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Anthracnose Disease and Physiological Aspects of Soybean in Saturated Soil Culture in Tidal Land

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Abstract Saturated soil culture in tidal land could be an alternative measure for achieving sufficient soybean production to meet demand and reduce the current 56% dependence on imports. Therefore, this study aims to investigate disease development and the nitrogen fixation rate of soybean in water-saturated cultivation on tidal land. It was conducted in Simpang Village, Berbak District, East Tanjung Jabung, Jambi Province, Indonesia, and subjected to a randomized block design as an environmental design. The treatment consisted of 3 water inundation levels in the trench, namely, 15, 20, and 25 cm from the soil surface. The results showed that the height of standing water in a water-saturated cultivation system affected the soil moisture. Furthermore, high soil moisture results in a high anthracnose disease in soybean caused by the fungus Colletotrichumdematinum var. truncatum. The disease attack rate reached 15.7% with 72% soil moisture at a depth of 15 cm below the soil surface. There was also an attack rate of 16.3%, with soil moisture of 70.5% at a depth of 20 cm from the soil surface. The highest percentage of fallen pods in the treatment plots was up to 15cm and 20 cm in standing water, which was 17.3% and 16.7%, respectively. The root nodules' weight decreased in height by 25 cm from the soil surface. Furthermore, the rate of nitrogen fixation decreased, as indicated by a reduction in the activity of the root nodules' acetylene, which decreased at the height of the waterlogging 25 cm from the soil surface. Soybean crop yield was affected by the height of standing water in the trench. The numbers of filled pods, the weight of dry seeds per plant, and the weight of 100 seeds were lower at the height of water 25 cm from the soil surface. Additionally, disease severity was strongly correlated with root nodules and seed/plant dry weight. Increased disease severity significantly contributed to the decline in dry weight of root nodules, very strongly to dry seed/plant weight and moderately to acetylene-reducing activity.

Keywords Plant Disease, Soybean, Saturated Soil Culture, Tides

1. Introduction

Soybean, also known as *Glycine max* L, is Indonesia's third most important national food crop after rice and corn. It is the source of raw materials for various staple foods, contains the highest protein, and is also rich in active compounds which are beneficial for human health. However, soybean productivity in Indonesia is still very low, as it only meets one-third of national needs. To meet the national demand for soybean, serious and integrated efforts are required to increase plant productivity and expand production locations. Furthermore, a wetting-drying cycle can improve the national soil structure from compaction pressure [1]. Saturated soil culture (SSC) is a cultivation innovation that provides continuous irrigation, maintains a constant water depth in the soil, and makes the layer saturated, which is required by plants to grow [2]. The controlling factors for the vertical flow of water in an area include the physical and macrospore properties of the tidal soil [3].

Soybean crops can be cultivated in tidal fields, and the yield is quite adequate. However, the cultivation method differs from irrigated and dry land. This plant does tolerate inundation, hence, it is recommended to plant soybean in tidal fields of type C water overflow and not A, which flows during high and low tides. Tidal land is sub-optimal due to its limitations that require careful handling. This includes the presence of a pyrite layer (FeS2), which produces harmful sulfate compounds in plants when oxidized. The increase in acidity in acid sulfate tidal soils is due to the direct or indirect oxidation of the FeS2 mineral. According to a study, soil acidification occurs when the amount of acid produced exceeds its buffering capacity [4]. The results of [5,6] proved that O_2 and Fe^{3+} are the main oxidizing agents in natural conditions against FeS_2 minerals. The O_2 molecule acts as the dominant oxidizing agent at the start of the reaction, which runs slowly at a neutral pH. According to [7], the Fe^{3+} in the soil solution can oxidize FeS_2 directly at a pH <4.0, and this process runs faster than oxidation by O_2. The oxidation process of FeS_2 by Fe^{3+}can continues even in the absence of O_2, thereby the soil remains acidic and becomes increasingly acidic [7].

Many plants including banana, avocadoes, olives, cereals are susceptible to one or more Colletotrichum spp., causing anthracnose (concave dead spot) and blight (brown tissue) in aerial tissues. Colletotrichum can also be present latently causing post-harvest damage and decay, damaging tissue before harvest but not developing markedly until after harvest [8,9,10,11,12]

In acid-reacting soils at low pH, the development and activity of several disease-causing pathogenic fungi are generally higher. One of the fungi reported to frequently attack soybean plants in the tidal zone in the study area is the fungus *Colletotrichumtruncatum*, which causes anthracnose disease. The symptoms can occur in the stems, petioles, and soybean pods. Symptoms in pods are round, irregular, and brown to black, and those in flower cause rot and fall off. Both acidic soil and disease disturbance caused low soybean productivity on tidal land.

Managing tidal fields with the right technology can increase productivity. One way to overcome this problem is by flooding the trenches, which is called saturated soil culture (SSC) in this study. Furthermore, the rhizosphere or root area is always in a reductive atmosphere with a saturated soil culture system, hence pyrite (FeS_2) is not oxidized. The growth and yield of inundated soybean in trenches increased following an increase in nitrogen-fixing and the growth of above-ground water table root. Inundation in trenches or wet cultivation can increase soybean yields in the field by up to 20% [13,14] or 80% [15].

