OPEN ACCESS Asian Journal of Plant Sciences

ISSN 1682-3974
DOI: 10.3923/ajps.2018.

Research Article

# Photosynthetic Apparatus of Soybean Exposed to Drought Due to Application of Arbuscular Mycorrhiza

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## Abstract

**Background and Objective:** Water shortage is a common condition in most rainfed area in the tropics especially when irrigation is limited. Response of plants to water shortage is related to physiological, biochemical and morphological adjustments. A study aimed to investigate the changes in physiological behaviour of drought stressed soybean plants applied with arbuscular mycorrhizal fungi. **Materials and Methods:** The study was conducted at the Screen House Faculty of Agriculture, University of Hasanuddin in Makassar, Indonesia. Three water supply levels were used consisted of 80-90, 60-70 and 40-50% of field capacity and four dosages of mycorrhizal fungi were applied consisted of 4 levels, control, inoculation of mycorrhizal fungi 0.05, 0.10 and 0.15 g/pot, respectively. Data were analyzed using a factorial ANOVA and least significance difference (LSD) test. In addition, a correlation analysis was performed to observe the relationship between parameters. **Results:** Results showed that drought decreased photosynthetic capability of soybean in terms of leaf chlorophyll index, the rate of light intensity acceptance and the percentage of energy absorption. In addition, stomatal density also declined in the stressed plants when water supply was limited to 40-50% of field capacity. However, application of the fungi as much as 0.15 g/pot in the stressed plant can retard the decline of leaf stomatal density. **Conclusion:** Hence, it can be concluded that application of mycorrhiza fungi improve photosynthetic capability of soybean experiencing drought stress.

Key words: Water shortage, arbuscular mycorrhizal fungi, leaf chlorophyll index, stomatal density, stomatal aperture

Received: Accepted: Published:

Citation: Nasaruddin and Ifayanti Ridwan, 2018. Photosynthetic apparatus of soybean exposed to drought due to application of arbuscular mycorrhiza. Asian J. Plant Sci., CC: CC-CC.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

## INTRODUCTION

Limitation of water is an important environmental factor which may result in water stress on plants and could be more pronounced in the future with global climate change. This condition is a major obstacle to the growth and productivity of plants especially in rain-fed areas of Indonesia where water shortage is a common phenomenon with limited and poor quality of irrigation. On this type of land, any water conservation strategies or management to increase plant water use efficiency are necessary to maintain high productivity especially for the second crop.

Plant response to water shortage is a very complex trait involving multiple genetic, morphological, physiological and biochemical mechanisms[1,2]. Plants generally have different morphological or physiological mechanism that allows a better production under limited water supply[3]. Plants respond to limited water condition through a variety of mechanisms to maximize the absorption of water by the root system and to minimize water loss by stomata closure and reduction of leaf area[4]. Plants are experiencing a shortage of water around the roots and responds immediately by sending chemical signals to shoots to launch a wide range of adaptive responses including reduced leaf area and modified stomatal characteristics[4,5]. The response of plants to water shortage can be done through the setting of various aspects of their physiology and metabolism[6]. The density of stomata, stomatal conductance and stomatal openings can be reduced with no reduction in leaf water potential[7,8], although the possibility of net photosynthesis and transpiration rate of the plants was reduced[9,10].

Soybean (*Glycine max* L. Merr.) is one of the major sources of protein for human nutrition and animal. In addition, it also has potential as source of vegetable oil. Optimum growth of the plant can be achieved when soil moisture was adequate during growing period in order to obtain its potential yield[11]. In Indonesia, soybeans are generally planted following rice by utilizing residual soil moisture at the end of the rainy season so that the plants might experience drought stress, especially when the rain stopped early at the end of the rainy season with sandy soil conditions. Soybean regarded as a species sensitive to some abiotic stresses[12], when compared with other tropical beans. Soybeans are very sensitive to lack of water in the reproductive phase (phase R1 and R2) and the phase of growth and seed filling (phase R3-R6)[13,14], therefore, water stress during these phases may result in drastically declined production and productivity of the crop.

