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Fruit and Seeds Development and Seed Germination of Andaliman Pepper (*Zanthoxylum acanthopodium* DC.)

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Abstract

Andaliman is a species found in the Lake Toba region of North Sumatra, Indonesia. To determine its optimal harvest time and the length of its physiological maturity, it is necessary to observe fruit development in new locations outside its natural habitat. Andaliman seeds are known for their low germination rates and are classified as endospermic seeds. This study aims to analyze the stages of fruit and seed development, evaluate the effects of seed treatments, and identify the most effective germination test methods for andaliman seeds. Seeds were treated with a 50% H_2SO_4 solution for 15 min and with a 200 ppm GA_3 + 100 ppm kinetin solution for 48 hr. The seeds were then tested using pleated paper and top paper media to assess germination response. Changes in fruit coloration indicated various stages of development: intense yellowish-green at 17 weeks after anthesis, deep yellowish-green at 20 weeks, deep red at 23 weeks, and dark greyish-yellowish-brown at 26 weeks. During development, some fruits experienced embryo abortion, resulting in many empty or wrinkled seeds. The seeds reached physiological maturity at 23 weeks after anthesis, as indicated by a deep red fruit color, increased seed dry weight, and enhanced germination and seed growth rates. The treatment with GA_3 and kinetin yielded the highest germination rate of 20%, with the pleated paper method proving to be the most effective for germination testing.

Keywords: gibberellic acid, imbibition, phenology, seedling, viability

Introduction

Andaliman (*Zanthoxylum acanthopodium* DC of the Rutaceae family), in Indonesia, is known as 'Batak pepper', a cultural endemic species around Lake Toba, North Sumatra Province, Indonesia. andaliman can grow at altitudes of 1,000-1,400 m above sea level, in temperatures ranging from 15 to 20 °C, and in open land conditions with high sunlight intensity or secondary forest land with low to moderate acidity (Kholibrina & Aswandi, 2021). Andaliman has many benefits, including its use as a traditional medicine for pain and gastric disorders (Aswandi & Kholibrina, 2020), its anticancer properties (Anggraeni, 2020), its antimicrobial effects (Djuang et al., 2022), and its use as a seasoning (Kholibrina & Aswandi, 2021). The current market demand for andaliman is on the rise. The Samosir Regency Government, in collaboration with the Ministry of Villages and PT Astra International Tbk, successfully exported 770 kg of andaliman to Germany and France in 2024 (Iskandar, 2024).

Efforts to expand and improve andaliman production are necessary to meet market demand. Developing andaliman production outside the North Sumatra region presents both opportunities and challenges for meeting market demand for andaliman. However, a constraint in the development of andaliman production is low seed germination. Siregar (2013) reported that soaking andaliman seeds in warm water at 60 °C, allowing them to cool for 24 hr, and replacing the water led to a seed germination of 36.25% after

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100 days on a mixed medium of topsoil, sand, and chicken manure. Shofyani and Sujarwati (2020) also reported that soaking andaliman seeds in 75% H_2SO_4 for 60 min resulted in a seed germination rate of 6.68% after 30 days on a cotton medium moistened with distilled water. The thick, hard testa of andaliman seeds accounts for their low germination percentage (Nurlaeni et al., 2021). Soaking in almost-boiling water or acid is the recommended method for improving the germination of hard seeds (International Seed Testing Association, 2021).

Planting in locations other than where andaliman originates will be subject to environmental factors that can affect plant growth and/or fruit development. Growing environmental factors, such as temperature, soil moisture content, and irradiation duration, can influence fruit and seed development (Sripathy & Groot, 2023). Information on fruit development is an important aspect that can aid plant cultivation, specifically in terms of how long it takes for the fruit to reach physiological maturity of the seeds and achieve harvest maturity. Observations of andaliman fruit phenology across different locations are essential for determining the optimal harvest time. Observations of fruit development are also widely made on other crops. *Zanthoxylum bungeanum* fruit matures 100 days after anthesis and has a maximum seed dry weight of 115 days after (Lu et al., 2024). Seeds that have reached physiological maturity have achieved their maximum seed dry weight, germination, and vigor (Murrinie, 2022). Siregar et al. (2023) reported that the andaliman fruit reaches physiological maturity 28 weeks after anthesis, with signs of a red epicarp.

Increasing the germination of andaliman seeds needs to be a continuous pursuit. Some methods to increase germination include soaking seeds in water, using chemicals, applying growth hormones, stratification, and scarification (Rahmawan et al., 2023). Few studies have investigated GA_3 and kinetin treatments to improve the seed germination of andaliman seeds, and several have examined other *Zanthoxylum* species. GA_3 and kinetin are known to increase seed germination of *Zanthoxylum armatum* and *Zanthoxylum nitidum* (Datt et al., 2017; Wang et al., 2023). Additionally, different testing media must be used to assess their impact on germination results. Based on the International Seed Testing Association (2021) rules, there are four methods for testing germination: the paper method, the sand or organic growth media method, the combination method using paper and sand, and the soil media method. Seed testing of small seeds typically employs paper media, utilizing either the top paper or pleated paper method. Gundala et al. (2023) explained that the pleated paper method is more suitable for testing small, hard, and thick seeds, such as those of sambiloto. This study aims to determine the phase of fruit and seed development, as well as the effects of H_2SO_4 and GA_3 + kinetin treatments and germination test methods on the germination of andaliman seeds.

Materials and Methods

The research was conducted from February 2024 to April 2025. Observations of fruit development and harvesting were conducted at the andaliman farm in Cipelah Village, Rancabali District, Bandung Regency, West Java Province, Indonesia. The farm is located at an altitude of approximately 1,350 m above sea level, with an average temperature of 22.2 °C, a humidity level of 88.68%, and an annual rainfall of 3,877 mm (National Aeronautics and Space Administration, 2025). The harvested andaliman fruits were dried for approximately 1 week until the dry pericarp cracked, releasing the seeds. The seeds were then extracted and sorted by soaking them in water for two h to separate viable seeds from empty ones. The average percentage of viable seeds is about 41.06%. Viable seeds were selected for seed quality testing.