Regular irrigation by overflowing or watering is a common practice by local farmers in cultivating soybean in the study area. This condition causes the plants to experience excess and lack of water at different times. The provision of water intermittently greatly disrupts plant growth because it will experience stress when dry. The plants recover after receiving water, but they experience drought stress before their recovery. According to [16], intermittent flooding may also inhibit N fixation compared to non-flooding.

Saturated soil culture is a cultivation technology that provides water permanently and maintains a constant depth, thereby keeping the soil layer in a saturated condition. By maintaining a constant water table, soybean is able to avoid the negative influence of inundation and increase resistance to many plant diseases [2,13,14,17,18]. Furthermore, this technology is a method of planting on beds by providing continuous irrigation in the trench to saturate the soil under the roots with water but not stagnate [19]. This is achievable by continuous irrigation from two weeks of age until the pods reach physiological maturity. The water level in the canals is maintained at ± 5 cm below the soil surface to create a saturated planting plot [20]. Consequently, the water level is maintained, and the subsoil is saturated with water. Fixed water levels can eliminate the negative effect of excess water on plant growth because soybean will acclimate and flourish. According to [20], saturated aquaculture developed in semi-arid tropical Australia can increase soybean yields compared to conventional irrigation.

2. Materials and Methods

Site and Study Time

This study was carried out in Simpang Village, Berbak District, East Tanjung Jabung Regency, Jambi Province, Indonesia, from May to October 2019. The study location is a type C tidal area with an altitude of 1 m above sea level. The soil type is Alluvial, with a pH of 5.7.

Materials and Instruments

The materials used include an anjasmoro soybean cultivar, agriculture lime, NPK basic fertilizer, and a water pump of 9 pH (Honda). Furthermore, the gas chromatograph (GC) was used in models 263-50 (Hitachi) with a flame detector or FID (Flame Ionization Detector). The GC was conditioned for approximately 3 hours before sample injection. Gas chromatography was adjusted, and injector temperature was 400°C, 35°C and 2,000°C column and detector temperatures, respectively. The carrier gas and the flow rate were nitrogen at 40 psi, hydrogen at 1.5 kg/Cm^2, and 0.5 kg/Cm^2 air.

Experiment Design and Execution

The saturated soil culture system used various water levels arranged as randomized block designs. The water levels used were 15, 20, and 25 cm from the soil surface, as shown in Figure 1. Each treatment was repeated six times resulting in 18 experimental plots. Land preparation started with clearing the experimental area of the existing weeds and then making a plot measuring 3 x 2 m. A trench was made around the plot measuring 30 cm wide and 30 cm deep. The trench was flooded continuously with inundation heights according to the treatments from the soil surface. Planting began with making a hole with a depth of 2-3 cm and a spacing of 30 x 20 cm. Based on the determined size of the experimental plot, the number of plants per plot was 100. Replanting was performed at the age of 7 days, when the newly grown seeds die after planting (DAP).

Observation Variables

1. Soil Moisture

Moisture content was observed in the center of the experimental plot after four weeks of planting (WAP), using the Soil pH and moisture meter ETP 302. Observations were made at 12.30 noon in sunny weather conditions.

