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ABSTRACT

In addition to land conditions, microorganism activities such as rhizosphere fungi found in marginal lands can also influence the growth and production of soybean plants (Suharta, 2010). This research aims to determine the differences in viability, vigor, development, and production of 4 soybean varieties cultivated in marginal lands, identify variations in the abundance of rhizosphere fungi among the 4 soybean varieties present in marginal lands, and ascertain the correlation between observed variables of soybean plants and the abundance of rhizosphere fungi. Soil samples were taken from around the roots of the 4 different soybean varieties, namely Argomulyo, Anjasmoro, Devon-1, and Dena-1, each with 4 replications. Each sample was extracted using the Promega (Germany) method. The extracted DNA was amplified using the Polymerase Chain Reaction (PCR) method based on the Internal Transcribed Spacer (ITS) sequence (White et al, 1990). The amplified results were visualized using the capillary electrophoresis digital method. The visualized DNA bands were around 500 bp, consistent with the expected target amplification size variation in the thickness of the visualized bands indicating the abundance of rhizosphere funding. PCR visualization during the germination period showed that the Devon-1 variety had the thickest bands, while the Anjasmoro variety had the thinnest bands. At 100 days after planting, PCR visualization indicated the absence of DNA bands in all four varieties. The variety with the highest viability and vigor index was Devon-1, while the lowest was Anjasmoro. The variety with the highest growth and production was Dena-1, while the lowest was Argomulyo.

Keywords:DNA, Electrophoresis, Polymerase Chain Reaction (PCR), Fungi Rhizosphere, Soybean

Introduction

Soybeans considered as one of the plants that has the potential to be developed in Indonesia. Soybeans also have high protein. Protein in soybeans plays an important role in fulfilling the nutrition of people in Indonesia (Rifqi and Dewi, 2018). The demands of soybeans continues to increase, consequently soybean production in Indonesia must also be increased. The condition of the planting land and plant growth must be considered in order to support production increases.

Fertile land that contains the nutrients needed by the plants is one of requirements that is supports the growth of soybean. Adequate and balanced nutrients will grows the plant well and fertile (Dewi, 2016). In spite of those, most of the land in Indonesia is dominated by marginal land. According to Suharta (2010), marginal land is land that is poor in nutrients, so the usage of this land is less than optimal because it contains only small amount of organic material. Land conditions with small amount of organic material content derive low activity of microorganisms which take a role as nutrient providers (Directorate General of Plantations, 2010).

Microorganisms in the soil, which is one of them is rhizosphere fungi, they influence soil fertility and play a role in providing nutrients for plants. Rhizosphere fungi also help sulphur transport and nitrogen to plants (Igiehon & Babalola, 2017). An example of a rhizosphere fungus that is beneficial to soybean plants is Glomus mosseae, where this fungus can help soybean plants in drought stress conditions (Chen et al, 2017). In Miranda's (2008) research, it was showed that the rhizosphere fungi G. eunicatum and E. colombiana could help soybean plants produce higher production. Based on the important role of rhizosphere fungi for plant growth, the abundance of rhizosphere fungi on marginal land should influence plant growth. However, there is still limited research explained abundance of rhizosphere fungi on marginal land. Limited information on the abundance and diversity of rhizosphere fungi on marginal land was the lead for conducting research on the abundance of rhizosphere fungi on marginal land which was analyzed using the Polymerase Chain Reaction (PCR) method to determine its effect on the growth and production of 4 varieties of soybean plants (Glycine Max (L.) Merill) planted on marginal land.

