



## **Soybean Adaptation to Water Stress on Vegetative and Generative Phases**

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### **Abstract**

Soybean is rich in protein and is one of the main sources of vegetable protein which essential in enhancing public nutrition. Climate change is the main trigger of the occurrence of extreme weather events makes plants become more vulnerable to drought. Drought stress significantly affect the decline in soybean production, especially when it occurs during the reproductive phase. This research aimed to identify the response of soybean to water stress as a reference for determining the adaptive and tolerant varieties. The research was arranged in split-split plot design, with main plot was varieties (Dering and Argomulyo), the development phase (vegetative and generative phases) as the subplot, and water stress in the form of irrigation intervals (2, 5, and 10 days) as the sub-sub plots. The results showed that water stress during the vegetative phase has not statistically significant effect on soybean production. Soybean crop adapted to water stress by reducing the number of leaves, the leaf area, stomatal openings, as well as doing motion response by folding leaves. This crop adaptation mechanisms affecting the formation of dry matter quantity, seeds yield, water use efficiency, and radiation use efficiency

**Keywords:** adaptation response, generative phase, soybean, vegetative phase, water stress

## A. Introduction

Soybean is one of the main food commodities after rice and corn that rich in protein and has diverse uses, especially as food and industrial raw materials as well as for the livestock feed industry (Zakaria, 2010). Soybean is an essential source of oil, protein, macronutrients and minerals (Clemente & Cahoon, 2009). The content of vegetable protein in soybean is essential for improving public nutrition. Vegetable protein, besides safety also relatively cheaper compared to animal protein, therefore, the soybean demand continues to increase in line with population growth.

Soybean consumption in Indonesia in 2015-2019 is expected to increase at around 2,77 million tons 2015 and 3,25 million tons in 2019. Meanwhile, projection of production for 2015-2019 based on the Ministry of Agriculture is about 93 thousand tons in 2015 and 1,22 million tons in 2019 (Bappenas, 2013). Based on these data, the soybean production is still in deficit and have not been able to filled needs of domestic consumption. To fill with domestic soybean and achieve self-sufficiency it is necessary to increase soybean planting acreage.

Potential land for soybean cultivation is quite extensive but it face difficulties, especially in the dry season is very vulnerable to drought, therefore, the water availability for the growth of soybean becomes limited. Climate change also causes extreme weather changes. A shift of season affects the planning of the agricultural activity. The planting schedule will be disturbed and decrease the production and cause crop failure. Drought reduces crop productivity especially in arid and semi-arid regions (Razzaghi, Ahmadi, Jacobsen, Jansen, Andersen, 2012). The rapid population growth also becomes a major concern, since it increases the food demand, especially soybean while the agricultural productivity decrease due to the climate change.

Drought stress during the reproductive phase causes the soybean production decreases more than 40%. In fact, soybean plantation in Indonesian is largely (65%) in the rice fields during the dry season. In this condition, the cultivation of soybean is often faced the risk of drought. The dry condition during flowering period cause flowers and young pod fall and reduce the number of pods and seed size. While, in the filling phase, the beans are not formed perfectly, that causes the soybean becomes smaller and the dry matter becomes lower, that cause the production decrease up to 40%. To anticipate the drought impact, the more adaptive variety is developed such as use old varieties of early maturing or tolerant to drought stress (BPTP, 2013). Drought has an influence in decreasing the soybean crop growth for the stems and the roots which lead to the decreasing of crop total dry matter (Hamim, Sopandie, & Jusuf, 1996). The photosynthesis rate of the crops that suffer from drought decrease sharply and is lower compared to the crops that do not suffer from drought (Liu, Jensen, & Andersen, 2004). The production of the soybeans is decreased when the water stress is increasing (Candogan, Sincik, Buyukcangaz, Demirtas, Goksoy, & Yazgan, 2013).

