The Effect of Gibberellin Extracted from *Eichhornia crassipes* Root on the Viability and Duration of Hard Seed Germination

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Abstract. The germination process is influenced by external and internal factors including the type of seed. Hard seeds require special treatment in stimulating the germination process such as scarification and stratification (for example applying exogenous hormones to seeds during the germination process). This study aimed to describe the effect of exogenous gibberellin hormone extracted from *Eichhornia crassipes* root on the viability and duration of germination of three different hard seeds. This study used RBD (Randomized Block Design) with two treatment factors, namely root extract concentration from *Eichhornia crassipes* (0 ppm, 200 ppm, 300 ppm, 400 ppm, and 500 ppm) and hard seed types (sapodilla seeds = *Annona muricata*, soursop seeds = *Manilkara kauki*, and longan seeds = *Dimocarpus longan*) with three replications. The observed parameters for seed viability were germination percentage and vigour index, while duration of germination parameters was germination index. Data were analyzed using ANOVA and followed by Duncan Test. The results showed that there was a significant effect of the root-extracted gibberellin concentrations and types of seeds on viability and duration of germination seeds. The optimal concentration of root-extracted from *Eichhornia crassipes* was 500 ppm, with the highest germination percentage, vigour index, and germination index were 80.55%, 387.33%, and 0.254%/etmal respectively. The longan seeds revealed the best viability and shortest duration of germination due to applying the exogenous gibberellin hormone extracted from *Eichhornia crassipes* root. The findings revealed that there was a positive synergism between exogenous and endogenous gibberellin on the effect of germination process in three different hard seeds tested.

1. Introduction

Every plant has the ability to reproduce, to maintain its type and offspring. Angiosperms are able to reproduce sexually or asexually, leading to the formation of seeds that enable them to survive by producing a new generation [1]. Seed germination is important life stages which contribute to plant distribution and adaptation [2]. The germination processes are influenced by internal and external factors. Cardoso *et al.* [3] report that the presence of light and temperature are the external factors that influenced the seed germination. Other external factors are salt stress and pH [4]. Rezvani and Zaefarian [5] stated that germination was restricted by pH lower and higher than 5 and 8, respectively. Another factor that has an effect on seed germination is endogenous hormone. The endogenous hormones were reported to influence plant growth and development such as seed germination [6, 7].

One of the endogenous hormones that influenced on seed germination is gibberellin [7, 8]. Gibberellic acids are a group of plant hormones that act in the elongation and cell division [9]. In addition, Zang *et al.*[10] stated that Gibberellic acid (GA₃) have an important role in many plant growth and development...
processes such as increasing chlorophyll content, the level of chlorophyll a and b, individual fruit weight, and the number of fertile seeds. The seed germination is a natural process in plant life stage, however, in the coat seeds (hard seeds) have to undergo pretreatment to accelerate the germination [11]. The pretreatment that can be given to coat seeds (hard seeds) are scarification treatments as well as by low-pH solutions to increase the permeability of seed coat [12]. Beside the scarification, another pretreatment that accelerates coat seeds germination is stratification. Stratification is a pretreatment to break down seed dormancy [13].

Based on that description, this study was conducted to determine and to describe the effect of the application of exogenous gibberellin on hard seed germination. In this study, gibberellin was obtained from the extraction of *Eichhornia crassipes* root. Lalitha et al. [14] reported that gibberellin was found on *Eichhornia crassipes* root. Although the roots of the *Eichhornia crassipes* plant contain gibberellin, it needs to be studied further to find out and describe the effect of gibberellin from *Eichhornia crassipes* root extract on viability and duration time of hard seed germination.

2. **Methods**

2.1 **Extract of Eichhornia crassipes Root**
The extraction process started with the sorting of the water hyacinth (*Eichhornia crassipes*) roots and then the roots were washed and dried under sunlight. After that, the water hyacinth roots were dried in oven for 3 days at 60°C. Then the roots were grained to become Simplicia, macerated with a ratio of 1:4 (500g: 2000mL of methanol 60%). After that, the roots were extracted using NaHCO₃ and ethyl acetate to separate chlorophyll compounds and other polar compounds.

2.2 **HPLC Test (High-Performance Liquid Chromatography)**
HPLC test was carried out to detect the gibberellins with a wavelength of 230 nm, then dissolved in 10 ml methanol-water with the ratio of 1:1 with result 2995.50 ppm.

