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Acceleration of Nutmeg (*Myristica fragrans* Houtt.) Seed Germination by Scarification and Gibberellin Application

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ABSTRACT
Nutmeg (*Myristica fragrans* Houtt.) is a native commodity of Indonesia. Propagation of this plant is relatively difficult and takes a long time for germination because of its hard seed coat. This study aimed to evaluate the effect of scarification and gibberellin (GA) application on the nutmeg seed germination. Factorial completely randomized design (CRD) was used in this study. The first factor was scarification (S0: without scarification, S1: scarification at part of the radicle out, S2: scarification at all the seed surface, and S3: seed coat wounding) and the second factor was GA soaking (0, 50, 100, 150 ppm) for 24 hours, each of combination treatments with six replications. The parameters of germination (days to germinate, germination, growth potential, percentage of germination), growth (root length, plant height, number of leaves, and girth), the morphological and anatomical structure of nutmeg seed were observed. Data of germination and growth were analyzed using Anava to DMRT at the level of 95% using SPSS, while data of morphological and anatomical structure were analyzed descriptively. The results showed that scarification (seed coat wounding) was the best treatment to enhance germination, while 100 and 150 ppm GA application of the decreased effect on all parameters of germination and growth. Combination of scarification (wounding seed coat) and soaking on 150 ppm of GA (G_3S_3) was the best treatment for improving germination parameters (day germinate until 9,50 day after plating, 88,88% of germination ability, germination percentage to 91,66%, growth potential to 97,22%) and growth parameters (root length to 13,67 cm, plant height to 24,95 cm, leaf number to 3,75, and girth stem to 1,94 cm). The main barrier of nutmeg germination was caused by a hard seed coat. That consists of ligneous sclerenchyma cells.
Keywords: *germination, seed, nutmeg (*Myristica fragrans *Houtt.), scarification, gibberellin*

I. INTRODUCTION
Nutmeg (*Myristica fragrans* Houtt.) is a native Indonesian commodity and is the oldest and most important spice product in the history of international trade [1]. Until now, Indonesia is the main producer and supplier of nutmeg and mace seeds, which is around 70% of the world's needs. Propagation of nutmeg seeds can be done by vegetative and generative techniques. Fegetatively multiply (graft or grafting) has the advantage of being faster growth but has a weakness with a weak root system and smaller stems. So far, most farmers use generative propagation because it has a strong rootstock advantage and is long-lived, but experiences constraints during long germination times, with a fairly low success rate of around 60% [2].

The alleged cause of slow nutmeg germination is the possibility of seeds in a dormant state. Dormancy is a condition of germination that is caused by the embryo experiencing several obstacles such as hard or thick seed coat and the presence of substances or material that covers the seed tissue, or an embryo that has not been fully developed. Also, another factor that has been known to influence seed dormancy and germination is the abscisic hormone (ABA) which plays an important role in maintaining dormancy and inhibits seed germination while gibberellins (GA) encourage seed germination [3].

This study aims to determine how the effect of scarification and administration of gibberellins (GA) as well as a combination of both in accelerating nutmeg seed germination.

II. METHOD
This study uses a completely randomized design (CRD) factorial pattern, the first factor is scarification (scarification, scarification of the radicular outlet, scarification of the entire surface of the seed, and wounding of the seed coat), the second factor is immersion

GA (0, 50, 100, and 150 ppm) for 24 hours, each wound combination with 6 replications to obtain 96 experimental units. The parameters observed include germination (germination days, germination, and growth potential) and growth (root length, hypocotyl height, number of leaves, and girth) as well as morphological and anatomical structure of nutmeg seeds. Data analysis used ANOVA followed by DMRT test at 95% with SPSS, while data in the form of images were described based on observations.

III. RESULTS AND DISCUSSION

Germination Parameters

Results of the study on germination parameters Table 1 shows that the combination of scarification treatment (seed skin injury) and GA 150 ppm (G_3S_3) is the best treatment combination because it increases the germination parameters (germination days up to 9.50 HST, germination capacity 88.88%, and growth potential 97. 22%).

Research on palm sugar by Saleh [4], shows a low percentage of germination without scarification [5]. The results of the study by Juhanda *et al*. [6] showed that scarification caused an increase in permeability of seed coat so that the rate of imbibition was high. Leguminosae plants experience physical dormancy due to the impermeable seed coat. Likewise, nutmeg shells that are impermeable cause water and air cannot be absorbed into the seeds and oxygen diffusion does not occur [7]. The high rate of imbibition is followed by the assessment of high food reserves [6]. Scarification has been shown to increase the germination of palm seeds [8], Sabal palmetto seeds and Thrimax morris [8]. Gibberellins act as the activation of the α-amylase enzyme to convert starch into glucose which is used as energy to start germination [9]. Research on Pistascia by Abu-Qaoud [10], shows that the highest germination is obtained by a combination of scarification treatment and GA. Seeds that have low germination power, other than because the seed coat is hard and impermeable, can also be caused by the presence of inhibitor compounds such as polyphenols and flavonoids produced in fruit or seeds [10].