Plants have evolved two major mechanisms to cope with water deficit: stress avoidance and tolerance. Stress avoidance is achieved by the formation of seeds before drought conditions prevail and specialized in the plant architecture. Morphological adaptations are, for example, the development of specialized leaf surfaces to decrease the rate of transpiration, the reduction of leaf area, sunken stomata or an increase in root length and density to use water more efficiently (Ramanjulu & Bartels 2002). The objective of this research is to identify the soybean adaptation responses to water stress as a reference to determine the adaptive and tolerant varieties.

## B. Methodology

The research was conducted in Cikabayan field Experiment, Bogor Agricultural University from April to June 2015. This research used experimental design of split-split plot design in three replications with the main plot was varieties (V1= Dering variety, V2= Argomulyo Variety). The subplot was the development phase (F1= stress during vegetative phase, F2= stress during generative phase). The sub-subplot was water stress in the form of irrigation intervals (I1= every 2 days, I2= every 5 days, I3= every 10 days). This research used the shade

of bamboo with a transparent plastic cover to be protected from contamination of rain to the water stress treatment.

Water stress treatment for vegetative phase was started after 2-week-after planting (Week After Planting=WAP) and stopped at the time of entering of generative phase or flowering stage and at the same time, treatment for generative phase was started and finished at harvest. At the beginning of planting until 2 WAP, all crops were irrigation every day. The crops were given the saturated water and left for some time until the condition is reached field capacity. The weighing method was done to determined water loss and soil water content during the stress period.

The variables observed in order to identify the adaptation responses to water stress are the number of leaves, the leaf area, the stomatal openings, the total dry matter, the seed yield, water use efficiency and radiation use efficiency. The number of leaves was observed every week until the age of 7 WAP. The leaf area was calculated every two weeks using the gravimetric method. The stomatal opening observations were done using samples of a replica of stomatal and observed under a trinocular microscope with 10x40 magnifying, which is equipped with Olympus DP25 digital camera types and DP2-BSW application. The dry matter was measured every 2 weeks using destructive sampling method as much as 3 crops for each treatment and oven dried at 80°C for 48 hours. The seed yield was observed at harvest and oven dried at 80°C for 48 hours. The data analysis was performed using F-test (ANOVA).

Water use efficiency (WUE) was calculated using the equation:

$$WUE = dW / \text{Crop water use (ETA)}$$

where, WUE is water use efficiency ( $\text{kg/m}^3$ ),  $dW$  is the addition of the dry matter ( $\text{kg}$ ), and ETA is crop water use (actual evapotranspiration) ( $\text{m}^3$ ) The amount of water loss from the pots, represented the evapotranspiration.

Radiation use efficiency was calculated using the equation :

$$\varepsilon = dW / Q_{int}$$

where,  $\varepsilon$  is globally radiation use efficiency ( $\text{g MJ}^{-1}$ ),  $dW$  is the addition of the dry matter ( $\text{g m}^{-2}$ ), and  $Q_{int}$  is cumulatively of solar radiation intercepted by crop canopy ( $\text{MJ m}^{-2}$ ).

Radiation interception was calculated using Beer's Law which is the function from the leaf area index, extinction coefficient and the intensity of solar radiation above the canopy. Extinction coefficient value used is the average value in various leaf area index. The intensity of radiation measurement was performed using a solarimeter sensor which has been calibrated by a standard solarimeter and connected to the data logger. The equation used is (Handoko, 1994):

$$Q_{int} = Q_0(1 - e^{-kLAI})$$

Where,  $Q_{int}$  is radiation interception ( $\text{MJ m}^{-2}$ ),  $Q_0$  is radiation above the canopy ( $\text{MJ m}^{-2}$ ),  $k$ : is extinction coefficient (0,7), and LAI is leaf area index.

## C. Results and Discussions

### 1. Relation Of Water Stress With Soil Water Content (Swc)

Water stress treatment provided information about the soil water content (SWC) on each irrigation intervals (Figure 1).

