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NITRATE REDUCTASE ENZYME ACTIVITY AND ITS CORRELATION WITH SOYBEANS YIELD IN SATURATED SOIL CULTURE AND WATERING CULTIVATION IN TIDAL LAND

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ABSTRACT
This study aimed to determine the Activity of Nitrate Reductase (ANR) of soybean in Saturated Soil Culture (SSC) and Watering Cultivation (WC) in tidal land and its correlation with aspects of soybean yield of the Anjasmoro cultivar. Treatment applications were arranged in a randomized block design (RBD) with three replications. The height of standing water in the trench in the Saturated Soil Culture system (SSC) as a treatment in this study was 15 cm, 20 cm, and 25 cm from the soil surface. WC actors were selected from 3 farmer groups. Soybean seeds were planted in experimental plots measuring 3 m x 2 m with a spacing of 30 cm x 20 cm, according to the spacing used by farmers. SP36, KCl, and Urea fertilizers were given as basic fertilizers at the recommended dosage. Observation variables included: ANR, number of filled pods/plant, dry seed weight/100 seeds, and dry seed weight/plant. The results showed that the height of standing water in the ditch in SSC affected the ANR and soybean yield. There was no difference in ANR for the height of the water in the ditch 15 cm and 20 cm from the soil surface, 12.76 mol NO_2, respectively $g^{-1}hour^{-1}$ and 13.43 mol NO_2. g^{-1}. $hour^{-1}$. The lowest ANR was obtained at the treatment level with the height of the puddle in the ditch 25 cm from the soil surface, namely 11.52 mol NO_2. $g^{-1}.h^{-1}$, but still higher than the ANR in WC. There was no difference in the number of filled pods/plant, dry seed weight/100 seeds, and dry seed weight/plant between the height of standing water in the 15 cm trench and 20 cm from the soil surface.

KEY WORDS
Nitrate reductase, saturated soil culture, soybean, Tidal land.

Soybean plant (*Glycine max* (L)) is one of the important food crops after rice and corn which is being developed in Indonesia. At this time soybean development has led to land use problematic (marginal) including tidal land which is estimated at 20.13million hectares (Subagyo, 2006). To reduce dependence on soybean imports, which were recorded at 1.17 million tons in 2018 (Central Bureau of Statistics, 2018), the cultivation of soybeans continues to be developed. In Jambi Province, soybeans are mostly developed in tidal areas, especially in Simpang Village and Rantau Makmur Village, Berbak District, East Tanjung Jabung Regency on overflow type C land. Based on the inundation pattern or the range of the tide, overflow type C is not inundated but the depth of groundwater at high tide is less than 50 cm (Ar-Riza and Alkasuma, 2008).

Soybean cultivation which is usually done by farmers is to provide intermittent irrigation (conventional) which is carried out every 2 or 3 weeks, in this study, it is called watering cultivation (WC). Intermittent provision of water interferes with plant growth because while in a state of dryness it will experience stress and when water is given a recovery occurs, but with watering intervals of 2 - 3 weeks, before recovering the plant will experience drought stress again. Possibly with this irrigation cultivation where water is less available, it interferes with metabolic processes in plants such as nitrate reductase activity which plays an important role in nitrogen fixation and affects plant growth. According to Ali et al. (2007); and Ali (2020) the nitrate reductase enzyme is one of the plant enzymes that has been very intensively studied because its activity is a limiting factor in the nitrate assimilation process which plays an important role in plant growth and productivity.

The application of saturated soil culture technology (SSC) is one of the efforts to overcome the low productivity of soybeans on tidal land, namely by irrigating plants continuously through waterlogging in ditches. With this technology, the groundwater table can be maintained at a certain depth or height, so that it can support metabolic activity, achieving high soybean growth and yields. The availability of sufficient water in the plant environment has a relationship with the nitrate reductase enzyme as a provider of protons and electrons for its activity. According to Salisbury and Ross (1992) available water molecules donate protons and electrons through photosynthesis which produces $NADPH_2$ during the light reaction. The resulting $NADPH_2$ is sufficient to support the activity of the nitrate reductase enzyme when reducing nitrate to nitrite. In connection with this (Ghulamahdi, et al., 2009; Sagala, et al., 2011), suggested that saturated water cultivation can improve growth, increase nutrient uptake and increase soybean production compared to dry cultivation.