Research Materials and Methods

This research used 4 soybean varieties, they were Argomulyo, Anjasmoro, Devon-1 and Dena-1 varieties. The tools used during germination and cultivation on marginal land are germinators, shovels, hoes, buckets, scrapers, tape measure, stationery and sacks. The materials required are seeds of the Argomulyo, Anjasmoro, Devon-1 and Dena-1 varieties, straw paper, plastic, urea fertilizer, KCL, SP-36, insecticide and water. The tools used for the PCR analysis and eletrophoresis stages were cool boxes, tissue paper, autoclave, oven, Eppendorf micropipettes, Centrifuge tools (Hybrid refrigerated Centrifuge CAX-370), Tissue Lyser (Tissue Lyser LT Qiagen), Vortex (Bio Vortex V1), Incubation machine (Thermo-shaker TS-100 AND CH-100), Spin machine (mIcro one), Nanophotometer, cuvettes, PCR machine (Labcycler), well buffers, electrophoresis machine (Qiaxsel advanced 12352 from Qiagen), and freezers. The materials used are rhizosphere soil extraction of 4 varieties of soybean plants 0.05 gr/variety, ice cubes, alcohol, DNA extraction kit, sterile microtube, sterile tip in 1000µL, 200µL, 10µL sterile tip, PCR reagent kit, and electrophoresis reagent kit .

This research is a quantitative descriptive study using a Completely Randomized Design (CRD). This experiment was a non-factorial experiment which consisted of 4 treatments and was repeated 4 times, which was conducted in Way Kanan District, there were 16 experimental units. The data obtained then analyzed using the Rstudio statistical program and GerminaQuant online software. Parameters of soybean plant growth were tested by homogeneity testing using the Barlet test which was then carried out by analysis of variance. If there is a significant difference, then a Least Significant Difference (LSD) test is carried out at a level of 5%.

Research Implementation

1. Planting Seeds

Planted 2 seeds/hole in the planting hole then sprinked with soil, then sprink enough furadan and cover with soil.

2. Plant Maintenance

Includes (1) replanting, replacing dead plants with new seeds one week after planting so that plant growth occurs simultaneously; (2) Weeding and watering are carried out routinely by clearing weeds that grow around the plants in order to avoid competition for nutrients between plants. Watering was done according to weather conditions, if the soil is dry then water is carried out; (3) Fertilization was done at the age of 2 DAP and 4 DAP using urea fertilizer 1.2 kg/land area, SP36 1.6 kg/land area, and Kcl 1.6 kg/land area. Fertilization is done by making an array near the soybean plants.

3. Sampling Rhizosphere Fungi of Soybean Plants

Soil samples took around the roots of 4 different varieties of soybean plants,they were Argomulyo, Anjasmoro, Devon-1, Dena-1 with 4 replicates for each variety. Five samples were taken from each replication from each variety randomly and homogenized. Soil samples taken by 1 gram/sample .

4. Fungi DNA Extraction

DNA extraction of fungal isolates was carried out following the Promega kit protocol with several adjustment modifications. In the first stage, 1 mL of fungal isolate was put into a 2 mL microtube. Then the samples were centrifuged for 5 minutes at 13,500 rpm at 4°C. The supernatant formed was then removed. Next, 1 mL of TAE 10x was added, the sample was vortexed for a while. Samples were centrifuged for 5 minutes at 13,500 rpm at 4°C. The supernatant formed was then removed immediately. Put 1 mL of PBS into the tube and then vortex briefly in order to homogenized them. Add 10 µL of lytic enzyme along with the vortex pellet for 2-3 minutes. Incubate at 37°C for 30 minutes without any homogenized treatment, then remove and incubate again at 55°C for 30 minutes. Every 5 minutes back and forth. After incubation, the sample was discarded, the sample was transferred to a new 2 mL microtube. The stage continues with destroying the nucleus using the addition of 300 µL of Nucleic Lysis Solution, then inverting it and leaving it for a moment. The sample was vortexed for 30 seconds. Then, 100 µL of Protein Precipitation Solution was added and then homogenized with a vortex for 30 seconds and left for 5 minutes. Next, incubate in an ice box for 5 minutes. Samples were centrifuged for 5 minutes at 13,500 xg at 4°C. Next, the supernatant was transferred into a taper tube containing 300 µL of isopropanol to precipitate the DNA. Samples were centrifuged for 5 minutes at 13,500 xg at 4°C. then the supernatant was removed. The DNA was washed from the remaining isopropanol by adding 300 µL of ethanol, then inverted. Next, the sample was centrifuged for 5 minutes at a speed of 13,500 xg at 4°C. Then, the ethanol is removed horizontally slowly and dried on tissue paper. After drying, 15-25 µL of DNA Rehydration

Solution was added to the sample and incubated at 65°C for 15 minutes (slowly tapping every 5 minutes).