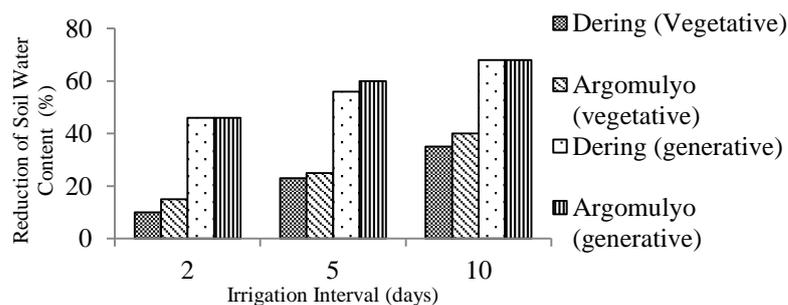


Figure 1. Percentage reduction of soil water content (SWC) on each irrigation intervals.

The reduction of SWC is not very large in the vegetative phase because crop water requirement in this phase is still relatively small, but the water requirements is rising in line with the growth of the crop, so that the SWC reduction become larger in generative phase. Water loss caused by transpiration very influenced by canopy cover stated in the leaf area index (LAI). Water loss increased with increasing value of LAI.

## 2. Responses of Soybean to Water Stress

Water stress at vegetative phase has not significant effect on some observation variables, meanwhile at generative phase influence all of observation variables. Period of water stress at vegetative phase is more shorter compared generative phase, thus cause moderate stress on vegetative phase and severe stress on generative phase. Short-term, moderate drought stress during vegetative growth stages generally does not impact soybean yield. Conversely, longer-term severe drought stress can cause irreversible plant cell death causing low growth yield. Soybean yield is most sensitive to water deficits during reproduction (Lenssen, 2012).

Table 1. Summary of results of ANOVA soybean responses due water stress on vegetative and generative phases.

Variables	Varieties Development (V)	Phase (F)	Water Stress (I)	Interaction (V x F)	Interaction (V x I)	Interaction (F x I)	Interaction (V x F x I)
Leaf number 4 WAP	0.524 <sup>ns</sup>	0.1099 <sup>ns</sup>	0.4822 <sup>ns</sup>	0.6355 <sup>ns</sup>	0.5493 <sup>ns</sup>	0.751 <sup>ns</sup>	0.5035 <sup>ns</sup>
Leaf number 7 WAP	0.017*	0.0004*	0.0000*	0.2522 <sup>ns</sup>	0.0026*	0.000*	0.1873 <sup>ns</sup>
Leaf area 4 WAP (cm <sup>2</sup> )	0.031*	0.0107*	0.0025*	0.5361 <sup>ns</sup>	0.3630 <sup>ns</sup>	0.001*	0.1526 <sup>ns</sup>
Leaf area 6 WAP (cm <sup>2</sup> )	0.036*	0.0023*	0.0000*	0.4526 <sup>ns</sup>	0.9526 <sup>ns</sup>	0.000*	0.1415 <sup>ns</sup>
Leaf area 6 WAP (cm <sup>2</sup> )	0.254 <sup>ns</sup>	0.0009*	0.0000*	0.8070 <sup>ns</sup>	0.0510 <sup>ns</sup>	0.000*	0.4715 <sup>ns</sup>
Total dry matter (g)	0.114 <sup>ns</sup>	0.0001*	0.0000*	0.2559 <sup>ns</sup>	0.0690 <sup>ns</sup>	0.000*	0.9569 <sup>ns</sup>
The seed yield (g)							
Stomatal opening (veg) (µm)	0.003*	0.0045*	0.0004*	0.9901 <sup>ns</sup>	0.9506 <sup>ns</sup>	0.0015*	0.7019 <sup>ns</sup>
Stomatal opening (gen) (µm)	0.464 <sup>ns</sup>	0.0098*	0.0112*	0.9955 <sup>ns</sup>	0.4495 <sup>ns</sup>	0.0077*	0.9650 <sup>ns</sup>

\*) significant at  $P \leq 0.05$ ; ns = not significant; Veg = observed on vegetative phase; Gen = observed on generative phase; WAP = week after planting.