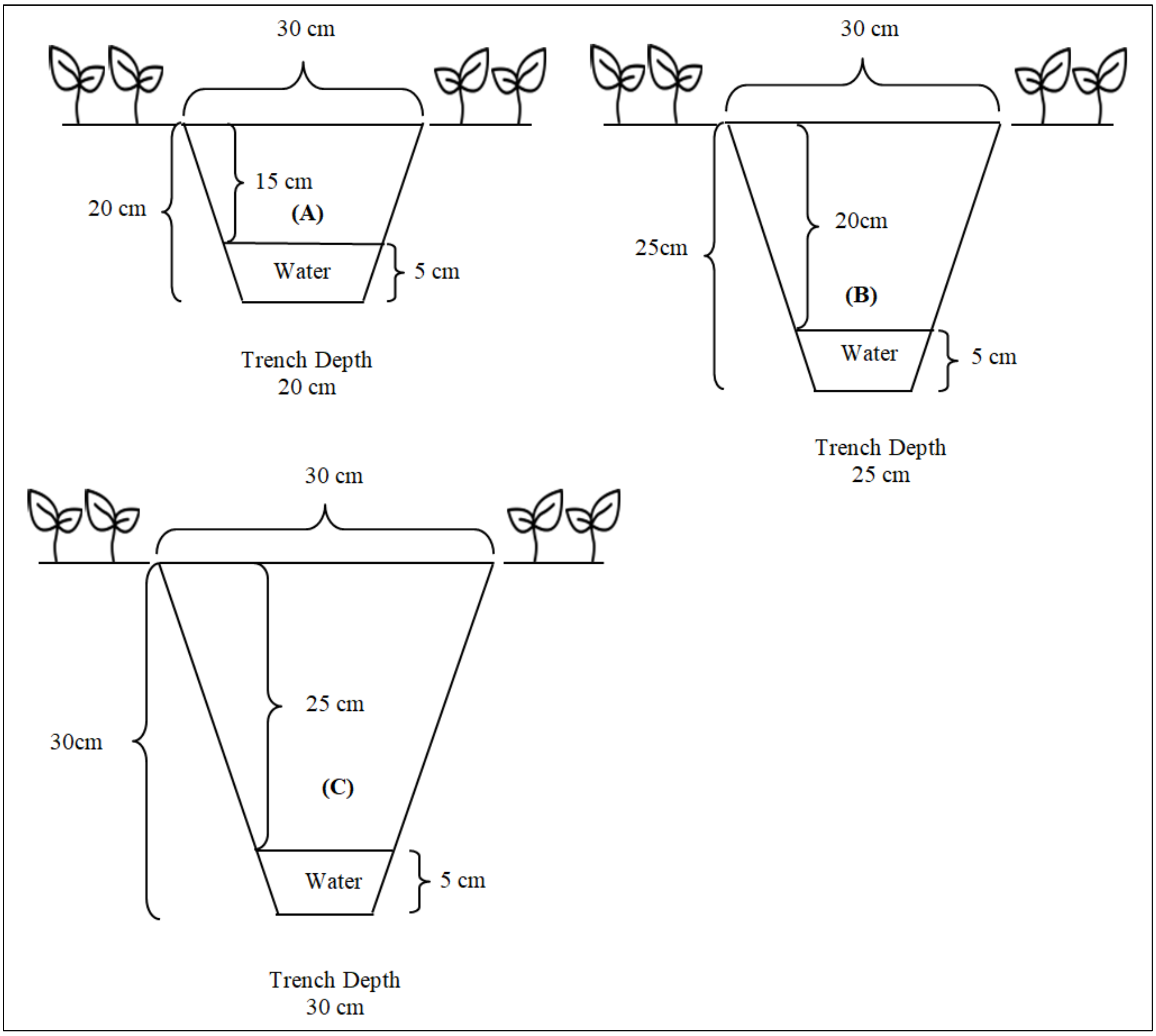


Figure 1. SSC profile and height of inundation in the trench. The height of water in the trench is 15 cm from the ground (A); The height of water in the ditch is 20 cm from the ground (B); The height of water in the trench is 25 cm from the ground (C)

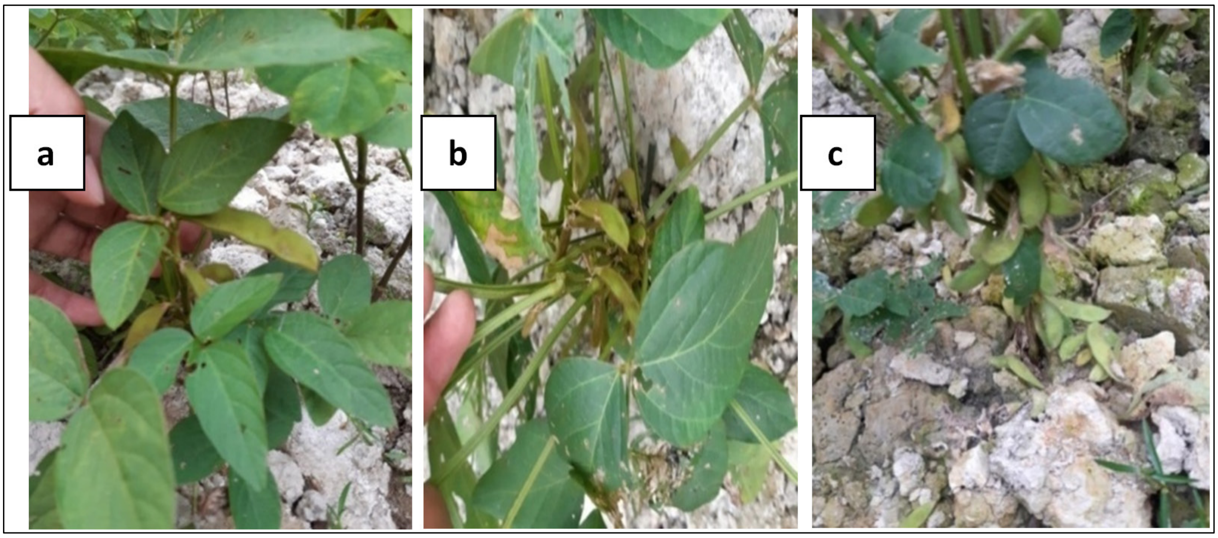


Figure 2. Symptoms of disease in soybean cultivar Anjasmoro in water-saturated cultivation with various water levels, a). 25 cm, b). 15 cm,c). pods fall off

2. Disease Incidence

The percentage of infected plants was observed when they began to show spots on the affected soybean leaves. The percentage of affected plants was calculated using the formula [22] as follows:

$$P = \frac{a}{b} x\ 100\%$$

Information:

P = percentage of affected leaves

a = Number of plants having leaves showing symptoms h the

b = Nber of plants observed

3. Disease Severity

Disease severity was calculated based on the scale of damage with the following formula:

$$P = \frac{\sum(nxv)}{NxV}\ x\ 100\%$$

Information:

KP = Severity of plant disease

n = Number of plant samples observed at each damage scale

v = Damage scale value

N = Total number of observed plant samples

V = Highest damage scale value

4. Observation of Physiological Variables

The physiological observations include root nodules' dry weight and nitrogen fixation rate. The nitrogen fixation rate was expressed based on the acetylene reduction activity of the root nodules. The acetylene reduction method was used to measure nitrogen-fixing activity at 6 WAP, as in [21].

5. Observation of Yield Variables

The yield components observed include total dry seed weight, seed weight per 100 dry seeds, and the number of filled pods.

6. Data Analysis

The collected yield data and components, as well as root nodules weights, were subjected to analysis of variance (ANOVA) and then continued with the least significant difference test (LSD) to determine the differences between treatments. Furthermore, laboratory analysis of the acetylene reduction activity of the root nodules was carried out to determine the nitrogen fixation rate. The data on the acetylene reduction activity of root nodules were displayed in graphical form. Meanwhile, data from soil moisture observations are presented in narrative form. Minitab software version 18 was adopted for the correlation and regression analysis.

3. Results and Discussion

Soil Moisture

The disease development on soybean of the Anjasmoro cultivar in water-saturated cultivation on tidal land was monitored from the beginning of growth to the pod-filling phase. Furthermore, pathogen development is influenced by environmental conditions, improper plant, and management. The plants are infected in environmental conditions that support the pathogen development. Differences in the height of puddles in the cultivation technology of saturated water cause differences in environmental conditions where they grow, especially soil moisture. The observation results showed that the soil moisture in the experimental plot with a puddle 25 cm below the soil surface was lower (53.3%). Meanwhile, the 15 cm and 20 cm trenches were higher and relatively the same, namely 72% and 70.5%, respectively. In this condition, the symptom of the disease found was irregular, blackish-brown patches on the pods and stalks. The infection began to occur from the formation phase until it ripened, causing pod loss, as shown in Figure 2.

Plant Disease

Disease Incidence

Anthracnose disease caused by *Colletotrichumdematinum* var. *truncatum* varied with the height of inundation in saturated culture systems. The results indicated that the highest percentage of infected plants was found at a depth of 15 cm below the soil surface, which was 15.7%. This is followed by the 20 cm depth of standing water in the trench, which was 16.3%. Furthermore, the percentage of affected plants was lower at 25 cm water depth below the soil surface, which was 10.4%, as shown in Figure 3.

High humidity was a very favorable environment for the fungus *Colletotrichumtruncatum*. This was proven in the treatment plots of 15 cm and 20 cm water height from the soil surface because the soil moisture was higher. Therefore, high humidity favored spore germination.