5. Analysis of Fungi Isolate DNA Extraction Results

The purity and concentration of DNA is determined using a Nanophotometer. The sample is dropped into the cuvette as much as 1.5 µl and then placed on the examination site. DNA purity is determined by calculating the absorbance ratio of A260 to A280 (A260/A280 ratio) as the absorbance ratio of nucleic acids to proteins, and A260/A230 as an indication of the presence of other components such as phenol. Before examining the sample, the blank was examined with DNA rehydration solution which was dropped into the cuvette as much as 1 µL first. Every changed of the sample, the cuvette is cleaned with a clean tissue. 6. Amplification of fungal ITS1 – ITS4 sequences using PCR instruments

Fungal growth was analyzed by comparing the thickness of the ITS sequence amplification with electrolysis PCR results. Fungi were amplified by PCR using internally transcribed spacer (ITS) primers. Each PCR mixture contained 10.5 µL of mastermix, 0.25 µL of Forward (F) primer, 0.25 µL of Reverse (R) primer, 8 µL of ddH2O, and 2 µL of DNA sample placed in a 0.2 mL microtube. After all the reagents were put into the microtube, the sample was homogenized by tapping and spinning. The thermocycling program used was predenaturation stage (95°C for 5 minutes), a denaturation stage (95°C for 1 minute), a primary attachment stage (54°C for 1 minute), and an extension stage (72°C for 1 minute). The denaturation, primary attachment, and elongation stages were repeated for 35 cycles. The next step was stabilization (72°C for 5 minutes) (Dong et al.

7. Electrophoresis of PCR Results

PCR samples were electrophoresed with the QiaxCel Advanced digital electrophoresis tool from Qiagen, Germany using the DNA HIGH RESOLUTION KIT. The procedure used follows the QiaxCel Advanced manual from Qiagen, Germany.

Results

1. Germination of Four Soybean Varieties

Based on the results of seed germination presented in Table 1 and Figure 1, the differences in varieties did not have a significant impact on the viability test outcomes. Nevertheless, the Devon-1 variety showed the highest viability, while the Anjasmoro variety showed the lowest viability. Furthermore, the vigor index also indicated no significant variation among the varieties. Vigor testing using the vigor index variable revealed that the Devon-1 variety had the highest vigor index, and the Argomulyo variety had the lowest vigor index.

Table 1. Results of the Least Significant Difference (LSD) Test on Viability (V) and Vigor Index (VI) of Soybean Seed Germination of 4 Varieties

Varieties	V	VI
Argomulyo	97.5a	54.0b
Anjasmoro	95.5a	60.0ab
Devon-1	99.5a	69.5a
Dena-1	99.0a	64.5ab
LSD	2,178	15,029

Note: Numbers followed by the same letters are not different based on the 0.05 LSD test

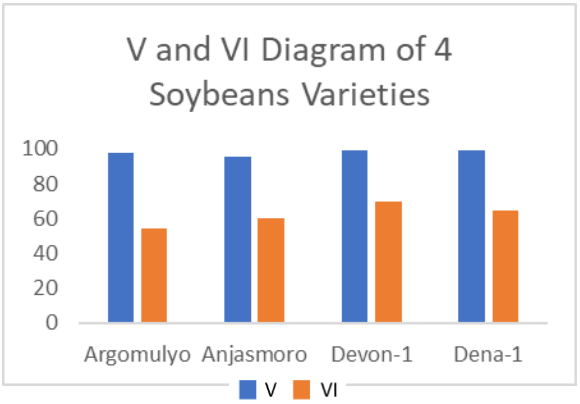


Figure 1. Viability (V) and Vigor Index (VI) of 4 soybean varieties

Then, the germination percentage graphs per day in the field (Figure 2) for the Argomulyo, Devon-1 and Dena-1 varieties almost simultaneously reached 100% germination which was marked by lines that were faster than other varieties and almost coincided. The Anjasmoro variety in viability measurements showed less germination compared to other varieties.

