# Effect of Seed Biopriming with Pseudomonas fluorescens for Increasing Seed Quality of Kenaf (Hibiscus cannabinus L.) Cultivar KR 14

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# Effect of Seed Biopriming with *Pseudomonas fluorescens* for Increasing Seed Quality of Kenaf (*Hibiscus cannabinus* L.) Cultivar KR 14

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**Abstract**. Kenaf is a seasonal plant that produces fiber with biomass that has the potential to be developed as animal feed at the Animal Feed Tefa of Jember State Polytechnic. The quality of seed that planted can affect crop yield and quality. Seed priming can stimulate germination to overcome the problem of low physiological quality of kenaf seeds due to storage. Trichoderma is one of the biological agents that can be used as a seed priming material. This study aims to determine the effect of biological agents for seed priming on increasing the quality of kenaf seeds. The research was conducted from June to August 2024 at Seed Technology Laboratory of Jember State Polytechnic. The research was arranged using a nonfactorial completely randomized design. The treatment applied was control (without soaking agent), 1 hour seed soaking with *Pseudomonas fluorescens* isolate, 2 hours seed soaking with *P. fluorescens* isolate, and 3 hours seed soaking with *P. fluorescens* isolate. The observations made were Seed Germination (%), Growth Rate (%), Vigor Index (%), sprout shoot length (cm) and root length (cm). The results showed that seed biopriming using *P. fluorescens* increasing seed germination and seed vigor percentage. The recommended seed soaking treatment for seed priming kenaf seeds with low initial viability is soaking for 1 hour.

## Introduction

Kenaf is a seasonal plant that produces fiber as raw material for the paper, textile and automotive parts industries. Currently, kenaf biomass is developed as animal feed. Kenaf can replace alfalfa straw or crude protein from soybean meal up to around 66% in dairy cattle feed [1]. Research on several kenaf lines in Malaysia showed that the leaves crude protein content ranged from 13.6% to 22.3% [2]. Kenaf flour has the potensial for replacing soybean and fish meal as a protein supplement for livestock [3]. Kenaf also has the potential as feed because of its ability to grow on marginal, dry, acidic land [4] and ex-mining land [5].

Jember State Polytechnic as a vocational college has various Teaching Factories (Tefa) that support student learning with conditions that are adjusted to small-scale industries. Dairy Cattle Tefa is one of the Tefa managed by the Integrated Agricultural Production UPA. The problems in managing Dairy Cattle Tefa are increasing dairy cattle productivity and developing types of green fodder. The development of kenaf plants also has the potential to be developed as feed in Tefa Animal Feed.

Developing kenaf plant as feed material can be started with the development of kenaf plant cultivation, especially the availability of kenaf seeds. The simultaneity of seed growth is the quality of

seeds that needs to be considered because it is related to the need for planting materials. Seeds with low physiological quality can be caused by suboptimal seed filling, seed harvest age and seed deterioration during storage. Kenaf seeds can still survive for up to 5 years at 0°C temperature storage, while viability decreases when seeds are stored using 12% Relative Humidity and temperature higher than 10°C [6]. Seed priming is carried out to overcome the problem of low physiological quality of kenaf seeds because storage uses materials that can stimulate germination. Seed priming of kenaf using GA3 50 ppm for 3 hours can improve seed physiological quality [7]. In addition to the use of synthetic gibberellin, research is needed on the application of natural PGPR from Trichoderma sp and *Pseudomonas fluorescens* which are environmentally friendly and can suppress pathogen attacks.

Bio-priming is a priming activity that uses beneficial microorganisms to protect seeds to control diseases and improve physiological quality such as hydrating seeds. The Biopriming method is generally carried out by moistening the seeds and then mixing the formulated bioagent product with the previously imbibed seeds [8]. Seed soaking using *Pseudomonas fluorescens* reduced the number of nematode eggs the most (86.39 %) and suppressed disease intensity by 71.95 % when compared to the control. [9]. Fungal and bacterial bioagents whether they are biofertilizer or biopesticide such as Azotobacter, Rhizobium, Arthrobacter, Agrobacterium, Azospirillum, Enterobacter, Streptomyces, Bacillus, Burkholderia, Klebsiella, PSB, Pseudomonas fluorescence, Trichoderma viride, Trichoderma harzianum and Vesicular Arbuscular Mycorhiza may be useful as biopriming agents. Seed biopriming is useful in almost all crops and ecologically friendly for chemical fungicides substitute [10].The application of biological agents in seed priming is expected to improve the physiological quality and simultaneity of seeds that play a good role in crop production.

