.18 ^a	3.88±1.41 ^c
Biomatriconditioning LA2-E	6.33±0.88 ^{abc}	5.42±1.38 ^c
Biomatriconditioning RJ6-R	4.77±1.14 ^{bc}	9.58±1.01 ^b

Means in the same column suffixed with different letters are different at 5% levels of significance according to DMRT, L1-R: Rhizosfer, M5-R: Rhizosfer, LA6-R: Rhizosfer, LA2-E: Endofit, RJ6-R: Rhizosfer and Mean±Standard error

Table 2: Stem diameter of areca nut seedlings on scarification and seeds biomatriconditioning using endophytic-rhizobacteria

Endophytic-rhizobacteria	Treatments	
	Without scarification	Scarification
Control	3.33±0.39 ^c	3.53±1.15 ^{bc}
Biomatriconditioning L1-R	4.83±0.21 ^{ab}	6.23±0.08 ^a
Biomatriconditioning M5-R	4.10±0.77 ^{bc}	5.97±0.96 ^a
Biomatriconditioning LA6-R	5.40±0.76 ^a	2.73±0.15 ^c
Biomatriconditioning LA2-E	3.73±0.24 ^{bc}	5.57±0.23 ^a
Biomatriconditioning RJ6-R	4.53±0.20 ^{abc}	5.07±0.63 ^{bc}

Means in the same column suffixed with different letters are different at 5% levels of significance according to DMRT, L1-R: Rhizosfer, M5-R: Rhizosfer, LA6-R: Rhizosfer, LA2-E: Endofit, RJ6-R: Rhizosfer and Mean±Standard error

Table 3: Root length of areca nut seedlings on scarification and seeds biomatriconditioning using endophytic-rhizobacteria

Endophytic-rhizobacteria	Treatments	
	Without scarification	Scarification
Control	4.70±0.52 ^d	4.35±0.66 ^d
Biomatriconditioning L1-R	11.12±0.91 ^a	12.78±0.91 ^a
Biomatriconditioning M5-R	7.92±0.51 ^{bc}	10.22±1.75 ^b
Biomatriconditioning LA6-R	9.57±0.93 ^{ab}	4.80±0.95 ^d
Biomatriconditioning LA2-E	9.50±0.50 ^{ab}	7.33±0.43 ^c
Biomatriconditioning RJ6-R	6.48±0.78 ^{cd}	8.22±1.78 ^{bc}

Means in the same column suffixed with different letters are different at 5% levels of significance according to DMRT, L1-R: Rhizosfer, M5-R: Rhizosfer, LA6-R: Rhizosfer, LA2-E: Endofit, RJ6-R: Rhizosfer and Mean±Standard error

Scarification treatment and seed biomatriconditioning with endophytic-rhizobacteria also significantly affected the root length of areca nut seedlings. In the seed without scarification, seeds biomatriconditioning using rhizobacteria L1-R showed better root length compared to the control and other treatments but not significantly different from LA6-R and LA2-E. Meanwhile, in scarified seeds, seed biomatriconditioning using L1-R showed better root length of areca nut seedlings and was significantly different from the control and other treatments (Table 3). The growth performance of areca nut seedlings on the treatment of scarification and seed biomatriconditioning using endophytic-rhizobacteria is shown in Fig. 1. In Fig. 1a without scarification, it appears that rhizobacteria L1-R, L6-R and LA2-E showed

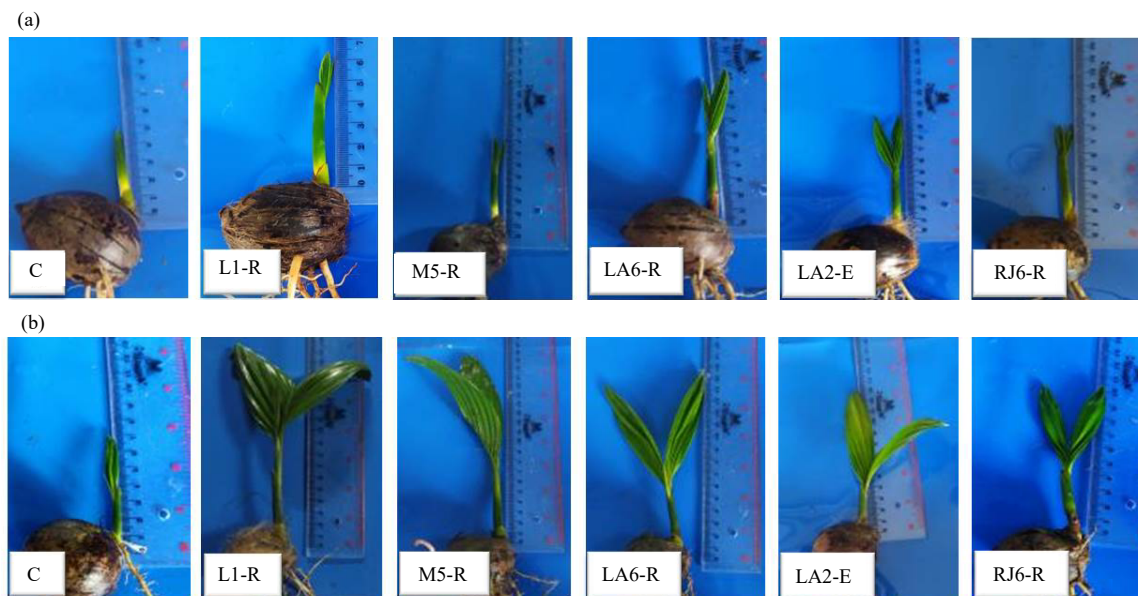


Fig. 1(a-b): Growth performance of areca nut seedlings on seed biomatriconditioning using endophytic-rhizobacteria, (a) Without scarification and (b) Scarification