Experiment 1. Preparation for Andaliman Fruits and Seeds

Observation of fruit development using observation samples from six parent trees, with five flowers selected from each tree.

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Observations of fruit development were carried out from July 2024 to January 2025, including the formation, development, and ripening phases of the fruit. Parameters observed included: length of fruit development period; fruit and seed colour observed qualitatively using RHS colour chart; fruit diameter and seed diameter (transverse side); 100 fruit weight and 100 seed weight; and analysis of growth hormone content using the HPLC (High-Performance Liquid Chromatography) method, following the procedure described by Bhalla et al. (2009).

Hormone analysis was performed at Starlab Analitik Indonesia at room temperature. A total of 500 mg of seeds was pulverized using a mortar and pestle with liquid nitrogen. The samples were then extracted using 20 ml of 65% methanol for gibberellin (GA), a 1:1 methanol–ethanol solution for abscisic acid (ABA), and 20 ml of 80% methanol for cytokinin. The extracted samples were centrifuged at 4000 rpm for 30 min The supernatant was separated from the debris using Whatman No. 42 filter paper and filtered through a Millipore syringe filter. An aliquot of 5-10 µl of the resulting supernatant was injected into the HPLC system for analysis.

Seed viability and vigour tests to determine whether the seeds have reached physiological maturity. The experiment followed a one-factor, completely randomized design, with maturity levels assessed at 20 weeks after anthesis (WAA), 23 WAA, and 26 WAA. Growth measurements included seed moisture content, seed dry weight, seed germination, maximum growth potential, and seed growth rate.

Seed moisture content was measured at harvest. Moisture content was tested at 103 ± 2 °C for 17 ± 1 hr. Each maturity level was repeated four times, with each repetition using ±0.6 g of seeds.

$$\text{Seed MC }(\%) = \left(\frac{(\text{Ww} - \text{Wd})}{\text{Ww}}\right) x100$$

Seed dry weight was conducted at 103 ± 2 °C for 17 ± 1 hr (Ismaniza et al., 2024; Siregar et al., 2023). Seed dry weight was measured after oven-drying.

Seed germination is the percentage of total normal seedlings (NS) that grow up to 60 days after planting (DAP). Each maturity level uses three replicates, each with 100 seeds. The seeds are germinated using the pleated paper method with two layers of filter paper moistened with distilled water in a plastic box measuring 17.5 cm × 12 cm × 4.8 cm. The germination boxes were then placed in a room with a temperature of 25 ± 2 °C, an RH of 60%-70%, and 24 hr of daily lighting. Normal germination criteria were determined based on the completeness of the seedling structure. Germination (%) was calculated by using the formula by Purohit et al. (2015):

$$\text{Seed germination (SG)} = \left(\frac{\Sigma\text{ normal seedlings}}{\text{total seeds sown}}\right) x100$$

The maximum growth potential (MGP) is the percentage of all seedlings that grow both normal and abnormal seedlings, calculated using the formula below. The germination method used is the same as for seed germination variables.

$$\text{Maximum growth potential (MGS)} = \left(\frac{\Sigma\text{ seedlings}}{\text{total seeds sown}}\right) x100$$

The germination rate was measured by counting normal seedlings every day (24 hr) from the day after planting until the last day of observation, calculated using the formula proposed by Sadjad (1994) and Fridayanti et al. (2023). The germination method used is the same as for seed germination variables.

$$\text{SGR} = \sum_{0}^{t} (\% \cdot 24\text{hr}^{-1})$$

t = observation time
% = percentage of normal seedlings at each observation time
$24hr^{-1}$ = observation time every 24 hr

Experiment 2. Treatments to Increase Andaliman Seed Germination

The experiment followed a two-factor, completely randomized design. The first factor was seed treatment consisting of control: soaking with 50% H_2SO_4 solution for 15 min (Purohit et al., 2015), soaking 200 ppm GA_3 +

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100 ppm kinetin for 48 hr (Datt et al., 2017), and a combination of soaking 50% H_2SO_4 solution for 15 min followed by soaking 200 ppm GA_3 + 100 ppm kinetin for 48 hr. The second factor was the germination method using filter paper, consisting of pleated paper (PP) and top paper (TP). Each treatment combination used 100 seeds with three replications. Seeds germinated in a plastic box (17.5 cm × 12 cm × 4.8 cm) at 25 ± 2 °C. The seeds used for testing came from fruits that had reached physiological maturity, as determined by the results of Experiment 1. Observations include determining the normal seedling (as described above), seed germination, maximum growth potential, seed growth rate, and seed weight gain during imbibition.

Seed weight accumulation during imbibition was calculated from measurements taken at specific intervals. Imbibition testing used treatments involving immersion in 50% H_2SO_4 for 0 min (control), 10 min, 15 min, and 20 min. The test was repeated three times, each using 500 mg of seeds.

Statistical Analysis

The data were analyzed using ANOVA in STAR and Minitab. If the test results showed a significant effect, further tests were conducted at the 5% level using the Honest Significant Difference (HSD) and Least Significant Difference (LSD) tests.

