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## Effect of Seed Soaking with Natural Growth Regulators on Germination of Red Rice Line SF 12-2-12

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### Abstract

Rice cultivation in Indonesia involves different types of rice, including red rice, which is known to have higher nutritional value compared to white rice. However, the germination rate of red rice seeds has decreased due to poor storage conditions. Nature Growth Regulators play a critical role in the success of seed germination, seedling establishment and subsequent plant growth. To improve the quality of red rice seeds, natural growth regulators can be used. In a recent study, researchers aimed to determine the best combination of natural growth regulators and concentration level for germination of red rice line. This research was conducted from July to October 2023 at the seed technology and agronomy laboratories of the Faculty of Agriculture, Universitas Andalas. This is a factorial experiment consisting of two factors arranged in random groups design and repeated four times. The first factor was the type of natural growth regulators, namely young coconut water and shallot extract. The second factor was the concentration level, namely 0%, 35%, 70%. The data were analyzed using the F-test with a 5% level of significance. If significant differences were found, Duncan's New Multiple Range Test (DNMRT) was conducted at a 5% level. The observation parameters for the germination test carried out were the maximum growth potential (%), seed germination (%), and hard seeds (%). The application of young coconut water on concentration 70% gave the best percentage of maximum growth potential and was not different with shallot extract on concentration 70%. The application of young coconut water on concentration 70% gave the best percentage of seed germination compare to other treatment.

**Keywords:** Concentration, red rice, shallot extract, young coconut water.

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### INTRODUCTION

Rice (*Oryza sativa* L.) is the main food crop Indonesians consume as a staple, providing carbohydrates and energy. As the demand for rice increases, the quantity and quality of rice consumed is crucial (Yusrin, 2023). In Indonesia, there are various types of rice, one of which is red rice. Public awareness of health is increasing, particularly in urban areas where red rice is being consumed due to its higher nutritional value compared to white rice (Swasti *et al.*, 2020).

Red rice, which is rich in nutrients and minerals, is believed to help combat stunting in children. Stunting is a growth disorder caused by chronic malnutrition that results in height below the average standard (WHO, 2015). Children who are malnourished during pregnancy and early life are particularly vulnerable to stunting, especially due to zinc (Zn) and iron (Fe) deficiencies (Dewi & Nindya, 2017). Red rice has higher crude protein, fiber, antioxidant, and phenolic content than white rice. Red rice has a lower glycemic index than white rice. Red rice content is 10.49% crude protein, 2.71% crude fiber, 143.38 mg Gallic acid equivalent /100 g phenolic content, 120 mg Retinol equivalent/100 gm flavonoid content, and 63.15±2.63 mg/dl glycemic index (Raghuvanshi *et al.*, 2017). In a study conducted by Swasti *et al.*, (2020) on the F5 generation of local red rice, it was found that the protein content ranged from 12.24% to 17.56%, while the food fiber level varied from 1.05% to 4.31%. Moreover, the carbohydrate content was observed to be 66.5122% to 71.987%, and the glycemic index was found to be low, ranging between

46.56% to 54.98%. Compared to commonly consumed white rice, 100 g contains 360 kcal energy, 6.6 g of protein, 0.58 g of fat, and 79.34 g of carbohydrates (Suliartini *et al.*, 2018).

West Sumatra is one of the regions in Indonesia that produces rice, besides Java. As per the latest data from BPS in 2021, West Sumatra contributed 1,450,849 t of rice to the national rice production, with a paddy field area of 3,086.68 km<sup>2</sup>. Although data on red rice production is not yet available, Sumatra is known for its diverse topography and genetic diversity of rice, including red rice. Researchers Swasti *et al.*, (2007) conducted a study on the local rice of West Sumatra and found that there is a wide genetic diversity in both ordinary and red rice. They further characterized ten red rice genotypes based on morphological and agronomic traits (Swasti & Putri, 2010), some of which can be used as gene sources for the assembly of red rice varieties (Swasti & Putri, 2011).

