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Morphophysiological characteristic responses of Soybean (*Glycine max* L.) grobogan variety in waterlogging stress

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ABSTRACT

Soybean (*Glycine max* L.) has important role to fulfill food necessity in order to improve people nutrition because of its extra nutrition. A Problem in the production of soybean are environmental factors such as rainfall that can lead to waterlogging. To cope with growing population and environmental problem, improving soybean (*Glycine max* L.) variety to increase tolerance to waterlogging has become the consensus goal of global soybean research. The soybean observed was Grobogan variety that very potential for Indonesian people. Therefore, information about tolerance and response of the morphophysiological characteristics of soybean (*Glycine max* L.) Grobogan variety in waterlogging stress was important. The research was carried out for 14 days in vegetative stadia with the concentration of waterlogging were 100% (G1), 150% (G2), 200% (G3) and control (G0). Based on the results, the highest plant was in 150% (G2) with the value 27.14 cm and getting decreased in 200% (G3). In other hand, leaf Nitrogen, wet weight, dry weight and root length were decline as the waterlogging concentration increased. Ethylene increased significantly in the concentration of 200% (G3), it reached 14,878 ppm, 5 times higher than control. The highest adventitious root was in 200% (G3), 18 times higher than control. Grobogan variety showed still survive but its weight loss up to 60.41% after 14 days

Keywords: *Ethylene, Physiology, Grobogan, Waterlogging, Soybean (Glycine Max* L.).

Introduction

Soybean (*Glycine max* L.) is one of the most important edible oil producer legumes plants because of its high nutritional value (Waqas *et al*., 2014). Soybean containing protein, oil, soluble and insoluble carbohydrates, the water content and various functional materials such as anthocyanin,isoflavon, saponin, and fibers food (Thomas *et al*., 2003; Bellaloui *et al*., 2013; Waqas *et al*., 2014).

The amount of nutrient content and benefits obtained from soybean plants causes soybean demand increase, but the increase in soybean demand is not followed by an increase in production rate. The soybean production deficit can be caused by environmental factors. One of the main factors in decrement of soybean production is climate change including the prolonged periods of rainy season and distribution of rainfall that carries broad impact on various sectors.

Rainfall provides a supply of water to the soil for growth but if the amount of water exceeds the absorption average capacity of land could potentially causing waterlogging (Chen *et al*., 2011). Standing upon the conditions of water, the excessive water can be classified into two. (1) water-saturated condi-

tion (waterlogging) where only root crops are flooded, and (2) the conditionof the whole part of the plant is flooded (complete submergence) (Shimamura *et al*., 2007 and Striker and Mworia, 2012). In this research, the treatment that will be applied is waterlogging condition. The waterlogging condition is suspected to trigger various problems and a serious barrier to increase soybean productivity in cultivated land.

The main problems in waterlogging stress is O_2 deficiency. This is a major factor that causes damage to the soybean crop, both physiological and physical. Physiologically, the consequences of waterlogging are the emergence of gas stress which is characterized by the emergence of excessethylene, decreased root permeability due to the deterioration of root hydraulic conductivity (Jitsuyama, 2017), early leaf senescence causes chlorotic leaves and plant growth is inhibited which ultimately decreases productivity.

The soybean varieties that will be observed is Grobogan variety. This variety has the advantages of short life (76 days), large pod size, high production, high protein content which reaches 43.9 % (Balitkabi, 2008).

Based on the background, it is necessary to carry out research on the waterlogging stress response in Grobogan variety by focusing on morphophysiological characters. This information is important in development of resistant varieties of soybean to waterlogging stress.

Materials and Methods

Measurement of Field Capacity

Measurement of field capacity was aimed to determine the water volume as a line mark of the waterlogging treatment. Furthermore, the planting medium was weighed by wet weight and dry weight. Wet weight was measured after no water dripped from polybags. Whereas, dry weight was measured after the planting medium was dried in oven at 105°C until a constant weight was obtained.