Results and Discussion

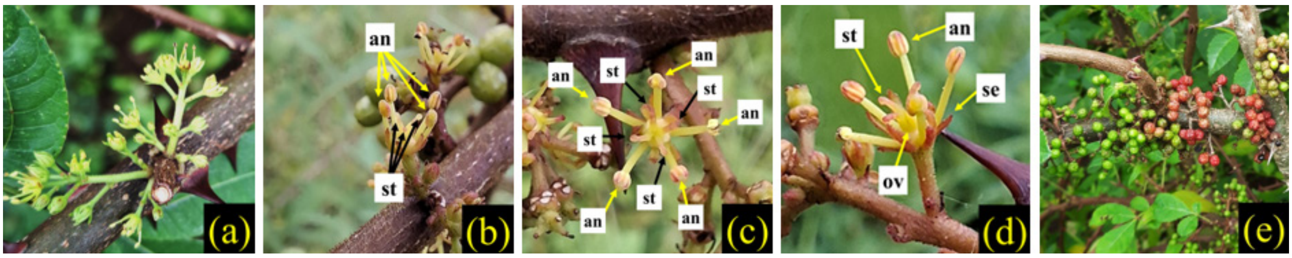
Preparation for Andaliman Fruits and Seeds

Andaliman flowers grow in clusters in a single inflorescence, and there are 7-11 flowers with two lateral flowers facing each other (Figure 1a). According to Endress (2010), this type of flower is classified as a limited dichasial compound flower. Andaliman flowers consist of several carpels attached in a single flower, each with a style and stigma. There are variations in the number of stigmas and anthers, namely flowers with three stigmas and four anthers (Figure 1b) and flowers with four stigmas and five anthers (Figure 1c). The structure of the andaliman flower consists of anthers, stigmas, ovaries, and sepals (Figure 1d), which is in accordance with the research by Siregar et al. (2019) regarding the structure of the andaliman flower. The anthers and stigma are moderate red (GRG 179 A), the carpels are brilliant yellow green (YGG 149 B), and the sepals are deep red (GPG 185 A). Andaliman flowers are tiny, measuring 3-5 mm in diameter. Andaliman plants flower throughout the year, so that on one branch of the tree, there are flowers and fruits in different stages of growth (Figure 1e). Generally, the flowers appear sequentially from the base of the branch to its tip.

The determination of the physiological maturity of seeds is carried out by observing the development and formation of fruit (Table 1). Fruit formation and development co-occur with seed

Figure 1

The Structure of the Andaliman Flower (a, b, c), the Flower Raceme (d), and the Fruit Set Growing on One Branch (e)



Note. st = stigma, an = anther, se = sepal, ov = ovary.

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development inside the fruit, so changes in fruit morphology can serve as an indirect indicator of the stage of seed development until physiological maturity is reached. Seed development consists of three phases: histodifferentiation, food reserve accumulation, and desiccation-ripening. The tissue differentiation phase, which begins fruit formation, starts with fertilization and continues through cell division and embryo formation, causing the fruit to swell (Oballim et al., 2023)

Table 1

Andaliman Fruit Development at Cipelah Farm, Bandung Regency, Indonesia

Fruit formation and development	Description
 0 WAA  2 WAA  6 WAA  10 WAA  12 WAA  14 WAA  17 WAA	Fruit formation: Flower anthesis (0 WAA). Stamen and stigma dry up at 2 WAA but do not fall off. The stamen has fallen off, and the ovary has enlarged at 6 WAA. The fruit enlarges, reaching a diameter of approximately 1 mm at 10 WAA and 1.5-2 mm at 12 WAA, when the fruit is intensely yellow-green (GG 143 C). Fruit size increases rapidly from 12 WAA to 14 WAA. Fruit enlargement slowed down, reaching 4-4.5 mm in diameter and an intense yellow-green (GG 143 B) color at 17 WAA.
 17 WAA  20 WAA  23 WAA	Fruit development: Fruit diameter did not increase significantly, but the colour changed to deep yellowish green (GG 141 A) at 20 WAA and deep red (GPG 185 A) at 23 WAA.
 ≥ 26 WAA	Fruit ripening: After 26 MSA, andaliman fruit begins to enter the maturation drying stage, characterized by a dry pericarp that is dark greyish-yellowish brown (BG N200 A). The dry pericarp will split open (dehiscence), allowing the seeds to separate from it. Dried fruit easily falls from the stem.

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until it reaches its maximum size. Flowers ready for pollination can be identified by their broken anther and bright yellow pollen. After pollination, the stamens and pistils are dry at 2 WAA, then fall off at 6 WAA, and the fruit begins to enlarge. The fruit continues to enlarge significantly until 17 WAA. The food-reserve accumulation phase is the fruit-development stage. This phase starts at 17-23 WAA. Embryo and endosperm enlargement within the seed occurs due to assimilate accumulation in the endosperm, which acts as a food reserve storage tissue to support embryo growth. Therefore, the seed's dry weight continues to increase, and its water content decreases, causing metabolism to slow (Hanapiah et al., 2022). Fruit size has enlarged considerably in this phase, and various physiological changes occur, including color changes. At 26 WAA, desiccation ripening occurs, when the water content of the fruit and seeds decreases further, marked by a dry pericarp that is dark grayish yellowish brown in color. The desiccation ripening phase involves a significant loss of water (Hanapiah et al., 2022) and a decrease in metabolism (Oballim et al., 2023; Pagalla, 2024).

The results of this study's observations showed a shorter fruit development period than that reported by Siregar et al. (2023) in Lingga Raja 2 Village, Dairi Regency, North Sumatra Province, where andaliman fruit reached red colour at 28 WAA and black colour after 30 WAA. Although the planting locations are different and geographically distant, both places have almost identical agroclimatic factors. Cipelah Village, located at 7.19° south latitude and 107.29° east longitude, with an altitude of approximately 1,390 m above sea level, has an average temperature of 22.2 °C, a humidity of 88.68%, and an annual rainfall of 3,877 mm. Lingga Raja 2 Village, situated at 2.83° north latitude and 98.42° east longitude, with an altitude above 1,300 meters above sea level, has an average temperature of 21.9 °C, a humidity of 88.84%, and an annual rainfall of 2,637 mm (National Aeronautics and Space Administration, 2025). The factor that distinguishes the two locations is the cultivation method. The andaliman plantation in Cipelah Village is cultivated more intensively, including garden maintenance, pruning, and scheduled fertilization. Meanwhile, cultivation in Lingga Raja 2 Village, according to Siregar (2022), is carried out traditionally using simple agricultural practices, with almost no fertilization and pruning performed only if branches hinder fruit harvest. Pruning and fertilization are known to affect fruit quality and quantity. Intensive pruning can increase the number of flowers and fruits harvested. Pruning can also increase chlorophyll in fruit and improve fruit quality, while unpruned plants maintain a more constant vegetative growth rate (du Toit et al., 2020; Parniani et al., 2022). Fertilization can increase the age at which flowers open, fruit weight, fruit volume, fresh weight, and dry weight (Sitorus et al., 2023). Fertilization can also increase chlorophyll content in leaves and fruit, thereby enhancing plants' photosynthesis rates (Arifin et al., 2023). Further research is needed to determine the effect of intensive cultivation on the development, quantity, and quality of andaliman fruit.