Silopuk is a type of local red rice with a firm texture, with an amylose content of 31.7% and a protein content of 13.3%. However, the plant is tall and deep, with many tillers and small grain size, as observed by Wahyuni in 2017. On the other hand, a new superior variety called Fatmawati has been identified. This variety has the characteristics of early maturity, few tillers, high productivity, a grain size of 29 g per 1000 grains, and sturdy stems (Canto, 2003). In 2016, a crossbreeding experiment was conducted between Silopuk and Fatmawati, which resulted in several recombinants. One of the recombinants has a seed size similar to Fatmawati and a red color like Silopuk. The resulting cross exhibits a wide genetic diversity for protein content 7.08%-16.14% and anthocyanin content 0.04-1.12 ng.mL<sup>-1</sup> (Swasti *et al.*, 2017).

Before 2020, F7 hopeful strains were created by crossing Silopuk (S) and Fatmawati (F). One of the F7 generations produced was the SF 12-2-12 line, which is a type of red rice. However, these red rice seeds have been stored under poor conditions for a long time, causing a decrease in their germination rate. Seeds that are kept in storage for extended periods of time tend to lose their quality gradually. The viability of the seeds is also slightly but noticeably decreased during storage. The embryonic tissue inside the seeds may suffer damage during storage, which does not cause the seeds to die but affects their ability to germinate effectively (Castellón *et al.*, 2010).

Seeds with decreased viability can be improved through invigoration. Seed priming, also known as invigoration, is a method that can help overcome poor germination and crop establishment, particularly in unfavorable growing conditions. Seed soaking is a technique that can be used to invigorate seeds. By soaking seeds, seed respiration can be triggered, which can help plants grow faster (Putra *et al.*, 2012). This technique involves treating seeds in water or an osmotic solution before sowing, allowing them to absorb water and initiate the first and second phases of germination without breaking through the seed coat (Dahamarudin & Rivaie, 2013). Priming with plant growth regulators and various organic sources in low concentration can enhance germination, growth and yield uniformity (Farooq *et al.*, 2009). Additionally, growth regulators can be used to invigorate seeds. When natural growth regulators are applied to seeds, they can help the development of seed embryos and stimulate seed germination (Rusmin *et al.*, 2020). A plant growth regulator is a natural or synthetic organic compound that controls physiological processes in plants. If the compound is naturally produced within the plant, it is called a plant hormone (Ogunyale *et al.*, 2014). One advantage of natural growth regulators is that they are cheaper and easier to obtain compared to artificial growth regulators (Hariani *et al.*, 2018).

Two natural growth regulators that can be used to improve seed quality are young coconut water and shallot extract. Young coconut water is a natural substance that can stimulate and regulate plant growth. It contains two types of hormones called cytokinin and auxin, which have been found to promote the production of plant buds. In 2013, research conducted by La Dahamarudin and A. Arivin Rivaie showed that invigorating seeds with young coconut water improved the germination percentage in several rice varieties as compared to the control group (Dahamarudin & Rivaie, 2013). According to Rosniawaty *et al.* (2018), the cytokinins found in coconut water include kinetin and zeatin, along with auxin, which is essential for encouraging healthy plant development. Similarly, a study conducted by Aisyah *et al.* in 2020 found that a 30% treatment of young coconut water significantly impacted the level of germination and uniformity of growth (Aisyah *et al.*, 2020). According to Aishwarya *et al.* (2022), In plant tissue culture and micropropagation, coconut water was traditionally used as a growth supplement. Its unique chemical combination of carbohydrates, vitamins, minerals, amino acids, and phytohormones can be supported by

the many different uses of coconut water. Because of the significant phytohormones it contains, the plant can develop healthier both now and in the future.