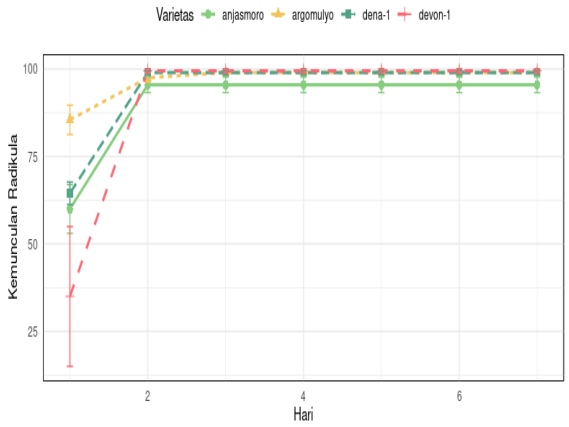


Figure 2. Graph of soybean seed germination percentage in the field for 7 days on 4 varieties

2. Measurement of plant height, number of leaves and greenness of leaves

Based on the observation variables of plant height and number of leaves in the 7th week, the results of the follow-up test on observation of plant height and number of leaves (Table 2) showed that the 4 varieties, Argomulyo, Anjasmoro, Devon-1 and Dena-1, were in accordance with the description varieties, where the Argomulyo variety had the lowest plant height and number of leaves and the Dena-1 variety had the highest plant height and number of leaves. Based on the results of leaf greenness in Table 4, the variety has no significant effect on the results of observing leaf greenness. However, the Dena-1 variety had the highest leaf greenness followed by the Devon-1, Anjasmoro and Argomulyo varieties with the lowest.

Table 2. Analysis of Variance using Least Significant Difference Test in Plant Height, Number of Leaves and Greenness of Soybean Leaves of 4 Varieties

Treatment	Week Plant Height	Number of Week 7th Leaves	Greenness of Leaves
Argomulyo	39.54b	15,95b	39,12a
Anjasmoro	54.75a	17,60ab	40,62a
Devon-1	47.98ab	21.25ab	42.27a
Dena-1	56.60a	23.00a	43.22a
LSD	12015	6,912	6,161

Note: Numbers followed by the same letters are not different based on the LSD test of 0.05

3. Measurement of the number of pods per plant, the number of filled pods per plant and the number of empty pods

Based on Table 3, observing the number of pods per plant, the number of filled pods per plant and the number of empty pods shows that the results of the 4 varieties, Argomulyo, Anjasmoro, Devon-1 and Dena-1 varieties, are in accordance with the variety description. The Dena-1 variety had the highest number of pods per plant, the number of filled pods per plant and the number of empty pods and the Argomulyo variety had the lowest number of pods per plant, the number of filled pods per plant and the number of empty pods.

Table 3. Analysis of Variance using Least Significant Difference Test in leaf greenness, number of pods per plant and number of filled pods per plant for 4 soybean varieties.

Treatment	Number of pods per plant	Number of filled Pods Per plant	Number of Empty Pods
Argomulyo	34.95b	29.20b	5.75a
Anjasmoro	60.35a	54.10a	6.25a
Devon-1	57.75ab	54,25a	3.50a
Dena-1	67,85a	60.30a	7.55a
LSD	23,354	22,551	5,555

Note: Numbers followed by the same letters are not different based on the LSD test of 0.05

4 Measurement on the Weight of 100 Grains and the Dry Weight in Numbers

Based on the results of the 100 grain weight and dry weight of the fruit in Table 4, the variety did not have a significant effect on the results of the observation of the weight of the 100 grain and the dry weight of the fruit. Based on the variable weight of 100 grains and dry weight in numbers, the results of further tests observing the weight of 100 grains and dry weight in numbers (Table 4) showed that the results of the 4 varieties, Argomulyo, Anjasmoro, Devon-1 and Dena-1 varieties, were in accordance with the variety description. The results of dry weight of upper parts (leaves and stem) and root are closely related to plant height and number of leaves, because plant height and number of leaves can influence the results of dry weight of upper parts (leaves and stem) and root.