Water requirement based on the field capacity was calculated by the following formula (Thien and Graveel, 2008):

$$FC\ (\%) = \frac{Tb - Tk}{Tk} x\ 100\%$$

FC: Field Capacity; *T*b: Wet weight of soil; *T*k: Dry weight of soil.

Seedling of seed, Media Preparation and Acclimatization

The seeds of Grobogan variety was soaked for 6 hours using distilled water and then drained. The seeds were grown in pottery containing planting mediaand sown until 2 leaves appeared then planted in a polybag with a composition of 2 kg of soil, 0.5 kg of husk charcoal and 0.5 kg of organic fertilizer so that a total weight was 3 kg/ polybag. The acclimatization process of soybean (*Glycine max*) was carried out for 10 days.

Waterlogging Selection

The soybean plants that had been acclimatized for 10 days were given the treatment of waterlogging stress according to the calculation obtained. The waterlogging was carried out for 14 days at all treatment levels by giving water into each polybag as much as the concentration determined based on the preliminary field capacity study results.

Observation Parameters

Leaf Nitrogen (N) Analysis

Nitrogen analysis were obtained from the leaves of the first and second branches from the point of growing. The method used was Kjeldahl method.

a. Destruction Stage

The leaf plant material that was dried in an oven at 110 °C for 24 hours (1g) put into 100 ml Kjeldahl flaskcontaining 30 mg selenand 5 mL H_2SO_4 96%. The material was heated at 70 °C for ± 15 minutes or the liquid was brown, then the temperature was raised to 140 °C for 15-30 minutes. The destruction process was completed when the solution becomes clear or colorless.

b. Distillation Stage

In the distillation stage, ammonium sulfate was broken down into ammonium by adding NaOH to alkaline and heated. The contents of the crushed flask were removed and transferred to a distillation flask which was added with 50 mL of distilled water and 20 to 30 mL of 30% NaOH. Then, the contents of the distillation flask were distilled. The distillation flask was connected to a distillation device to refine N. Before the distillation was started, a 150 mL Erlenmeyercontained 25 mL of 4% H_3BO_3 (boric acid) was prepared as a container of N which was released. Distillation was carried out until the vol-

ume in the Erlenmeyer became 150 mL.

c. Titration Stage

The Erlenmeyer liquid was titrated with HCl 0.02 N. The end of the titration was marked by changes in the color of the solution from blue to pink. The difference in the number of sample and blank titrations was the Nitrogen equivalent amount. The determination of blanks was done in the same waybut did not use plant material. The Nitrogen content was obtained by the formula:

$$\%\ Nitrogen = \frac{(t-b)x\ N_{H_2SO_4}\ x\ 0{,}01401}{W}\ x\ 100\%$$

t = H_2SO_4(ml) for the sample
b = H_2SO_4(ml) for blank
N = Normality H_2SO_4 used
W = Weight of plant material used.

Chlorophyll content

Soybean leaves at each concentration of the waterlogging were weighed around 0.1 g with analytical balance. Chlorophyll content was extracted by rinsed in 10 mL ethanol 96% for 24 hours. The extract was filtered using Whatman 42 filter paper then measuring its absorbance using UV-V is spectrophotometer in 649 nm and 665 nm wavelength. The calculation was as follows:

Total Chlorophyll Content:

[20.0 x A649 + 6.10 A665] g/mL

Wet weight and dry weight (Productivity)

The procedure of measuring the wet weight of the plant was cutting the plant based on each organ including leaves, stems, and roots, then weighted using analytical balance. The wet weight of the plants was the accumulation of the total number of all plant organs. While, dry weight was done by drying the plants using oven until the constant weight obtained.