Shallot extract is a natural regulator growth can stimulation germination seed. Soaking rice seeds in shallot solution improves the uniformity, speed of growth, root length, and stem height of local Toraja rice seedlings (*Hanifa & Maintang, 2017*). In 2023, *Prastio et al.* reported that using shallot extract and young coconut water with a concentration of 25% was able to increase the viability and vigor of rice seeds that had deteriorated (*Prastio et al., 2023*). Shallots contain two compounds, auxin and allithiamin. Auxin spurs root growth in plant cuttings while allithiamin, formed from the mashing of shallots, accelerates plant tissue metabolism and acts as a fungicidal and bactericidal agent (*Mariana et al., 2023*). The study was conducted to determine the best type and concentration of natural growth regulators for germination of red rice line.

## MATERIALS AND METHODS

### Experiment Site

This research was conducted from July to October 2023 at the seed technology and agronomy laboratories of the Faculty of Agriculture, Universitas Andalas.

### Materials and Equipment

The materials used in this study were SF-12-2-12 red rice line (F7 generation from a cross between Silopuk and Fatmawati), young coconut water, shallots, aquadest, and soil.

The tools used in this study were scissors, label paper, glass bottles, beaker glass, filter paper, bucket, seed tray, sprayer, knife, aluminum foil, forcep, measuring cylinder, erlemeyer flask, and documentation tools.

### Research Methods

The study was designed as a two-factor experiment with four replications in a completely randomized design. The first factor was the type of natural growth regulators, namely young coconut water and shallot extract. The second factor was the concentration level, namely 0% (aquadest steril), 35%, and 70%. The data were analyzed using the F-test with a 5% level of significance. If significant differences were found, Duncan's New Multiple Range Test (DNMRT) was conducted at a 5% level.

### Research Procedure

#### Seed preparation

The SF 12-2-12 line, an F7 red rice derivative of Silopuk and Fatmawati cross, was used. The line had been stored for about 3 years, and 1200 seeds were treated.

#### Procedure for treating young coconut water

1 liter of young coconut water was mixed with the endosperm. The soft and thin endosperm is blended with the coconut water, resulting in a mixture of endosperm and young coconut water. Making coconut water solution with a concentration of 35% and 70%, by diluting the young coconut water as much as 35 mL added 65 mL of distilled water so that the volume becomes 100 mL (*Viza & Ratih, 2018*). As for the 70% concentration, it is done in the same way by mixing 70 mL of young coconut water with 30 mL of distilled water so that the volume becomes 100 mL.

#### Procedure for treating shallot extract

Shallot bulbs were washed and mashed using a blender in a ratio of 1:1, which means 1000 g of shallots were added to 1000 mL of distilled water without removing the skin. The extract was then poured into an Erlenmeyer flask and left for 24 hr. The extract was filtered using gauze and Whatman filter paper no. 1 to obtain a stock solution of shallot bulb water extract with a concentration of 100%. To make a 35% and 70% solution of shallot extract, the extract was diluted by adding 65 mL and 30 mL of distilled water to 35 mL and 70 mL of shallot extract, respectively, so that the final volume becomes 100 mL (*Fitriah et al., 2020*).

### Seed testing

The seeds are soaked for 6 hr, depending on the type and concentration of natural growth regulator being used. For conducting seed germination tests, soil media is used. The soil media is prepared by mixing soil and manure in a 1:1 ratio in the nursery container.

### The Observed Parameters

The observation parameters for the germination test carried out were the maximum growth potential (%), seed germination (%), and hard seeds (%). Observation of maximum growth potential is done by counting the number of sprouts that grow normally or abnormally. The criteria for normal germination are sprouts that have good root system development, especially primary roots, and for plants that normally produce seminal roots, these roots should not be less than two. Hypocotyl development is good and perfect without any damage to the tissues. Perfect plumula growth with green and well-developed leaves or emerging from the coleoptile. Perfect epicotyl growth with normal buds. And has one cotyledon for monocotyledon sprouts. The criteria for abnormal sprouts are damaged sprouts, without cotyledons, broken embryos, and short primary roots. Sprouts that are deformed, weak, or unbalanced development of important parts. Twisted plumules, hypocotyls, epicotyls, swollen cotyledons, short roots. Broken cotyledons or no leaves or stunted sprouts, soft sprouts (Sutopo, 2002).

