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Nitrogenase Activity and Plant Physiological Process of Soy Bean Under Saturated Soil Culture on Mineral and Peaty Mineral Soil

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Abstract

Development of soybean (Glycine max L.) production in sub-optimal land like tidal land is still less performed. Soybean productivity on tidal land is still low due to the application of conventional farming technologies, so it is not able to mitigate the negative effects of high levels of pyrite, Al, Fe, and Mn and the low availability of P and K. The possible cultivation technique is saturated soil culture (SSC) that able to influence the conditions below the root zone, physiological activity, adaptability and acclimatization of soybeans. The research objective was to study the physiological characteristics and growth of soybean under water-saturated conditions in minerals and peaty mineral soils on tidal land. The experiment was carried out in plastic house, experimental station of Cikabayan, Bogor Agricultural University from December 2014 until March 2015.

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A three factorial completely randomized design was used in this experiment. The first factor was cultivation technique, consisted of dry and saturated soil culture. The second factor was varieties (Willis and Tanggamus) and the third factor was soil type (mineral and peaty mineral soil). The result showed that saturated soil culture increases the number of stomata per plant, chlorophyll (a and b), root volume, number of root nodules, roots ethylene content, plant biomass dry weight and grain per plant, and number of filled pods. Interaction of saturated soil culture technique and mineral soil produce the highest number of stomata per plant, number of nodules, plant biomass dry weight and number of filled pods. Interaction between saturated soil culture and Tanggamus resulted on the highest number of nodules and filled pods. Dry cultivation increase the density of stomata, but the plant growth at soil moisture below field capacity (up to 40% field capacity), continually lowering the growth and production of soybean. Tanggamus has higher number of stomata per plant, total chlorophyll, root volume, plant biomass weight and number of filled pods, while Willis has higher density of stomata, the rate of roots ethylene formation, number of nodules and dry weight of grain per plant. Ethylene formation in the root zone supports the formation of new roots and subsequently increase plant nutrient uptake, thus increasing the dry weight of grains per plant. Mineral soil increase root ethylene content, root volume, plant biomass dry weight, number and weight of filled pods per plant. Peaty mineral soil increase the number of root nodules and stomata density but they are not significantly different from those of the mineral soil.

Keywords: chlorophyll; ethylene; Glycine max L.; root nodules; stomata.

1. Introduction

Reduced of productive land for planting lead to the expansion of planting area toward suboptimal land including tidal land that still widely available and are not cultivated. Tidal swamp land area in Indonesia is around 20.13 million ha [1]. Around 9.53 million hectares are potential for agricultural land and 2 million hectares is suitable for soybean [2]. The type of soil in the tidal area can be mineral or peaty minerals soils. Naturally, peat soils have lower fertility due to low nutrient levels and contain a variety of organic acids that toxic for most plants [3]. Development of soybean crops in suboptimal land like the tidal area is still less performed. Soybean productivity in tidal land is still low due to the application of conventional cultivation technology, so it is not able to mitigate the negative effects of high levels of pyrite, Al, Fe, and Mn and the low availability of P and K. One of cultivation technology solutions that can be developed is saturated soil culture (SSC). Saturated soil culture is performed by providing continuous irrigation from planting to harvest time with stable water table to make the layer below the rooting area saturated with water [4].

Response of soybean varieties to water-saturated is differing. Soybean longetivity have better growth and production than the early maturing soybean old [5, 6]. Saturated soil culture is able to increase the activity of nitrogenase, uptake of N, P, K leaves, dry weight of nodules, roots, stems, leaves, pods, and grain as compared to dry farming system. Soybean growth under saturated soil culture continue to be better than dry-saturated cultivation and dry cultivation [7].

Changes in below the root zone conditions of soybeans into water saturated will affect plants physiological activity, adaptability and acclimatization of plants to the root zone conditions with high soil moisture conditions.

Physiologically, adaptation of soybeans in water saturated conditions begin with the formation of ACC (1-amino-siklopropane-1-carboksilik acid) and roots ethylene formation stimulates aerenchyme network of roots and new roots, thus enhancing root nodule formation and nutrients absorption. The ethylene content in the roots of long lived soybean is higher than short-lived soybean [8]. The plant is expected to utilize water saturated conditions to perform the process of normal growth.

Plants adaptability that supported by physiological processes and good root conditions can accelerate optimal growth, in turn produce maximum production. Water saturated conditions is able to improve nodule dry weight around 411.76%, and increase leaf nutrient N, P, and K uptake respectively by 310.71%, 272.03%, and 452.28% compared to dry conditions [8]. Saturated soil culture can increase the dry weight of roots and root nodules and N improve bacterial activity when compared to regular irrigation method [9].

Application of saturated soil culture is expected to overcome the constraints of soybean development in mineral and peaty mineral soil that have suboptimal land characteristics. One indicator is the improvement of plant physiological processes that can support crop production. The purpose of this research is to study the physiological characteristics, including nitrogenase activity of soybean in water-saturated conditions in minerals and peaty mineral soil.