Table 4. Analysis of Variance using Least Significant Difference Test in Weight of 100 Grains and Soybean Dry Weight of 4 Varieties

variety	Weight 100 items	Aged dry weight
Argomulyo	16.45a	185.38b
Anjasmoro	15.38a	237.38ab
Devon-1	15.08a	225.93ab
Dena-1	16.90a	341.15a
LSD	2.26101	139.4546

Note: Numbers followed by the same letters are not different based on the LSD test of 0.05

5 Analysis of the Abundance of Soybean Rhizosphere Fungi

5.1 Soybean Rhizosphere Fungi DNA Extraction

Extraction was carried out by taking samples from the isolates of the fungi KAr, KAn, KDv, KDn with a volume of 1 mL for each sample. Taking the same volume aims to convincing that there are no other variables that influence the result. These are the results of successive DNA extraction of fungal isolates analyzed for purity using a nanophotometer. Based on the results of the Nanophotometer on DNA

KAr fungi at wavelengths (λ) 260 and 280 nm DNA obtained purity of 1.759. The results of the nanophotometer on KAr DNA showed a concentration of 50.9 µg/µL in Table 5 and Figure 3 below.

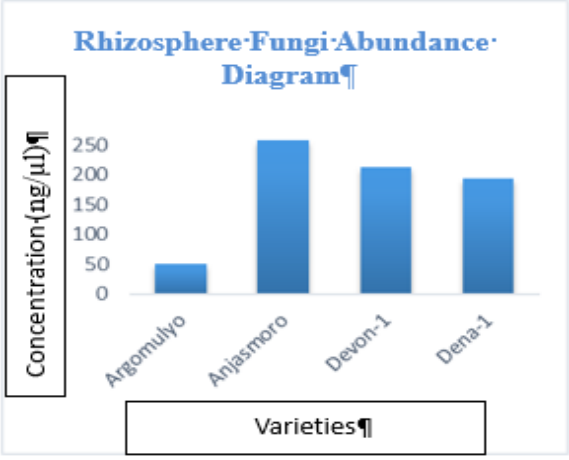
Figure 3. Rhizosphere Fungi Abundance Diagram

Table 5. Results of Concentration and Purity Analysis of Fungi DNA Extraction Results

Number	Sample	Concentration (ng/µl)	Purity (A260/A280)
1	KAr	50,9	1,759

2	kan	259	1,958
3	KDv	212	1,801
4	KDN	193	1,800
5	KC	124	1,754

Information:
KAr = Argomulyo rhizosphere fungi
KAn = Anjasmoro rhizosphere fungi
KDv = Devon-1 rhizosphere fungi
KDn = Dena-1 rhizosphere fungi
KC = control fungus (rhizosphere without plants)



Based on the DNA extraction results from Table 7 and Figure 5 above, it shows that the Anjasmoro variety has the highest fungal abundance and is significantly different from the Argomulyo variety with a standard deviation of 211 but not significantly different from the Devon-1, Dena-1 and Control varieties. Therefore, it can be concluded that based on the results of DNA extraction, the Anjasmoro, Devon-1 and Dena-1 varieties are preferred by rhizosphere fungi compared to the Argomulyo variety.

5.2PCR Genes and Visualization of PCR Results

PCR results were analyzed using electrophoresis. Visualization of the PCR results of the KAr, KAn, KDv, KDn, KC samples is shown in Figure 4 below.

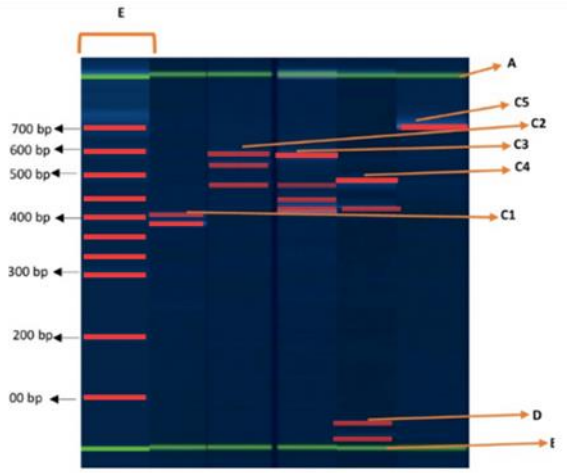


Figure 4. Results of electrophoresis visualization during germination, A = Alignment marker 3000 bp; B = alignment marker 15 bp; C1 =±400 pb (KAr); C2 =± 600 pb (KAn); C3 =±600 pb (KDv); C4 =± 450 pb (KDn); C5 =±700bp (KC); D = Dimers; E = Column Size Marker