## 3. Morphology Response

### Leaf Number and Leaf Area

Water stress at vegetative phase has not given statistically significant effect on the number of leaves (Table 1). The stressed soybean in the vegetative phase and observed at the age of 7 WAP showed decrease in the number of leaves but not significantly different when compared to with stresses 2 days (Figure 2). This was caused by the plant ability to restore growth after passing the stress condition called recovery mechanism. Meanwhile, the stress on the generative phase gave a different effect on each variety (Figure 2). The number of leaves decreased with increasing periods of stress. Dering variety had a greater reduction than Argomulyo in the same stress conditions. The lack of leaves during stress is caused by disturbance of growth and adaptation mechanisms of plants through the defoliate of leaves to reduce transpiration.

Water stress treatment also gave different effect on the leaf area on vegetative and generative phase (Table 1). While stressed, the crop reduces leaf area to reduce the field transpiration. Leaf area decreased with increasing periods of water stress. Dering variety decreased leaf area is greater than the Argomulyo in the same stress conditions (Figure 3).

Defoliation and leaf area reduction is one of the mechanisms of plant adaptation due to water stress. This mechanism is called dehydration avoidance which is the plant ability to keep the tissue potentials by reducing the water loss. At this mechanism the plants usually have the ability to reduce in surface evapotranspiration through reduction of the leaf area and defoliation the old leaves (Fukai & Cooper, 1995). Water stress affects the growth and development of the

canopy (Stone, Wilson, Jamieson, & Gillespie, 2001). Sulistyono, Suwarno, Lubis, & Deni. (2012), stated that the first physiological processes that occur as impact of the drought is the reduction of leaf area, which can cause a decrease in stomatal conduction and photosynthesis rate. The reduction of leaf area and stomatal opening is the mechanism to avoid drought by reducing transpiration.

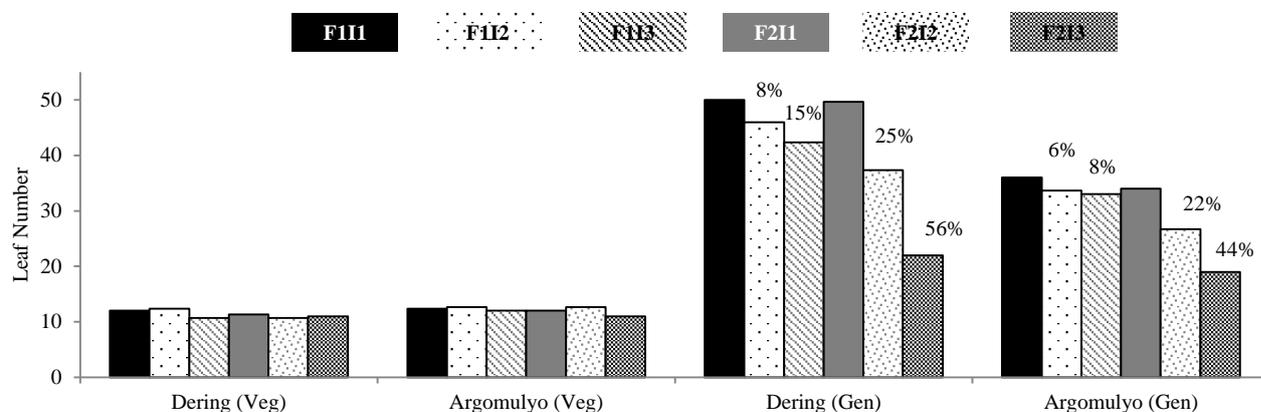


Figure 2. The number of leaves of soybean at age of 4 and 7 WAP. F1 = vegetative phase, F2 = generative phase; I1, I2, I3 = water stress by irrigation intervals of 2 days, 5 days, and 10 days. Numbers above diagram showed percentage reduction of the number of leaves by compared irrigation interval 2 days.