2. Materials and Methods

This research was conducted at the experimental station of Cikabayan (plastic house), Department of Agronomy and Horticulture, Faculty of Agriculture, Bogor Agricultural University from December 2014 to March 2015. Materials and tools used in this study were soybean seed of Tanggamus and Willis varieties, soil (mineral and peaty mineral), lime, cow manure, urea, SP-36, KCl, rhizobium inoculants, insecticides, poly bag (30 cm x 40 cm), syringe, Chromatograph Gas, stereo / electrical microscope, electrical scales and oven.

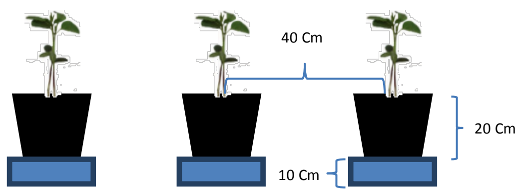

Figure 1: side position and distance of polybag media

2.1 Research Design

The research design is factorial completely randomized design with 3 factors and three replications. The first factor is dry cultivation with 80-40% moisture content of field capacity and saturated soil culture (water depth is 20 cm from the soil surface). The second factor is the soybean varieties ie Willis and Tanggamus and the third factor is the type of soil namely mineral soil and peaty mineral soil. Each treatment combination consists of 6 units of observation so that there are 144 experimental units. Soybeans were planted in a polybag, 30 cm x 40 cm in size (soil volume 10 L). Each poly bag consists of two plants. The data were analyzed using analysis of variance and Tukey different test at level α = 0.05 using the Statistical Analysis System (SAS) software version 9.1.

2.2 Measurement of Nitrogenase Activity (ethylene content)

Nitrogenase activity was measured with ARA method (Acetylene Reduction Assay) [10]. Roots and nodules were taken by cutting from the root neck. Root were put in 310 mL bottles and closed with rubber. As much as 10% by volume of air inside the bottle removed using a syringe. Furthermore, as much as 10% by volume of acetylene inserted into the bottle and incubated for 30 minutes. Furthermore, the air in the bottle is taken as much as 1 mL using syringe to measure asetilen reduction with Gas Chromatograph.

2.3 Stomata Observations

Sampling for stomata observation was conducted at 10-11 am by determining observed leaf samples, namely 5th leaves from the top. The selected leaves are smeared with nail liquid for 2 cm long and 2 cm wide on the lower surface of leaves and left for 5 minutes or until almost dry up. Clear plastic tape was attached and pressed on the leaves surface that has been smeared with polish. Then the tape was removed from leaf and placed on glass slide. Stomata number or density was observed under electric microscope at 40 times magnification with a field of view of 0.19625 mm^2.

2.4 Measurement of chlorophyll content

Measurement of chlorophyll content is using soybean mature leaves or fully developed. Chlorophyll is extracted using standard methods developed by [11]. Fresh leaves as much as 2 g diced and crushed into powder in a mortar, then add acetone tris 85/15 as much as 2 mL using autometric buret and stirred to make homogeneous solution. Then, the solution is inserted into the micro tube to centrifuge for 30 seconds at a speed of 14,000 rpm. After that, 1 mL of centrifuge solution collected using a micro pipette and dissolved again in a test tube with 2 mL of acetone tris 85/15. The extract absorbance was measured at 663 nm, 647 nm and 537 nm with a spectrophotometer. Chlorophyll content is determined by the following equation:

Chlorophyll a = $0.01373 \times A_{663} - 0.0000897 \times A_{537} - 0.003046 \times A_{647}$

Chlorophyll b = $0.02405 \times A_{647} - 0.004305 \times A_{537} - 0.005507 \times A_{663}$

3. Results and Discussion

3.1 Density and Number of Stomata per Plant

Cultivation techniques and varieties treatment significantly affect leaf stomata density, while cultivation techniques and soil types and their interactions at 8 weeks after planting (WAP) affected the number of stomata. Table 1 shows that soybean with dry cultivation techniques (moisture content 80-40% of field capacity) has higher stomata density (broad view of 0.19625 mm2), but not followed by the higher number of stomata per plant with saturated soil culture. The significant difference of stomata number per plant is caused by wider leaf per plant under saturated soil culture than under dry cultivation.

Genetic differences in the two varieties affect the number of stomata. Willis variety has higher stomata density, namely 27.32% (P> 0.05) and number of stomata per plant, namely 4.23% (P <0.05) compared with Tanggamus variety. Soil type did not affect the density of stomata, however, soybean planted in mineral soil has higher number of stomata per plant compared with that in peaty mineral soil.