All four varieties have band lengths ranging ±500 bp with significantly varying thicknesses. PCR visualization results during germination showed that the Devon-1 variety had the thickest band with the length of approx±600 pb. The Anjasmoro variety has the thinnest and longest ribbons±600 bp and the Argomulyo variety has the shortest DNA band, which is approx±400 pb. Meanwhile, the control has a band length ranging from± 700 pb pb. This indicates that the PCR process using primers ITS 1 and ITS 4 went well even though there were different band thicknesses. The PCR reaction that produces amplicons is shown by a clear and distinct DNA band that fluoresces when analyzed via

electrophoresis. Figure 5. It is clear that the control has the longest band±700 PB.

Figure 5. Electrophoresis visualization results at 100 days after planting, A1= Size Marker, A2= KAr (no band out), A3= KAn (no band out), A4= KDv (no band out), A5= KDn (no band out ribbon), A6= KC (no ribbon exit)

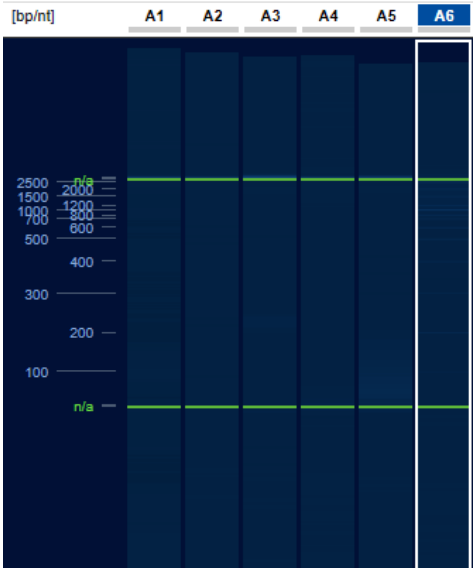
PCR visualization results 100 days after planting showed that the 4 varieties did not show any DNA bands.

Discussion

Based on viability testing, Devon-1 had the highest viability and Anjasmoro the lowest among the other varieties, although they were not significantly different. In the vigor index test, the Argomulyo variety had the highest vigor index and the Devon-1 variety had the lowest vigor. Differences in varieties may affect the viability and vigor index of seed germination. As in the study of Astuti et al (2020), the Devon-1 variety has the highest viability compared to Argomulyo.

Based on plant height measurement, in the 7th week the highest number found in the Dena-1 variety. This may be due to the different genetic characteristics of the 4 varieties, as in the study of Marliah et al (2012) which explained that the highest plant height at 15 HST was found in the Anjasmoro variety. While at the age of 30 HST the highest plant height was obtained in the Grobogan variety, the difference in response shown in soybean plant height due to differences in varieties, was thought to be due to differences in the genetic characteristics of these varieties. This is also in accordance with the opinion of Sadjad (1993) that differences in growing power between varieties are determined by the genetic factors of these varieties.

Based on measurement of the number of leaves in the 7th week the highest number of leaves was again found in the Dena-1 variety.



This is presumably due to differences in absorption of the needs of growth elements and growth factors such as absorption of light. According to Elizabet et al (2013), the formation of new leaves is influenced by the plant's adequate needs for growth elements. The absorption and change of light energy in the formation of seeds for crop yields is largely determined by the plant leaves themselves, because plant leaves are the biological site for photosynthesis.

Based on measurement of leaf greenness, the highest intensity of leaf greenness was found in the Dena-1 variety, followed by the lowest in the Devon-1, Anjasmoro and Argomulyo varieties. The difference in the amount of green leaf intensity is thought to be influenced by the amount of chlorophyll content that is different in the leaves of each variety. According to Ningsih et al (2012), chlorophyll content plays an important role as a medium for capturing sunlight energy which in photosynthesis will produce ATP (adenosine-5'-triphosphate) and NADPH (nicotinamide adenine dinucleotide phosphate).

