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Effectiveness of Gallic Acid Seed Priming on Germination and Early Seedling Growth of Purple Waxy Corn (*Zea mays* var. *ceratina* Kuleshov)

Candra Aya Sofia, Sri Darmanti*, Nintya Setiari
Biology Department, Faculty of Science and Mathemathics, Diponegoro University
Jl. Prof. Jacub Rais, Tembalang, Semarang, 50275, Indonesia.

Corresponding author*
darmantisri@gmail.com

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Abstract

Purple waxy corn (*Zea mays* var. *ceratina* Kuleshov) is a functional food crop with high nutritional value due to its anthocyanin content. Successful crop cultivation is strongly influenced by seed quality, particularly during the germination stage. One approach to improving seed quality and germination performance is seed priming using gallic acid. This study aimed to evaluate the effects of gallic acid seed priming at different concentrations on the germination and early seedling growth of purple waxy corn and to determine the optimal concentration that produces the best response in seed germination and early seedling development. The experiment was arranged in a completely randomized design with a single factor, namely gallic acid concentration, consisting of five levels: 0, 50, 100, 150, and 200 ppm. Each treatment was replicated five times. Data were analyzed using analysis of variance (ANOVA), followed by Duncan's Multiple Range Test (DMRT). The germination parameters observed included seed viability, vigor index, germination acceleration index, percentage of normal seedlings, and percentage of abnormal seedlings. The early growth parameters consisted of radicle length and shoot length. The results showed that gallic acid seed priming at concentrations ranging from 0 to 200 ppm had no significant effect on the germination characteristics of purple waxy corn. However, it significantly affected early seedling growth. The optimal concentration for enhancing seedling growth was 150 ppm.

Keywords: anthocyanins; functional food; gallic acid; seed viability; vigor.

INTRODUCTION

Waxy corn (*Zea mays* var. *ceratina* kuleshov) is a local corn variety originating from South Sulawesi. In Indonesia, this commodity is considered a strategic crop with high economic value and has the potential for further development (Farmansyah et al., 2025). The starch content in the endosperm of purple waxy corn is comparable to tapioca flour from cassava, so this purple waxy corn can be used as a rice substitute (Paweningsih, 2019). According to Mufidah et al. (2021), purple waxy corn has a high anthocyanin content, ranging from 54.00 to 115.05 mg per 100 g. This makes purple waxy corn potentially useful as a functional food source of antioxidants. Seed storage ability is a trait that is genetically controlled and influenced by seed quality at the time of storage, environmental factors before storage (pre- and post-harvest periods), seed moisture content, storage temperature, and storage duration. Seed quality deterioration caused by decreased seed vigor and viability results in low germination ability and seedling growth. Seed deterioration can occur during the pre-harvest, harvest, or post-harvest periods (Khan et al., 2017). To improve seed viability, invigorating treatments can be applied, which are physical or chemical treatments on seeds aimed at improving the vigor and viability of seeds that have deteriorated. Invigoration treatments can be done using priming, coating, or pelleting methods, which serve to increase germination rates and protect seeds from pests or pathogens (Sonhaji et al., 2013). One commonly used method to improve seed quality is priming, also known as osmoconditioning. Priming is a slow hydration process aimed at balancing the water potential of seeds and activating seed metabolism before the germination process starts (Kurnia et al., 2016).

Gallic acid (3,4,5-trihydroxybenzoic acid) is a phenolic compound that is widely found in plants and can act as a strong antioxidant. Phenolic compounds are considered great antioxidants and have been proven to be more effective than vitamins C, E, and carotenoids. Gallic acid protects plants from lipid peroxidation and reduces cell membrane damage by controlling H_2O_2 production and boosting the activity of enzymes that neutralize oxidizing compounds like H_2O_2 (Zeid et al.,

2019). Some studies on the effect of phenolic compound priming on germination and early seedling growth show that priming corn seeds with salicylic acid at a dose of 0,5 mM can improve germination under suboptimal, optimal, and above-optimal temperatures (Bahrabadi et al., 2022). Zeid et al. (2019) reported that priming cowpea seeds with ascorbic acid and gallic acid at doses of 50–300 ppm can increase hydrolytic enzyme activity and radicle length. Shao et al. (2024) stated that the combination of gallic acid priming at a dose of 0 – 20 µM and zinc ferrite nanoparticles affects increasing biomass, chlorophyll content, photosynthesis, and nutrient accumulation so that the plants are more tolerant to salt stress.