One of efforts made to overcome the problem of water shortage was the utilization of arbuscular mycorrhizal fungi (AMF). The AMF plant symbiosis is known to improve mineral nutrition of host plants, improving resistance to environment stress especially drought stress[15]. This symbiotic action can improve water plant relation result in more tolerant plant during lack of water condition[16,17]. The AMF infected plants often show different photosynthetic characters. Wang and Blatt[18] reported that AMF infected plants showed higher photosynthesis, stomatal conductance and transpiration rate than plants without AMF. This can be attributed to the level of radiation energy absorbed, chlorophyll and leaf stomata characteristics as an important component in the process of photosynthesis. Information about radiation characteristics, energy absorption and leaf chlorophyll and leaf stomatal characteristics in plants infected by AMF especially in soybeans was still relatively limited. In addition, while many researches had studied the plant-mycorrhiza fungi interaction in drought condition, only few studies include the effect of the fungi on photosynthetic apparatus especially on soybean plant in the tropics. The aim of this study was to evaluate the effect of AMF on the absorption of radiation energy, chlorophyll index and stomatal characteristics of soybeans in water limited conditions.

## MATERIALS AND METHODS

The study was conducted at the Screen House Faculty of Agriculture, University of Hasanuddin, Makassar Tamalanrea Unhas Campus, from July-October, 2014 as a controlled environment experiment. This recent study emphasizes on the indirect effect of mycorrhizae fungi on photosynthetic characteristics of soybean plant especially in the tropics. In addition, some parameters of the plant in regards to radiation acceptance and energy absorption were measured as response of the plant in adapting to drought condition as altered by AMF. Previous studies had rarely taken into account these parameters despite the importance of these parameters in determining the level of photosynthesis by the plant. Observation of stomatal characteristics were done at the Laboratory of Plant Physiology and Nutrition Hasanuddin University.

The plant material used was soybean variety of Willis obtained from Peas, Beans and Tuber Crops Research Institute in Malang. The isolate of mycorrhizae fungi was obtained from Plantation Crops Research Institute in Bogor in the form of compost. Growth media was taken from experimental farm

Land of Corn and Cereals Crops Research Institute in Bajeng district of Gowa Regency. The growing medium consisted of clay = 11%, dust = 26%, sand = 64%, pH ($H_2O$) = 5.37, C organic = 19% N, total = 0.14%, P bray = 19.75 ppm, K = 0.51 cmol+ $kg^{-1}$ and CEC = 6.22%. Previously the growing medium was evenly mixed with cow manure obtained from the Faculty of Animal Husbandry Unhas with ratio of 2:1. The media were crushed and then air dried before distributed into plastic pots with 5 kg growing media per pot.

The experiment was arranged in a factorial design patterns based on split plot design. Water supply level was set as the main plot (MP) consisted of 3 levels, 80-90% of field capacity (k1), 60-70% of field capacity (k2) and 40 -50% of field capacity (k3). Inoculation of AMF was set as sub plot (SP) consisted of 4 levels i.e., control (c0), inoculation AMF 0.05g per pot (c1), 0.10 g/pot (c2) and 0.15 g per pot (c3).

Two seeds of soybean were planted in each plastic pot and for each of treatment combination was consisted of 4 pots and replicated 3 times. Distance between pot was set at $30\times30$ cm. Parameters observed consist of acceptance level of radiation intensity and the percentage of radiation absorption by using a spectrophotometer miniature CI-710, leaf chlorophyll index using CCM+200, stomatal characteristics using nail polish method and observed under the microscope with magnification of 400-1000 times, leaf stomatal conductance, temperature and humidity of the leaf measured using Porometer and leaf nutrient content.

**Statistical analysis:** Observations data were tabulated then analyzed using a factorial analysis of variance (ANOVA) followed by the least significance difference (LSD) test ($p\leq0.05\%$). A correlation analysis was performed using Excel program Microsoft Office version 2010.