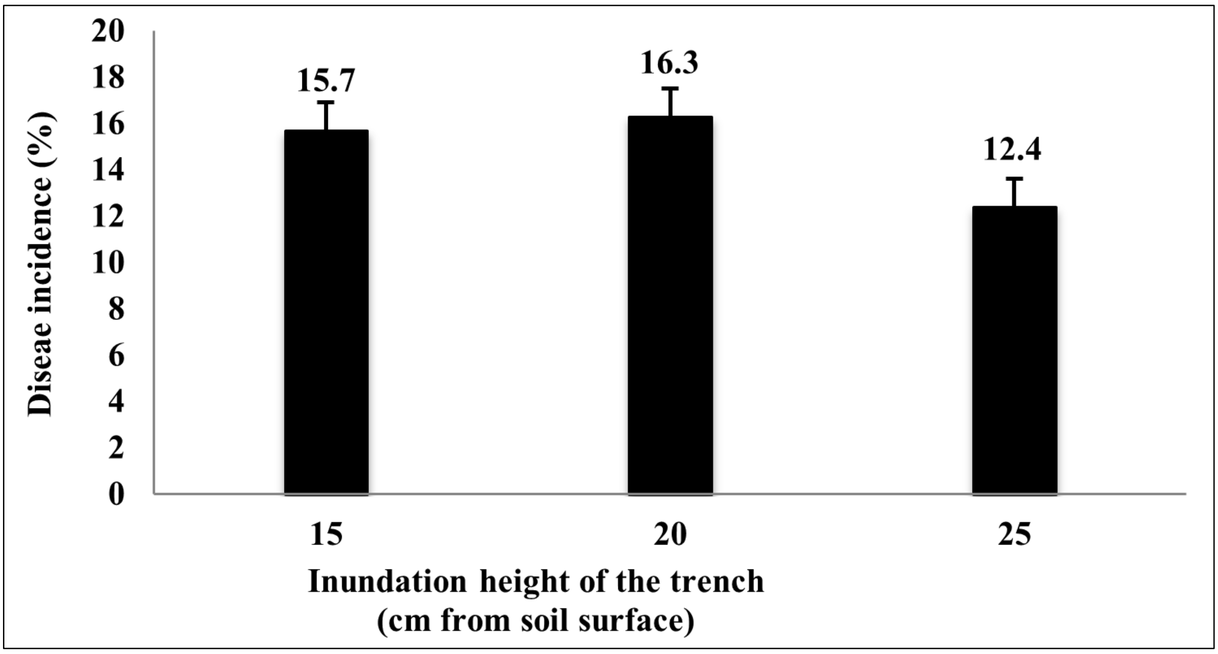


Figure 3. The disease incidence of anthracnose on soybean cultivar Anjasmoro based on various water levels in saturated soil culture on tidal land

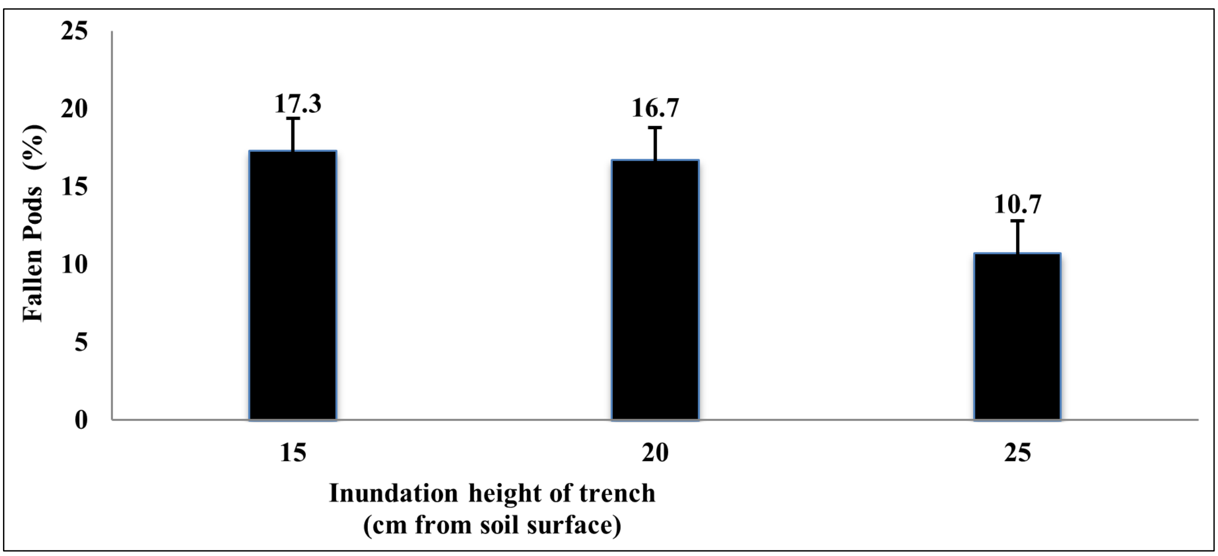


Figure 4. Percentage of pod loss in soybean cultivar Anjasmoro in the various water levels in saturated soil culture on tidal land

Disease Severity (DS)

The severity of the disease was affected by the depth of the waterlogged ditch in the water-saturated cultivation system. At the height of water inundation 15 cm and 20 cm below the soil surface, the severity of the disease increased by 30.02% and 29.5%, respectively. Meanwhile, at 25 cm, the severity of the disease was lower at 9.5%. Environmental conditions such as soil moisture and air humidity affect the development of the disease. The microclimate's high humidity affected the spores' development, severely impacting crop production. Furthermore, the percentage of fallen pods shown in the treatment with 15 cm and 20 cm depth was 17.3% and 16.7%, respectively. The lowest percentage of pod loss was obtained in the treatment of 25 cm puddle height, which was 5.6%, as shown in Figure 4.