Table 1: Average of stomata density and stomata number per plant by cultivation techniques, varieties and soil types

Treatment	Stomata density (per 0.19625 mm^2)	Stomata number per plant
Cultivation techniques		
Dry	247.13 a	127,789,928 b
Saturated soil culture	163.48 b	354,921,778 a
Varieties		
Willis	237.79 a	246,571,652 a
Tanggamus	172.82 b	236,140,054 a
Soil type		
Mineral	202.55 a	299,826,555 a
Peaty Mineral	208.07 a	182,885,151 b

Note: Numbers followed by the same letter are not significantly different at the Tukey test level α = 0:05.

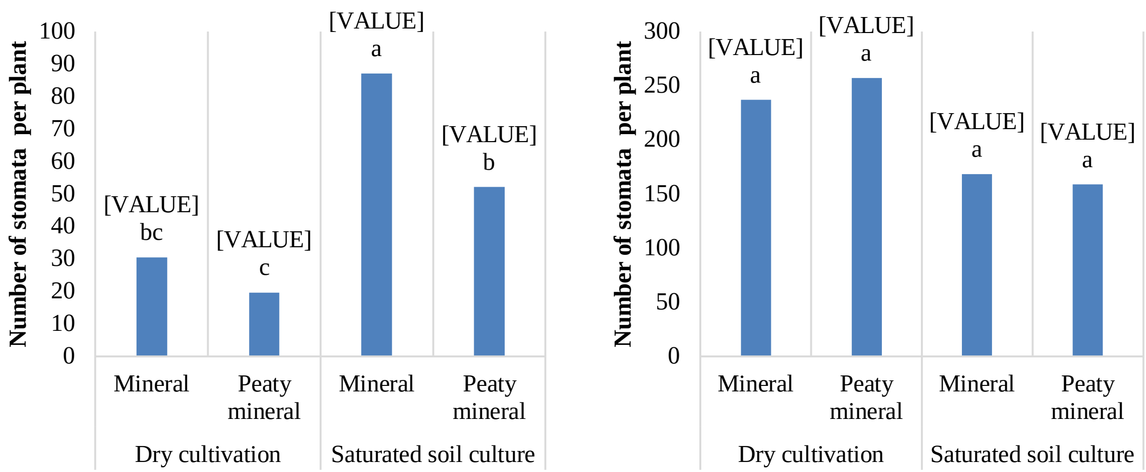

Figure 2: the number of stomata per plant and stomata density with interaction between soybean cultivation techniques and soil type

Interaction between cultivation techniques and soil types has significant effect on the number of stomata per plant. Interaction between saturated soil culture technique with mineral soil resulted higher number of stomata per plant at of 8 WAP and significantly different compared to other interactions. Saturated soil culture technique in mineral soil increase the number of stomata per plant by 65% compared with saturated soil culture interaction with peaty mineral soil as much as 62%. Saturated soil culture technique can multiply the number of stomata per plant amounted to 63.65% but lower stomata density by 51.47%. The higher decrease in stomata density occurs in the interaction between saturated soil culture with peaty mineral (Figure 2).

Visualization of leaf stomata observed under microscope 40x magnification (broad view of 0.19625 mm^2) is shown in Figure 3. The density of stomata under saturated soil culture is lower, but the number of stomata per plant is higher due to better vegetative growth that produces higher number of leaves and in turn wider total leaf area. Plants that suffer from drought have narrower stomata holes, as a natural process to reduce the loss of water through transpiration. Shrinking of stomata hole will reduce the influx of CO_2 into the leaf thus lowering photosyntate assimilation process, which in turn slows the growth of plants. Stomata plays an important role as a tool for the process of adaptation to drought stress. Drought stress will make stomata closes to restrain the rate of transpiration. Some plants adapt to drought stress by reducing the size of the stomata and stomata number [12].

The research of [13] on comparative leaf anatomical study of some sensitive and tolerant soybean genotypes to drought stress showed differences in stomata density of the two genotypes. Watering or no the drought stress treatment does not cause any change in stomata density. The reduced water availability due to soil drying causes decreased in water absorption by plant roots, in turn will lower the water content of the plant, the water potential of the plant (leaf water status), turgor pressure and conductivity of stomata, so that plant growth is inhibited [14].

3.2 Content of Chlorophyll a and b

Measurement of chlorophyll content is one approach to study the effects of water shortages on plant growth and production because closely related to the rate of photosynthesis. The results of measurements of chlorophyll content of leaf sheets in the middle of the fifth leaf from the top position indicates that the cultivation techniques, varieties and soil types did not significantly affect the chlorophyll content at 8 WAP. The analysis showed that the ratio of chlorophyll a and b in leaves from dry cultivation and saturated soil culture is unchanged at 3:1. Chlorophyll content of leaves from saturated soil culture tends to increase around 4.53% compared with those from dry cultivation. Willis variety contains more chlorophyll than Tanggamus, while cultivation in peaty mineral soil has lower average chlorophyll content around 3.15% than mineral soil.