## RESULTS

**Acceptance level of radiation intensity, energy absorption percentage and leaf chlorophyll index:** Statistical analysis showed that the level of water supply and application of AMF significantly affect plant leaf chlorophyll index ($p<0.05$) in the vegetative phase, reproductive phase and the phase of maximum pod formation. The treatments had significant effect on the level of radiation acceptance and the percentage of energy absorption of photosynthetic active radiation (PAR). No significant interaction of the water supply level and application of AMF on all parameters (Table 1).

The water supply level of 80-90% of field capacity and the AMF inoculation of 0.150 g/pot show highest leaf chlorophyll index at vegetative phase, reproductive phase and the phase of maximum pod formation. Similarly, the acceptance level of solar radiation of the plant subjected to the treatments was at the highest which impacted on the percentage of the PAR energy absorbed. The higher the level of water supply and dose of AMF inoculation, better the effect on the level of acceptance of the radiation intensity, the percentage of PAR radiation energy absorbed and leaf chlorophyll index.

The PAR intensity was positively correlated linearly with the absorption of the radiation energy and the average of leaf chlorophyll index. Increasing light intensity resulted in greater absorption of radiation energy following equation of y = 0.0004x-20.254, r = 0.83** and higher leaf chlorophyll index following equation of y = 0.1387x+102 570, r = 0.84** (Fig. 1a). Average leaf chlorophyll index was positively correlated linearly with leaf temperature and humidity. The higher the leaf temperature and humidity, the higher the leaf chlorophyll index by following equation of y = 0.0026x -233.8, r = 0.85** and y = 0.0008x-61.791, r = 0.92**, respectively for leaf temperature and humidity relationship with leaf chlorophyll index (Fig. 1b).

Table 1: Effect of various levels of water supply and application of arbuscular mycorrhizal fungi on physiological characteristics of soybean

| Treatments of water supply | Observation parameters LCi P. Veg | LCi P. Rep | LCi P. MP | IS (Watt $cm^{-2}$) | Ae (%) |
|---|---|---|---|---|---|
| 80-90% Fc (k1) | 121830.46[a] | 114577.84[a] | 117661.32[a] | 113,545.91[a] | 28.59[a] |
| 60-70% Fc (k2) | 119716.10[ab] | 113526.53[ab] | 116958.35[a] | 99,607.48[b] | 22.9[b] |
| 40-50% Fc (k3) | 118011.32[b] | 112602.20[b] | 113687.51[b] | 96,177.33[b] | 18.31[c] |
| $LSD_{\alpha=0.05}$ | 2590.52 | 1297.61 | 848.36 | 5809.28 | 2.69 |
| **Inoculation AMF** | | | | | |
| 0.000 g/pot (c0) | 118986.94[c] | 113269.59[b] | 115783.49[b] | 93967.03[b] | 21.76[b] |
| 0.050 g/pot (c1) | 120086.31[b] | 113392.60[b] | 115845.72[b] | 104949.56[a] | 23.01[b] |
| 0.100 g/pot (c2) | 120484.63[b] | 114044.37[ab] | 116677.98[ab] | 106746.34[a] | 24.52[ab] |
| 0.150 g/pot (c3) | 121500.41[a] | 114709.87[a] | 117645.27[a] | 106778.02[a] | 25.84[a] |
| $LSD_{\alpha=0.05}$ | 992.02 | 1074.52 | 1343.00 | 4916.04 | 2.83 |

Numbers followed by different letters in the column, were significantly different at the level of 95%. Fc: Field capacity, LCi: Average of leaf chlorophyll index, P.Veg.: Vegetative phase, P.Rep.: Phase reproduction, P.MP: Phase formation maximum pods, IS: Rate of acceptance of light intensity and Ae: Percentage of energy absorption

