

## ***Accelerating Tamarind (*Tamarindus indicu* Linn) Seed Germination by Soaking Treatment Method***

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### **ABSTRACT**

*Tamarind constitutes orthodox seed which is impermeable to water so that it is difficult to be penetrated by water in its imbibitions process. Consequently, the seeds have longer dormancy time so that special treatment is needed to accelerate its seed development. This research was done by soaking the seeds in the PGPR solution with the concentration and the soaking duration which are different from the purpose to accelerate the tamarind seed germination of the tamarind. The research used RAL (complete random sampling) consisted of soaking time (L) – L<sub>1</sub>: 2 hours, L<sub>2</sub>: 4 hours, L<sub>3</sub>: 6 hours, and PGPR concentration (P) which was P<sub>0</sub>; without PGPR concentration (control), P<sub>1</sub>: 10 ml/L of water, P<sub>2</sub>: 20 ml/L of water, and P<sub>3</sub>: 30 ml/L of water. The combination of treatment was 12 combinations, and each of them was repeated 3 times. The tamarind seeds were soaked according to the treatment, dried up, and germinated. The result of the research showed that germinating the seeds in 6 hours with PGPR concentration of 30 ml/L of water could accelerate the seeds until 4 days, 100% of sprouting power, 2.5% of vigor index, and 1% of germination rate.*

*Keywords: Tamarind, PGPR, Seed Soaking*

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### **PRELIMINARY**

Tamarind is a tropical plant which has multifunction as furniture material, charcoal, and spices. It is the plant which yields fruit (Situmorang *et al.*, 2015), and it can be reproduced by its seeds (Tandon *et al.*, 2019) which are included in orthodox seeds with their hard seed coat (Fernanda Imansari & Haryanti, 20017) so that they have impermeable characteristic. The result is that water cannot penetrate the germination process so that it is difficult for the seeds to geminate although they are germinated in the germinating area, and the tamarind seeds will undergo dormancy so that special treatment is needed to break them (Situmorang *et al.*, 2015), This treatment is a pre-treatment of the seeds before they are germinated so that the seeds can grow (Fathurrahman; I Gde Adi Suryawan Wangiyana, 2018) to make them germinate well. Germinating seeds are indicated by the emergence of plumule and radicle of seeds after some processes of metabolism, imbibitions process which can soften the seed coat, activate their hormone and radicle, and breaking food reserves until the embryo establishes plumule and radicle (Gumelar, 2015). During the metabolic process takes place, respiration also continues. Our results confirm that seed pretreatment is an effective technique to increase germination percentage, germination rate, seedling growth, and seed yield. However, if sowing as soon as possible, pre-soaking is recommended to harvest better benefits compared to the hydration-dehydration method (Tian *et al.*, 2014).

A treatment which can accelerate the germination of tamarind seeds is needed to handle their germination. One of the treatments is by soaking the seeds with effective technique in increasing the percentage of germination using plant growth promoting rhizobacteria. The purpose of soaking the seeds



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before germination is done is that endosperm, which is food reserves, can easily be digested after the imbibitions occur in seeds because water will affect the work of alpha amylase. Water can also arrange growth regulating substance to make it balanced. It carries food reserves from endosperm to the point where embryo shaft grows. It is highly needed by seeds for the process of germination because germination will occur when water penetrates to the seeds (Marthen & Rehatta, 2013). Research on PGPR have been done to accelerate germination and vigor index in seeds. Some bacteria found in PGPR, among others, are *Pseudomonas*, *Bacillus*, *Flavobacterium*, *Azospirillum*, *Acetobacter*, *Azotobacter*, *Beijerinckia*, *Rhizobium*, and *Arthrobacter*. PGPR plays its role in synthesizing growth hormone in plants. One of the growth hormones produced is gibberellins, auxin, and cytokinin hormone. The advantage of gibberellins hormone is that it can be used in the process of germination in seeds. The research conducted by Putri, *et. al.*, (2018) reveals that interaction of GA3, gibberellins acid 500 ppm will yield the fastest germination of 6.6 days.