Currently, the Sweetener and Fiber Crop Instrument Standard Testing Center is one of the centers that provides certified kenaf seeds and has not met Indonesian market demand. This research needs to be done to improve the quality of kenaf seeds through seed priming and kenaf seed production with fertilization so that seeds with high production and quality are obtained. The quality seeds produced will later be used for research in the following year on kenaf cultivation as animal feed that can be developed in Tefa Animal Feed.

**Method**

The research was conducted from June to August 2024, at the Seed Technology Laboratory of Jember State Polytechnic. The materials used in the study included Kenaf seeds of Foundation Seed Class KR 14 from BSIP-TAS, germination paper, plastic bags, *Pseudomonas fluorescens* isolate, distilled water, labels, and 70% alcohol. The tools used in the study included hand sprayers, scales, measuring glasses, beaker glasses, digital cameras, laminar air flow, autoclaves, petri dishes, micropipettes, pipette tips, Erlenmeyers, and stationery

Seed priming is a method of increasing germination and seed growth simultaneity using materials that can stimulate seed germination. The research was designed with a non-factorial completely randomized design. The treatments applied were control (without a soaking agent), 1 hour seed soaking with *Pseudomonas fluorescens* isolate, 2 hours seed soaking with *Pseudomonas fluorescens* isolate, and 3 hours seed soaking with *Pseudomonas fluorescens* isolate. The treatments were repeated 5 times to obtain 20 experimental combinations. The observations made were Seed Germination (%), Growth Rate (%), and Vigor Index (%), sprout shoot length (cm) and root length (cm). The data were analyzed using ANOVA, followed by a Tukey test at the 5% significance level if significant effects were found.

The propagation of *Pseudomonas fluorescens* isolates was carried out by growing pure isolates in King`s B liquid media (contain of 20 gram protease peptone, 1.5 gram K2HPO4, 1.5 gram MgSO4.7H2O, Glycerol 15 ml, and 1 liter of distilled water) and incubated at room temperature for 24 hours. The purity of the bacterial isolates was indicated by colonies that produced yellowish green pigments as an indication of antibiotic and siderophore compound production. The application of *Pseudomonas fluorescens* isolates for seed soaking was carried out at a concentration of 10% (100 ml isolate/1L water).

Seed quality was assessed using the between-paper method. Seeds that had been given soaking treatment with *Pseudomonas fluorescens* were planted on germination paper. In each replication, 50 seeds were planted. The seeds that had been arranged were covered and rolled in plastic and placed on the germinator. The germinator was set with lighting and was in humid conditions. Germination observations were carried out 7 days after planting. Observation of vigor index (first count vigor index) was conducted 5 days after planting, and observation of growth rate was conducted 6 days after planting. Observation of sprout shoot length (cm) and root length (cm) was conducted on sprouts aged 7 days after planting.

**Result and Discussion**

Research shows that seed soaking treatment using *Pseudomonas fluorescens* isolates has an effect on kenaf seed germination with initial viability having decreased. Commercial kenaf seeds typically have a germination rate of more than 90%. The seeds used in the test had undergone initial testing with an average viability result of 84% and an average vigor index (first count vigor index) of 70%.

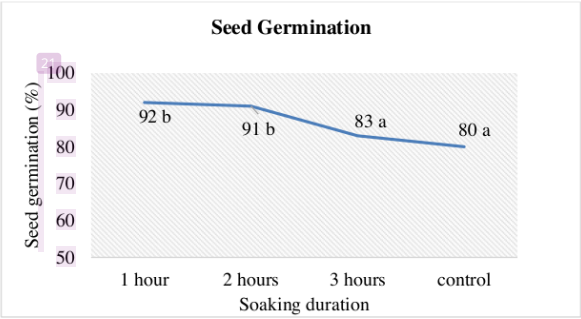


**Figure 1.** Effect of seed soaking using *P. fluorescens* isolate on seed germination

Seed germination is an observation parameter to determine the quality of seeds to be planted. The results of the study in **Figure 1** show that soaking for 1 and 2 hours can increase seed germination compared to the control. Priming can stimulate enzyme activity, metabolism, and promote plant grow faster. Biopriming using *P. fluorescens* enhances seed germination and seedling vigor in chili by improving the germination rate, percentage, mean root length, mean shoot length, and biomass production when compared to seeds that have not been prime [11]. Biopriming with P. fluorescens ensures strong bacterial establishment and adhesion to the seed before planting, making it a recommended treatment to enhance the seed index and improve the growth of sunflower seedlings [12]. Seed priming is an effective, cost-efficient method to boost germination, growth, and the productivity of crops. It involves using various priming agents such as water, inorganic salts, sugars, solid media with water and nutrients, beneficial microbes, micronutrients, hormones, rhizobacteria, and organic substances. Seed priming not only enhances plant growth and yield but also boosts tolerance to abiotic stress [13]. Biopriming seed is a promising and environmentally friendly method to prevent the transmission of bacterial fruit blotch from seeds to seedlings. Liquid or solid matrix priming using *Pseudomonas fluorescens* improved the germination energy for watermelon and germination rate of melon compared with unprimed seeds [14].

