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Viability Test and Polyethylene Glycol (PEG) Soaking Factor of Soybean Seeds Due to Shading Stress

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Abstract

Soybeans are a type of agricultural crop commodity in Indonesia that has benefits for society, especially as a source of vegetable protein. The aim of this research is to measure the viability test of soybean seeds and the soaking factor of polyethylene glycol due to shade stress. The research was carried out in the Tissue Culture Laboratory at Asahan University from December 2023 to January 2024. The researchers used a completely randomized factorial design (CRD) with 2 factors: first, soybean variety due to shade stress consisting of four levels, including V1 = Dena 1, V2 = Dena 2 , V3 = Denasa 1 and V4 = Denasa-2. The second factor is the duration of soaking in polyethylene glycol with a concentration of 7.5% consisting of four levels (P0) (control), (P1) 3 hours, (P2) 5 hours, (P3) 7 hours. Observed variables consist of maximum growth potential, germination capacity (%), growth simultaneity (%) and vigor index. Research data was analyzed using variance analysis procedures to determine whether there was an effect of each treatment. The soybean seed variety treatment was effective on the maximum growth potential of the Dena-1 V1 variety (96.67%), germination capacity (57.08%), growth simultaneity (59.17%) and vigor index (70.83%). The long soaking treatment of Polyethylen Glycol (PEG) had no significant effect on maximum growth potential, germination capacity and vigor index. but the real influence on the simultaneity of growth with results is P2 (5 hours) 44.17%. There is no interaction various seed varieties and the length of soaking in Polyethylen Glycol (PEG)

Keyword: *Polyethylene Glycol; Soybean Seeds; Shade Stress; Viability*

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INTRODUCTION

Soybeans are a Species of agricultural crop commodity in Indonesia that has many benefits for society, especially as a source of vegetable protein. Soybeans are one type of commodity that the government is working on to achieve the food self-sufficiency target (Ponto et al., 2017) . The national demand for soybeans in 2020 will reach 2.87 million tons per year, but soybean production in Indonesia is only 0.96%, according to the demand figures taking into account domestic soybean production capabilities, there will be an increase in the soybean supply deficit in Indonesia. The soybean supply deficit in Indonesia reached 1.91 million tonnes in 2020 (Kementerian Pertanian, 2016). Soybean productivity in Indonesia from 2015 to 2020 continues to decline every year. In 2015 soybean productivity reached 964,183 tonnes, until 2019 there was a very significant decline where national soybean production was only able to obtain 424,190 tonnes and increased again in 2020 to 613,300 tonnes (Setyawan & Huda, 2022).

Soybean plants are short-day plants and require high light intensity, so soybeans are classified as C3 plants. If during soybean planting there is a decrease in solar radiation for a long time it will affect the growth and yield of soybeans. C3 plants experience photorespiration which results in lower net results of photosynthesis than C4 plants. C3 plants have a lower net photosynthesis rate than C4 plants because their respiration results depend on light so they lose more CO2 than C4 plants. The higher the shade intensity can increase plant height and specific leaf area, but the number and area of leaves, light absorption rate, photosynthesis rate, leaf chlorophyll index, number of filled pods, and seed weight per soybean plant decrease (Humoen et al., 2020).

The natural process of plant growth begins with seed germination, namely the stage of emergence of the radicle in the seed testa. One of the factors that influences the failure of generative plant propagation through seeds is the low ability of seeds to germinate (Dewi & Miftakhurrohmat, 2022). Reducing seed quality (deterioration) can have an indirect impact on crop production. The decline in seed quality begins when the seeds are physiologically mature. Physiological damage to seeds is characterized by decreased seed viability and vigor. The level of physiological maturity of seeds can also influence growth potential. The decrease in seed viability and vigor during storage is due to the breakdown of food ingredients, causing the seeds to lack food reserves and increasing protein synthesis. Soybean seeds are orthodox seeds which have high protein content so they are susceptible to physiological damage if post-harvest application is not appropriate (Lestari et al., 2020).

Invigoration is seed conditioning to improve the physiological and biochemical processes of seeds. Methods for invigorating seeds include osmoconditioning/osmopriming by soaking the seeds in a PEG (Poly Ethilyne Glycol) solution. PEG which is used in various seed research plays a very important role in increasing viability and the time used for the germination process becomes faster and this PEG compound has been used in seeds of food and horticultural crops. Soaking that is too short is thought to not be able to induce seed germination, so it is not able to display good viability performance, however, if soaking is too long, the absorption

of the solution with osmotic pressure can have a negative effect on seed viability (Wahdah & Susanti, 2020).