Weather data show that temperature around the plastic house is 30°-32°C and even higher in the plastic house, thus affecting the formation of chlorophyll. Temperature can affect enzyme performance in chlorophyll biosynthesis. At high temperature, enzymes work will be disrupted and inhibits the formation of chlorophyll. The low total chlorophyll content in dry farming is due to low soil moisture content. Lack of water affects all aspects of plant growth, which includes the process of physiology, biochemistry, anatomy and morphology [15]. Lack of water will inhibit the synthesis of chlorophyll in the leaves due to decreased photosynthetic rate and the increase in temperature and transpiration which causes chlorophyll disintegration [16]. Plants that experience water shortages generally have smaller size organs than plants under suitable condition [17].

Figure 3: leaf stomata appereance by cultivation technique, varieties and soil type

Table 2: The content of chlorophyll (a, b and total) of soybean in the interaction between cultivation technique, varieties and soil types

Treatment	Content (mg g^{-1})		
	Chlorophyll a	Chlorophyll b	Total chlorophyll
Cultivation techniques			
Dry	2.8825 a	1.16417 a	4.0450 a
Saturated soil culture	3.0475 a	1.19000 a	4.2367 a
Varieties			
Willis	3.0225 a	1.20833 a	4.2283 a
Tanggamus	2.9075 a	1.14583 a	4.0533 a
Soil type			
Mineral	3.0158 a	1.20083 a	4.2150 a
Peaty Mineral	2.9142 a	1.15333 a	4.0667 b

Note: Numbers followed by the same letter are not significantly different at the Tukey test level $\alpha = 0.05$

3.3 Root Ethylene Content, Number of Nodule and Root Volume

Root volume and the number of root nodules correlated to the formation of ethylene which in turn affects nitrogenase activity of roots. Cultivation techniques and soybean varieties have significant effect on the root volume. Table 3 shows that the roots of soybean increase in volume by 84.92% in saturated soil culture technique. Sufficient soil moisture at water saturated condition and low soil temperature around 25 °C strongly supports the growth of roots and root nodules. There are differences in varieties response as representation of plant genetic influence on the root volume. Tanggamus varieties have larger root volume that affected by massive root growth compared with Willis. The number of root nodules that affect root volume increase was relatively similar between the two varieties. Root development has increased relatively well on mineral soil even though the volume was not significantly different with crops cultivated on peaty mineral soil.

Table 3: Root volume, nodule number and levels of ethylene of soybean in cultivation techniques, varieties and soil types

Treatment	Variable		
	Ethylene content(ppm)	Number of root nodule	Root volume (cm^3)
Cultivation techniques			
Dry	2.469 b	5.42 b	0.4083 b
Saturated soil culture	22.798 a	39.50 a	2.7083 a
Varieties			
Willis	13.053 a	22.54 a	1.4175 b
Tanggamus	12.214 a	22.38 a	1.6992 a
Soil type			
Mineral	15.173 a	14.04 b	1.6158 a
Peaty Mineral	10.094 a	30.88 a	1.5008 a

Note: Numbers followed by the same letter are not significantly different at the Tukey test level $\alpha = 0.05$

Interaction of cultivation techniques, varieties and soil types has significant effect on the volume of soybean roots at 8 WAP. Roots volume experienced marked improvement in the interaction of saturated soil culture with Willis and Tanggamus varieties in minerals and peaty mineral soil (Figure 4). Interaction of saturated soil culture technique, Tanggamus varieties and peaty mineral soils produce the highest root volume and if compared with interaction of dry farming techniques with the same varieties and soil type, the root volume increased approximately 89.19%. The highest increase in root volume, 89.29%, was found in Willis varieties grown in peaty mineral soil with saturated soil culture techniques.

Increased roots volume on the two varieties and two soil types is greatly influenced by cultivation techniques.

On average, interactions of the three factors are affecting the increase in roots volume from dry cultivation to saturated soil culture in the range of 85.00%. Although it was not significantly different. In dry cultivation, Tanggamus variety has higher root volume than Willis and consistently mineral soil types produce greater volume for the two varieties. In saturated soil culture, root volume of Wilis variety tends to decline while Tanggamus variety show increased in the root volume in peaty mineral soil at 8 WAP.