Availability of water determines productivity and the activity of the enzyme nitrate reductase is positively correlated with productivity. However, the activity of nitrate reductase and its relationship with the soybean yield of Anjasmoro cultivar on SSC and WC in tidal land is not widely known. Therefore, it is necessary to research to determine the activity of the soybean nitrate reductase enzyme in SSC and WC on tidal land. Apart from that, this study also aims to determine the correlation between nitrate reductase activity and the yield aspects of the Anjasmoro cultivar soybean.

METHODS OF RESEARCH

This research was conducted in Simpang Village, Berbak District, East Tanjung Jabung Regency from May 2019 to October 2019. The research location is a tidal area of overflow type C and alluvial soil type. Based on the inundation pattern or the range of the tide, type C overflow is not flooded but the groundwater depth at high tide is less than 50 cm (Ar-Riza and Alkasuma, 2008).

The material used was soybean seed cultivar Anjasmoro, SP36, KCl, Urea, insecticide, acetone, $KHCO_3$, K_2CO_3, leaf sample, buffer $Na_2HPO_4.2H_2O$, and $NaH_2PO_4.2H_2O$, $NaNO_3$, SA (sulfanilamide), NED (napthylethylene diamide), HCl_3N, and H_2O. The tools used are scales with an accuracy of 0.001g and 100g, a thermometer, pH meter, spectrophotometer, and measuring cup.

This research was conducted in parallel (series) between the WC group (farmer's version) and the SSC group. Both groups used a randomized block design (RBD) as an environmental design with 3 replications. The height of standing water in the ditch as a treatment for the SSC was 15 cm, 20 cm, and 25 cm from the soil surface. In the WC group, 3 farmer groups are designated as WC actors (data source). Soybean seeds were planted with spacing according to the spacing used by farmers, namely 30 cm x 20 cm in the experimental plot measuring 3 m x 2 m. SP36 and KCl fertilizers as basic fertilizers were given simultaneously at the time of planting according to the recommended doses of 150 $kg.ha^{-1}$ SP36 and 100 $kg.ha^{-1}$ KCl. Urea fertilizer 50 $kg.ha^{-1}$, given twice, i.e. 1/2 dose at planting time and the remaining 1/2 dose given 30 days after planting.

The variables observed in SSC and WC in this study were soil moisture, nitrate reductase activity, and aspects of soybean yield which included: the number of filled pods/plant, dry seed weight/100 seeds, and dry seed weight/plant.

Measured according to the method described in Hartiko (1983): fresh leaves (3rd leaf from the tip/top of soybean plant) were picked around 9-10 am at 6 weeks after planting (WAP). The leaves were washed with distilled water until clean then the leaf bones were removed to obtain leaf blades. Leaf-blades weighing 500 mg were cut into thin strips of about 1 mm using a sharp knife. The leaf pieces were put into 5 ml of phosphate buffer solution in a dark tube. After soaking for 24 hours, the buffer solution was replaced with a new one. Phosphate buffer was made from a mixture of 0.1 M NaH_2PO_4 and 0.1 M Na_2HPO_4. $NaNO_3$ 0.1 ml was added with a micropipette and the incubation time was recorded for 2 hours. A dye reagent consisting of 0.2 ml of 0.02% N-naphthylenedi amine solution and 0.2 ml of 1% sulphanil amide in 3 N HCl was prepared. After being incubated for 2 hours, 0.1 ml of incubation liquid was taken from a dark tube and put into a test tube containing a dye reagent, then waited for a pink color to occur as a sign that nitrate was reduced to nitrite by the enzyme nitrate reductase. One test-tube was not given the filtrate and was used as a blank. After the color change occurred, 2.5 ml of distilled water was added, and then transferred to a cuvette to measure the absorbance in a spectrophotometer at a wavelength of 540 nm.