Research result Sa'adati et al., (2023) showed that a concentration of 7.5% and a soaking time of 5 hours of Polyethylene glycol (PEG) was able to increase the viability of soybean seeds that had been stored for 2 years. However, no research has been conducted to test the viability of soybean seeds subjected to shade stress. Based on the description above, the author is interested in conducting research on the Viability Test of Soybean Seeds and the Polyethylene Glycol (PEG) Soaking Factor of Soybean Seeds Due to Shade Stress.

METHOD

The research was carried out in the Tissue Culture Laboratory, Faculty of Agriculture, Asahan University. The research was carried out from December 2023 to January 2024. The materials used in this research were soybean seeds of the agromulyo, anjasmoro, dena-1 and denasa-1 varieties resulting from research that were exposed to shade stress, distilled water, straw paper, label paper and The materials used in this research were soybean seeds of the agromulyo, anjasmoro, dena-1 and denasa-1 varieties resulting from research that were exposed to shade stress, distilled water, straw paper, label paper and a calculator. The tools used are analytical scales, culture racks, measuring cups 500 ml, measuring flasks 250 ml, straw paper, plastic, tweezers and timers.

Trial Design

This research used a Factorial Completely Randomized Design (CRD) with 2 treatment factors: the first factor, namely: soybean variety due to shade stress, consisted of four levels, including V1 = Denasa 1, V2 = Denasa 2, V3 = Dena 1 and V4 = Dena- 2. The second factor is the length of soaking in polyethylene glycol with a concentration of 7.5% consisting of four treatment levels including (P0) without soaking (control), (P1) 3 hours, (P2) 5 hours, (P3) 7 hours. Observed variables consist of maximum growth potential, germination capacity (%), growth simultaneity (%) and vigor index. Research data was analyzed using the ANOVA variance analysis procedure to determine whether there was an effect of each treatment. If it is known that there is a real difference in the effect of the treatment, continue with the DMRT (Duncan's Multiple Range Test) test at the 5% level.

Research Implementation

The tools that will be used in this research are cleaned or sterilized first. Then prepare the materials to be used such as soybean seeds, PEG solution, straw paper, distilled water, label paper, stationery and other materials. The soybean seeds used were the result of previous research due to shade stress from coconut trees with various varieties used, namely the Denasa 1 variety, Denasa 2 variety, Dena-1 variety and Dena-2 variety. Then the seeds are collected in the Tissue Culture Laboratory. Then wash the seeds with running water so that they are clean and there is no dirt attached, choose good seeds for planting. The concentration of the PEG solution is done by

weighing 7.5 grams of the solution then adding 1 liter of distilled water and homogenizing it in a measuring flask.

Seed germination testing was carried out by soaking each seed variety according to the treatment time with PEG concentration of 7.5%. Each treatment consisted of 2 replications and each treatment used 12 soybean seeds. The seeds are germinated for 7 days using the UKDdp method Paper Rolled in plastic test), then placed in a storage box.

Data Analysis

Maximum growth potential includes soybean seeds that germinate and grow normally or abnormally 7 days after sowing, calculated using a formula on (Tefa, 2017).

$$\text{PTM} = \frac{\sum \text{growing seeds}}{\sum \text{seeds planted}} \text{x } 100\%$$

The germination capacity of soybean seeds that have been treated is calculated by the number of seeds that germinate normally and expressed in percent. Germination power measurements were carried out when the soybean plants were 3 and 5 days after sowing. Germination power is calculated using the following on (Tefa, 2017).

$$\text{DK} = \frac{\sum \text{KN Count I+KN Count II}}{\sum \text{seeds planted}} \text{x } 100\%$$

Description:
KN =normal sprouts

Simultaneity of growth is calculated based on the percentage of normal sprouts of soybean seeds on the 7th day. Simultaneity of growth is calculated using the following formula on (Tefa, 2017).

$$\text{DK} = \frac{\sum \text{KN day 7}}{\sum \text{seeds planted}} \text{x } 100\%$$