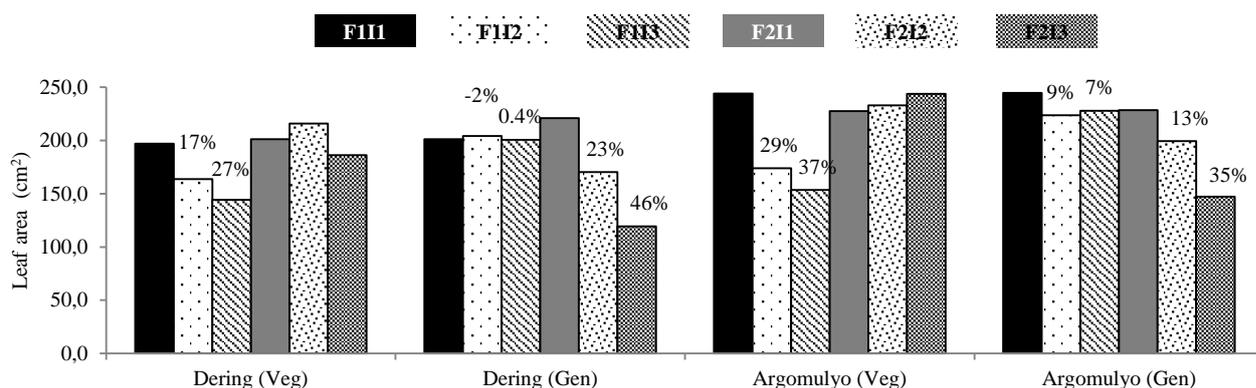


Figure 3. Leaf area of soybean at age 4 and 6 WAP. F1 = vegetative phase, F2 = generative phase; I1, I2, I3 = water stress by irrigation intervals of 2 days, 5 days, and 10 days. Numbers above diagram showed percentage reduction of leaf area by compared irrigation interval 2 days.

#### 4. Physiology Response

##### Stomatal Openings

Water stress on the vegetative and generative phases gave a different effect on stomatal opening (Table 1). Water stress for 5 days and 10 days caused the crops perform adaptation response by reduced stomatal openings at vegetative and generative phases (Figure 4). Stomatal opening was decreased when the water stress is increasing. Reduction of stomatal opening is one of the mechanisms of plant tolerance to stress to reduced water loss. Argomulyo variety decreased stomatal openings is greater than Dering variety at vegetative and generative phases. Sudarsono & Widoretno (2003); Purwanto & Agustono (2010); Permanasari & Sulistyansih (2013) research results that the width of the stomatal openings become smaller with increasing water stress. Plants which suffer from drought will closure of stomata to reduced water loss through transpiration.

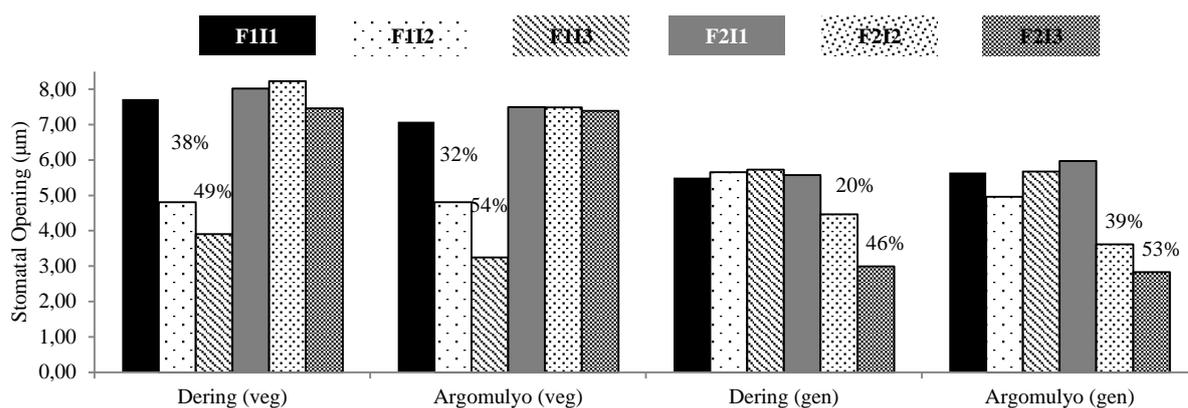


Figure 4. stomatal openings of soybean at vegetative and generative phases. F1 = vegetative phase, F2 = generative phase; I1, I2, I3 = water stress by irrigation intervals of 2 days, 5 days, and 10 days. Numbers above diagram showed percentage reduction of stomatal opening by compared irrigation interval 2 days.