Data collection for yield aspects was carried out after harvest, including the average number of filled pods per plant, dry seed weight/100 seeds, and dry seed weight/plant.

Environmental observations in the form of soil moisture content were carried out in the center of the plot. Soil moisture was measured in the morning at 7.00, in the afternoon at 12.30, and in the afternoon at 16.30 WIB using a soil moisture meter (ETP 302) at a depth of 15, 20, and 25 cm from the soil surface. Observations started when the plants were 2 weeks after planting until the plants were 10 WAP.

Data were analyzed with Anova, and if the test results were significant, continued with the BNT test at a 5% significance level to determine the difference in effect between treatments. A T-test at a 5% significance level was used to compare the effect of SSC with WC on all observed variables. Correlation and regression analysis of nitrate reductase activity to aspects of the results using Minitab software version 18.

RESULTS AND DISCUSSION

The depth of the water puddle in the ditch at the SSC affects soil moisture. The highest soil moisture was achieved at a height of 15 cm and 20 cm from the ground and the lowest was obtained at 25 cm from the soil surface (Figure 1).

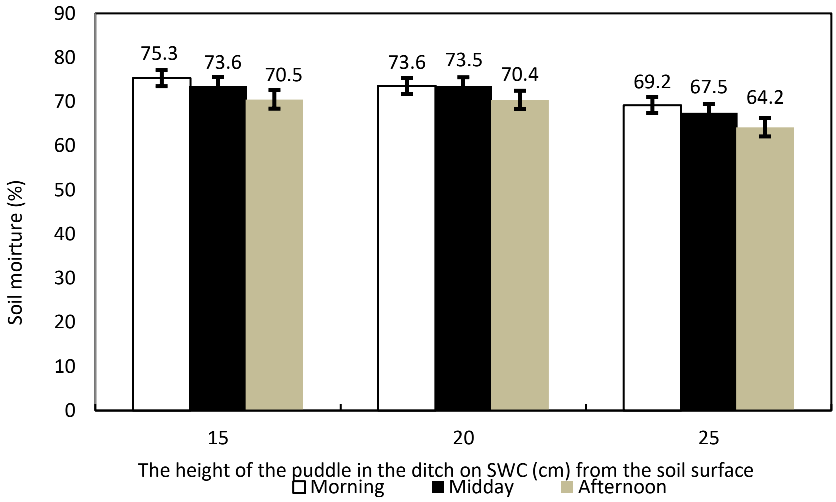

Figure 1 – Average soil moisture in experimental plots in saturated soil culture from June to August

Based on the group comparison test (T-test) between soil moisture in the SSC group and soil moisture in the WC group, there was a significant difference in soil moisture, both in the morning, afternoon, and evening. Soil moisture in SSC was higher than that in WC (Figure 2).

The results showed that the difference in height of standing water in the ditch in the SSC system affected the activity of nitrate reductase (ANR). The highest nitrate reductase activity was achieved at the treatment level of high standing water in the trench 15 cm and 20 cm from the soil surface, respectively 12.76 mol $NO_2.g^{-1}.hour^{-1}$ and 13.43 mol $NO_2.g^{-1}.hour^{-1}$

and not significantly different between the two. The lowest ANR was obtained at the height of the puddle in the ditch 25 cm from the soil surface, namely 11.52 mol $NO_2.g^{-1}.hour^{-1}$ (Figure 3A). Overall the ANR for SSC was higher than the ANR for WC (Figure 3B).