Description:
KN =normal sprouts

The Vigor Index is calculated based on the number of normal sprouts of soybean seeds on the 3rd day after sowing. The vigor index is calculated using the following formula on (Tefa, 2017).

$$\text{IV} = \frac{\sum \text{KN Count 1}}{\sum \text{seeds planted}} \text{x } 100\%$$

Description:
KN =normal sprouts

RESULTS AND DISCUSSION

Maximum growth potential

From the results of the analysis of variance, it can be seen that the treatment of various varieties showed a real effect on the 7th day. Meanwhile, the length of soaking in Polyethylen Glycol (PEG) did not show a significant effect on the 7th day of observation. The interaction between the two treatments showed no significant effect on the observed age.

Table 1. Average Maximum Growth Potential for Various Soybean Varieties and Soaking Time for Polyethylen Glycol (PEG) 7th day

Treatment	Average
V_1	96.67 a
V_2	89.16 b
V_3	96.67 a
V_4	94.17 a
P_0	95.00
P_1	92.50
P_2	94.17
P_3	95.00

Description: The same letter in the same column indicates no significant difference at 5% DMRT.

It can be seen that, giving various varieties to maximum growth potential shows that the V1 treatment is 96.67%, not significantly different from V3 96.67% and V4 94.17% but significantly different from V2 89.16%. In the long soaking treatment, Polyethylen Glycol (PEG) showed P3, namely 95.00%, which was not significantly different from P0 95.00%, P2 94.17% and P1 92.50%. The interaction between the treatments of various soybean varieties and the length of soaking in Polyethylen Glycol (PEG) showed no significant difference.

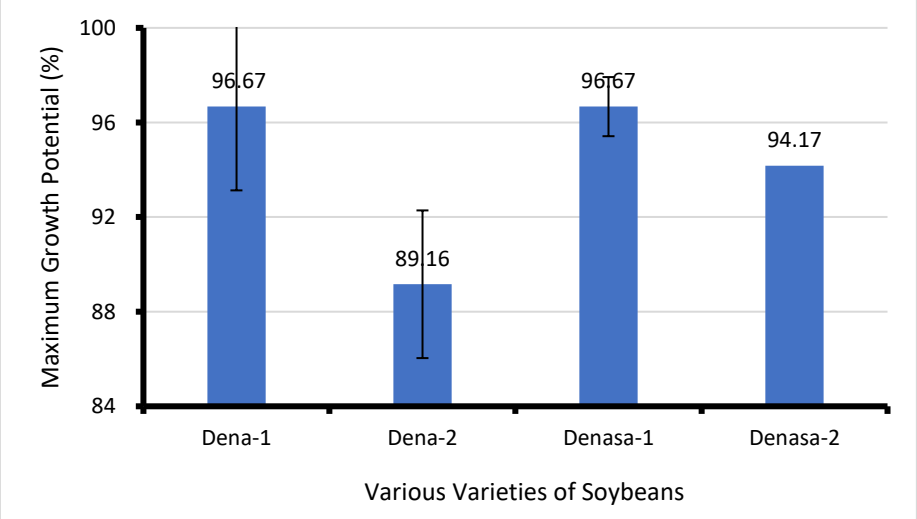

Figure 1. Histogram of Various Soybean Varieties Against Maximum Growth Potential

The results showed that the Dena-2 variety had a higher vigor index than the Dena-1, Denasa-1 and Denasa-2 varieties. Supported by research results Khairani et al., (2016) The Dena-2 variety has the highest score on several benchmarks, this is because the Dena-2 variety is a variety that has many advantages, one of which is that it is somewhat resistant to drought. Soaking soybean seeds for 3, 5 and 7 hours was not significantly different from soaking for 0 hours using Polyethylene glycol (PEG) and did not reduce maximum growth potential. Soaking seeds does not reduce maximum growth potential, indicating that during soaking the seeds absorb water normally so that water and oxygen requirements are sufficient for seed germination. The addition of Polyethylene glycol (PEG) does not poison the seeds because it has a large molecular weight which does not penetrate the seed tissue so it does not interfere with seed germination (Bismindar et al., 2018). Apart from that, the seeds also have maximum good growth potential, which means that the seed viability is relatively high. Invigoration treatment is only carried out on seeds that have low viability (Aisyah et al., 2018), to repair the cell membrane in the seed so that the seed can imbibition normally (Asih, 2020), and there is no leakage of seeds so that it does not cause a lack of material to be broken down into energy during germination (Sari et al., 2020). The influence of various soybean varieties on maximum growth potential on day 7 is presented in Figure 1.