## 5. Motion Response

### Leaf Folding

One of the adaptation response of crop on water stress that the folding of leaves to reduce exposure of radiation heat on the leaves surface which can accelerate the transpiration rate. This response occurs when generative phase. In Figure 5, there was difference between the position of the leaves of crop stress and not stress. The crop control (irrigation interval 2 days) showed grew freshly and open wide the leaf surface, whereas in crop stress folding their leaves. Fukai & Cooper (1995) states that the plant will roll or fold their leaves to reduce absorption heat radiation and reduces transpiration through the leaf surface.



Figure 5. A. not stress (irrigation interval 2 days) B. folding leaves of soybean on water stress condition (irrigation intervals 5 and 10 days).

## 6. Adaptation Responses Influence of Production

### Total Dry Matter and Seed Yield

Total dry matter is produced from the net photosynthesis. Water stress treatment at vegetative phase has not significant effect on the total dry matter (Figure 6A) and the seeds yield (Figure 6B). Meanwhile, giving stress on the generative phase affects the decrease in dry matter production and seeds yield. Dry matter and seeds yield of soybean decreased with increasing periods of water stress. Dering variety decreased production is greater than Argomulyo at the same stress conditions.

The low dry matter production at the stress conditions caused by the crop adaptation mechanism that reduced the number of leaves and leaf area so that the surface of photosynthesis is also reduced. The crops are also adapted by reduced stomatal openings and fold the leaves so that the exchange of CO<sub>2</sub> and H<sub>2</sub>O in the leaves are obstructed. Mechanisms of crop adaptation to stress caused obstruction of the photosynthesis process and therefore contributes to the production of dry matter and seed yield of soybean. Based on Sopandie (2014), that the drought stress reduced the growth and photosynthesis. A decrease in photosynthesis in water stress conditions caused by stomatal closure and metabolic influences. Water deficit caused the closure of stomata which reduced the CO<sub>2</sub> concentration, whereas the dehydration of the leaf mesophyll cells can cause damage to photosynthesis organs. The tolerant crops are able to maintain their biological function in the low water potential condition although with limited growth.