Ethylene

The ethylene of soybean plants which had been waterlogged by water for ± 14 days was measured with HITACHI 263-50 Gas Chromatography. The total fresh roots were taken from the root neck (Yamamoto *et al.*, 1995) and put into an aerated tube vaccum then incubated for 6 hours. Ethylene (1 µl) was taken with a syringe to be measured by gas chromatographyusing a Flame Ionization Detector. The sample was separated by a capillary gas column (30 m x 0.25 µm). The injector, column and detector temperatures were 110 °C, 70 °C, 110 °C, respectively. The carrier gas was He with a flow rate of 27 mL/min. The standard used was ethylene 0.5%.

Soybean (*Glycine max*) Growth Parameters

Soybean growth parameters includeplant height, root length, leaf area, and adventitious root. Measurement of plant height was carried out once every 2 days for 14 days of waterlogging stress treatment. Soybean height was measured from the base of the stem to the tip of the stem or growing point. While leaf area, root nodule and adventitious root were carried out after the process of giving a waterlogging stress for 14 days finished. Leaf area measured by the gravimetric method. The calculation formula was as follows:

$$LD = \frac{Wr}{Wt}\ x\ LK$$

LD = Leaf area (mm^2), Wr = Leaf weight replica paper (g), Wt = Total paper weight (g), LK = Total paper area (mm^2)

Design of Experiment and Data Analysis

Experiments were carried out with 4 treatment levels, control (G0), 100% (G1), 150% (G2) and 200% (G3). Data of wet weight and dry weight parameters were analyzed by statistical analysis, ANOVA (Analysis of Variance) one factor at 95% confidence leveland Duncan Multiple Range Test (DMRT) at the 5%. Data of ethylene, chlorophyll and leaf Nitrogen levels will be analyzed descriptively.

Results and Discussion

Effect of Waterlogging Stress on Soybean Leaf Nitrogen

Nitrogen is a major component of protein, chlorophyll, enzymes, hormones andvitamins.The results of leaf Nitrogen parameter presented in figure 1.

The results showed that leaf Nitrogen in control (G0) was more compared to waterlogging treatment at the level of 100% (G1), 150% (G2), and 200% (G3). Total leaf Nitrogen (N) concentration in control (G0) reached 2.45%, whereas in G1 (100%), G2 (150%), and G3 (200%) were 1.31%; 1.16% and 1.34%, respectively. From these data, leaf Nitrogen concentrations in the waterlogging treatment G1 (100%), G2 (150%), and G3 (200%) did not differ significantly, but decreased approximately 1.11% to 1.29%, compared to control.

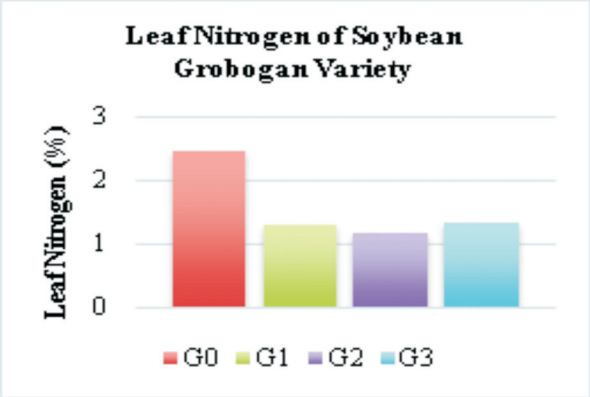

Fig 1. Leaf Nitrogen of Soybean Grobogan Variety (G0: Control, G1: 100%, G2: 150%, G3: 200%).

Nitrogen content higher in the control due to differences in absorption of Nitrogen (N) elements. Waterlogging causes a significant decrease in N content and rate of N accumulation in plants due to reduced root activity (Sigua *et al.*, 2012). Roots in control plants can absorb Nitrogen better because it was probably supported by uninterrupted root permeability, whereas in waterlogging treatment, root permeability decreases due to reduced hydraulic conductivity of soybean roots (Jitsuyama, 2017) that affected Nitrogen uptake. In addition, distribution of roots also affected the amount of Nitrogen uptake (Wang *et al.*, 2014).