Germination Power (%)

From the results of the analysis of variance, it can be seen that the treatment of various varieties showed a very real influence on the days observed. Meanwhile, the long soaking treatment for Polyethylen Glycol (PEG) did not show a significant effect on the days observed. The interaction of the two treatments showed no significant influence on the observed parameters.

Table 2. Average Germination Power in Various Soybean Varieties and Soaking Time in Polyethylen Glycol (PEG) Days Observed

Treatment	Average (%)
V_1	57.08 a
V_2	31.66 c
V_3	25.00 d
V_4	45.00 b
P_0	37.91
P_1	37.50
P_2	37.08
P_3	46.25

Description: The same letter in the same column indicates no significant difference at 5% DMRT.

It can be seen that, giving various varieties of germination showed that the V1 treatment was 57.08% significantly different from V4 45.00%, V2 31.66% and V3 25.00%. In the long soaking treatment for Polyethylen Glycol (PEG), P3 46.25% showed no significant difference to P0 37.91%, P1 37.50% and P2 37.08%. The

interaction between the treatments of various soybean varieties and the length of soaking in Polyethylen Glycol (PEG) showed no significant difference.

The concentration of the PEG P3 solution caused the percentage of germination to produce the highest amount, namely 46.25%, while the lowest was in the PEG treatment (P2 = 3 hours) namely 37.50%. This can occur allegedly because the PEG concentration applied is appropriate to the type of seed used (seed size, thickness of the seed coat and whether or not the seed absorbs a solution easily). According to Aisyah et al., (2018), that for varieties whose seeds have a larger surface area, the absorption or imbibition of water and other gases will be greater, so they require an osmoconditioning solution with a higher concentration.

From this experiment, it can be seen that the soaking time P3 (7 hours) caused the highest percentage of abnormal sprouts, namely 46.25%, while the lowest was the soaking time P1 (3 hours), namely 37.50%. This indicates that soaking time that is too long causes increased growth of abnormal seed sprouts. Utomo (2006) states that water is absolutely necessary for germination, however soaking for too long can cause anoxia (loss of oxygen), thus limiting the respiration process. Respiration is a stage of the germination process that occurs after the water absorption process. If the respiration process is limited, the germination process will be slow. The effect of various soybean varieties on germination power on the days observed is presented in Figure 2.

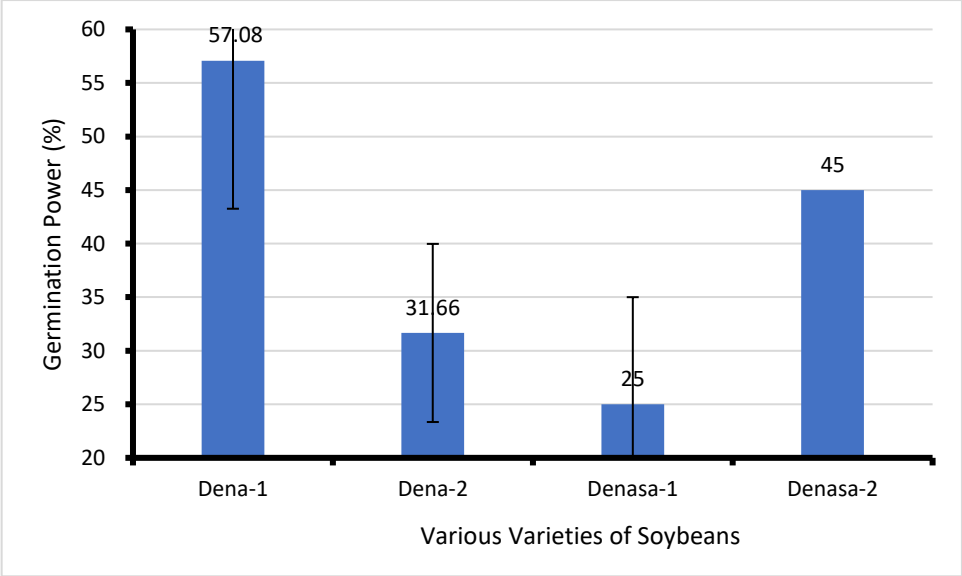

Figure 2. Histogram of Various Soybean Varieties Against Growth Simultaneity

Simultaneity of Growth (%)