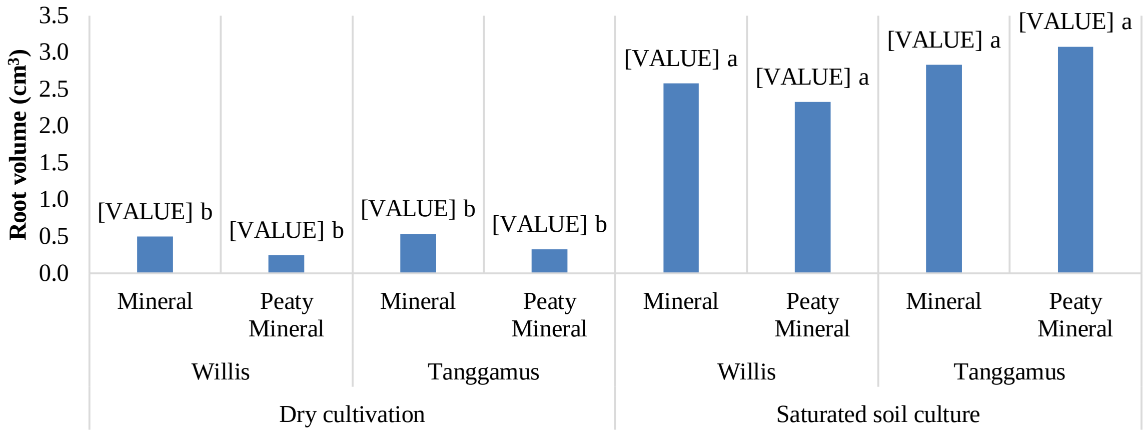

Figure 4: roots volume of soybean with the interaction of cultivation techniques, varieties and soil types

Root volume is determined by root growth and massive root nodule formation. The more roots and nodules developed, the greater root volume. Water saturation create favor conditions for the growth of roots and nodules (Figure 5).

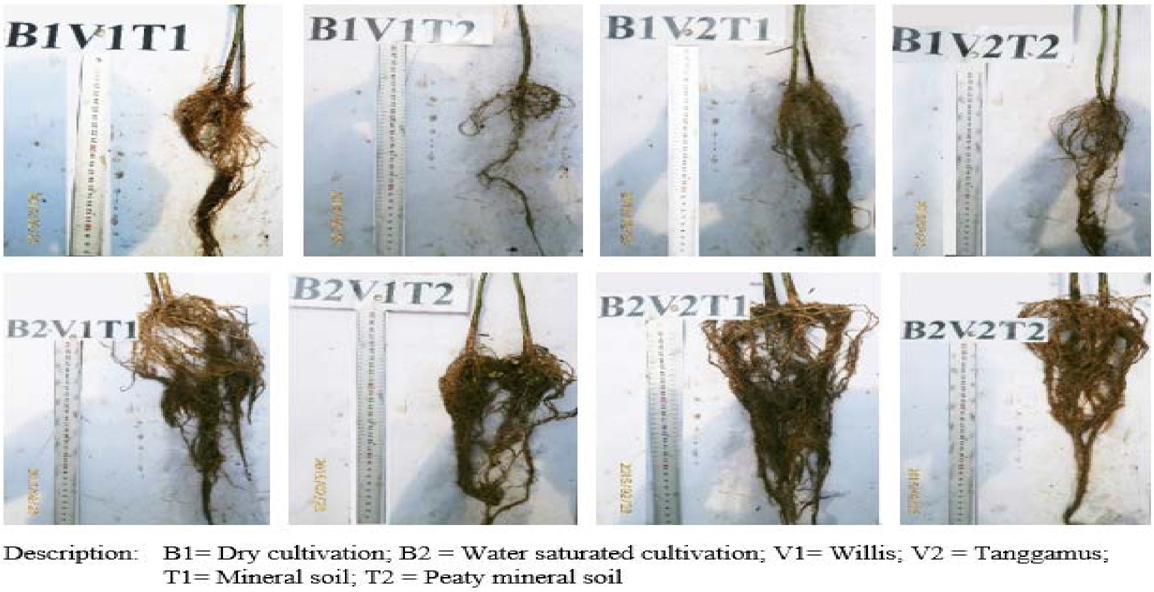

Figure 5: root appearance by cultivation technique, varieties and soil type

Interaction of cultivation techniques and varieties as well as with soil type has significant effect on the number of root nodules. Changes in dry farming to saturated soil culture increase the number of root nodules of Willis and Tanggamus, namely 84.15% and 88.36%, respectively (Figure 6A). There was no difference in the number of root nodules between the two varieties in each cultivation techniques. Changes occur in the interaction of saturated soil culture technique with minerals and peaty mineral soil. Figure 6B shows that the number of nodules formed on the interaction of saturated soil culture technique and peaty mineral soil higher than interaction of saturated soil culture and minerals soil. Increasing in the number of root nodules from interaction of dry cultivation and mineral soil to interaction of saturated soil culture reached 70.38%, lower compared to interaction with peaty mineral soil, which reached 92.30%. There is no difference in the number of root nodules from dry cultivation between mineral and peaty mineral soil. Marked differences in the number of root nodules occur in saturated soil culture between mineral and peaty mineral soil at 8 MST. Interaction of saturated soil culture and peaty mineral soil produces the highest number of root nodules among other treatment combinations.