2.3 **Dilution of Giberelin Extract of Eichhornia crassipes**
*Eichhornia crassipes* root extract was diluted to become 500 ppm (0.5 g diluted in 1 L of water); 400 ppm (0.4 g diluted in 1 L of water); 300 ppm (0.3 g diluted in 1 L of water); 200 ppm (0.2 g diluted in 1 L of water).

2.4 **Treatment of Seed Scarification**
Three different hard seeds (sapodilla seeds, soursop seeds, and longan seeds) were rubbed using sandpaper around the eye of the seed (seed embryo) until they were brownish white.

2.5 **Immersion Seeds (Stratification of seeds)**
Three different hard seeds (sapodilla seeds, soursop seeds, and longan seeds) were immersed in solution of gibberellin extracted from water hyacinth root (*Eichhornia crassipes*) for 24 hours; 50 mL each solution.

2.6 **Maintenance of Seeds in Planting Media**
Seeds were planted in media sand and soil with a ratio of 1:1 in a polybag of 3 kg, each polybag contains 4 seeds with a period of observation for 30 days.

2.7 **Data analysis**
The data parameters (germination percentage and vigour index, and germination index) were analyzed by Two-Way Variant Analysis (ANOVA), then followed by Duncan Test.

3. **Results**
The application of gibberellin extract from water hyacinth root (*Eichhornia crassipes*) at various concentrations (0 ppm, 200 ppm, 300 ppm, 400 ppm and 500 ppm) have an effect on germination percentage of three hard seeds (sapodilla seeds, soursop seeds, and longan seeds) (Figure 1).

![Figure 1](image1.png)

**Figure 1.** Effect of Gibberellin Hormone Extracted from *Eichhornia crassipes* Root on the Germination Percentage of Three Hard Seeds. Data represent mean values and standard error of the means (n=3)

The highest percentage of seeds germination at a concentration of 500 ppm of 80.55%, while the lowest percentage of seed germination was indicated at concentrations of 0 ppm and 200 ppm, with the percentage of 16.66% and 27.77% respectively (Figure 1). Longan seeds showed a greater value of germination percentage compared to sapodilla seeds and soursop seeds.

![Figure 2](image2.png)

**Figure 2.** Effect of gibberellin Hormone Extracted from *Eichhornia crassipes* Root on the Vigor Index of Three Hard Seeds. Data represent mean values and standard error of the means (n=3)

Figure 2 reveals that the highest vigour index was the application of 500 ppm concentration of gibberellin hormone extracted from root with a percentage of 387.33% and the low vigour index value was shown by the application of 0 ppm and 200 ppm concentration of gibberellin hormone extracted from root with percentages of 25.55% and 58.66% respectively. Longan seeds showed the highest vigour index of 193.73% compared to sapodilla seeds and soursop seeds with the percentages of 135.60% and 174.53% respectively. Based on ANOVA followed by Duncan Test, it was known that the application of the gibberellin hormone extracted from water hyacinth root with various concentrations (0 ppm, 200
ppm, 300 ppm, 400 ppm, and 500 ppm) have a significant effect on the vigour index in three hard seeds tested.

Duration of germination of three hard seeds was obtained by the parameter of germination index by calculating the total germination days in the three types of hard seeds (Table 1).

### Table 1. Effect of Gibberellin Hormone Extracted from *Eichhornia crassipes* Root on the Germination Index of Three Hard Seeds

| Extract Concentration (ppm) | Types of seed (%)/etmal | Average (%)/etmal |
|-----------------------------|-------------------------|-------------------|
|                             | Sapodilla Seeds         | Soursop Seeds     | Longan Seeds     |
| 0                           | 0.00±0.00<sup>a</sup>   | 0.042±0.006<sup>ab</sup> | 0.043±0.003<sup>ab</sup> | 0.028 |
| 200                         | 0.0407±0.004<sup>ab</sup> | 0.0403±0.002<sup>ab</sup> | 0.058±0.024<sup>ab</sup> | 0.046 |
| 300                         | 0.0817±0.027<sup>bc</sup> | 0.099±0.022<sup>bcd</sup> | 0.0993±0.010<sup>bcd</sup> | 0.093 |
| 400                         | 0.146±0.025<sup>d</sup> | 0.139±0.032<sup>cd</sup> | 0.1483±0.024<sup>d</sup> | 0.144 |
| 500                         | 0.1573±0.017<sup>d</sup> | 0.258±0.054<sup>e</sup> | 0.3467±0.101<sup>f</sup> | 0.254 |
| Average (%)/etmal           | 0.0851                  | 0.1156            | 0.139            | 0.113 |

Different notations (abc) indicate a significant difference between treatments, 0.05 significance level.