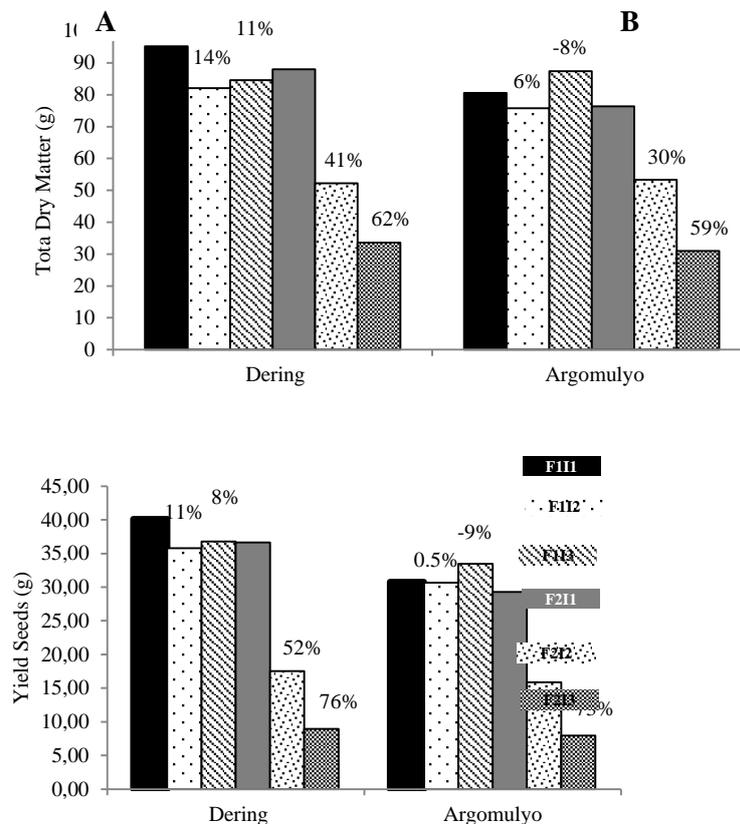


Figure 6. The total dry matter (A) and the seeds yield (B) of soybean plants on water stress condition. F1 = vegetative phase, F2 = generative phase; I1, I2, I3 = water stress by irrigation intervals of 2 days, 5 days, and 10 days. Numbers above diagram showed percentage reduction of dry matter and seeds yield by compared irrigation interval 2 days.

Harnowo (1992), stated that the water stress on reproductive phase inhibit distribution of assimilates to the reproductive parts, reducing the number of pods, seeds and seed weight per crop. Water stress also affects the decrease in the percentage of active roots, dry matter, number of leaves and pods, and plant height. The research also concluded that the stress will reduce leaf area, accelerates senescence of leaves, reduce the number of pods and seed yield per hectare. Drought stress at 50% under the condition of water available during the vegetative phase did not affect the yield. According to Pramono, Ratresni, Kamal, & Nurmauli. (1993), the effect of lack of water that occur in the generative phase is more significantly affect reducing the yield than it occur in the vegetative phase.

## 7. Water Use Efficiency (WUE)

Crop tolerant reduced the rate of water loss that improved the water use efficiency. Water stress affected of water use efficiency in both varieties (Figure 7). Water use efficiency decline with increasing water stress on Dering variety, whereas in Argomulyo variety have increased the efficiency. This indicates that the Argomulyo variety more efficient using water to form biomass. The value of water use efficiency for soybean based Steduto et al. (2012), is 1.2 to 1.6 kg/m<sup>3</sup>.

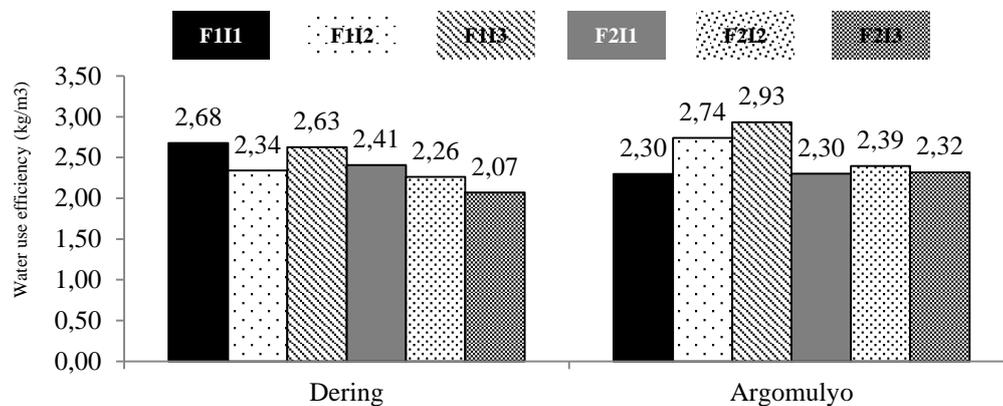


Figure 7. Water use efficiency of soybean on water stress conditions. F1 = vegetative phase, F2 = generative phase; I1, I2, I3 = water stress by irrigation intervals of 2 days, 5 days, and 10 days. Numbers above diagram showed the value of water use efficiency.

On the water stress conditions, water use efficiency were higher in genotype tolerant effect that genotypes is able to maintain their generative growth and suppress yield loss lower than the sensitive genotype (Efendi & Azrai 2008). Blum (2005); Sinaga (2008) stated that the reduction water requirement by crops is useful to increasing the water use efficiency.