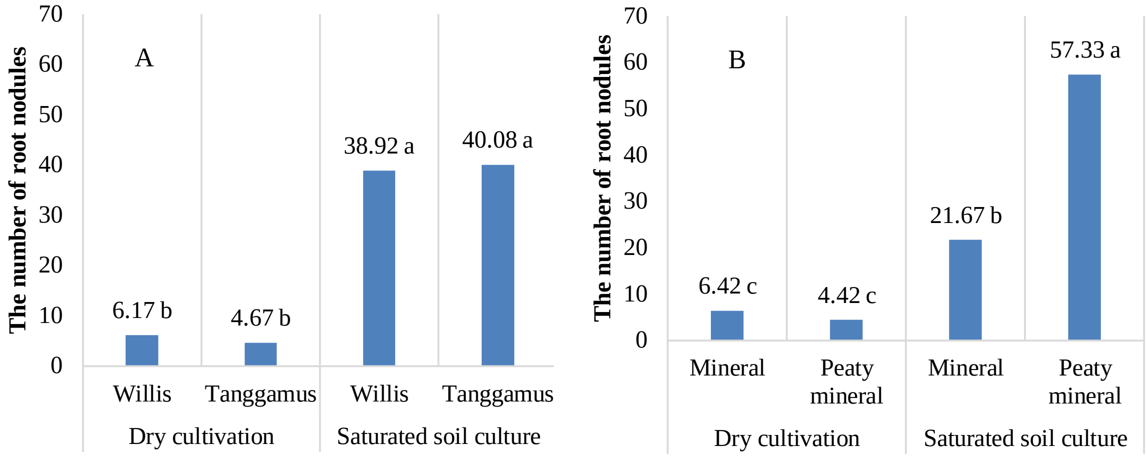

Figure 6: number of soybean root nodule in interaction between cultivation technique with varieties (A) and interaction between cultivation technique with soil type (B)

Growth and development of roots and root nodule formation affect nitrogenase activity. Nitrogenase activity can be measured with ARA (Acethylene Reduction Assay) method. The formation of ethylene gas as result of acetylene reduction was detected by gas chromatography. Theoretically, reduction of 3 moles of ethylene is equivalent to the transfer of 1 mole of nitrogen into ammonia. This ratio means that the speed of acetylene reduction in one area or volume is based on nitrogen fixation rate [18, 19].

Saturated soil culture will stimulate rapid growth and development of roots and root nodules and in turn the formation of ethylene gas in roots is higher. Active formation of ethylene will increase the activity of nitrogenase and nutrients absorption, especially nitrogen. Table 2 shows increased ethylene levels of soybean roots in saturated soil culture techniques. The addition of 20.329 ppm ethylene concentration in saturated soil culture is higher than dry cultivation. Although not significant, Willis and planting in mineral soil tends to increase the levels of ethylene by 6.43% to 33.47% compared to levels of ethylene in Tanggamus in peaty mineral soil.

Roots ethylene production is a response to anaerobic state. Changes in the dry state into anaerobic condition will change SAM (Sadenosil methionine) to ACC (1 amino cyclopropane-1-carboxylic acid), conversely in sufficient oxygen condition, ACC will be changed to ethylene [20]. The formation of ethylene on soybean roots will affect the formation of plant roots that is more horizontally above the water surface. [4] and [9] state that saturated soil culture can increase the activity of nitrogenase, and an increase in the activity of nitrogenase can reach 8-fold compared to regular irrigation [4].

The effectiveness of the roots and root nodules in the process of nitrogenase activity (ethylene formation) is better in Willis than Tanggamus. Similarly, the nitrogenase activity of soybean roots in mineral soil is more effective than in peaty mineral soil, although the root volume is inversely proportional to the root ethylene levels as described previously. Root ethylene formation is influenced by moist conditions in the root zone due to high water levels below the root surface and the presence of oxygen for oxidation processes, making it easier for the adaptation process. Initial growth of soybean is preceded by adaptation mechanisms in meeting the nutrient needs, especially nitrogen for root growth requirements [8]. Adaptation mechanism begins with increasing the content of 1-aminocyclopropane-1-carboxylic acid (ACC) in root followed by increased roots ethylene content. Roots ethylene will increase aerenchyme network and new rooting. Growth of new roots will enhance root nodule formation [21] which in turn increases the activity of nitrogenase and nutrient uptake by the roots [7].

3.4 Dry weight (Plant biomass, grain per plant) and number of pods

Cultivation techniques, varieties, soil types and their interactions affect the vegetative phase and subsequently affect the generative phase of the plants. Good vegetative growth can support growth and development variables such as dry weight of plant biomass and yield (number of pods and grain weight). Tanggamus under saturated soil culture techniques in mineral soil has higher number of pods, weight of plant biomass and grain weight per plant. Willis variety tends to have heavier grain per plant than Tanggamus (Table 4).