The research conducted by Widawati & Suliasih (2018) reveals that the germination and the growth of sorghum planted in the marginal land can be optimized by using PGPR because PGPR solution can decompose polysaccharide walls of the seed cells to become permeable so that imbibitions process in seeds can occur well. O<sub>2</sub> which enters the seeds can be used in the embryo respiration (Rachmaati & Korlina, 2016). The result of the research conducted by Walida, *et. al.* (2018) shows that the application of soaking by using PGPR can accelerate seed germination 2 days faster than that soaked by using PGPR and 11 days faster than soaked by using water.

This research was conducted to overcome the problem of germination of Javanese tamarind seeds, by soaking the seeds in PGPR solution using different concentrations and soaking time to accelerate germination of tamarind seeds up to 4 days faster than germination without treatment so that the time needed to germinate the seeds could be more fast.

The objective of this research was to solve the problems of tamarind seed germination by using seed soaking treatment in PGPR solution using different concentration and soaking duration to accelerate tamarind seed germination 3 days faster than germination without treatment so that the time used for germination can be faster

## **RESEARCH METHODS**

The research as conducted in the Laboratorium Teknologi Benih (Seed Technology Laboratory), Faculty of Agriculture, Methodist Indonesia University, from January until February 2019. The equipment used in this research was a germination tub, a calibrated beaker, hand sprayer, analytic scale, and a ruler. The materials used were tamarind seeds, PGPR, topsoil, sand, compost, and water. The research used RAL (Complete Random Sampling) consisted of soaking duration (L): L<sub>1</sub>: 2 hours, L<sub>2</sub>: 4 hours, and L<sub>3</sub>: 6 hours, with PGPR (P) concentration: P<sub>0</sub>: without PGPR (control), P<sub>1</sub>: 10 ml/L of water, P<sub>2</sub>: 20 ml/L of water, and P<sub>3</sub>: 30 ml/L of water. The whole combination of treatment consisted of 12 combinations and each of them was repeated 3 times. When the result of the analysis of the variance had significant influence, the next test would be done by using Duncan test in which thin, flat, and elliptical, blackish brown seeds so that when they were soaked in water they will be submerged and not defect. The activities of observation were done, among others, during the time of seed germination (days), the power of germination (%), vigor index (%), IKP (Growth Acceleration Index), and the potency of maximum growth (%). The duration of soaking with different concentration can be seen in the following Figure:

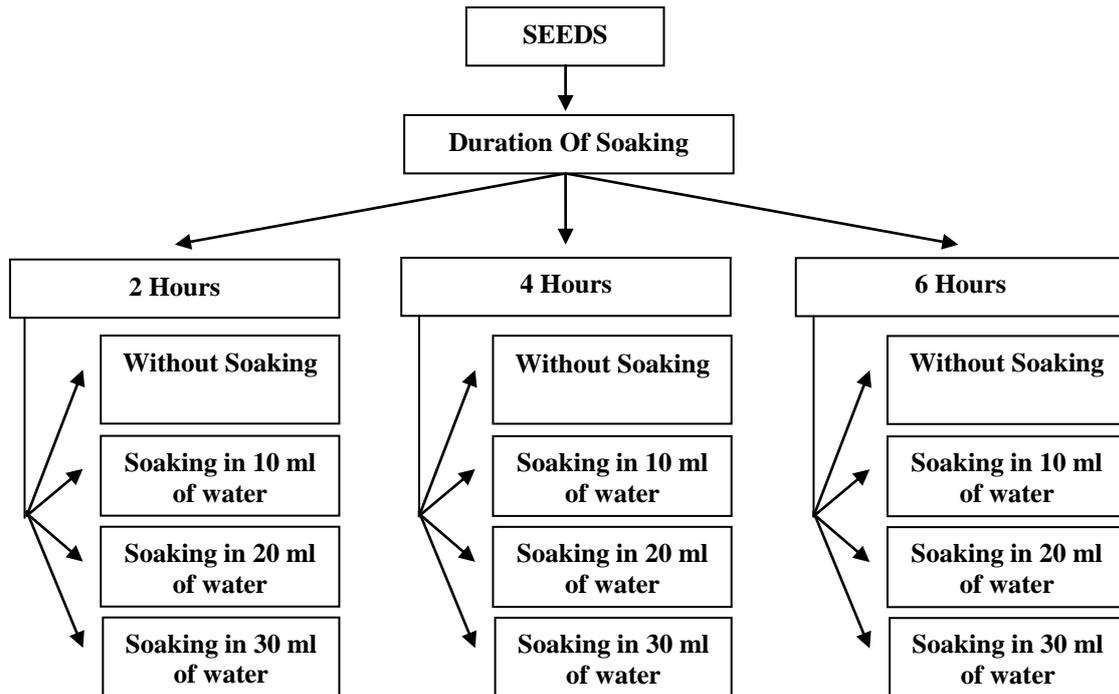


Figure 1. Seed Soaking with PGPR solution and different concentration.

## RESULTS AND DISCUSSION

Germinated seeds constitute a series of changes in metabolism which occurs in morphology, physiology, and biochemistry of the seeds. Good process of metabolism will yield good germination because when the process of germination occurs, the seeds can use food reserves (endosperm) optimally (Juhanda *et al*, 2013). The content in endosperm is one of the internal factors which influence seed germination.

### 1. Seed Germination Time (Days)

Figure 1 shows that the treatment of seed soaking in 6 hours in PGPR with concentration of 30 ml/L of water can accelerate the germination of tamarind seeds until 3 days after the planting (days), compared with soaking treatment within 2 hours without PGPR (7 days). The time of this germination can be accelerated by applying the seeds in solution containing PGPR (Rachmawati & Korlina, 2016) since PGPR contains *Pseudomonas fluorescense* and *Bacillus bacteria*. The immersion carried out allows bacteria to enter through the imbibition process in the seeds (Mangmang *et al.*, 2015b).. Seed immersion treatment in PGPR aims to allow the bacteria contained in PGPR to colonize the seeds as early as possible (Baihaqi A. F; W. S. D. Yamika; N. Aini, 2018). PGPR is a type of bacteria that lives in colonies (Nasib *et al.*, 2016). The bacteria in the PGPR can synthesize various phytohormones such as gibberellins, IAA, and cytokinins. Some phytohormones such as gibberellins will stimulate seeds to germinate so that the breaking or dormancy will occur fast (Lestari, R. Linda, and Mukarlina, 2016).

From the initial figure, it can be seen that the soaking of seeds within 6 hours in the PGPR solution with the concentration of 30 ml/L of water has the time of germination until 4 days and 4 days faster than that of the treatment of soaking time in 2 hours in PGPR solution with the concentration of 10 ml/L of water. This is because the greater the concentration of PGPR, the microbial population contained in PGPR will increase (Marom *et al.* 2017) and the longer the immersion of the seeds will cause the seed coat to become cracked and soft, water containing PGPR will enter the seeds through the seeds. imbibition process so that the plumule and radicle formed are easier to get out of the seed.

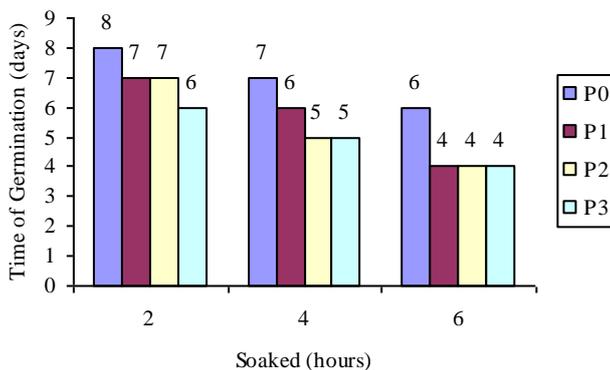


Figure 2. Time of Tamarind Seed Germination.