Based on measurement of the number of pods per plant, the Dena-1 variety had the highest number of pods, followed by the Anjasmoro, Devon-1 and Argomulyo varieties. The difference in the number of pods is thought to be caused by differences in the characters determined by the genetic factors of these varieties. According to Sadjad (1993), differences in growing power between varieties are determined by the genetic factors of these varieties. Based on measurement of the number of filled pods per plant, the Dena-1 variety had the highest number of filled pods, followed by the Devon-1, Anjasmoro and Argomulyo varieties with the lowest. According to Gumilar et al (2013), the formation of soybean pods depends on the number of flowers present, not all flowers become pods even though complete pollination has occurred.

Based on measurement of the number of empty pods per plant, the Dena-1 variety had the highest empty pods followed by the lowest Anjasmoro, Argomulyo and Devon-1 varieties. The difference in the number of empty pods is thought to be due to soybean plants being attacked by pod ladybugs. This is in accordance with the research of Hadi et al (2018) during the pod filling process at the age of 45-60 DAP soybean plants were attacked by the pod ladybug (Ritortus liniari) which occurs during seed development and pod formation which causes the pods to become dry and empty. Apart from being attacked by pests, empty pods may be caused by harvesting which is carried out simultaneously where the ripening age of the pods for each variety is different.

Based on measurement of the weight of 100 seeds, the Dena-1 variety was found to have the highest weight, followed by the Argomulyo, Anjasmoro and Devon-1 varieties, the lowest. This is presumably due to the influence of environmental factors in various growth areas. According to Gumilar et al (2013), variations in soybean seed size within a variety occur due to variations in environmental conditions in various growing areas. Based on dry weight observations, the Dena-1 variety had the highest dry weight, followed by the Anjasmoro, Devon-1 and Argomulyo varieties with the lowest. Dry weight of shoots is determined by plant genetics and is closely related to the results of plant height, number of leaves and soybean pods, where varieties have plant height,

Based on the results of the analysis of the abundance of rhizosphere fungi in the germination phase in this study, it can be concluded that the rhizosphere fungi in the Devon-1 variety are indicated to be the most abundant compared to other varieties, followed by the Dena-1, Argomulyo and Anjasmoro varieties. However, when compared to the control, the abundance in the control area is indicated to be more abundant. This is indicated by the thickest or intensely colored ITS 1 and ITS 4 primer amplified DNA bands. In the results of the analysis of the abundance of rhizosphere fungi in the generative phase, rhizosphere fungi were not identified in the 4 soybean varieties. Li Yan et al (2022) stated that many rhizosphere fungi are found in the vegetative phase and at least when they come to the generative phase.

Based on the results of PCR visualization, it shows that differences in the abundance of fungi found in the rhizosphere of soybean plants can be caused by various factors. It is suspected that this difference in abundance is caused by the possibility that the fungal community in the rhizosphere suppresses fungal growth by releasing antifungal compounds. As in research by Mahartha et al (2017), the rhizobacteria tested were able to suppress pathogenic fungal colonies by releasing antifungal compounds. Reddy

(2014) stated that the bacterial species Pseudomonas spp. and Bacillus spp. Able to suppress the growth of fungi by producing antibiotic compounds that are antifungal. Such as knowing the diversity of fungi, the types of fungi and their roles in the rhizosphere of the soybean plants of the 4 varieties, because this study only looked at their abundance. As in Ulwiyah's research (2021) which states that the importance of identifying fungi is carried out to find out the type of fungus in order to know the benefits of these fungi. In addition, identification of fungi can also be done to determine the level of diversity of fungi in an ecosystem.

Conclusion

Based on the results of research and discussion, the following conclusions can be drawn:

1. The soybean plant variety with the highest viability is the Devon-1 variety and the lowest is the Anjasmoro variety. The soybean plant variety with the highest vigor index is the Argomulyo variety and the lowest is the Devon-1 variety. The soybean plant variety with the highest growth and production was the Dena-1 variety and the lowest was the Argomulyo variety.
2. The highest abundance of rhizosphere fungi in the vegetative phase of each variety was in the Devon-1 variety and the lowest in the Anjasmoro variety. Furthermore, the abundance of rhizosphere fungi in the generative phase of each variety was no longer identified.

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