Table 1. Effects of Scarification Interaction and Giving GA on Germination

Sample Code	Germination Parameters		
	Germinating day (%)	Germination (%)	Growing Potential (%)
G_0S_0	79.16^{a}	0.00^{f}	19.43^{h}
G_0S_1	61.83^{d}	0.00^{f}	30.55^{fg}
G_0S_2	40.83^{f}	44.44^{e}	47.22^{de}
G_0S_3	29.50^{f}	61.10^{cd}	63.88^{bc}
G_1S_0	72.66^{c}	0.00^{f}	22.21^{gh}
G_1S_1	61.66^{d}	0.00^{f}	38.88^{ef}
G_1S_2	23.83^{g}	49.99^{de}	55.55^{cd}
G_1S_3	17.75^{hi}	66.66^{bc}	72.21^{b}
G_2S_0	73.58^{bc}	0.00^{f}	24.66^{gh}
G_2S_1	58.16^{e}	0.00^{f}	58.33^{c}
G_2S_2	19.66^{h}	66.66^{bc}	72.21^{b}
G_2S_3	16.41^{i}	83.33^{a}	88.88^{a}
G_3S_0	76.00^{b}	0.00^{f}	24.99^{gh}
G_3S_1	56.33^{e}	5.55^{f}	58.33^{c}
G_3S_2	12.91^{j}	77.77^{ab}	91.66^{a}
G_3S_3	9.50^{k}	88.88^{a}	97.22^{a}

Information: * G0: GA 0 ppm, G1: GA 50 ppm, G2: GA 100 ppm, G3: GA 150 ppm. S0: without scarification, S1: partial scarification of the surface of the seed coat, S2: scarification of the entire surface of the seed coat, S3: injury to the seed coat.
* Numbers followed by the same letter are not significantly different at the 95% confidence level of the Duncan Test.

Growth Parameters

After the nutmeg seeds germinate, the parameters of growth will be observed. The results showed that the scarification and provision of GA significantly affected all growth parameters. Increasing GA concentration increases growth parameters that are significantly different from controls. The best treatment with the highest mean value was scarification treatment (seed coat treatment) and giving 150 ppm GA with average growth parameter values: root length 13.67 cm, hypocotyl height 24.95 cm, number of leaves 3.75, and stem wound 1.94 cm.

After germinating GA plays a greater role than scarification, because scarification plays a role in breaking dormancy at the beginning of germination, intending to expand the surface of the seeds so that imbibition occurs more quickly than without clarification. Seeds that have thick skins, such as nutmeg seeds can be accelerated by filing their skin so that they become permeable and germination can begin [11].

Table 2. Effect of Scarification Interaction and Giving GA on Growth of Sprouts

Code Sample	Growth Parameters			
	Root Length (cm)	High Hypocotyl (cm)	Number of Leaves	Twisted Rod (cm)
G_0S_0	1.26^{gh}	0.18^{l}	0.00^{d}	0.23^{j}
G_0S_1	2.15^{fgh}	2.63^{j}	0.00^{d}	0.50^{fg}
G_0S_2	3.23^{ef}	5.80^{h}	1.00^{c}	0.65^{f}
G_0S_3	2.87^{cd}	9.08^{g}	2.33^{b}	0.81^{e}
G_1S_0	1.43^{gh}	0.53^{l}	0.00^{d}	0.45^{ghi}
G_1S_1	2.75^{efg}	3.21^{j}	0.00^{d}	0.55^{ef}
G_1S_2	4.80^{c}	11.16^{f}	2.50^{b}	0.91^{d}
G_1S_3	12.15^{b}	15.90^{e}	2.66^{b}	1.31^{c}
G_2S_0	1.00^{h}	0.46^{l}	0.00^{d}	0.38^{i}
G_2S_1	2.08^{fgh}	2.45^{jk}	0.00^{d}	0.51^{fg}
G_2S_2	10.96^{bc}	18.24^{d}	2.82^{b}	1.66^{b}
G_2S_3	10.27^{c}	19.30^{c}	2.41^{b}	1.69^{b}
G_3S_0	1.50^{gh}	1.55^{k}	0.00^{d}	0.41^{hi}
G_3S_1	7.20^{d}	4.35^{i}	0.00^{d}	0.55^{ef}
G_3S_2	11.83^{b}	21.49^{b}	2.83^{b}	1.85^{a}
G_3S_3	13.67^{a}	24.95^{a}	3.75^{a}	1.94^{a}

Information: * G0: GA 0 ppm, G1: GA 50 ppm, G2: GA 100 ppm, G3: GA 150 ppm. S0: without scarification, S1: partial scarification of the surface of the seed coat, S2: scarification of the entire surface of the seed coat, S3: injury to the seed coat.
* Numbers followed by the same letter are not significantly different at the 95% confidence level of the Duncan Test.