## 2. Germination Power (%)

Seed germination power is the benchmark of seeds to germinate optimally. It reflects the result of the seedbed from sprouts. The increase in the power of sprouts is influenced by food reserves which can support the germination process. The result of observation in Figure 2 indicates that the treatment of soaking with PGPR concentration of 30 ml/L of water within 6 hours has the percentage of the tamarind sprout power of 100%, and the parameter for sprout power shows the response which has significant influence on the length of soaking and the PGPR concentration. This indicates that there is water absorption which has contained PGPR solution with good imbibitions by seeds. Seed coat which consists of cellulose bonds of polysaccharides on the walls of seed coat will soften so that there will be hydration from protoplasm. It can be seen from the cracked seed coat which makes the seeds enlarged. Seed coat which consists of cellulose bonds of polysaccharides will cause the coat to be hard so that its permeability becomes low to absorb water and oxygen which can curb germination (Lestari, R. Linda, and Mukarlina, 2016).

Along with the process of imbibitions, respiration rate will increase. Rhizobacteria in PGPR solution will help synthesize hormone in seeds so that it can trigger the phase of seed germination (Mardiah, et. al, 2016). One of the hormones which is activated is gibberellins hormone. The activated gibberellins will be translocated to aleuronic layers which yield  $\alpha$ -amylase enzyme which will enter seed endospere (food reserves) and catalyze the process of the changing from starch to glucose which yields energy; it is translocated to the growth point for the growth of sprouts. Amylase enzymes break down starch into glucose, lipase enzymes break down fats into fatty acids and glycerol and protease enzymes break down proteins into amino acids which are then used to form new proteins such as -amylase. In addition, the formation of -amylase is also influenced by gibberellins contained in the embryo (Indriana K. R, 2017). This overhauled food reserves will produce energy which is then translocated to the growing point area for the growth of seed germination.

In Figure 3 it can be seen that the seed soaking treatment for 6 hours had the best germination compared to other treatments. The longer the seeds are soaked, the more they soften the tough seed coat, making it easier for the seeds to germinate. Because the hard seed coat in general can inhibit seed germination. So that before the seeds are germinated, a preliminary treatment is carried out, namely by soaking the seeds in a PGPR solution. By softening the seed coat, the PGPR solution will enter the seeds and accelerate the germination of the seeds.

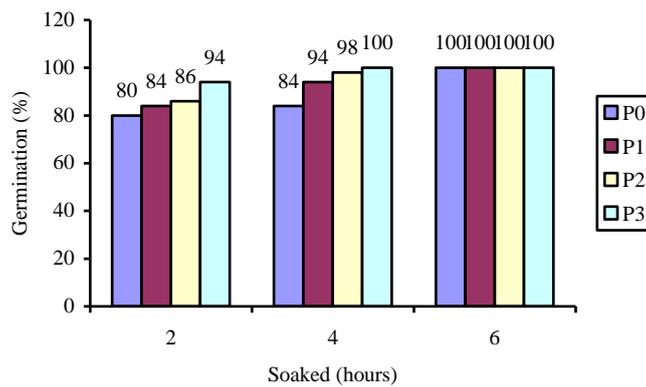


Figure 3. Sprout Power of Tamarind Seeds

### 3. Germination Acceleration Index (%)

The time it takes for a seed to germinate is called the germination rate of the seed. The speed of a seed to germinate can vary depending on the physical, physiological and genetic conditions of the seed, as well as the environmental conditions in which the seed is germinated. One of them is due to the thickness of the seed. Pre-treatment can be given to seeds to break dormancy in seeds. One of the initial treatments carried out was soaking the seeds (Fathurrahman; I Gde Adi Suryawan Wangiyana, 2018). Soaking the seeds serves to soften the seed coat so that the water absorption process in the seeds takes place properly (Melasari et al., 2018). Seed immersion treatment was carried out with the aim of increasing the speed of germination (Walida et al, 2019).