The results of the analysis of variance, it can be seen that the treatment of various varieties and the length of soaking in Polyethylen Glycol (PEG) showed a very real influence on the observation of growth synchrony. The interaction between the two treatments showed no significant effect on the observed age. Table 3 showed that, giving various varieties to the synchronization of growth showed that the results of treatment V1 were 59.17% significantly different from treatments V4 39.16%, V2 20.00% and V3 11.66%, while treatments V2 and V3 were not significantly different.

In the long soaking treatment for Polyethylene Glycol (PEG), P2 44.17% was not significantly different from P3 35.75%, P1 28.33% and P0 21.66%, while treatments V1 and V0 were not significantly different. The interaction between the treatments of various soybean varieties and the length of soaking in Polyethylen Glycol (PEG) showed no significant difference.

Table 3. Average Simultaneous Growth in Various Soybean Varieties and Duration of Polyethylen Glycol (PEG) Soaking Day 7

Treatment	Average (%)
V_1	59.17 a
V_2	20.00 c
V_3	11.66 c
V_4	39.16 b
P_0	21.66 c
P_1	28.33 c
P_2	44.17 a
P_3	35.83 b

Description: The same letter in the same column indicates there is no significant difference at 5% DMRT

The seeds of the Denasa-1 variety have a lower growth simultaneity value than the seeds of the Dena-1 variety. The Denasa-1 variety has a growth simultaneity value at the end of planting of 11.66%, while the Dena-1 variety is 57.08%, Wahyuni et al., (2022) states that the value of growth simultaneity in seeds indicates the seed vigor parameters and shows the growth potential of the seeds to grow, uniform growth and normal seed development in various environmental conditions. According to Prabhandaru & Saputro (2017), Simultaneous growth indicates that plants grow synchronously, uniformly and with greater vigor under stress treatment, so that they can produce plants that are more tolerant to stress and increase plant yields. Based on the measurement of growth synchrony, the Dena 1 variety has better vigor than the Denasa-1 variety. The Dena-1 variety has more uniform and synchronous growth and has higher growth strength. Growth strength is demonstrated by the simultaneous growth of seeds. Simultaneity of seeds that have a value of more than 50% indicates that the seeds have high seed growth strength.

According to Arthawijaya et al., (2022) Brief soaking of seeds results in slowing of seed metabolism due to lack of water. Soaking seeds for 3 hours with a concentration of Polyethylene glycol (PEG) did not affect the synchrony of seed growth, whereas soaking seeds for 5 hours with an additional concentration of Polyethylene glycol (PEG) increased the synchrony of seed growth, but the 7 hour soaking treatment tended to reduce the synchrony of seed growth. This result shows that soaking soybean seeds for 3 hours and 5 hours with the concentration of Polyethylene glycol (PEG) given is still within the tolerance levels of the seeds so that it does not poison the seeds and the seeds can still germinate well. Soaking the seeds for 7 hours with the given concentration of Polyethylene glycol (PEG) causes the seeds to experience anoxia (loss of oxygen) (Afdharani et al., 2019). Availability of oxygen needed in the seed respiration process. Oxygen in the respiration process is needed for the process of

breaking down food substances to obtain energy, so that the respiration process is not optimal causing the energy produced to be reduced, as a result germination and growth are hampered (Yuanasari et al., 2015). Simultaneous growth of seeds is influenced by the ability of the seeds to utilize energy reserves, where in one lot they are able to utilize energy reserves well so that they can grow into strong seeds simultaneously (Aisyah et al., 2018). The influence of various soybean varieties on growth synchrony on day 7 is presented in Figure 3.