## 8. Radiation Use Efficiency (RUE)

Water stress greatly affected the radiation use efficiency of soybean (Figure 8). The low value of the efficiency in the stress condition is caused by the reduction of photosynthesis rate due to lack of water and then it impacts on the crop growth. Rusmayadi, Handoko, Koesmaryono, Hadjar, (2008), stated that the effect of soil water deficit can be attributed directly to radiation use efficiency (RUE), crop growth and yield. Water deficit reduced the RUE due to the decrease of the photosynthesis activity. RUE measurements is helpful to understand the consequences of the drought stress for the crops and its variations based on the age. The low RUE on stress conditions caused by the interception of the radiation is strongly influenced by the structure of the canopy through the leaf area index and extinction coefficient of the canopy. Crop seized has a value of leaf area index were low because the crops folding the leaves and reduced leaf area. Salvagiotti & Miralles (2008) stated that crop production is determined by the partition and the accumulation of plants biomass. The process depends on the role of the canopy in the interception of radiation which is affected by leaf area index (LAI), the canopy structure and the process of converting the radiation into biomass accumulation. LAI decline effect on the radiation use efficiency because to the interception of the radiation is determined by LAI (Bonhomme, 2000).

RUE value based on various literatures are 1.32 - 2.52 g/MJ PAR (Sinclair & Muchow 1999), 2.04 g/MJ PAR (Singer, David, Thomas, John, & Jerry, 2010). In this reseach, the radiation use efficiency value with global radiation is quite big, it mean that the crop is very efficient in using the solar radiation to form dry matter. It is caused by the used of pots/polybags method give the optimum growing environment for the crops and minimizing the other factors other than water stress treatment. The used of pots also cause the crop competition with the surroundings can be reduced so that the crop can grow optimally with high production compared to the crops that are planted in field.

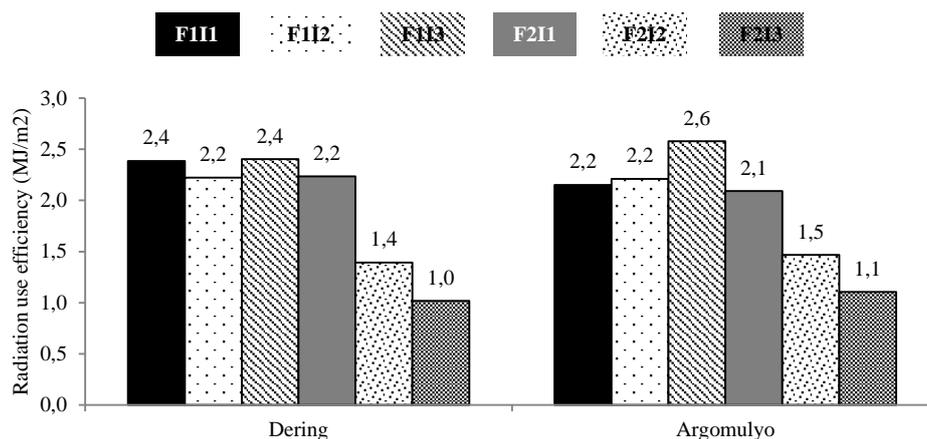


Figure 8. Radiation use efficiency of global radiation at water stress conditions. F1 = vegetative phase, F2 = generative phase; I1, I2, I3 = water stress by irrigation intervals of 2 days, 5 days, and 10 days. Numbers above diagram showed the value of radiation use efficiency.

#### D. Conclusion

Dering and Argomulyo adapted to the water stress by maintaining high water potential although they lack of water (dehydration avoidance) through the defoliating of leaves, leaf narrowing, reducing stomatal opening, and folding the leaves to reduced water loss through transpiration. Soybean adapted by recovery mechanism when water stress occur at vegetative phase so that it is did not significant effect on the reduce production. Based on the mechanism of adaptation both varieties it can be concluded that both soybean varieties tolerant to drought stress. Argomulyo is more tolerant because at the same water stress conditions decreased production less than Dering variety. It also improved water and radiation use efficiency on water stress conditions.

The selection of variety for cultivation in dry season is recommended to use Argomulyo because it is more adaptive and tolerant to water stress. But for cultivation in the normal condition, it is better to use dering variety because this variety have a higher production potential than Argomulyo.

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