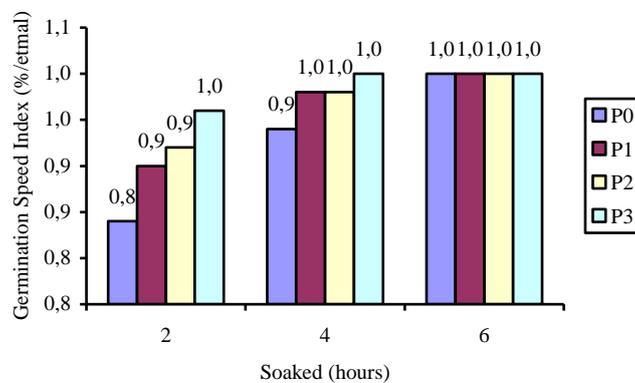


Figure 4. Germination Accelerate Index (%etmal) of Tamarind Seeds.

The result of the research in Figure 4 shows that the highest rate of seed germination acceleration index with the soaking of 6 hours with PGPR concentration of 30 ml/L of water has 1 %/etmal value and the lowest rate is found in the soaking time of 2 hours with PGPR concentration of 10 ml/L of water at 0.8%/etmal value. Seed immersion application in PGPR solution affects the germination rate index. Soaking the seeds in PGPR solution makes the seed coat soften so that it is easier for prospective plumules to penetrate. At the beginning of germination, seeds need water by absorbing water from the environment around the seeds through the imbibition process, then there will be an increase in respiration rate and activate enzymes and hormones contained in the seeds. One of the hormones contained in PGPR is the hormone gibberellins. The gibberellin hormone can accelerate cell division in seeds (Setiawan et al. 2021) so that it can stimulate seed germination (Pertiwi et al., 2016). In the germination process, the hormone gibberellins

will be translocated to the aleurone layer to produce the enzyme amylase. The amylase enzyme will enter the food reserves and convert starch into sugar so that energy is produced followed by the formation of protein compounds and can then be used for the activity of forming new cells in the embryo. After the new cells are formed, the differentiation of cells takes place to form the plumule (future stem) and radicle (future root) (Wahdah et al. 2018).

4. *Vigor Index (%)*

Seed vigor is the characteristics of seeds which provide the indication of normal sprout growth and development in sub-optimal environment (Indriana K. R. & dan R. Budiasih, 2016). A high percentage of seed vigor index indicates that the seeds have good growth abilities (Zakia et al., 2021). The seeds which have high vigor will germinate faster (Gumelar. 2015). Seeds with high vigor will germinate faster. The high seed vigor can be seen from the ability of the seeds to survive various limiting factors that affect seed growth (Latue et al. 2019) such as the longevity of the seeds being stored, preventing pathogens (Wahdah et al. 2018), pest attacks, uniform growth and high production. both in sub-optimal environmental conditions (Kolo & Tefa, 2016).

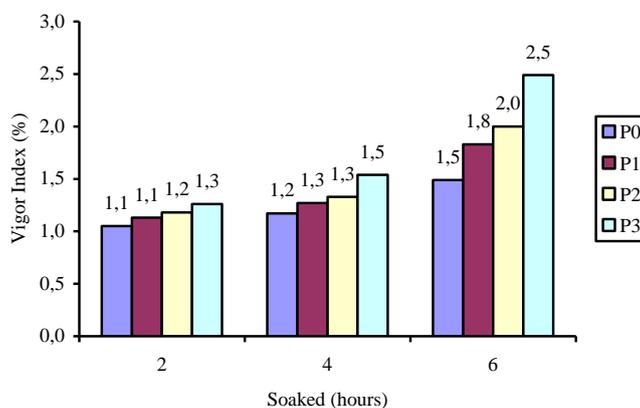


Figure 5. Vigor Index (%) of Tamarind Seeds

Figure 5 shows that soaking treatment with PGPR concentration of 30 ml/L of water within 6 hours can increase vigor index to 2.5% while seed soaking treatment within 2 hours with PGPR concentration of 100 ml/L of water can decrease vigor index to 1.1%. Higher vigor index value can be caused by auxin found in PPR content. Auxin is one of the hormones which can accelerate the establishment of roots in seeds. It is known that PGPR contains *Rhizobium sp.*, *Pseudomonas fluorescens*, and *Bacillus polymyxa* bacteria which can yield IAA to stimulate the growth of roots. The established roots will absorb water and nutrient which will be used in the process of metabolism of the plant.