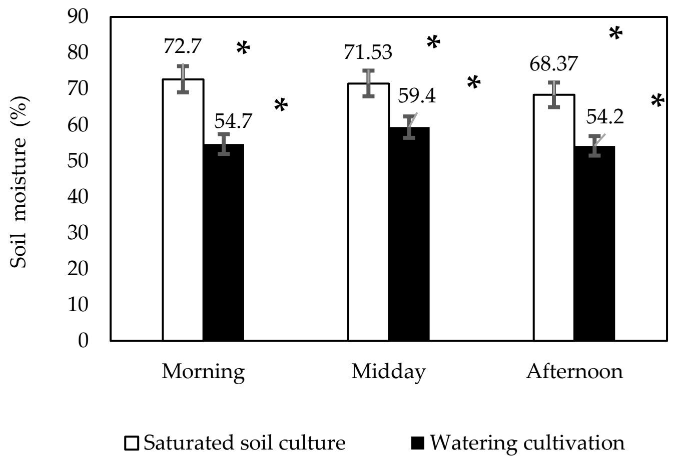

Figure 2 – Soil moisture in saturated and aquaculture watering (processed data). The * sign indicates the difference significant based on the T-test at a 5% significance level

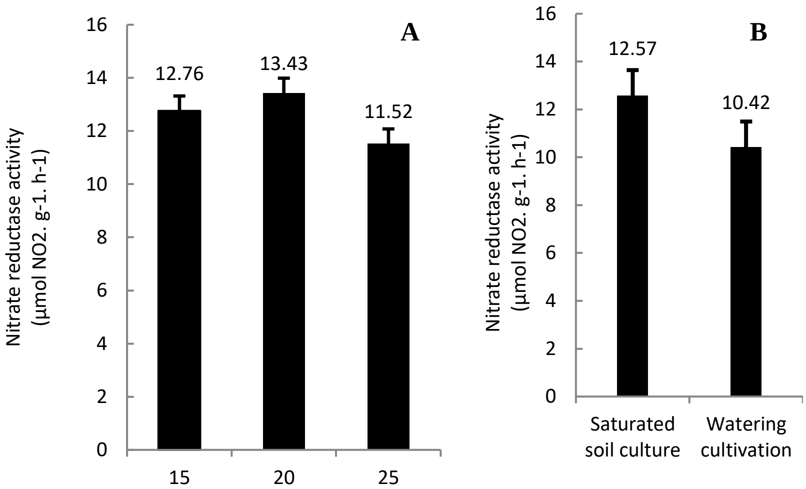

Figure 3 – Nitrate reductase activity of soybean cultivar Anjasmoro aged 6 WAT at saturated soil culture (A) and watering cultivation (B). The * sign indicates a significant difference based on the T-test at a 5% significance level

Aspects of the results in this study included the number of filled pods/plant, dry seed weight/100 seeds, and dry seed weight/plant. Differences in the height of waterlogging in the ditch at SSC affected the number of filled pods/plant, dry seed weight/100 seeds, and dry seed weight/plant of the Anjasmoro cultivar. There was no difference in the number of filled pods/plant, dry seed weight/100 seeds, and dry seed weight/plant at a height of 15 cm and 20 cm of waterlogging from the soil surface. The value of the three variables decreased significantly at the height of the puddle in the ditch 25 cm from the ground surface. However, even though there was a decrease in the number of filled pods/plant, dry seed weight/100 seeds, and dry seed fallow/plant, they were still higher than those obtained in watering cultivation (Table 1). In general, SSC is better than WC. The results of the comparison test (T-test) between SSC and WC on the number of filled pods, dry seed weight/100 seeds, and dry seed weight/plant showed that the three variables were significantly higher in SSC than in WC (Figure 4 A, B, and C).