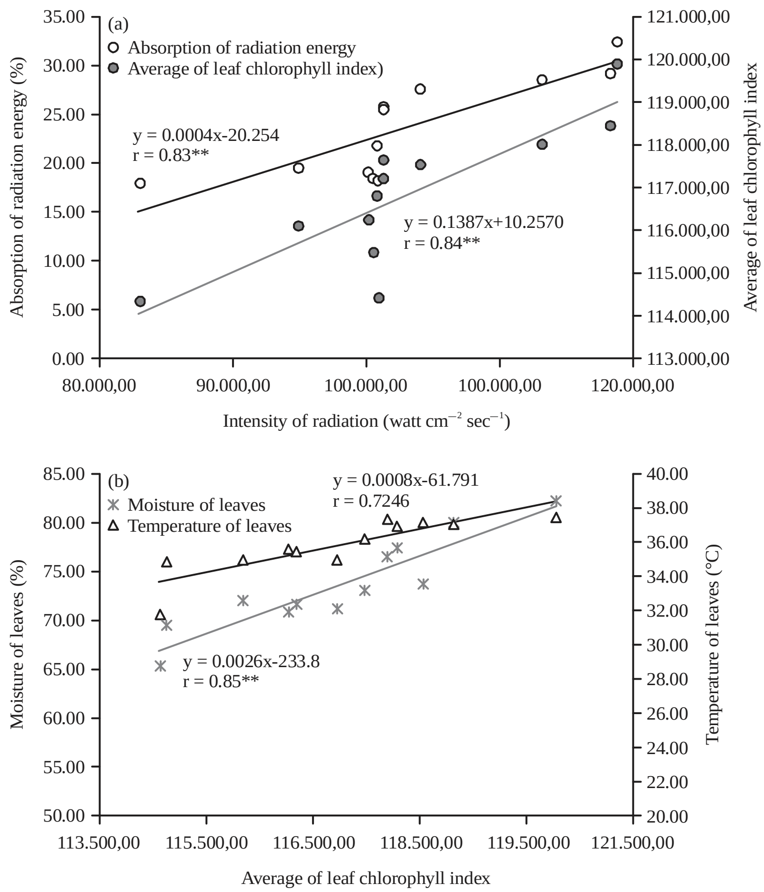


Fig. 1(a-b): (a) Correlation of radiation intensity with radiation energy absorption and average of leaf chlorophyll index of soybean plant and (b) Correlation between the average of leaf chlorophyll index with leaf temperature and humidity of soybean plant

Table 2: Average stomatal density of soybeans on the levels of water treatment and inoculation of AMF

| | Water treatment | | | | | | |
|---|---|---|---|---|---|---|---|
| | Density stomatal of upper epidermal cell (n mm$^{-2}$) | | | | | | |
| Dose of inoculation AMF | 80-90% | Fc (k1) | 60-70% | Fc (k2) | 40-50% | Fc (k3) | $LSD_{AMF\ dosage\ inoculation\ (\alpha = 0.05)}$ |
| 0.000 g/pot (c0) | 306.10 | $a^x$ | 253.65 | $a^x$ | 158.06 | $b^y$ | |
| 0.050 g/pot (c1) | 342.32 | $a^x$ | 272.54 | $a^x$ | 181.40 | $b^{xy}$ | 88.03 |
| 0.100 g/pot (c2) | 350.10 | $a^x$ | 284.21 | $a^x$ | 192.51 | $b^{xy}$ | |
| 0.150 g/pot (c3) | 369.21 | $a^x$ | 329.21 | $ab^x$ | 260.29 | $b^x$ | |
| $LSD_{water\ supply\ level\ (\alpha = 0.05)}$ | | | 78.89 | | | | |
| **Density stomatal of lower epidermal cell (n mm$^{-2}$)** | | | | | | | |
| 0.000 g/pot (c0) | 453.37 | $a^y$ | 293.82 | $bc^y$ | 232.85 | $c^x$ | |
| 0.050 g/pot (c1) | 508.93 | $a^y$ | 359.37 | $ab^{xy}$ | 277.74 | $b^x$ | 161.86 |
| 0.100 g/pot (c2) | 520.04 | $a^y$ | 416.04 | $ab^{xy}$ | 324.52 | $b^x$ | |
| 0.150 g/pot (c3) | 714.48 | $a^x$ | 461.60 | $bc^x$ | 326.30 | $c^x$ | |
| $LSD_{water\ supply\ level\ \alpha = 0.05}$ | | | 169.09 | | | | |

Numbers followed by different letters in the column (a,b,c) and row (x,y), were significantly different at the level of 95%, Fc: Field capacity

**Leaf stomatal density:** Statistical analysis showed that the water supply treatment interacted with the dose of AMF inoculation rates in affecting the density of stomata on the upper and lower epidermis of the leaves. The water supply of 80-90% of field capacity and AMF inoculation dose of 0.150 g/pot (k1, c3) results in the highest stomatal density, both on the upper epidermis cells and the underside leaf epidermis cells (Table 2).