5. *Potency of Maximum Growth (%)*

Seed germination potential describes the ability of seeds to germinate normally or abnormally. The ability to germinate can be affected by maturity, size, or the presence of dormancy in the seed. A seed is said to have a good maximum growth potential if the seeds can germinate (Fathurrahman; I Gde Adi Suryawan Wangiyana, 2018). The potency of maximum growth is one of the indicators to find out the high viability of seeds (Putri et al. 2018). In this viability it is also seen the ability of growth of normal and abnormal seeds in a certain limit. The increase in the potency of maximum growth is followed by the increase in seed viability (Kolo & Tefa, 2016). The viability of seeds can be seen from the potential value of their maximum growth. This value is influenced by the length and the soaking concentration applied in seeds. Figure 5 indicates that

the treatment of the length of soaking within 6 hours with PGPR concentration of 30 ml/L of water has the potential value of maximum growth up to 100%. The seeds which are sown will grow normally in a certain limit. PGPR will increase induction into the emergence of seeds to 100%. This is caused by the existence of gibberellins and auxin hormones found in PGPR so that they can increase the activity of enzymes such as amylase enzyme in seeds which can increase the availability starch assimilation in the initial seed germination. The capacity of seed to sprout fast is in accordance with the power of sprouts so that they will yield high viability.

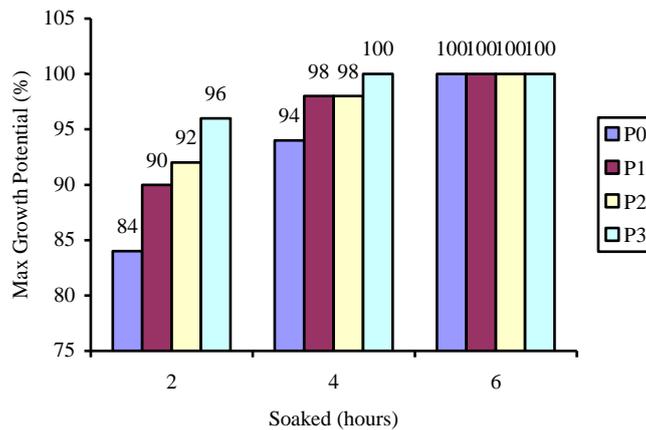


Figure 6. Potency of Maximum Growth (%) of Tamarind Seeds.

From the picture, it can be seen that the best maximum growth potential is found in the treatment of soaking the seeds for 6 hours in a PGPR solution of 30 ml/L of water. The soaking treatment for 6 hours can accelerate the germination time of seeds up to 4 days when compared to soaking for 2 hours with a PGPR concentration of 10 ml/L of water.

## CONCLUSION

The research conclusion is that the treatment tamarind seed soaking in PGPR solution can accelerate the process of seed germination compared with germination without the soaking treatment in PGPR. The application on the seeds for 6 hours with 30 ml/L water concentration of PGPR could fasten the germination process up to 4 days compared to the application on the seeds for 2 hours with 10 ml/L water of PGPR concentration. Seed soaking within 4 hours with the concentration of 30 ml/L takes 5 days while the soaking treatment without PGPR within 4 hours takes 7 days to germinate, and the soaking treatment without PGPR within 2 hours takes 8 days to germinate. The longer the soaking is, the longer the PGPR penetrates to the seeds in the imbibitions process. PGPR application can help accelerate the germination time of the seeds (3 days), increased germination capacity (100%), vigor index (2.5%), germination speed index 1%/etmal and maximum growth potential (100%).

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