Table 1 was shown that the highest germination index value was at 500 ppm concentration of gibberellin hormone extracted from root with 0.254%/etmal. The low value was indicated at 0 ppm and 200 ppm concentration of gibberellin hormone extracted from root with percentages of 0.028%/etmal and 0.046%/etmal respectively. Longan seeds showed the highest germination index value of 0.139%/etmal compared to sapodilla seeds and soursop seeds, 0.085%/etmal and 0.115%/etmal respectively. Based on ANOVA followed by Duncan Test it was revealed that the application of the gibberellin hormone from water hyacinth root extract with various concentrations (0 ppm, 200 ppm, 300 ppm, 400 ppm and 500 ppm) have a significant effect on the germination index of three hard seeds.

### 4. Discussions

Based on the data analyzed, the viability and the duration of the germination of three hard seeds were influenced by the application of the gibberellin hormone extracted from water hyacinth root in various concentrations (0 ppm, 200 ppm, 300 ppm, 400 ppm and 500 ppm) (Fig. 1, 2 and Table 1). These results showed that gibberellins extracted from water hyacinth root has a significant effect on hard seeds germination. Gibberellins are growth hormones that accumulate in the fibre root [15]. Gibberellins are one of the hormones that are needed by plants in their development and growth, especially in the process of germination of seeds before becoming new individual. Gibberellin is an isoprenoid (diterpenoid) compound which is a derivative of the entangling process [16]. The efficiency of exogenous gibberellin via seed treatment may improve seedling initial growth, where by gibberellin stimulates the break down of endosperm of the seed and stimulates cell divisions and proportion of stem and root part of seedlings [17]. It means that there is a positive synergy between endogenous and exogenous gibberellin to stimulate the germination process in hard seeds tested.

It is known also that gibberellin also triggers the formation of the enzyme α-amylase which will break down the starch so that the sugar levels in the cell will increase and cause the water outside of the cell to enter the cell and the cells will be extended. The results of the breakdown of starch will also be used for the process of respiration by mitochondria so that it will produce ATP which will be used in the process of cell growth and growth and cell expansion [18]. The addition of exogenous gibberellins...
derived from the extraction of water hyacinth roots containing gibberellin [19] will be able to increase the synergy between exogenous gibberellins and endogenous gibberellins that are in the seeds so that it will be able to influence seed viability and seed germination time.

The seed viability is the ability to grow through the germination process. The seeds viability can be demonstrated through metabolic symptoms or growth symptoms [20]. Parameters for seed viability that used were germination percentage and germination vigour index. The germination of seed normally has a difference in the level of viability of these seeds [21]. These measured parameters were obtained by comparing between one sprout and the other sprouts based on several criteria, namely normal sprouts, abnormal sprouts, and dead sprouts [22]. It can also be influenced by several factors, namely the level of seed maturity, seed size, and seed dormancy period. In this case, these parameters were not recorded yet as supporting data.

To determine seed viability also followed by the duration of seed germination with germination index parameters. Germination index used to determine the speed of seeds in starting and doing the germination process by calculating the duration for seeds to begin to accentuate the radicle and to do the germination process [23]. In this study also showed that longan seeds and soursop seeds were not significantly different based on the germination index value compared to sapodilla seeds. As it was known that germination process was affected by internal factors and external factors [24]. One of internal factors that affect the germination seed was the condition of seed itself. If the seeds have not reached ripe physiologically it will have low viability. Even in certain plants, these seeds will not be able to germinate [25]. Besides that the addition of exogenous gibberellin extracted from the roots where the gibberellin was produced also affect the germination process.

The results of this study indicated that the application of the exogenous gibberellin hormones extracted from water hyacinth root has an effect on the germination process of three types of hard seeds. The greater amount of gibberellin concentration applied on seed will have a greater effect on the germination. These findings revealed that exogenous and endogenous gibberellins can be a synergy to stimulate the physiological processes of seeds before they become new individuals by germination process [16].

5. Conclusion

Based on the results, it can be concluded that the application of the gibberellin hormone extracted from *Eichhornia crassipes* root in various concentrations (0 ppm, 200 ppm, 300 ppm, 400 ppm, and 500 ppm) showed a significant effect on the seed germination percentage, vigour index, and germination index of three hard seeds (sapodilla seeds, soursop seeds, and longan seeds). Longan seeds showed the highest value compared to soursop seeds and sapodilla seeds in germination percentage, vigour index, and germination index. The findings revealed that there was a positive synergism between exogenous and endogenous gibberellins on the effect of germination process in three different hard seeds tested.

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