Andaliman fruit and seeds undergo color changes as they develop (Table 2). At 17 WAA, the fruit is intense yellow-green, and the seeds are intense, reddish-orange with soft testa. When dried, the seeds inside the fruit become wrinkled, so seeds from strong yellow-green fruits cannot be used as seeds. Fruits at 20 WAA are deep yellowish green, and the seeds are black with a hard testa, while fruits at 23 WAA are deep red with black seeds. Fruits at 26 WAA are dark grayish yellowish brown due to the dried pericarp. The color change from green to red in *Zanthoxylum bungeanum* fruit occurs due to an increase in anthocyanin content (Zhang et al., 2024), because of chlorophyll degradation and carotenoid biosynthesis during fruit ripening (Chen et al., 2024). The same phenomenon is also thought to occur during the ripening of andaliman fruits. Fruit color is a reliable indicator for determining the optimal harvest time for seeds. Therefore, it is necessary to determine the fruit's color when the seeds reach physiological maturity.

Andaliman seeds are classified as endosperm seeds because the endosperm

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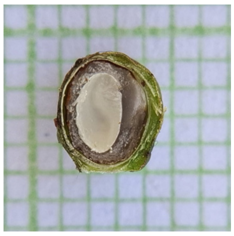
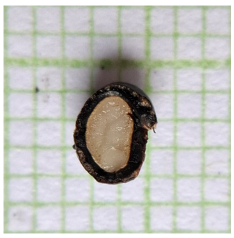
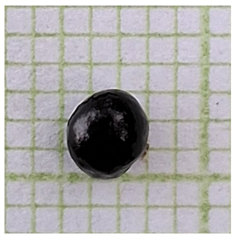
formed at fertilization persists or remains until the seed matures. The embryo has two cotyledons surrounded by a relatively thick endosperm. The embryo is tiny, surrounded by endosperm (Figure 2a). The andaliman seed embryo is white with round and flat cotyledons (Figures 2b, 2c). Endosperm seeds in dicotyledonous plants occur because the endosperm, as a food reserve storage tissue, is not entirely absorbed by the embryo when the seed reaches maturity. This is different from dicotyledonous seeds in general, whose cotyledons take over the function of storing food reserves after the endosperm is completely absorbed. Some dicotyledonous plants are known to retain endosperm that functions as a food reserve for germination, such as *Jatropha curcas*, *Ricinus communis*, and *Alrurites moluccana* (Setiawan et al., 2021).

Normal andaliman seeds contain endosperm and embryos (Figure 3a). However,

Table 2

Andaliman Fruit and Seed Development in Cipelah Farm, Bandung Regency, Indonesia

Maturity level (WAA)	Whole fruit	Fruit cut (longitudinal)	Seed
17	 Intense yellow green (GG 143 B)		 Intense reddish orange (GOG 169 A)
20	 Dark yellowish green (GG 141 A)		 Black (BG 203 A)
23	 Dark red (GPG 185 A)		 Black (BG 203 A)
≥26	 Dark greyish yellowish brown (BG N200 A)		 Black (BG 203 A)

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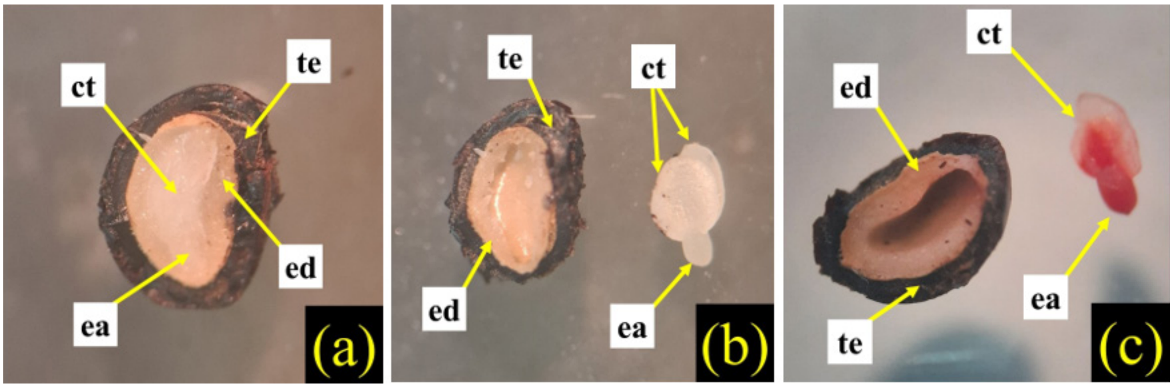
some seeds were found to have embryos with abnormal structures, namely a lack of a clear embryo axis and cotyledon (Figure 3b). Additionally, seeds with embryo sacs containing tissue fragments are believed to be remnants of embryo decay (Figure 3c). Therefore, seed sorting must be done carefully to separate seeds without embryos or with abnormal embryos from viable seeds. A species of the same genus as andaliman, *Zanthoxylum armatum*, also has a low percentage of viable seeds, ranging from 24% to 36.5% (Datt et al., 2017; Purohit et al., 2015). The large number of empty seeds in *Z. armatum* is caused by gene overexpression, which can trigger premature ripening, parthenocarpy, and modifications in the flower organs (Tang et al., 2022). Embryo abortion can also occur because of environmental stress, for example, in *Paeonia ludlowii* plants (Chen et al., 2022). More detailed research is needed to identify the causes of embryo abnormalities and empty seeds in andaliman grown in Cipelah Village.