Table 1 – Number of filled pods per plant, dry seed weight per 100 seeds, and dry seed weight per plant based on the height of the puddle in the ditch at SSC

Height of standing water in the trench (cm from ground level)	Number of filled pods/plant	Dry seed weight/100 seeds (g)	Dry seed weight/plant (g)
15	34,50 a	20,03 a	38,65 a
20	36,23 a	21,94 a	40,31 a
25	29,43 b	18,51 b	32,33 b

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

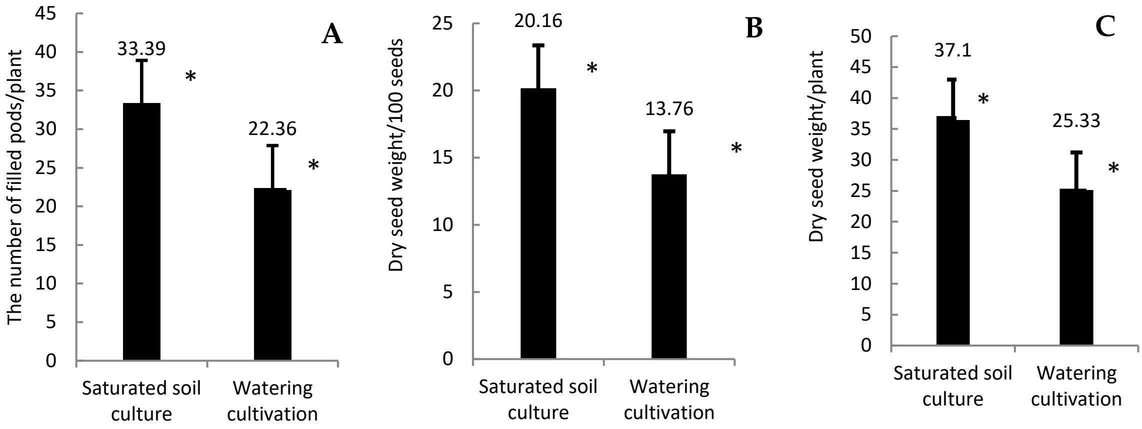

Figure 4 – The number of filled pods/plant (A), dry seed weight/100 seeds (B), and dry seed weight/plant (C) in SSC and WC. The * sign indicates a significant difference based on the T-test at the 5% significance level

The results of the correlation analysis (Table 2) showed a positive correlation between nitrate reductase activity and the results which included the number of filled pods/plant (r = 0.8541), dry seed weight/and 100 seeds (r = 0.7996), and dry seed weight/plant (r = 0.9143) soybean cultivar Anjasmoro. This means that the activity of nitrate reductase and soybean yield changed in the same direction. The higher the ANR value, the higher the average result, and vice versa. Based on correlation analysis between nitrate reductase activity and yield aspects, it can be stated that ANR is most closely correlated with dry seed weight/plant and the number of filled pods/plant compared to dry seed weight/100 seeds. The results of this study are in line with the results of research by Suherningsih (1990) that ANR has a positive correlation with production, dry weight, total nitrogen, and crop yield. Furthermore, it was said that the higher the ANR, the higher the dry weight of the plant.

Table 2 – Analysis of linear correlation between nitrate reductase activity and soybean production variables of Anjasmoro cultivar

Production variable	$r_{counted}$	r_{table}	Criteria
Number of filled pods/plant	0,8541	0,6664	Very strong
Weight of dry seeds/100 seeds	0,7996	0,6664	Strong
Weight of dry seed/plant	0,9143	0,6664	Very strong

The results of the regression analysis of the number of filled pods/plants were expressed by the equation model Y= -44,21 + 6,092ANR (R^2= 0,9219). This model shows that with a one-unit increase in nitrate reductase activity, the number of filled pods will increase by 6.092. The coefficient of determination (R^2) was 0.9219, indicating that 92.19% of the variation in the number of filled pods/plants (Y) was caused by nitrate reductase activity.