Physiological Aspects

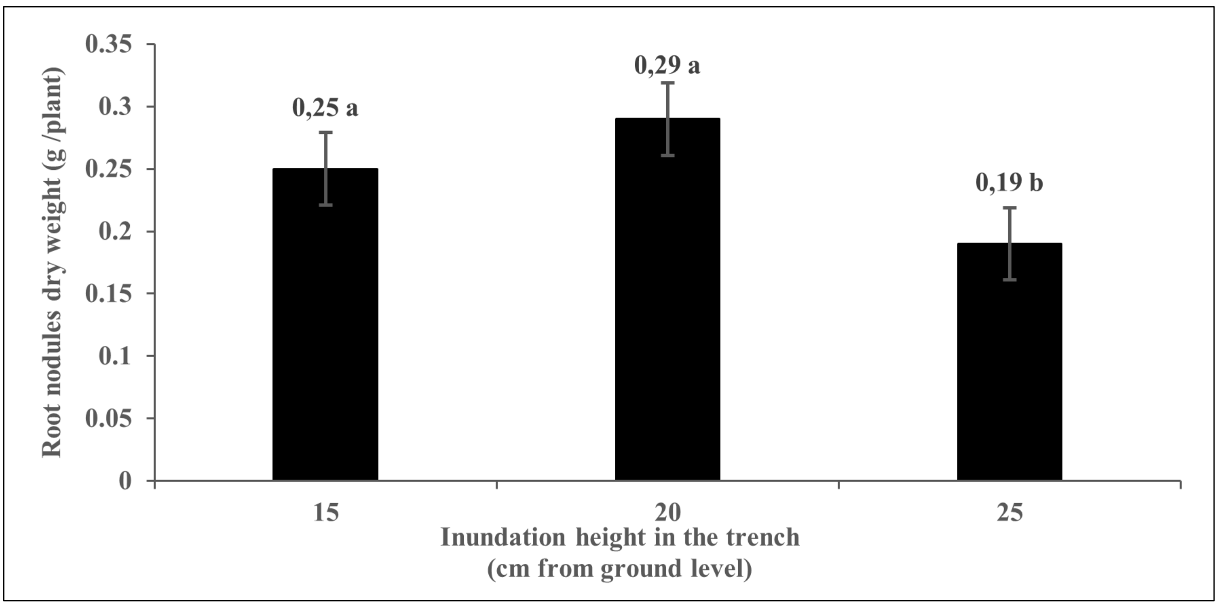
The height of standing water in the trench affects the root nodules' dry weight of the Anjasmoro soybean. The dry weight of root nodules at a depth of 25 cm below the soil surface was lighter than 15 cm and 20 cm. Furthermore, there were no significant differences in the dry weight of the Anjasmoro cultivar soybean root nodules at 15 cm with a waterlogged height in the trench 20 cm from the ground, as shown in Figure 5.

The rate of nitrogen fixation can be explained by the acetylene reduction activity of root nodules, as shown in Figure 6. The waterlogging of 15 cm and 20 cm did not affect the acetylene reduction activity. Hence, at that height, there was no difference in the nitrogen fixation ability for each nodule weight unit. Furthermore, the acetylene reduction activity decreased at the waterlogging height of 25 cm below the soil surface, showing that the rate of nitrogen fixation decreased. This study's results also indicate that drought causes nitrogen fixation to decrease. In line with this statement, [23] reported that nitrogen fixation had decreased under drought conditions before other processes, including photosynthesis and soil nitrogen uptake. Meanwhile, the ability to embed each nodule weight unit and per plant declined as plant age increased.

This experiment does not demonstrate that trench nodules inundation will be active when filling pods. The results showed that the ability of nodules to fill the pods reduced to the same level as the control. Although plants continuously inundated in the trench had heavier nodules dry weight due to the lower nodules activity, the nitrogen embedding rate in the treatment was not different from the control [23].

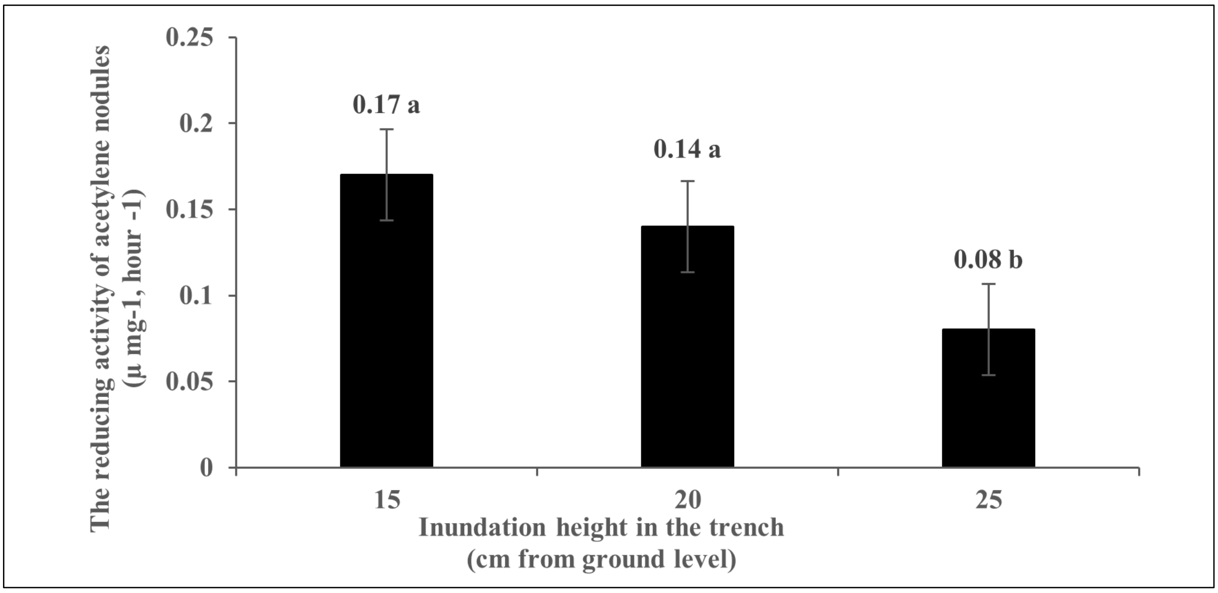
Correlation among DS and Dry Weight of Root Nodules, Acetylene-reducing Activity, Dry Weight of Seeds/Plants

Table 1 showed a strong correlation between disease severity and root nodules dry weight (r = 0.6530), acetylene-reducing activity (r = 0.4044), and dry seed/plant weight (r = 0.8465). Based on the correlation analysis, it can be stated that disease severity was most closely correlated with dry seed/plant and root nodules' dry weight compared to acetylene reduction activity.