According to the International Seed Testing Association (2012), the germination percentage is determined by counting the number of seeds that produce healthy sprouts within a specific time frame and under certain conditions, which vary depending on the type of seed. However, since not all seed types have information available in ISTA, germination can also be determined by counting the number of seeds that sprout normally until a certain time or until no more seeds sprout normally. Seeds that are still hard at the end of the test because they do not absorb water due to the impermeable skin are considered hard-shelled seeds (Sutopo, 2002).

## RESULTS AND DISCUSSIONS

### Zn and Fe Content in Red Rice Line

The SF-12-2-12 red rice line represents the F7 generation from a cross between the Silopuk and Fatmawati varieties. Among the four lines tested, only SF 12-2-12 was capable of germinating after 36 months of storage. This seed has the advantage of containing high iron (Fe) and zinc (Zn) content at the standard level. The results are presented in Table 1.

**Table 1. Zn and Fe Content in Red Rice Line**

Red Rice Line	Zn	Fe
SF 12-2-12	27.80	17.40

According to Table 1, the red rice line SF12-2-12 has a high level of iron content, 17.40 ppm. However, the zinc content of this line is standard 27.80 ppm. Indrasari (2006) reported that the content of Fe and Zn in both local and superior rice ranged between 9.4-16.2 ppm and 20.0-35.0 ppm, respectively. According to Yustisia et al. (2012), iron (Fe) and zinc (Zn) are crucial micronutrients that determine the nutritional quality of rice. However, even in superior-quality rice, these micronutrients are at low levels.

Zinc (Zn) and iron (Fe) are micronutrients that are essential for the growth and development of plants, especially during the process of germination. Zn and Fe play a key role in many metabolic processes in plants, such as photosynthesis, DNA synthesis, and respiration. Seeds that contain adequate amounts of Zinc usually have better germination rates, more vigorous seedlings, and higher yields. However, Zinc deficiency can lead to growth retardation, leaf chlorosis, and decreased nutrient intake (Rashid et al., 2019). Iron is also necessary for properly functioning processes like respiration and photosynthesis. It takes part in the electron transport chain and in the conversion between Fe<sup>2+</sup> and Fe<sup>3+</sup>. Additionally, it is a crucial element for carbon dioxide (CO<sub>2</sub>) fixation (Krohling et al., 2016).

**Maximum Growth Potential**

Based on the F-test analysis at the 5% significance level, it was found that there is interaction between the two types of natural growth regulator treatments and concentration (Table 2).

**Table 2. Percentage of Maximum Growth Potential**

Types of natural growth regulator	Concentration natural growth regulator		
	0 %	35 %	70 %
Young Coconut Water	24,00 a A	19,33 a A	32,00 a B
Shallot Extract	18,67 a A	24,00 a AB	28,67 a B
KK = 13,36%			

Notes: The numbers in the column followed by the same lowercase letter and the numbers in the row followed by the same uppercase letter was no significantly different according to the Duncan's New Multiple Range Test (DNMRT) advanced test at a 5% significance level.

Based on the data presented in Table 2, it can be concluded that the most effective treatment for promoting seed germination is the use of natural growth regulators derived from young coconut water at a concentration of 70%. This treatment resulted in a germination rate of 32%. On the other hand, the least effective treatment was observed at a 0% concentration of both types of natural growth regulators.