Regression analysis for dry seed weight/100 seeds is expressed by the equation model Y= -10,02 + 2,381ANR (R^2= 0,8582). The model explains that every one unit increase in nitrate reductase activity will be followed by an increase in dry seed weight/100 seeds of

2,381 units. The coefficient of determination (R^2) of 0.8582 showed that 85.82% of the variation in dry seed weight/100 seeds was caused by nitrate reductase activity. Furthermore, the results of the regression analysis of dry seed/plant weight were expressed by the equation model Y = -23.74 + 4.711ANR (R^2 = 0.8817). The model shows that every one unit increase in nitrate reductase activity causes an increase in dry seed/plant weight of 4.711 units. The coefficient of determination (R^2) of 0.8817 indicates that 88.17% of the variation that occurs in dry seed/plant weight is caused by nitrate reductase activity.

DISCUSSION OF RESULTS

Soil moisture states the amount of water that fills part or all of the soil pores. The high soil moisture at the height of the puddle in the trench 15 cm (75.3%) and 20 cm (73.6%) from the soil surface at the SSC indicated that most of the soil pores were filled with water. In other words, the availability of water in the plant environment at these two water levels in the trench is more than the availability of water at a height of puddles of water in a trench 25 cm from the soil surface with lower soil moisture (64.2%) (Figure 1). Overall soil moisture in SSC was higher than that in WC soil, both in the morning, afternoon, and in the afternoon (Figure 2). This also shows that the water content/availability of groundwater in the SSC is higher than in the WC. The availability of high soil moisture will affect the growth and yield of plants.

Based on the analysis of the variance test (ANOVA) on the activity of nitrate reductase (ANR) showed significant results. This shows that the variation of water inundation height in the ditch at the SWC significantly affects the ANR. From the results of the BNT further test, it was found that the highest ANR was achieved at the treatment level of the height of puddles in the trench 15 cm and 20 cm from the soil surface, both of which caused a significantly higher ANR than the treatment level for the height of puddles in the ditch 25 cm from the ground surface (Figure 3 A). Overall the ANR for SWC was higher than the ANR for WC (Figure 3B).

The high soil moisture in the SSC, both at a height of 15 cm and 20 cm from the soil surface allows for the availability of sufficient water for plant growth. In contrast to the low soil water content at the height of the puddle in the trench 25 cm from the soil surface, it is suspected that it cannot act as a provider of protons and electrons through the photosynthesis process, so it cannot support nitrate reductase activity. Likewise water cultivation with lower groundwater content.

High soil water content has a relationship with the activity of the enzyme nitrate reductase. In this case, water plays a role or functions as a provider of protons and electrons through the process of photosynthesis at the stage of the photolysis reaction of water during the light reaction. In line with this statement, Salisbury and Ross (1992) explained that the available water molecules donate protons and electrons through photosynthesis which produces $NADPH_2$ during the light reaction. The $NADPH_2$ produced is sufficient to support the activity of the enzyme nitrate reductase when reducing nitrate to nitrite. Furthermore, Lehninger and Thenawijaya (1994), and Chamizo-Ampudia et al. (2017) stated that the nitrate reductase enzyme gains electrons from nucleoside or $NADPH_2/NADH_2$ which transfers electrons to flavin adenine dinucleotide (FAD) then proceeds to molybdenum, and finally to nitrate reductase to reduce nitrate to nitrite. Each step in the process of converting nitrate to nitrite requires the transfer of six electrons for each molecule (Campbell et al.,1999).