Root and stem growth is controlled through the molecular mechanism of GA transduction signals by GA receptors and DELLA protein in the roots can inhibit germination because it plays a role in maintaining ABA, so GA plays an activating DELLA protein so that GA can be expressed [12], auxin plays a role in degradation DELLA in root cells due to responses from GA, so GA can be induced to lengthen roots [13]. The formation of shoots is influenced by the work of several hormones including IAA, cytokinins, and GA [14]. GA's main physiological role is to promote the growth of many plants. Gibberellins play an important role in the vegetative growth stage [15]. As reported by. [16] 500 ppm GA3 treatment before sowing can increase the hypocotyl length of apple plants. Dahayanake and Galwey [17], explained that GA increased the stem length of Brassica vary. Annual, through increased cell wall plasticity followed by the hydrolysis of starch to sugar thereby reducing the potential for water to enter cells and cell elongation [18]. Gibberellins have a role in differentiation, growth, and development [19]. With this special function, giving exogenous GA can increase the number of nutmeg sprout leaves. Equivalent to research by Wani et al. [16], that 150 ppm GA3 treatment increased the number, area, and length of leaves of apple plants. Other studies by Hasson et al. [20], reported that RGA plays a role in initiating leaf primordia by suppressing the activity of the KNOX1 protein which plays a role in activating GA by the ARP gene. Besides, when the GA leaves are formed it acts as a precursor to elongation of the stem [21]. Hormones generally work at low concentrations, the higher the concentration can act as an inhibitor for plants. But GA has a wide concentration range, even high GA content is not toxic. The use of GA can affect the size of plant organs through the process of cell division and enlargement [22]. So that in this study, the use of 150 ppm GA showed more optimal results compared to other GA treatments.

Morphological and Anatomical Nutmeg Seeds

Nutmeg is known as a plant that has seeds wrapped in fruit flesh and has arilus (mace) is a part that covers the shell, red, and fleshy and cover the skin of the seeds [23]. Arilus (seed coat) generally comes from funiculus which is the connecting part between the seeds and the ovaries. Nutmeg consists of hard skin and thick enough to reach 1-1.3 mm more shiny and slippery when cooking. When the seeds mature, the outer epidermal cells appear to elongate in the radial direction and the thickening of the walls in the direction of the cell length is seen at all corners of the cell. Epidermal cells are small, square cells with thick cuticles. The inner epidermis in the form of compact palisade cells stretches radially with thin cell walls. The parenchyma cells are round and contain tannins. While the sclerenkim cells contain lignin. Seed coat has a transport network that is spread on the parenchymal tissue. In young seeds, the endosperm is located inside the seeds, divided into several radial-shaped parts that enter the perisperm (ruminate endosperm), which is the soft part and is thin-walled parenchyma cells. It has calcium oxalate in the form of a prism, sometimes seen on the outer perisperm [24]. While in mature seeds, perisperm becomes separated due to loss of cell contents and cell walls thinning so that it merges with endosperm. Endosperm consists of large parenchyma cells and has a thin wall and contains a lot of starch.

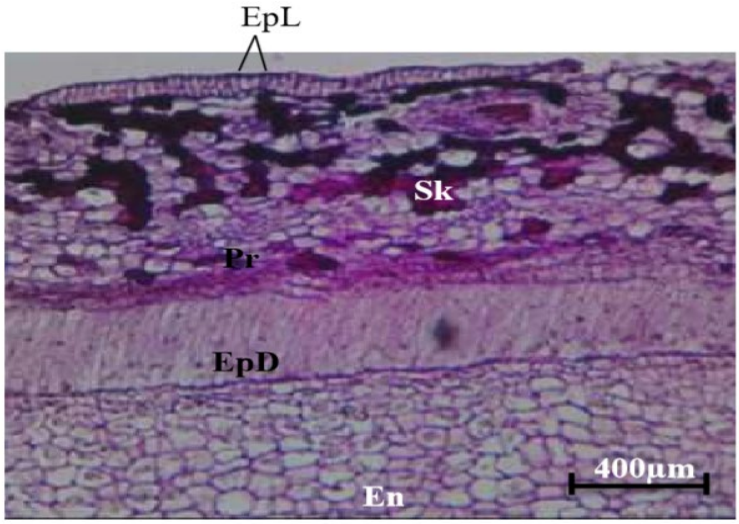

Fig 1. Transverse slices of nutmeg shell (Myristica fragrans Houtt.)
Note: EPL- (outer epidermis), Pr- (parenchyma), EPD- (inner epidermis), En (endosperm), Sk (sclerenkim).

Embryos in dicotyledonous plants, including nutmeg, have two cotyledons so that they divide symmetrically. The apex of the curve between the two cotyledons functions to compile an epicotyl apical meristem. The upper pole of the embryo will differentiate into protoderm, procambium, and radicles. The results of observations of the anatomical structure of adult nutmeg seeds that show a perfect development round (globular). The embryo is composed of parenchymal cells and is small in size with 1/8 of the length of the seed and the width of the embryo is about ¼ of the width of the seed.

IV. CONCLUSION

The conclusion of this research is the scarification and administration of GA and the combination of both spur nutmeg seed germination and germination growth, the main obstacle of nutmeg seed germination is caused by thick and hard seed coat because it contains a lot of lignin in the sclerenkim cells.

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