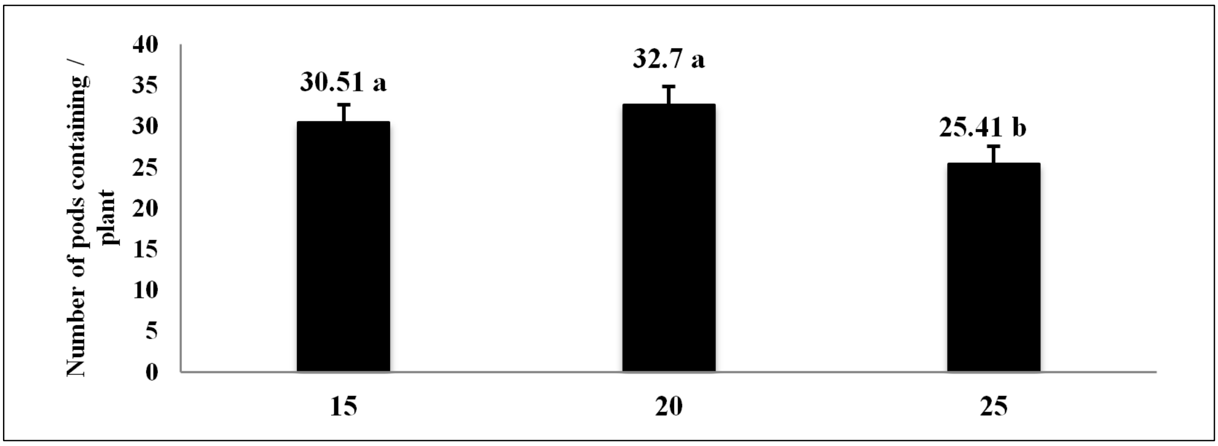
Note: The numbers followed by the same letter are not significantly different according to the LSD test at the 5% level

Figure 5. The dry weight of the Anjasmoro cultivar soybean root nodules at three high levels of standing water in the trench.



Note: The numbers followed by the same letter are not significantly different according to the LSD test at the 5% level

Figure 6. Reduction activity of acetylene root nodules of Anjasmoro soybean at three high levels of standing water in the trench



Note: The numbers followed by the same letter are not significantly different according to the LSD test at the 5% level.

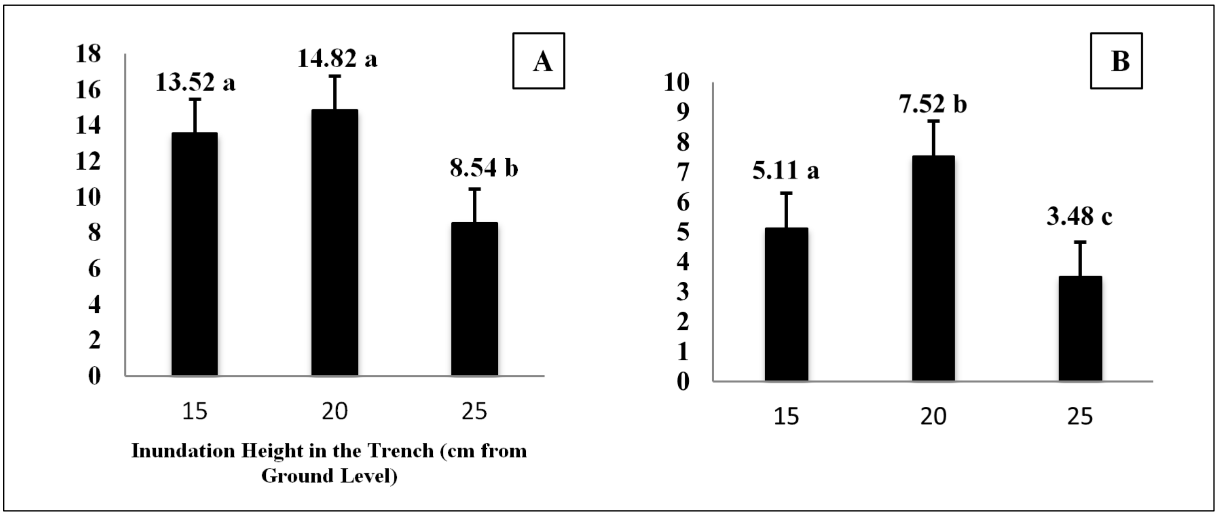
Figure 7. Several pods containing Anjasmoro soybean cultivar at three high levels of standing water in the trench