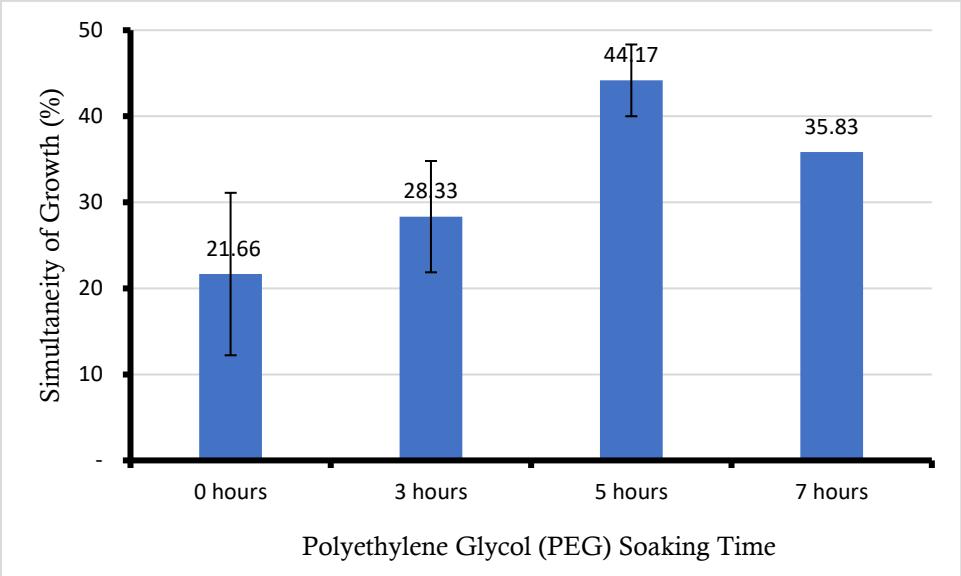

Figure 3. Histogram of Various Soybean Varieties Against Growth Simultaneity

The effect of soaking time in Polyethylene Glycol (PEG) on growth synchrony on day 7 is presented in Figure 4.

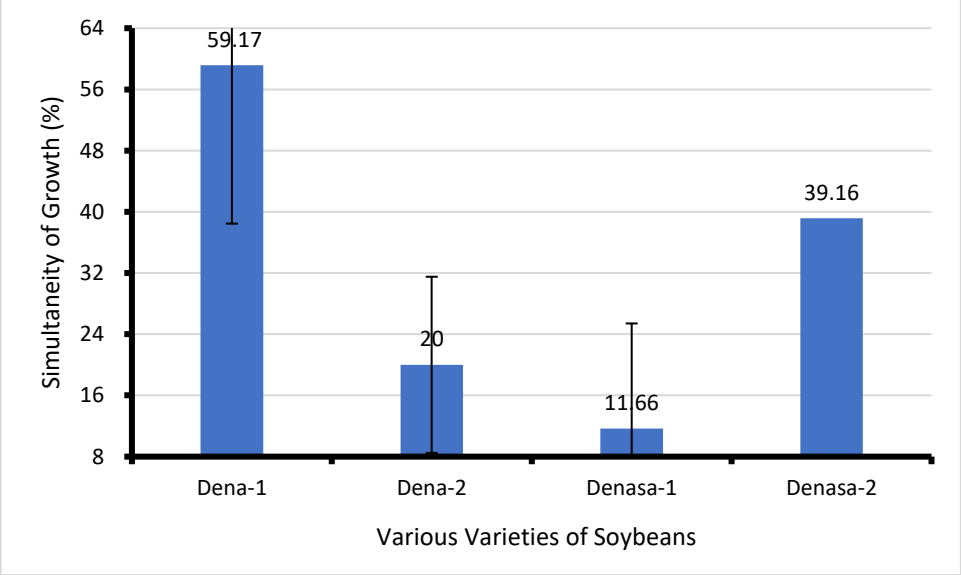

Figure 4. Histogram of Polyethylene Glycol (PEG) soaking time on growth synchrony

Vigor Index (%)

From the results of the variance analysis, it can be seen that the treatment of various varieties showed a very real effect on the 7th day. Meanwhile, the long soaking treatment for Polyethylen Glycol (PEG) did not show a significant effect on the 7th day of observation. The interaction of the two treatments showed no significant influence on the observed parameters.