Effect of Waterlogging Stress on Wet Weight and Dry weight of Soybean (*Glycine max* L.) Grobogan Variety

The results of photosynthesis could be seen from the wet and dry weight of plants and their relative growth rates (Figure 4).

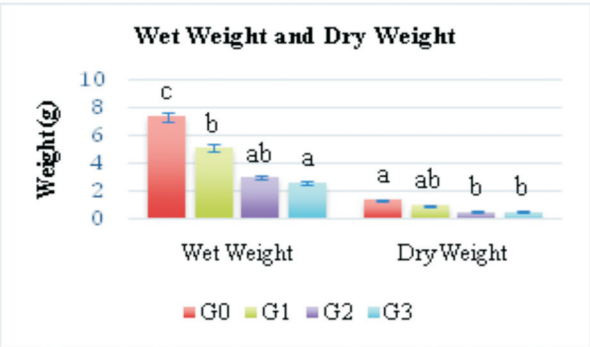

Fig 4. Wet Weight and Dry Weight of Soybean Grobogan Variety. Numbers marked with the same letters show no significant difference from the DMRT test at the level of 5%. (G0: Control, G1: 100%, G2: 150%, G3: 200%).

The highest wet weight was achieved in the control (G0) and the lowest was at a concentration of 200% (G3) with the value 7.42 g and 2.71 g, respectively. This was because the wet weight was influenced by plant morphology. In the control, the leaves and roots contributed greatly because they had size and length larger than waterlogging treatment. It affected the accumulation of water stored in these organs. Crop production is usually more accurately expressed in terms of dry weight. Dry weight can indicate plant productivity because 90% of photosynthesis results are in the form of dry weight as a manifestation of metabolic processes (Gardner *et al.*, 1991).

Based on the result, dry weight of soybean at 150% (G1) and 200% (G3) treatment significantly decreased compared to control (G0), it reached 1.44 g. This resultwas caused by decreased leaf area and chlorophyll as photosynthetic organelles and decreased primary metabolism caused by damaged root conditions. If the roots were damaged it will inhibit the absorption of water, nutrients and photosynthesis, resulting in decreased growth and low dry weight.

The results showed that soybean Grobogan variety was less tolerant because the research found that soybean Grobogan variety still survive on 14 days of waterlogging but the weight was reduced up to 60.41%.

Effect of Waterlogging Stress on Soybean (*Glycine max* L.) Grobogan Variety Growth

Waterlogging stress can affect plant growth, The measurement data of root length, leaf area, and plant height shown on Table 1.

The results showed that waterlogging significantly affected root length. The highest root length was at the control (G0), 42.850 cm. Root length de-

Table 1. Soybean (*Glycine max* L.) Grobogan Variety Growth

Treatment	Root	Height	Leaf Area
(G)	Length (cm)	(cm)	(mm^2)
G0	42.850[b]	24.50[a]	30.19[c]
G1	20.350[a]	26.19[a]	25.42[b]
G2	17.950[a]	27.14[a]	15.52[a]
G3	15.183[a]	25.98[a]	16.57[a]

Note: Numbers marked with the same letters in the same column show no significant difference from the DMRT test at the level of 5%. (G0: Control, G1: 100%, G2: 150%, G3: 200%).

creased significantly at 100% (G1), 150% (G2) followed by 200% (G3) with 20.35 cm, 17.95 cm and 15.183 cm, respectively.

Root length decreases during waterlogging because division or extension of root cells was inhibited. It was due to the limited availability of metabolic energy. Inhibition of division or extension of root cells was also caused by decreasing auxin and cytokinin. The decrease in the two hormones was caused the increased synthesis of ethylene (Table 3.2).

In terms of plant height. Waterlogging made soybean Grobogan variety perform a growth strategy to avoid stress by elongating the stems/shoot. Elongation of the stem was caused by the interaction between Giberellin Acid (GA) and ethylene. It was explained that internodal rapid growth resulted from ethylene which mediated the ratio of endogenous growth promoter Giberellin (GA) and growth inhibitors (ABA)(Benschop *et al*., 2006). One of the functions of GA was to stimulate stem lengthening.