Fruits at 23 WAA had the largest diameter and heaviest weight of 100 fruits and did not differ significantly from those at 20 WAA (Table 3). Fruit diameter at 26 WAA was not measured because the pericarp had already cracked and dried. Seed diameter at 23 WAA increased, higher

Figure 2

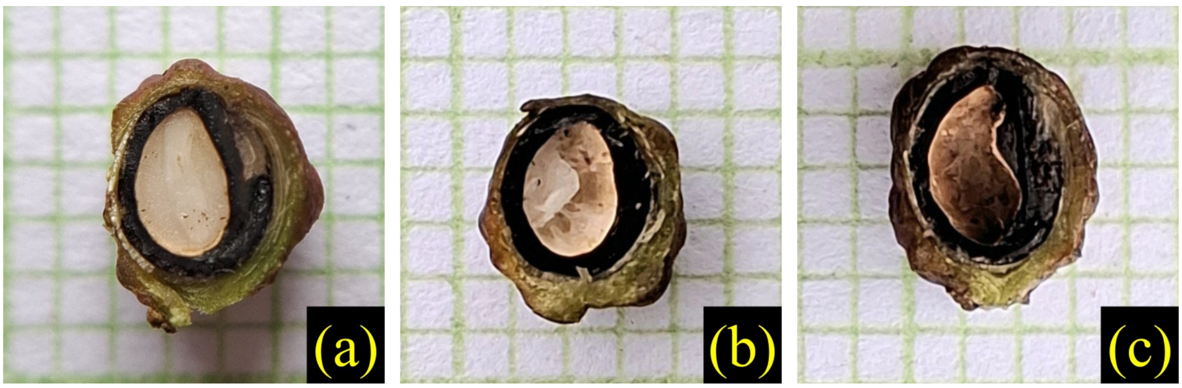
The Internal Structure of Andaliman Seed. Seed with Embryo Still Attached (a), Seed with Detached Embryo (b), and Seed with Detached Embryo that has been Tested for Tetrazolium (c)



Note. te = testa, ed = endosperm, ct = cotyledon, ea = embryo axis.

Figure 3

Andaliman Fruits with Normal (a), Abnormal (b), and Empty (c) Embryos



Note. ep = epicarp, me = mesocarp, te = testa, ed = endosperm, em = embryo.

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than at 20 WAA, and did not increase further at 26 WAA. This indicates that the accumulation of food reserves had slowed or even stopped. The weight of 100 seeds increased from 17 WAA to 23 MSA but decreased to 26 WAA, indicating the accumulation of food reserves up to 23 WAA. The decrease in the weight of 100 seeds at 26 WAA is thought to be due to desiccation during ripening. However, it could also be caused by the breakdown of food reserves during seed deterioration in the field. This deterioration occurs because the seeds have reached physiological maturity but have not yet been harvested. Prolonged delay in seed harvesting in the field can accelerate deterioration, reduce seed vigor and viability, and increase the risk of pathogen attack (Martins et al., 2024). Further research is needed to clarify this matter.

Fruits at 23 WAA and 26 WAA had a lower ABA/GA ratio than fruits at 20 WAA, but black fruits had a higher ABA/cytokinin ratio (Table 4). The roles of ABA and GA in seeds are opposite: ABA induces dormancy, and GA promotes germination. A high ABA/GA ratio will increase dormancy, and a low ABA/GA ratio will encourage seed germination (Toora et al., 2024). The role of cytokinin hormones is to inhibit ABA activity (Huang et al., 2018). Lower cytokinin hormone content cannot inhibit ABA, resulting in seed dormancy. The addition of exogenous growth regulator hormones is predicted to enhance germination of andaliman seeds.

Seeds used for viability testing were seeds at 20, 23, and 26 WAA (Table 5). Seeds at 23 WAA had the highest dry weight among the maturity levels. Dry weight is related to the availability of food reserves in seeds; the higher the dry weight, the greater the food reserves available. Nutrient absorption and accumulation in seeds increase during seed development. Seed dry weight increases rapidly when fruit growth begins to slow or stabilize, so dry weight accumulation is commonly used to reflect the stage of fruit maturity and development (Lu et al., 2024). Seeds at 26

Table 3

Fruit Diameter and Weight, Seed Diameter and Weight, Harvest Moisture Content, and Seed Dry Weight of Andaliman Seeds at Different Maturity Levels

Maturity levels (WAA)	Fruit diameter (mm)	100 fruits' weight (g)	Seed diameter (mm)	100 seeds weight (g)
17	4.03 b	3.72 b	2.23 b	1.11 b
20	4.24 a	4.08 a	2.26 b	1.22 ab
23	4.26 a	4.22 a	2.40 a	1.29 a
26	-	1.70 c	2.39 a	0.83 c

Note. Values followed by the same letter in one column indicate no significant effect on the HSD test at $p < 0.05$; WAA = week after anthesis.

Table 4

Gibberellic Acid, Abscisic Acid, and Cytokinin Content of Andaliman Seeds at Different Maturity Levels

Maturity levels (WAA)	Gibberellic acid (GA, ppm)	Abscisic acid (ABA, ppm)	Cytokinin (ppm)	ABA/GA ratio	ABA/cytokinin ratio
20	6.13	6.65	2.39	1.09	2.79
23	6.63	6.46	2.24	0.97	2.88
26	5.86	5.68	1.75	0.97	3.24

Note. WAA = week after anthesis.

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WAA have the lowest moisture content because they have been exposed to sunlight and wind, with the pericarp cracked open. Seeds at 23 WAA had significantly higher seed germination and KCT values than other maturity levels. The lowest seed germination and KCT values were observed in 20 WAA seeds, then increased to 23 WAA, and subsequently decreased to 26 WAA. The 23 WAA maturity level had the highest PTM value and was not significantly different from the 26 WAA maturity level. The seed germination rate within 60 days in this study was very low, indicating that seed treatment is necessary to increase germination. The results of Siregar's (2022) study using alluvial topsoil as a medium showed a germination capacity of 26.25% after 60 days of planting.