From the introduction above, it's important to study the effectiveness of gallic acid priming on the germination and early growth of purple waxy corn seedlings.

MATERIAL AND METHODS

The research was conducted in May 2026 at the Plant Structure and Function Biology Laboratory, Department of Biology, Faculty of Science and Mathematics, Diponegoro University. The materials used were purple waxy corn seeds produced by Panah Merah, distilled water, and gallic acid. The study used a Completely Randomized Design (CRD) with a single factor in the form of gallic acid doses, namely 0 ppm, 50 ppm, 100 ppm, 150 ppm, and 200 ppm. Each treatment had 5 replications.

Procedure

The Preparation of Gallic acid Solution

The preparation of gallic acid solution starts by making a 200 ppm stock solution by dissolving 150 mg of gallic acid in 750 mL of distilled water. Then, the stock solution is diluted according to other treatment doses, which are 50 ppm, 100 ppm, and 150 ppm.

Seed Selection

Before germination, the seeds are selected by soaking them in water for 1 minute. The seeds used are the ones that sink. The selected seeds are primed by soaking them for four hours in the gallic acid solution according to the treatment concentration.

Germination

Germination is done in 200 ml thinwall containers lined with five layers of coarse filter paper and given 3 mL of distilled water. Each treatment has 5 thinwall replicates. Each thinwall is sowed with 10 seeds.

Maintenance

Maintenance is done by adding 1 mL of distilled water at each observation time.

Observation

Observations are made every 6 hours until 100% of seeds in a treatment have germinated.

Variable

Viabilty

Seed viability describes the percentage of seeds that successfully germinate. According to Rusmin et al. (2016), the viability parameter is calculated using the formula:

$$V = \frac{\sum seeds\ that\ successfully\ sprouted}{\sum germinated\ seed} \times 100\%$$

Detail:
V : Viability (%)

Vigor Index

The vigor index describes the seed's ability to grow normally under suboptimal environmental conditions. According to Rusmin et al. (2016), the vigor index parameters are calculated using the formula:

$$VI = \%K \times p$$

Details:
VI : Vigor index
%K : percentage of normal sprouts
P : average sprout length (cm)

Priming Index

The priming index is a value that shows the speed and uniformity of a seedling over a certain period of time. According to Rusmin et al. (2016), it can be calculated using the formula:

$$PI = \sum\left(\frac{the\ number\ of\ new\ sprouts\ at\ time - t}{Time\ (Hour)}\right)$$

Detail :
PI : Priming Index

Length of Radicle and Plumule

The radicle length was measured using a ruler after 48 hours of observation. The length of the plumule was measured using a ruler observed after 96 hours of observation

Data Analysis

The data obtained were analyzed using Analysis of Variance (ANOVA) at a 95% confidence level. If there are significant differences, a follow-up test is done using DMRT (Duncan's Multiple Range Test)

RESULT AND DISCUSSION

The ANOVA test results at a 95% confidence level showed that the treatment did not have a significant effect on the viability of purple waxy corn seedlings.

However, the averages obtained (Table 1) show that the germination viability percentage tends to be higher at doses of 0 ppm, 100 ppm, and 150 ppm. The highest average vigor index also tends to be found at the 150 ppm dose, while the highest average germination index tends to be at the 200 ppm dose compared to the other treatments. Nugraheni et al. (2018) stated that seeds with high viability indicate that the seeds have sufficient food reserves in the endosperm, which are used as an energy source by the seed during the germination process.