The lower ANR at the height of the puddle in the trench 25 cm from the soil surface was thought to be due to lack of water which is characterized by lower soil moisture which can cause plants to experience water stress. Likewise in the WC where the provision of water through intermittent watering interferes with plant growth because when dry the plants experience stress and when water is given there is recovery, but before recovering the plants experience drought stress again. Nitrate reductase is a key enzyme for nitrogen assimilation and acquisition and plays a central role in plant biology (Wang et al., 2018; Xu G et al., 2012; Costa-Broseta, et al., 2020). According to Fu et al. (2018) ANR decreased in plants experiencing water stress. This can occur due to stomata closure thereby cutting or limiting the supply of CO_2 to mesophyll cells. As a result, the rate of photosynthesis in these cells decreases significantly (Fitter, 1991). The low rate/efficiency of photosynthesis affects the amount of reducing power (NADPH) formed in the light reaction (Salisbury and Ross, 1992). If there is not an adequate supply of $NADPH_2$ in the cytosol, it will cause a decrease in the activity of the nitrate reductase enzyme because $NADPH_2$ has an important role as a proton and electron donor that can stimulate the movement of electrons in the cytosol, it will cause a decrease in the activity of the nitrate reductase enzyme because $NADPH_2$ has an important role as a proton and electron donor that can stimulate the movement of electrons in the cytosol. This then causes the ANR at the height of the inundation in the ditch to 25 cm from the ground surface and at the measured WC to be low. The results of this study are in line with the opinion of Hale and Orcutt (1987); Larsson et al. (1989); Munjal et al. (1997) which stated that ANR decreased in plants experiencing water stress.

The activity of nitrate reductase (ANR) which was still detected at a puddle height of 25 cm from the soil surface and in the WC indicated a fairly high assimilate flow in the leaves. This is thought to be due to the assimilation of the division between roots, vegetative organs, and reproductive organs above the soil as a response of plants to water shortages (Goldsworthy and Fisher, 1992). Under conditions of water shortage, some of the products of photosynthesis are required to meet energy expenditures when reducing nitrogen by taking between 10% and 30% of the available assimilate supply.

The low soil water content at the height of the puddle in the trench 25 cm from the soil surface in the SSC and the WC, in addition to partially or completely closing the stomata, also results in increased mesophyll resistance. This condition limits the amount of CO_2 that can diffuse into the leaf and reduces the concentration of CO_2 in the intercellular space in the leaf. Reducing the amount of CO_2 entering the leaf will reduce the amount of CO_2 entering the Calvin cycle and increase the O_2/CO_2 ratio. This causes the enzyme ribulose bisphosphate carboxylase (RuBP carboxylase) not to fix CO_2, but O_2 and add it to the Calvin cycle so that the photorespiration process occurs. In this process, a molecule of a two-carbon compound is sent out of the chloroplast to the mitochondria and peroxisomes which then break down the molecule to CO_2 without producing ATP or assimilate. The absence of ATP or assimilate in photorespiration causes soybean growth and productivity at a height of waterlogging in a trench 25 cm from the soil surface and in the WC to be hampered or not optimal because the aging process and pod ripening occur faster so that the seeds formed are smaller and the average value of overall production is low.

Conditions of lack of water can also reduce water traffic and metabolism in the plant body. Decreased respiration will result in reduced energy produced in the form of ATP, $FADH_2$, and $NADH_2$. The indirect effect on production can occur due to reduced nutrient absorption. At low soil moisture, the availability of water in the soil is low, and nutrient absorption is also low (Jumin, 2005). In addition, lack of water also reduces the rate of photosynthate translocation when soybeans enter the pod filling phase. This causes the pods not to be filled. In addition, the seeds that are formed also have a low dry seed weight. This proves that the water content affects plant production. The lower the percentage of water content, the lower the average production produced.

CONCLUSION

Saturated soil culture (SSC) affects soil moisture, nitrate reductase enzyme activity, and aspects of soybean yield of the Anjasmoro cultivar. The height of standing water in the ditch at the SSC which results in nitrate reductase enzyme activity and aspects of high soybean yields was achieved at a height of 20 cm from the soil surface, followed by the height of the puddle in the ditch at 15 cm from the soil surface. Soil moisture in SSC is higher than soil moisture in WC. Similarly, nitrate reductase activity, number of filled pods/plant, dry seed weight/100 seeds, and dry seed weight/plant were higher in SSC than in WC. The activity of nitrate reductase in SWC was positively and strongly correlated with the number of filled pods/plant and dry seed weight/plant but strongly correlated with the dry weight of seeds/100 seeds.

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