Based on the maximum dry weight of the seeds and their higher viability and vigor compared to other stages of ripeness, it is estimated that the physiological ripeness of andaliman seeds is achieved at 23 WAA, when the fruit has turned deep red. Species of the same genus as andaliman, *Zanthoxylum armatum*, take about 6-8 months to ripen the fruit (Agnihotri et al., 2022), while the *Zanthoxylum bungeanum* takes about 115 days after flowering (Lu et al., 2024), during which the red colour of the fruit can be characterized (Zhang et al., 2024). The character of the andaliman, which flowers throughout the year and takes a long time to reach physiological maturity, causes fruit in one tree with a variety of ages, so the use of fruit color indicators is the easiest way used for sorting seed harvesting in the field.

Treatments to Increase Seed Germination

Andaliman seedlings are epigeal with the hypocotyl extending and pushing the cotyledons upwards. In its development, andaliman seedling takes 10 days from splitting the testa until the cotyledons open completely. To date, criteria for normal andaliman seedlings have not been established. However, in this study, normal andaliman seedlings were defined by referring to the general criteria for normal seedlings established by the International Seed Testing Association (2021), namely: seedlings with all important structures such as radicle, hypocotyl, and cotyledon, that are well developed and healthy; seedlings with minimal damage or deficiencies; and seedlings with secondary infections. Normal andaliman seedlings have healthy radicles, straight or slightly curved hypocotyls, and testa detached from the cotyledons, as well as fully open cotyledons (Figure 4a). Abnormal seedlings are damaged, deformed, disproportionate, and rotten. Sprouts that cannot grow normally include those with damaged hypocotyls, short radicles, and testa still attached to the cotyledon, causing the sprout to die (Figure 4b). Seeds that did not grow after the last day of observation were split open to determine their condition. Observation of seeds that did not grow showed fresh seeds with white embryos (Figure 4c) and dead seeds with decaying embryos (Figure 4d).

Evaluation of GP, MGP, and SGR variables identified the best germination test method and seed treatment. The study conducted an ANOVA

Table 5

Andaliman Seed Moisture Content at Harvest, Seed Dry Weight, Seed Germination, Maximum Growth Potential, and Seed Growth Rate at Different Maturity Levels

Maturity levels (WAA)	Seed moisture content at harvest (%)	Seed dry weight (mg per seed)	Seed germination (%)	Maximum growth potential (%)	Seed growth rate (% per 24 hr)
20	56.55 c	5.29 c	0.33 b	1.00 b	0.0123 b
23	41.31 b	7.57 a	4.67 a	6.67 a	0.1627 a
26	27.01 a	6.08 b	1.67 b	1.67 ab	0.0627 b

Note. Values followed by the same letter in the same column indicate no significant effect on the LSD test at $p < 0.05$; WAA = week after anthesis.

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www.j-tropical-crops.com
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test to determine the interaction effect between the germination method and seed treatments. The interaction between seed treatment and germination method had a highly significant effect on SGR. In contrast, the seed germination (SG) and maximum growth potential (MGP) variables had a very significant effect on a single variable. Tables 7 and 8 show the results of further testing using the HSD test on the three variables.

Seed imbibition testing was conducted to determine the water absorption process in andaliman seeds. Kholibrina and Aswandi (2021) stated that the hard testa structure in andaliman can inhibit germination by blocking water absorption and gas exchange. Based on the data in Table 6, the imbibition process in the control seeds, after 6 to 60 hr, resulted in the highest weight gain compared to seeds soaked in a 50% H_2SO_4 solution. Overall, all scarification treatments showed the same pattern in seed water absorption, with high water absorption occurring at 6 hr of soaking. The water absorption rate decreased and was very low at 60 hr of soaking. The test results indicate that andaliman seeds do not pose any obstacles to the water absorption process. The imbibition rate in andaliman seeds indicates that they can still absorb water despite their hard testa. A similar phenomenon also occurs in several other plants of the genus *Zanthoxylum*. Research by Mikell et al. (2022) on *Z. fagara* plants showed that scarified and non-scarified seeds did not differ significantly in water absorption. This indicates that *Z. fagara* seeds can absorb water normally and do not exhibit physical dormancy (Mikell et al., 2024). In contrast, in *Z. rhoifolium*, the imbibition process is hindered by the tegument tissue within the seeds (Corrêa et al., 2022).

Seed treatment with GA_3 + kinetin soaking had significantly higher SG and MGP values than other treatments (Table 7). GA_3 and kinetin treatments also affected the germination of seeds in the same genus as andaliman (Zanthoxylum). *Zanthoxylum armatum* seeds treated with 200 ppm GA_3 + 100 ppm kinetin produced an SG of 72.50% (Datt et al., 2017). Phuyal et al. (2022) also reported that a 1500 ppm GA_3 treatment yielded an SG value of 54.67%. Additionally, a

Figure 4

Normal Seedling (a), Abnormal Seedling (b), Fresh But Ungerminated Seed (c), and Dead Seed (d)