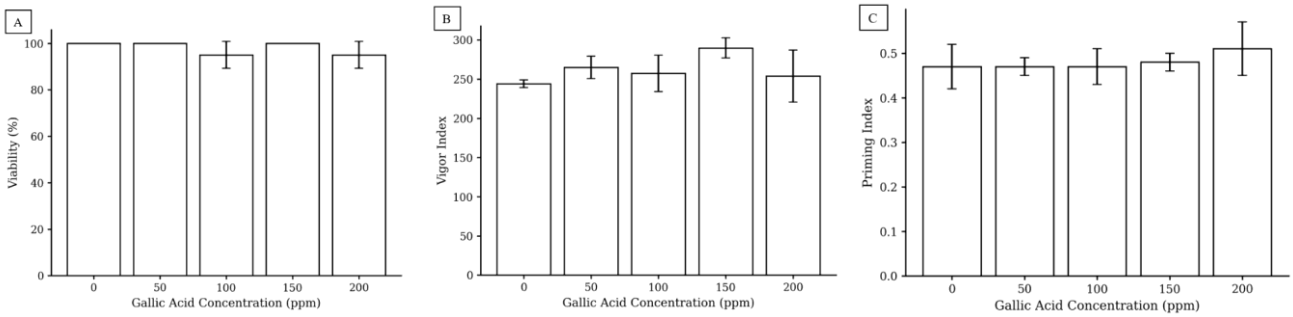


Figure 1. A. Effect of gallic acid concentration on seed viability of purple waxy corn (*Zea mays* var. *ceratina* Kuleshov), B. Effect of gallic acid concentration vigor index of purple waxy corn (*Zea mays* var. *ceratina* Kuleshov), and C. Effect of gallic acid concentration priming index of purple waxy corn (*Zea mays* var. *ceratina* Kuleshov).

As seen in (Figure 1.), gallic acid doesn't seem to affect viability, vigor index, or breaking dormancy index, which suggests that this compound probably isn't directly involved in the process of dormancy breaking or germination initiation. Xu et al. (2024) reported that applying gallic acid dose 0-500 µM on *Arabidopsis thaliana* doesn't affect seed germination, but it does influence the growth of the primary root after germination by regulating meristem cell division and auxin distribution. So, the effect of gallic acid is more noticeable during the seedling growth phase than in the early germination stage. The lack of a significant effect of gallic acid may be attributed to the already high seed viability.

To produce a noticeable effect, different seed types may have different sensitivities. Shumaila et al. (2023) stated that priming rice seeds with gallic acid at various concentrations of 10–60 ppm gave different responses in germination and antioxidant activity. The optimal concentration found was 60 ppm, showing that seed response is very dependent on the dose given. Ullah et al. (2023) stated that gallic acid priming combined with biochar under salinity and boron stress in eggplant (Solanum melongena) improved the morphological, anatomical, and physiological responses of the plants while reducing the impact of environmental stress. Jalil's (2024) study on various red onion varieties shows that the interaction between variety and seed priming treatment produces different responses in germination percentage, growth rate, vigor, and seedling growth, confirming that there are genetic sensitivity differences to the treatments given. Corso et al. (2020) found that each species has a different composition of endogenous phenolic compounds in their seeds. These differences can lead to different responses to the application of external phenolic compounds.

The Duncan test at a 95% confidence level showed that the treatment had a significant effect on radicle length. The radicle length with 150 ppm gallic acid was longer than at other concentrations. At 150 ppm, it produced the tallest shoot length, which was significantly different from 0 ppm, which had the shortest shoot length. However, treatments of 50, 100, and 200 ppm were not significantly different from either 0 ppm or 150 ppm because they were in the same homogeneous subset.

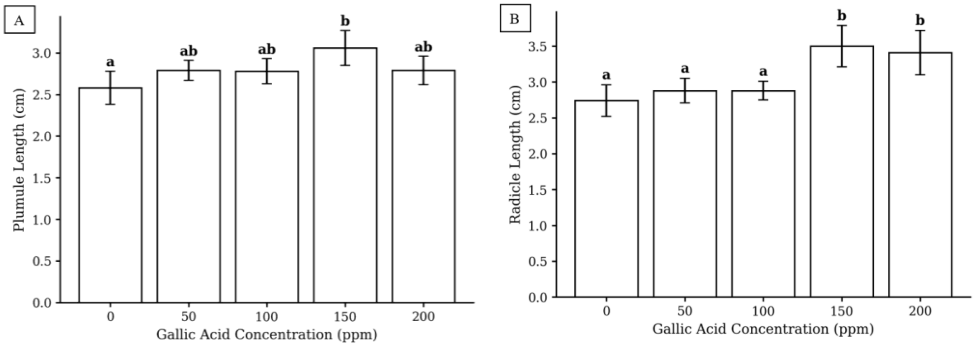


Figure 2. A. Effect of gallic acid concentration on radicle of purple waxy corn (*Zea mays* var. *ceratina* Kuleshov) at hour 48, B. plumule length of purple waxy corn (*Zea mays* var. *ceratina* Kuleshov) at Hour 96.