In Table 2, it is shown that there is no significant difference between the effects of young coconut water and shallot extract as organic growth regulators on the growth of rice seeds. To determine the maximum growth potential of the seeds, the number of normally and abnormally growing sprouts is counted. *Darmawan* (2007) suggests that seed germination is measured by the ratio of normally germinated seeds to the total number of seeds under specific conditions and duration. Rice seeds undergo deterioration over time, which can be identified by decreased metabolism. Biochemical analysis can reveal metabolic symptoms, while physiological indications such as maximum growth potential, normal sprout dry weight, and germination power can indicate growth symptoms (*Rozen et al.*, 2019). The research results are consistent with *Prastio's* 2023 research, which suggests that the maximum growth potential is not significantly different among the organic materials used for priming. However, the shallot extract stands out as the most effective among all the materials tested, including young coconut water, bean sprout extract, and tomato extract. All of the organic materials used for priming have hormone content that can stimulate seed germination. This is achieved through the reactivation of seed metabolic processes via respiration, which leads to the absorption of water by the seeds (*Prastio et al.*, 2023).

Based on the data presented in Table 2, it can be concluded that the most suitable concentration for processing young coconut water is 70%, as it results in the highest percentage of maximum growth potential compared to 0% and 35% concentrations. This finding is consistent with a study by *Putra et al.*, (2022), which also found that the treatment with a concentration of 75% resulted in the highest potential for soybean seed growth. The result obtained from the 70% concentration is significantly different from the result obtained from the 0% concentration treatment.

According to Table 1, there is a 70% effect of shallot extract concentration on maximum growth potential compared to 0% concentration. However, there is no significant difference in maximum growth potential between 70% and 35% concentrations. According to *Lestari et al.* (2020), higher concentrations resulted in both normal and abnormal sprout growth. The study found that different immersion times had no impact on increasing seed germination, vigor index, maximum growth potential, growth rate, plant height, and the number of leaves. According to *Prastio et al.*, (2023), the concentration treatment of shallot extract, it was observed that there was no significant difference between the 75% treatment and the 25% and 50% treatments. Hormones contained in shallot extract can help the metabolic process of seeds so that they can germinate optimally at maximum growth potential.

### Percentage of Seed Germination

Based on the F-test analysis at the 5% significance level, it was found that there is interaction between the two types of natural growth regulator treatments and concentration (Table 3).

**Table 3. Percentage of Seed Germination**

Types of natural growth regulator	Concentration natural growth regulator		
	0 %	35 %	70 %
Young Coconut Water	21,33 a A	18,00 a A	31,33 b B
Shallot Extract	17,33 a A	22,00 a AB	26,00 a B

KK = 12,99%

Note: The numbers in the column followed by the same lowercase letter and the numbers in the row followed by the same uppercase letter was no significantly different according to the Duncan's New Multiple Range Test (DNMRT) advanced test at a 5% significance level.

Based on the findings presented in Table 3, it can be concluded that the most effective treatment interaction is the use of a natural growth regulator type of young coconut water with 70% concentration. This treatment resulted in the highest percentage of seed germination, which was 31.33%. In contrast, the other treatments did not have a significant impact on the percentage of seed germination. *Primilestari et al.* (2019) found that coconut water and shallot solution invigoration improved the quality of local Jambi rice seeds by increasing sprout length and vigor index.