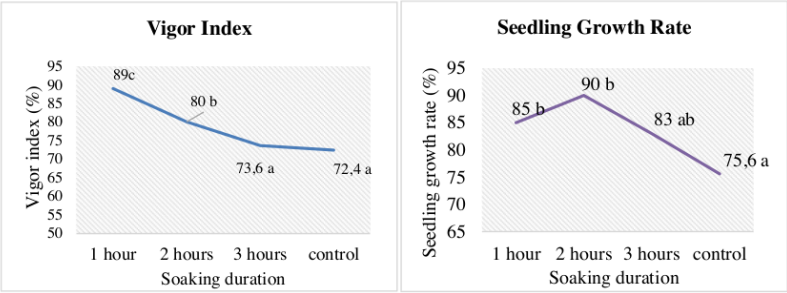


**Figure 2.** Effect of seed soaking using *Pseudomonas fluorescens* isolates on vigor and seedling growth index

Seed vigor refers to the seeds' capacity to thrive in less-than-ideal conditions. There are multiple methods to assess seed vigor, generally using the calculation of the first count vigor index and seedling growth rate. The results of the study in **Figure 2** show that the vigor index calculated at the first count in the seed soaking treatment using *Pseudomonas fluorescens* isolates showed the best vigor index number among the other soaking treatments. However, the seedling growth rate parameter for soaking treatments of 1, 2 and 3 hours did not show a significant result on seedling growth. Priming treatment can help produce uniform sprouts. By stimulating uniform germination, primed seeds produce seedlings with uniform size and growth. This provides an adaptive advantage for plants in dealing with adverse environmental conditions. [15]. Biopriming using *P. fluorescens* is recommended for control of seed-borne diseases. Biopriming chili seeds with *P. fluorescens* has shown effective suppression of diseases [16].

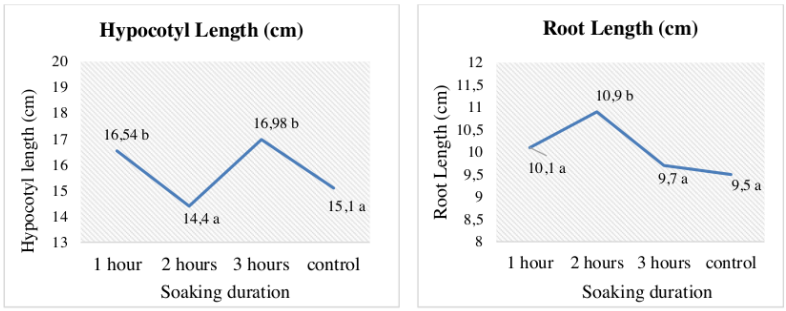


**Figure 3.** Effect of seed soaking using *Pseudomonas fluorescens* isolate on seedling hypocotyl length and seedling root length

Hypocotyl and root lengths are among the parameters used to assess the vigor of seeds being tested. Seeds with high vigor will grow faster than seeds with low vigor. The results of the study in **Figure 3.** show that the 1 and 3 hour soaking treatments affect the average hypocotyl length compared to the

control. The root length parameter shows that seeds soaked for 2 hours have better root lengths than other treatments, but the hypocotyl length is not better than the control.

Overall research results show a tendency that the longer the seed is soaked, the more the seed viability and vigor decreases. This occurs because the seed coat of long-stored seeds starts to deteriorate. Damaged seed coats when soaked in liquid will absorb water too quickly. The imbibition process that is too fast can actually injure the seeds and can actually inhibit germination. The imbibition process, which involves adding water to the seed to increase its water content to a certain level, is the first stage of germination. The imbibition process with the presence of water causes the outermost layer of the seed to rupture. After this procedure, the seed cells will divide and go through a number of biochemical processes until they finally develop into plants. Ideally, seeds undergo imbibition with a slow process so that the cells in the seed are not damaged.

## Conclusion

The study shows us that seed priming using *Pseudomonas fluorescens* can increase the percentage of seed germination and increase seed vigor compared to control. The recommended seed soaking treatment for seed priming kenaf seeds with low initial viability is soaking for 1 hour.

## Acknowlegment

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