Table 4. Test Results of Average Differences in Vigor Index on Various Soybean Varieties and Soaking Duration of Polyethylen Glycol (PEG) 7th Day

Treatment	Average (%)
V_1	70.83 a
V_2	40.00 c
V_3	31.66 c
V_4	55.00 b
P_0	46.66
P_1	46.66
P_2	46.66
P_3	57.50

Description: The same letter in the same column indicates there is no significant difference at 5% DMRT

It can be seen that, giving various varieties, the vigor index in treatment V1 was 70.83%, which was significantly different from treatment V4 55.00%, V2 40.00% and treatment V3 31.00%, while V2 and V3 were not significantly different. In the long soaking treatment for Polyethylene Glycol (PEG), P3 was 57.50%, not significantly different from P0, P1, and P2, namely 46.66%. The interaction between the treatments of various soybean varieties and the length of soaking in Polyethylen Glycol (PEG) showed no significant difference.

The Denasa-1 variety has the lowest vigor index value of 31.66% in contrast to the Dena-1 variety which has a vigor index value of 70.83%, this is supported by research results Wahyuni et al., (2022) that the seeds of the Dena-1 variety show faster seed germination and have strong seed vigor, Fatonah & Nalwida (2017) added stated that a high vigor index value indicates the speed of seed germination so that the seed can have good vigor. Growth speed is one of the parameters of seed vigor, namely the ability of seeds to grow and develop into normal plants in sub-optimum environmental conditions.

Soaking soybean seeds in Polyethylene glycol (PEG) for 0, 3, 5 and 7 hours reduces the seed vigor index because the seeds have a poor vigor index. Invigoration treatment will affect seeds that have low viability because the seeds have poor cell organelles and enzyme activity runs abnormally. Invigoration is better carried out on seeds with low viability (<72%) because cell organelles are starting to be damaged and invigoration treatment will help improve seed viability (Yuanasari et al., 2015). Different from research results Ernita & Mairizki (2019); Falahhosseini et al., (2017); Lei et al., (2021) which results in invigoration using Polyethylene glycol (PEG) increasing the vigor index of soybean seeds. This is because the soybean seeds used have low viability so that Polyethylene glycol (PEG) treatment can improve the cell

membranes in the seeds thereby increasing the seed vigor index. The influence of various soybean varieties on the vigor index on day 7 is presented in Figure 5.

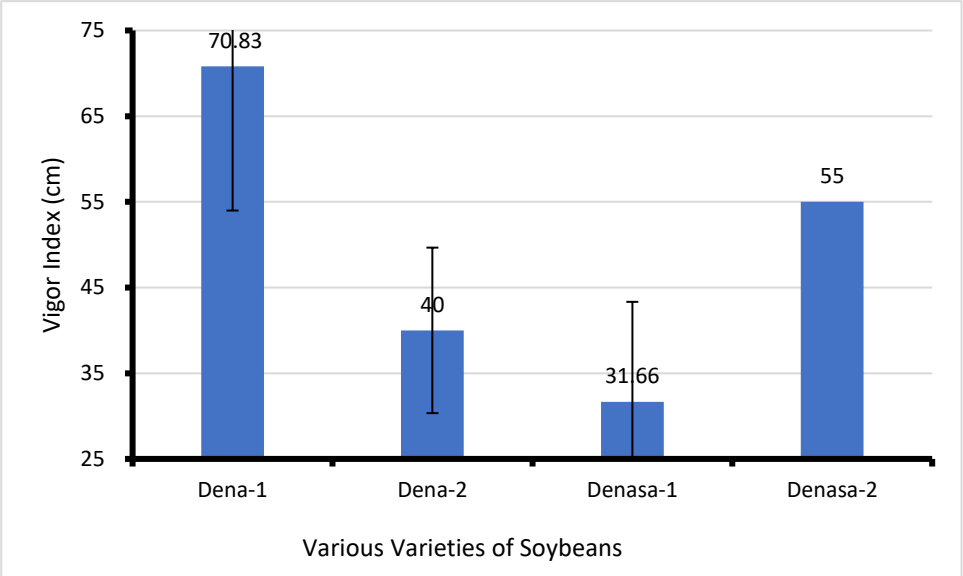

Figure 5. Histogram of Various Soybean Varieties Against the Vigor Index

CONCLUSION

Treatment of soybean seed varieties was effective for all observed parameters. The long soaking treatment of Polyethylen Glycol (PEG) did not have a significant effect on maximum growth potential, germination capacity and vigor index. However, simultaneous growth had a real influence with the best results respectively, namely P2 (5 hours) 44.17%. There is no interaction between giving various seed varieties and the length of soaking in Polyethylen Glycol (PEG).

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