Another parameter was leaf area. Based on the results, leaf area decreased with an increasing in waterlogging concentration. The result was also supported by Guang *et al*., (2012) stated that the presence of waterlogging stress will reduce the growth of leaf area caused by the ability of roots to absorb water and nutrients due to hypoxic or anoxic conditions. Besides that, factor that is influential was the synthesis of the cytokinin. It was showed that the levels and composition of endogenous cytokinins changed during leaf development.

Effect of Waterlogging Stress on Ethylene concentration and Adventitious Root of Soybean (*Glycine max* L.) Grobogan Variety

Physiological mechanisms that can improve plant resistance to waterlogging stress conditions include ethylene production and adventitious root (Table 2).

Based on the results, the highest ethylene concentration was in 200% (G3) waterlogging treatment, it reached 14,878 ppm, 5 times higher than control. Measurements of endogenous ethylene concentrations in flooded plant organs consistently report rapid (within 1 h) elevation of ethylene to physiologically saturating (1 μL L^{-1}) concentrations after the onset of flooding in *Rumexpalustris* (Voesenek and Sasidharan, 2013).

It was explained that accelerated synthesis of 1-aminocyclopropane-1-carboxylic acid (ACC) takes place in roots following the induction of ACC synthase genes by hypoxia. ACC (1-aminopropane-1-carboxylic acid) is precursor of ethylene. Step of ACC formation does not require oxygen. The ACC is then oxidised to ethylene by ACC oxidase (ACO). However, ACC oxidase (ACO) becomes a limiting factor in waterlogging condition because this conversion requires the presence of O_2. Under this condition, ACC is transported from the anaerobic root system through the xylem to the shoot where there is oxygen. Then, ACC rapidly converted to ethylene inducing leaf epinasty (Wen *et al*., 2017).

Table 2. Ethylene Concentration and Adventitious Root of Soybean (*Glycine max* L.) Grobogan Variety

Treatment (G)	Ethylene (ppm)	Adventitious root
G0	3,554	0.00 [a]
G1	2,424	0.00 [a]
G2	3,357	7.14 [b]
G3	14,878	18.00 [c]

Note: Numbers marked with the same letters in the same column show no significant difference from the DMRT test at the level of 5%. (G0: Control, G1: 100%, G2: 150%, G3: 200%)

Stress aeration induced the formation of adventitious roots. The results showed that the waterlogging stress affects the number of plant adventitious roots. Adventitious roots in 100% (G1) were not formed. Adventitious roots were significantly increased in 200% (G3) followed by 150% (G2) with the value 7.14 and 18.00, respectively (Table 2).

Adventitious roots can reduce the adverse effects of waterlogging by expanding the area of rooting into the air, increasing aerobic respiration, and oxidizing the rhizosphere (Bacanamwo and Purcell, 1999). The adventitious roots contain aerenchyma with the associated internal O_2 movement to the apex enabling growth (Herzog *et al*., 2016). Adventitious root formation occurs because of the interaction of plant hormones, auxin and ethylene (Verstraeten, 2014).

Conclusion

Based on the results we concluded that: (1) The highest plant was in 150% (G2) with the value 27.14 cm and getting decreased in 200% (G3); (2) Leaf Nitrogen, wet weight, dry weight and root length were decline as the waterlogging concentration increased, with leaf Nitrogen 2 times, chlorophyll content up to 1,8 times, wet weight 2 times, dry weight 2.5

times and root length up to 2.8 timesless than control; (3) Grobogan variety showed a weight loss up to 60.41% after 14 days treatment; (4)Ethylene increased significantly in the concentration of 200% (G3), it reached 14,878 ppm, 5 times higher than control; (5) The highest adventitious roots was in 200% (G3), 18 timeshigher than control.

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