Table 4: Average dry weight and the number of soybean pods per plant by cultivation techniques, varieties and soil types

Treatment	Number of Pod	Dryweight (g per plant)	
		Plant biomass	Grain
Cultivation Technique			
Dry	17.1 b	6.61 b	3.62 b
Saturated soil culture	78.3 a	28.15 a	16.01 a
Varieties			
Willis	40.9 b	15.79 b	10.02 a
Tanggamus	54.5 a	18.98 a	9.61 a
Soil Type			
Mineral	56.2 a	21.05 a	11.83 a
Peaty Mineral	39.3 b	13.72 b	7.80 b

Note: Numbers followed by the same letter are not significantly different at the Tukey test level α = 0.05

Number of filled pods of Tanggamus increased by 80.30% in saturated soil culture and the highest number of pods in interaction between cultivation techniques and varieties. Number of filled pods of Willis in saturated soil culture increased by 75.20% compared to that with dry cultivation. The effect of dry cultivation on the number of pods of Willis and Tanggamus varieties is relatively similar, but the Tanggamus is more responsive than Willis as shown by the number of pods under saturated soil culture (Figure 7A). Interaction of cultivation techniques and soil types showed response of plants to form pods on mineral soil is better although pod formation in dry cultivation is relatively the same. The difference is shown by soybean grown in minerals and peaty mineral soil under saturated soil culture. Number of pods has decreased by an average of 16.92 g or 50.63% in soybean grown in peaty mineral soil either in dry or saturated soil culture (Figure 7B).

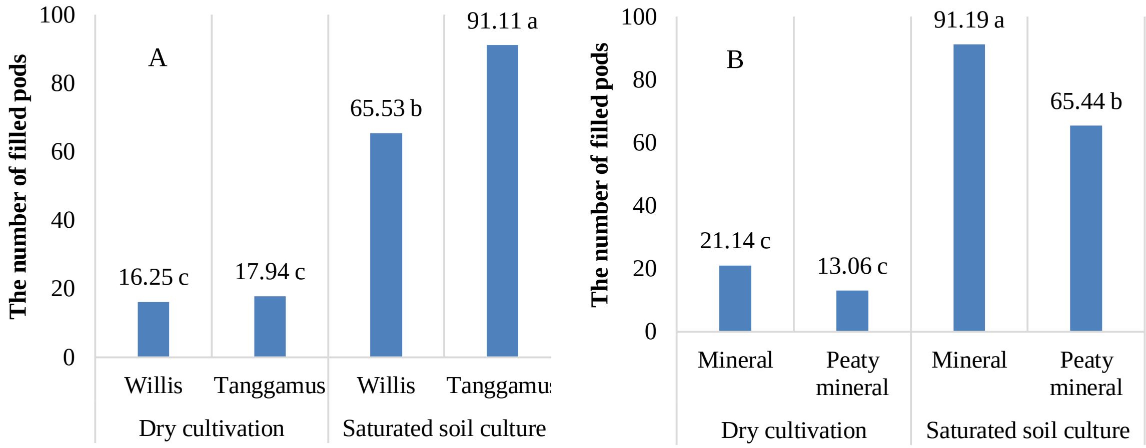

Figure 7: number of soybean filled pods in the interaction of cultivation techniques and varieties (A) and cultivation techniques with soil type (B)

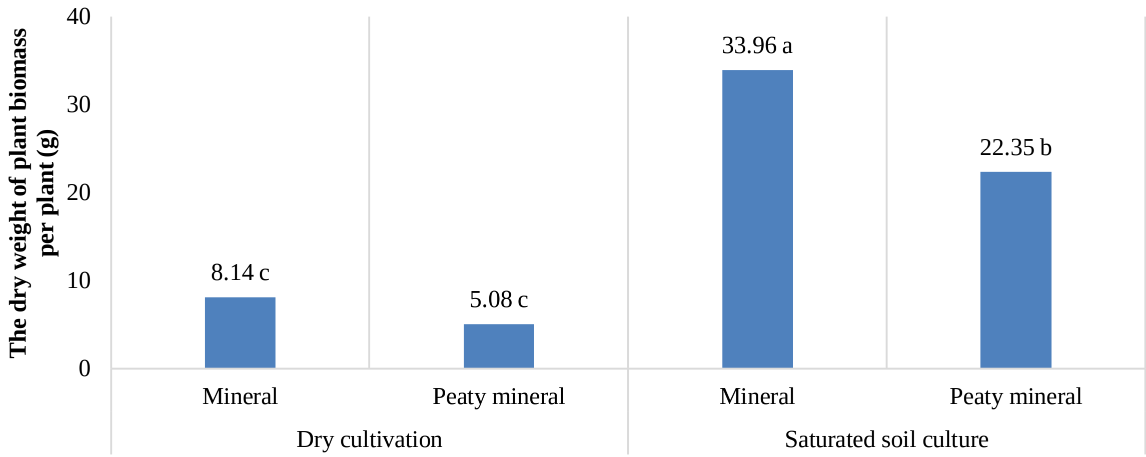

Figure 8: dry weight of plant biomass in the interaction between cultivation and soil type

Increasing the number of pods on saturated soil culture is due to the ability of plants to form pods in response to moisture and more effectively soil moisture to stimulate the formation of roots and nutrients absorption. Reference [22] shows that decreased levels of soil moisture content 75% of field capacity begin to inhibit plant high, dry weight of plants bud, number of pods per plant and grain weight per plant, but it the inhibition is not significantly different with soil moisture content 50% of field capacity.