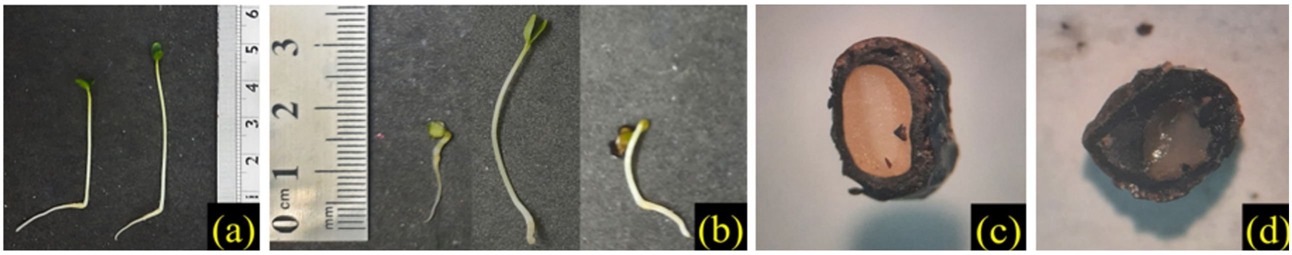

Table 6

Seed Weight Accumulation at Different Soaking Times

Seed treatments	Seed weight accumulation (g) at different soaking times (hr)						
	0	6	12	24	36	48	60
Control	0.500	0.584 a	0.608 a	0.632 a	0.651 a	0.664 a	0.668 a
H_2SO_4 10 min	0.500	0.565 b	0.583 b	0.612 b	0.629 b	0.640 b	0.641 b
H_2SO_4 15 min	0.500	0.567 b	0.584 b	0.614 b	0.631 b	0.644 b	0.644 b
H_2SO_4 20 min	0.500	0.566 b	0.581 b	0.615 b	0.628 b	0.639 b	0.641 b

Note. Values followed by the same letter in the same column indicate no significant effect on the HSD test at $p < 0.05$.

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100 mg/L GA_3 treatment significantly increased the seed germination of *Zanthoxylum nitidum* by 41.7% (Wang et al., 2023). GA_3 and kinetin are hormones that play a crucial role in germination and breaking seed dormancy. GA_3 promotes growth by increasing the plasticity of the cell wall, followed by hydrolysis of starch into sugar, which reduces the water potential in the cell and results in the entry of water into the cell for seed germination (Bareke, 2018), while kinetin, which is one type of cytokinin, has a role in regulating morphogenesis and cell division (Wulannanda et al., 2023). Although the SG value remains generally low, the higher SG value of the GA3 + kinetin treatment compared to the control indicates imbibition in andaliman seeds. Although the GA_3 + kinetin treatment yielded a higher germination rate than the control, it was still low overall. This is thought to be because andaliman seeds have a hard testa, causing mechanical resistance. 'Panggal Buaya' seeds, from the same genus as andaliman, have a hard and rigid testa due to their high lignin content (Purwaning, 2009). Lignin in cell walls provides mechanical support and resistance to various pressures (Liu et al., 2018).

The H_2SO_4 treatment had the lowest SG and MGP values and did not increase germination (Table 7). Shofyani and Sujarwati (2020) reported that soaking andaliman seeds in 75% H_2SO_4 for 60 min resulted in germination of 6.68%, which was not significantly different from the control after 30 days of planting. Research by Phuyal et al. (2022) on *Zanthoxylum armatum* plants also noted that treating seeds with an SG value of 5.33% with 60% H_2SO_4 for 5 min could not break dormancy after 45 days of planting and was not significantly different from the control. Meanwhile, research by Purohit et al. (2015) on *Zanthoxylum armatum* seeds treated with 50% H_2SO_4 for 15 min resulted in an SG of 93.3% after 5 months of planting. H_2SO_4 treatment was also successful in increasing the germination of seeds of another plant, *Ebenopsis ebano*, but was not successful in improving the germination of *Zanthoxylum fagara* (Luera & Gabler, 2022). H_2SO_4 treatment accelerates physical degradation or softens the impermeable seed coat (Luera et al., 2021; Phuyal et al., 2022). However, observing the testa surface of the treated seeds with a microscope showed no significant difference from the control. Seeds soaked with H_2SO_4 still have a smooth testa surface and intact testa structure. The low seed

Table 7

Andaliman Seed Germination (SG) and Maximum Growth Potential with Various Seed Treatments

Seed treatments	Germination methods					
	Pleated paper (PP)			Top paper (TP)		
	Seed germination (%)					
Control	0.75	(6.33)	bcA	0.73	(2.67)	bcB
H_2SO_4	0.72	(1.33)	cA	0.71	(0.33)	cB
GA_3 + kinetin	0.84	(20.00)	aA	0.77	(10.00)	aB
H_2SO_4 → GA_3 + kinetin	0.77	(10.00)	bA	0.73	(3.67)	bB
	Maximum growth potential (%)					
Control	0.76	(7.33)	bcA	0.73	(3.33)	bcB
H_2SO_4	0.72	(1.33)	cA	0.71	(0.67)	cB
GA_3 + kinetin	0.84	(20.67)	aA	0.79	(12.67)	aB
H_2SO_4 → GA_3 + kinetin	0.79	(12.00)	bA	0.74	(4.67)	bB

Notes. Values outside the brackets are the result of the transformation using the square root . Values inside the brackets are the original data; values followed by the same letter in the same column or row show no significant effect on the HSD test at $p < 0.05$; lowercase letters in the column indicate the effect of seed treatment; capital letters in the row indicate the effect of germination method.

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germination of seeds treated with H_2SO_4 is thought to be due to damage to the endosperm or embryo caused by acidic liquid seeping into the seed. A shorter soaking time with a higher concentration may produce different germination results.

The PP method had significantly higher SG and MGP values than the TP method (Table 7). The paper media is commonly used for small seeds. Few studies have applied it to SG tests on andaliman seeds. The PP method is suitable for seeds that require high humidity to germinate, such as *Andrographis paniculata*, which are small and have hard seed coats (Gundala et al., 2023). The PP method is also suitable for germinating seeds that require little light. Bowden and Landais (2018) reported that Radicle Emergence testing of radish seeds using the PP method yielded results comparable to those obtained with the TP method under dark conditions. The TP method is more suitable for seeds requiring light and low media humidity, such as *Eucalyptus pellita* seeds (Yuniarti et al., 2017). Further research is needed to investigate the effects of different germination media, including those beyond paper, on andaliman germination. Additionally, research on the behavior of andaliman seeds is also necessary. No study has classified andaliman seeds as recalcitrant, intermediate, or orthodox. However, in several plants of the same genus (*Zanthoxylum*) as andaliman, it is mentioned that the seeds are orthodox. Corrêa et al. (2021) noted that *Zanthoxylum rhoifolium* seeds are resistant to drought at moisture levels of up to 5% and can be stored at -20 °C, classifying them as orthodox seeds. Furthermore, research by Neya et al. (2017) reported that *Zanthoxylum zanthoxyloides* seeds retain their initial viability after drying at a moisture content of around 4.9%.