Table 2 indicates that the highest percentage of seed germination occurred with the young coconut water treatment at a concentration of 70%, as compared to treatments with concentrations of 0% and 35%. Furthermore, when the ratio between young coconut water and shallot extract was evaluated, it was found that young coconut water at a concentration of 70% had the highest percentage of seed germination. This is because the content of young coconut water at 70% concentration is higher than that at 35% concentration. According to *Hedty et al.* (2014), coconut water is a solution that can accelerate the germination process of plants. This is because it contains essential nutrients and growth regulators that play a crucial role in the germination process. The findings of *Putra et al.* (2022) that a 75% concentration of coconut water is particularly effective in increasing the germination of soybean seeds. This is because the hormone content in coconut water is higher in this concentration than in others, such as 0%, 25%, and 50%. Therefore, soaking soybean seeds in a 75% coconut water solution leads to a higher germination rate due to the higher concentration of essential nutrients and growth regulators. The composition of coconut water are IAA (auxin) at 11384.9 ng.mL<sup>-1</sup> and GA3 (gibberellin) at 38734.8 ng.mL<sup>-1</sup>. Additionally, various types of cytokinins were found in coconut water, including trans-zeatin at 7948.9 ng.mL<sup>-1</sup>, ribosyl cis zeatin zeatin glucoside at 4155 ng.mL<sup>-1</sup>, zeatin riboside glucoside dihydrozeatin at 932.7 ng.mL<sup>-1</sup>, dihydrozeatin riboside at 556.7 ng.mL<sup>-1</sup>, and dihydrozeatin glucoside at 930.9 ng.mL<sup>-1</sup> (*Putra et al.*, 2022).

The effects of a 60% coconut water concentration on seed germination, vigor index, and emergence speed are observed. Immersion times of up to eight hours can improve stem girth, fresh weight, dry weight, plant height, number of leaves per plant, and speed of emergence of seeds (*Zainudin & Adini*, 2019). These phytohormones aid in cell division in seeds, speed up the rate of water absorption, and support seed germination, as stated by (*Aisyah et al.*, 2020). In summary, young coconut water is a valuable source of phytohormones that are beneficial for seed germination and growth.

Table 3 indicates that 70% shallot extract concentration has a greater effect on the percentage of seed germination than 0% concentration. However, there is no significant difference in the percentage of seed germination between 70% and 35% concentrations. A study *Tammu & Bermuli* (2022) on the effect of natural growth regulators on seed germination, which includes the use of shallot extract, suggests that higher concentrations can improve germination rates. According to *Silomba* (2006), seed soaking activates the hydrolase enzyme, which hydrolyzes the food reserves in the seeds (endosperm) if there is sufficient water in the seeds. An investigation into the concentration and soaking duration in shallot extract shows that these factors significantly affect the germination power (*Dzakwan et al.*, 2023).

### Percentage of Hard Seeds

According to the results of the ANOVA test conducted at a 5% significance level, there was no interaction between the type and concentration of natural growth regulator on the percentage of hard seeds. Similarly, the Single-factor analysis indicated that there was no significant effect on the percentage of hard seeds (Table 4).

**Table 4. Percentage of Hard Seeds**

Types of natural growth regulator	Concentration natural growth regulator (%)			Mean
	0 %	35 %	70 %	
Young Coconut Water	38,67	50,67	32,67	40,67 A
Shallot Extract	34,67	35,33	39,33	36,44 A
Mean	36,67 A	43,00 A	36,00 A	
KK = 20,09%				

Note: The numbers were no significantly different according to the f test at a 5% significance level

The research shows that natural growth regulators found in young coconut water and shallot extract do not affect the softening of seeds, regardless of the concentration. The percentage of hard seeds ranges between 32.67% and 50.67%. This is likely due to the presence of zinc (Zn) and iron (Fe) in red rice.

Red rice varieties have been discovered to have different levels of essential micronutrients, including Zinc (Zn) and Iron (Fe). These micronutrients play a critical role in many physiological processes in plants, including the regulation of dormancy. Zinc and iron are metals that play important roles in seed germination and maturation. Zinc-containing enzymes such as SOD and catalase help control reactive oxygen species (ROS) levels, allowing seeds to break dormancy (Veena & Puthur, 2022). Iron is involved in metabolic processes such as respiration and photosynthesis and acts as a co-factor for many enzymes (Grillet et al., 2014).

### CONCLUSION

The application of young coconut water on concentration 70% gave the best percentage of maximum growth potential and was not different with shallot extract on concentration 70%. The application of young coconut water on concentration 70% gave the best percentage of seed germination compare to other treatment.

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