Interaction of cultivation techniques and soil types shows that the response of the vegetative growth in mineral soils better than in peaty mineral although in dry cultivation the dry weight plant biomass is relatively the same. The difference is shown by soybean grown in soil minerals and peaty mineral with saturated soil culture. Dry weight decreased by an average of 7.33 g or 56.07% on soybean grown in peaty mineral soil either in dry and saturated soil culture (Figure 8). Increased dry weight of plant biomass on saturated soil culture is due to the ability of plants to accumulate photosynthesis product and effectiveness of nutrient absorption and metabolism as a result of better vegetative growth (the growth of roots and root nodules, leaf area, number of stomata). Naturally peat soils have low fertility rates because of low nutrient content and contains a variety of toxic organic acids for most to plants [3].

Plant growth affects the level of crop production. Increased dry weight of plant biomass will affect soybean grain production. Grain weight has similar to response pattern dry weight plant biomass on the interaction of cultivation techniques and soil types (Figure 9), in addition influenced by the genetic potential of plants (produced grain size). Higher crop production in saturated soil culture technique in mineral soil caused by the availability of sufficient water and higher fertility rate as well as lower stress variations. Growth and productivity are closely related to water availability in the environment. Research conducted by [22] at different soil moisture on the growth and yield of soybean enriched by vesicular arbuscular mycorrhiza showed that reduction of soil moisture at a rate of 75% of field capacity is significantly reduce plant dry weight. Lowest plant dry weight was obtained at a rate of 25% of field capacity. Research [23] indicates that drought stress on generative phase (age 51-75 days) can reduce soybean yield by 62%, while [24] show that drought stress during pod filling period is lowering the yield by 55%.

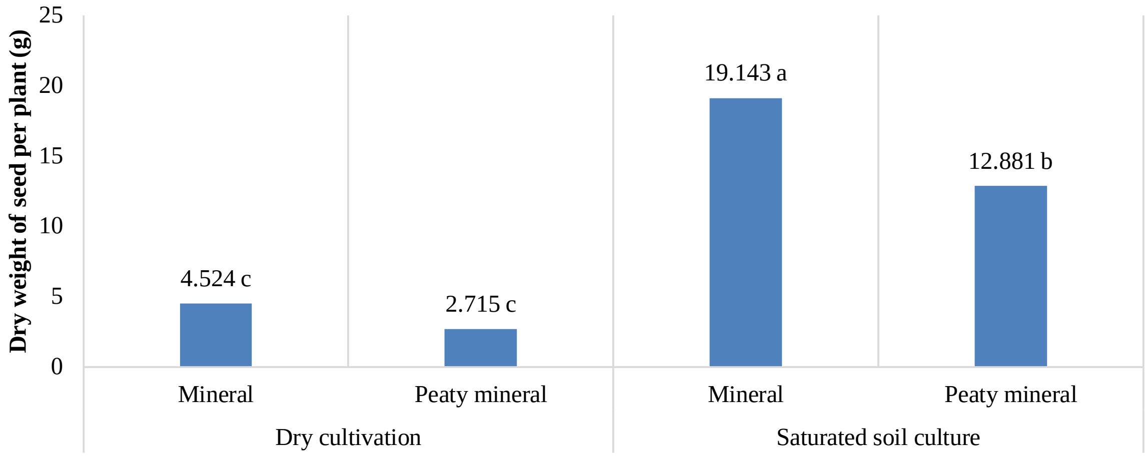

Figure 9: dryweight of seed per plant in the interaction between cultivation and soil type

4. Conclusion

Saturated soil culture technique increase the number of stomata per plant, chlorophyll (a and b), root volume, root nodule, root ethylene content, plant biomass dry weight, number of pods and grain dry weight per plant. Dry cultivation significantly affect on the increase of stomata density, but the growth of soybean on water conditions below field capacity (up to 40% field capacity), continually lowering the rate of growth and crop production.

Tanggamus varieties have higher stomata number per plant, total chlorophyll content, root volume, plant biomass dry weight, and number of filled pods, whereas Willis varieties have higher stomata density, the rate of root ethylene formation, number of root nodule and grain dry weight per plant. The formation of ethylene in the root zone will support the formation of new roots and subsequently increase plant nutrient uptake, thus increasing dry weight of grain per plant.

Mineral soil types increase levels of root ethylene, root volume, plant biomass dry weight, number of filled pods and grain weight per plant. Peaty mineral soil had greater impact on stomata density but not significantly different with mineral soil, but has significant effect on the number of root nodules.

Interaction between saturated soil culture technique and soil mineral increases the number of stomata per plant, root nodule, number of filled pods, and plant biomass dry weight. Interaction between saturated soil culture and varieties is able to increase the number of nodules and filled pods.

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