As shown in (Figure 2.) the 150 and 200 ppm treatments produced longer radicle lengths compared to the other treatments. These results indicate that those treatments were able to stimulate primary root growth during the early germination phase. The radicle is the first organ to appear during germination and plays an important role in water and nutrient absorption. According to Baroi et al. (2024), radicle growth is influenced by cell division and elongation activity that occurs in the root meristem tissue. Longer radicles indicate that metabolism and cell formation processes are more active, thus enhancing the seedling's ability to acquire resources for further growth.

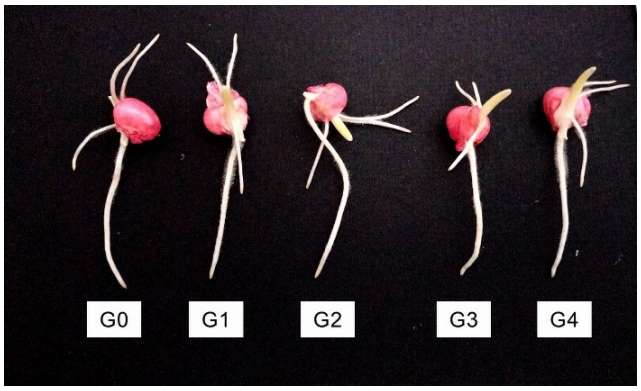


Figure 3. Observation Results of Purple Waxy Corn Germination (personal doc., 2026).

In the early growth of corn plants, the 150 ppm treatment significantly increased radicle length because gallic acid priming can reduce excessive ROS production, which in turn can boost gibberellin hormone production, resulting in maximum radicle and shoot growth. Castro-Camba et al. (2022) showed that early corn growth is determined by the success of cell division and elongation in the radicle and plumule meristem tissues. This process requires energy from the mobilization of food reserves, most of which is regulated by gibberellin hormones. Gibberellins play a role in activating germination metabolism and supporting embryo development during the early growth phase. Research by El-Maarouf-Bouteau and Bailly (2008) explains that the balance of ROS is also very important because ROS in controlled amounts act as signaling molecules that regulate cell growth and hormone interactions, while excessive accumulation can cause oxidative damage to seedling tissues. Ullah et al. (2023) mention that gallic acid, as an antioxidant phenolic compound, can reduce excessive ROS formation, protect cell membranes, and maintain metabolic activity during germination, thus supporting the growth of radicles and shoots.

According to MacDonald and Mohan (2025), gallic acid priming enhances the antioxidant defense system by increasing the activities of superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD), which reduce excessive reactive oxygen species (ROS) accumulation during seed germination. This antioxidant activity protects cell membranes, maintains metabolic processes, and promotes cell division and elongation in the radicle and plumule. Bailly et al. (2008) explained that maintaining ROS within the oxidative window is essential because controlled ROS levels act as signaling molecules that regulate embryo development, whereas excessive ROS causes oxidative damage and suppresses seedling growth. These mechanisms explain why the 150 ppm gallic acid treatment in the present study resulted in the highest radicle and plumule growth.

According to Rahmawati et al. (2025), seed priming with *Spirulina platensis* biostimulant significantly improved seed vigor, germination rate, and shoot length without causing adverse effects on seed germination. These findings indicate that an appropriate priming treatment can enhance early seedling growth by stimulating physiological and metabolic processes during germination. Similarly, the present study showed that priming with 150 ppm gallic acid significantly increased radicle and plumule lengths, suggesting that gallic acid also acts as an effective biostimulant for promoting early seedling development.

CONCLUSIONS

Priming with gallic acid at doses of 0, 50, 100, 150, and 200 ppm had no effect on seed germination, but it did affect the early growth of purple waxy corn sprouts. A dose of 150 ppm of gallic acid is the optimal dose for improving germination and early growth of purple waxy corn sprouts.

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