better growth compared to control and other isolates, whereas in (Fig. 1b) with scarification, isolate L1-R showed the best growth performance compared to control and other isolates.

DISCUSSION

The results showed that seed scarification was not able to improve the germination and vigor of areca nut seedlings. On the other hand, seeds biomatriconditioning using endophytic-rhizobacteria were significantly able to improve the vigor of areca nut seeds, both in non-scarified and scarified seeds. Endophytes-rhizobacteria have different abilities in increasing the vigor of areca nut seedlings. In the seed without scarification, endophytic-rhizobacteria L1-R, LA6-R and LA2-E showed better performance in increasing the vigor of areca nut seedlings compared to the control and other treatments. Meanwhile, in scarified seeds, L1-R rhizobacteria showed the best performance in increasing the vigor of areca nut seedlings compared to other endophytes. In unscarified seeds, endophytic-rhizobacteria inoculation was thought to be able to supply the hormones needed to initiate germination through the activation of enzymes which play a role in the seed germination process. As stated that rhizobacteria can synthesize hormones that play a role in seed germination^{25,26}. This can be seen from the results of the germination test which showed that the endophytic-rhizobacteria isolates which were not scarified were more able to increase the germination of areca nut seeds compared to controls²⁷.

The ability of endophytic-rhizobacteria in improving seed germination is related to the ability to synthesize hormones, also because these bacteria can fix nitrogen and dissolve phosphate^{26,28-31}. The endophytic-rhizobacterial isolate used in this study was proven to have the ability to fix nitrogen, dissolve phosphate and produce growth hormone IAA^{21,32}.

Inoculation of endophytic bacteria isolated from healthy cocoa plants, besides being able to increase cacao seed germination, can also increase cacao seedling height, root dry weight and shoot dry weight³³. It was further reported that this bacterial isolate was also able to fix nitrogen, dissolve phosphate and produce IAA^{34,35}. On the other hand, studies on rhizobacteria isolated from the roots of rice plants, when re-inoculated into rice seeds, were shown to be able to increase the germination of the rice seeds. This improvement in germination is also related to the ability of these rhizobacteria to produce growth hormones, dissolve phosphate and fix nitrogen³⁶. Furthermore, Afa *et al.*³⁷, also reported that rhizobacteria isolated from the rhizosphere of healthy shallots were able to produce growth hormones in the form of IAA, fix nitrogen and solubilize phosphates so that they have the potential to increase seed viability and vigor as well as plant growth and yield. The role of endophytic-rhizobacteria on seed vigor was also reported by Bhutani *et al.*³⁸ that the application of bacterial isolates to plant roots increased in root length and the number of lateral roots. The implication of this research is the discovery of seed treatments that can overcome dormancy in arecanut seeds

while increasing their viability and vigor. By moistening the seeds in the suspension of rhizobacteria (seed biotric conditioning), the dormancy of the areca nut can be overcome and the germination of the areca nut is more vigorous and uniform. The results of this study are useful for accelerating the breaking of areca seed dormancy caused by hard skin. Without scarification treatment, isolates L1-R, LA6-R and LA2-E were recommended as seed treatments to overcome the problem of dormancy and seed low vigor.

CONCLUSION

Scarification treatment did not affect the vigor of the areca nut. Seed biotric conditioning with endophytic-rhizobacteria was able to increase the vigor of areca nut seeds either without scarification or with scarification. There were 3 isolates of endophytic-rhizobacteria which were better able to increase the vigor of areca nut without scarification, namely L1-R, LA6-R and LA2-E. The 3 isolates were able to increase the vigor of areca nut (root length), respectively by 137, 104 and 102% compared to the control, while in the scarified seeds, L1-R isolates were more able to increase the vigor of areca nut by 194% compared to control.

SIGNIFICANCE STATEMENT

This study discovers the scarification and biotric conditioning treatment effective in breaking dormancy of Areca nut and that can be beneficial in increasing vigor and growth of areca nut seedlings. Areca nut has promising prospects for future development and the demand increasing constantly for both domestic needs and as an export commodity continues to increase from year to year. Our finding revealed that the scarification and biotric conditioning treatment with rhizobacteria was able to cover the problem of seed dormancy and increase the vigor of areca nut seeds. This study will help the researcher to uncover the critical areas of seedling dormancy using the scarification and biotric conditioning treatment.

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