The interaction between seed treatment and germination method had a highly significant effect on GR. GA_3 + kinetin treatment using the PP method was the most effective combination (Table 8). The combination of H_2SO_4 treatment with the top paper method showed the lowest SGR value. Seed germination rate is an indicator of seed vigor. Seeds that grow faster will respond faster to light to increase growth (Almanda et al., 2023).

The earliest normal seedling appeared in the GA_3 and kinetin combination under the PP method at 11 days after planting. Based on the data in Table 9, seeds treated with the PP method produce normal seedlings faster than those treated with the TP method. The accumulation of normal seedlings per day with GA_3 + kinetin treatment in the PP method increased until day 24, after which the accumulation rate tended to level off until the last day of observation (Figure 5). Although there was a peak in the accumulation of normal seedlings on day 24, the first count could not be determined because the seed germination was still low. The peak of the addition rate is commonly used as the first-count

Table 8

Andaliman Seed Growth Rates with Various Seed Treatments and Germination Methods

Seed treatments	Germination methods					
	Pleated paper (PP)			Top paper (TP)		
	$(\%.24hr^{-1})$					
Control	0.7693	(0.0929)	bc	0.7903	(0.1293)	bc
H_2SO_4	0.7825	(0.1151)	bc	0.7170	(0.0142)	c
GA_3 + kinetin	1.5157	(1.8072)	a	1.0231	(0.5523)	b
H_2SO_4 → GA_3 + kinetin	0.9703	(0.4519)	bc	0.8319	(0.2054)	bc

Notes. The values outside the brackets are the result of the transformation using the square root ($\sqrt{x+0.5}$); The values inside the brackets are the original data; values followed by the same letter indicate no significant effect on the HSD test at $p < 0.05$.

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benchmark for evaluating germination. Then, after the addition rate slows, it will be used as a reference for the final count (Ningsih et al., 2021).

Splitting the seeds that failed to grow by the last day of observation allowed for examination of their condition. The H_2SO_4 treatment had the lowest percentage of fresh seeds that did not grow and the highest rate of dead seeds compared to other treatments (Table 10). Splitting all of the seeds revealed that none were empty. That indicates that empty seeds resulting from embryo abortion did not cause the low SG value in this study. Therefore, using the tetrazolium test on fresh seeds that do not germinate is highly recommended to ensure their viability. This test can distinguish between fresh seeds that are completely dead and seeds that are still alive but unable to germinate under standard testing conditions. Seed sorting with water makes it easy to eliminate empty seeds from the test.

Table 9

Initial Emergence of Normal Andaliman Seedlings in Various Seed Treatments and Germination Methods

Seed treatments	Pleated paper (DAP)	Top paper (DAP)
Control	31	29
H_2SO_4	20	49
GA_3 + kinetin	11	12
H_2SO_4 → GA_3 + kinetin	12	14

Figure 5

Accumulation of Normal Seedling Growing In GA_3 + Kinetin Treatment

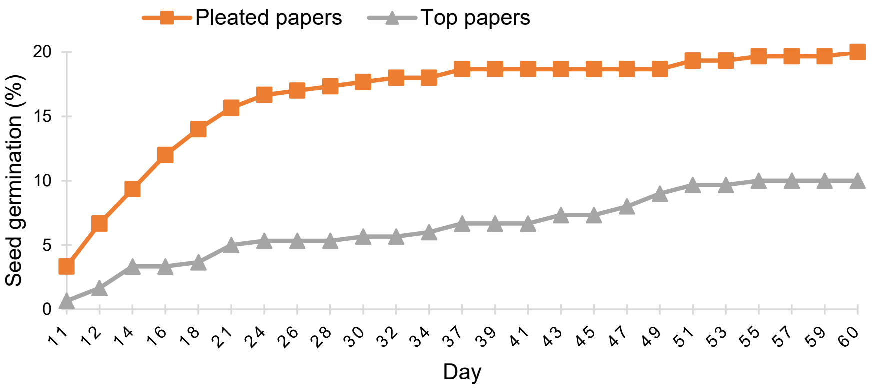

Table 10

Final Percentage of Fresh Non-germinated Seeds and Dead Seeds

Seed treatments	Fresh non-germinated seeds (%)	Dead seeds (%)
Control	95.97 a	4.03 b
H_2SO_4	90.76 b	9.24 a
GA_3 + kinetin	95.74 a	4.26 b
H_2SO_4 → GA_3 + kinetin	96.02 a	3.98 b

Notes. Values followed by the same letter in the same column indicate no significant effect on the HSD test at $p < 0.05$.

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Conclusions

Andaliman seeds reach physiological maturity at 23 weeks after anthesis, characterized by dark red fruit color, higher seed dry weight, and higher seed germination and growth rates compared to other maturity levels. Soaking seeds that had reached physiological maturity in a mixture of 200 ppm GA_3 and 100 ppm kinetin for 48 hr resulted in higher seed germination and greater growth potential than in other treatments. The pleated paper method is more suitable than the top paper method. Seed soaking in a GA_3-kinetin mixture for 48 hr, followed by germination using the pleated paper method, is the most effective method. Further research is needed to determine the cause of empty seeds. In addition, research to improve and accelerate germination, including seed treatment with different media combinations, must also be conducted, especially regarding light requirements during germination. Research on seed behaviour is also needed to determine the classification of Andaliman seeds, whether they are orthodox, intermediate, or recalcitrant.

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