Table 1. Analysis of linear correlation between disease severity and root nodules dry weight, reducing the activity of acetylene nodules, dry seed weight/plant

Variable	recounted	table	Criteria
Root nodules dry weight	0.6530	0.5760	Strong
Reducing the activity of acetylene activity	0.4044	0.5760	Moderate
Dry seed weight/plant	0.8465	0.5760	Very strong

The regression analysis of root nodules was expressed by the equation model Y= 0.5937 – 0.0229 DS, with an R^2 of 0.4265. This model shows that an increase of one unit in disease severity reduces the dry weight of root nodules by 0.229 units. The coefficient of determination (R^2) of 0.4265 indicates that 42.65% of the variation in the dry weight of root nodules (Y) is caused by the severity of the disease.

Regression analysis for acetylene reduction activity was expressed by the equation model Y= 0.3426 – 0.0141 DS, with an R^2 of 0.1636. The model explains that every one-unit increase in disease severity will be followed by a decrease in acetylene reduction activity by 0.0141 units. The R^2 of 0.1636 indicates that 16.36% of the variation in acetylene reduction activity is caused by disease severity. Furthermore, the regression analysis of the dry seed/plant weight was expressed by Y = 19.6952 – 0.8782 DS, where R^2 = 0.7166. This signifies that every one-unit increase in disease severity causes a decrease in dry seed/plant weight by 0.8782 units. The R^2 of 0.7166 implies that 71.66% of the variation in dry seed/plant weight is caused by disease severity.



Note: The numbers followed by the same letter are not significantly different according to the LSD test at the 5% level

Figure 8. Dry seed weight per 100 dry seeds (A) and dry seed weight per plant (B) Anjasmoro soybean cultivar at three high levels of standing water in the trench

Crop Yields

The water height in the trench significantly affects the number of filled pods/plants, dry seed weight/100 seeds, and dry seed weight/plants. There was no difference in the number of pods between the height of 15 cm and 20 cm of water from the ground. Furthermore, the number of filled pods at the height of water of 25 cm was significantly lower than 15 and 20 cm, as shown in Figure 7.

There were no significant differences between the weight of dry seeds/100 seeds and dry seeds/plants in the treatment of inundation of 20 and 15 cm. Furthermore, they were significantly higher than the depth of 25 cm below the soil surface, as shown in Figures 8 A and 8 B.

A height of 20 cm from the soil surface can efficiently produce optimal production. Furthermore, the soil moisture below 25 cm from the ground was drier, indicated by 58.3%. This condition causes soybean to experience disturbances in their physiological functions due to a lack of water. Lack or deficit of water in a plant closes its stomata. It also increases mesophyll resistance, thereby limiting the amount of CO_2 intake, which reduces the speed and efficiency of photosynthesis [24,25]. Furthermore, closing all or part of the stomata decreases CO_2 concentration in the intercellular space of the leaves. This reduces the amount of CO_2 entering the Calvin cycle and increases O_2/CO_2 ratio. The increase in the ratio causes the ribulose bisphosphate carboxylase (RuBP carboxylase) enzyme to tether and add O_2 (instead of CO_2) to the Calvin cycle. The product decomposes, and a molecule of a two-carbon compound is transferred from the chloroplast to the mitochondria and peroxisomes, breaking down these molecules into CO_2 without assimilating (photorespiration process) or producing ATP. Furthermore, the growth and productivity of soybean at 25 cm depth below the soil surface are inhibited or not optimal because ATP or assimilates are not produced in photorespiration.

Water deficiency conditions at a depth of 25 cm below the soil surface can reduce or affect metabolism in the plant. Furthermore, prolonged wilting will cause the cuticle to be less permeable to water. This situation reduces the speed of ion transport, respiration, enzyme activity, and protein synthesis and inhibits the translocation process. A decrease in respiration will produce reduced energy in the form of ATP, $FADH_2$, and $NADH_2$. The indirect effect on production occurs due to the reduced absorption of soil nutrients. Additionally, the low availability of water in the soil will lower the absorption of nutrients [26, 27].

4. Conclusion

The height of standing water in the trench in the saturated water culture system affected soil moisture. A high incidence of anthracnose disease, caused by the fungus *Colletotrichumdematinum* var. *truncatum*. was seen on soybean. The weight of the root nodules showed a decrease in the puddle height by 25 cm from the soil surface. Furthermore, the nitrogen fixation rate decreased, as indicated by a decline in the acetylene reduction activity of the root nodules, which decreased at the height of the water by 25 cm from the soil surface. The numbers of filled pods, the weight of dry seeds per plant, and the weight of 100 seeds were lower at a depth of 25 cm below the soil surface. Disease severity was strongly correlated with root nodules and seed/plant dry weight. The increase in disease severity contributed significantly to the decrease in the dry weight of root nodules, very strongly to dry seed/plant weight and moderately to acetylene-reducing activity.

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