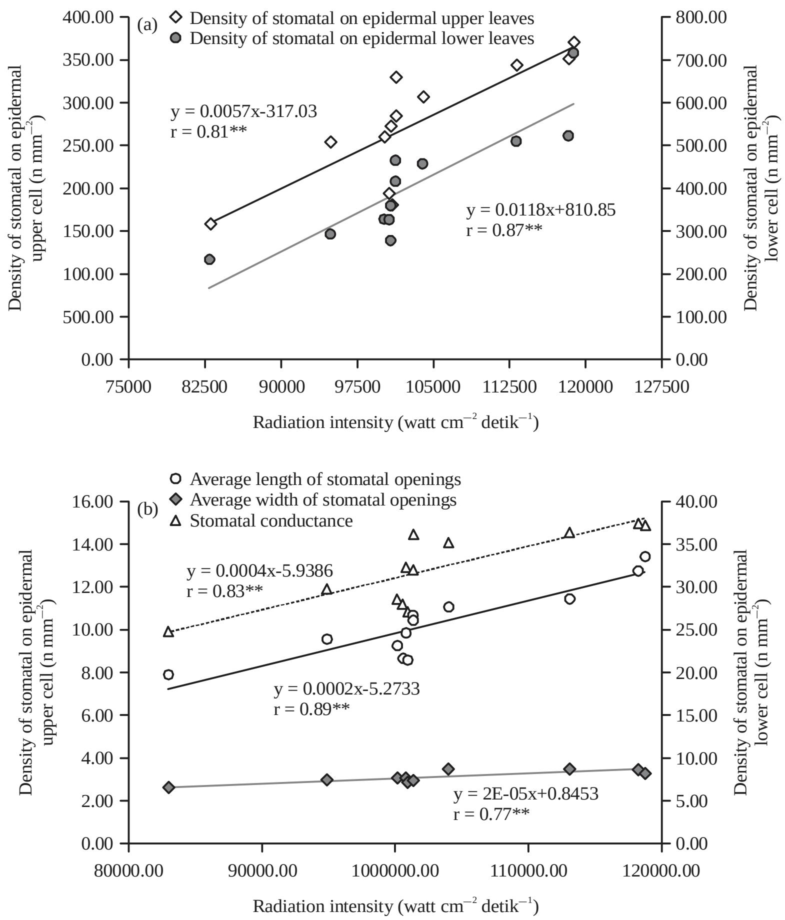


Fig. 2(a-b): (a) Graph correlation between radiation intensity and stomatal density of soybean leaves and (b) Correlation between radiation intensity and length and width of stomatal openings and stomatal conductance of soybean plant

Table 3: Average length and width of stomatal openings of soybeans on the levels of water treatment and inoculation of AMF

| Treatments of water supply | Length of stomatal openings: Upper epidermis (μm) | Length of stomatal openings: Lower epidermis (μm) | Width of stomatal openings: Upper epidermis (μm) | Width of stomatal openings: Lower epidermis (μm) | Stomatal conductance |
|---|---|---|---|---|---|
| 80-90% Fc (k1) | 12.65$^{a}$ | 10.78$^{a}$ | 4.13$^{a}$ | 4.67$^{a}$ | 36.29$^{a}$ |
| 60-70% Fc (k2) | 10.44$^{a}$ | 9.43$^{ab}$ | 3.24$^{ab}$ | 3.24$^{b}$ | 31.39$^{b}$ |
| 40-50% Fc (k3) | 8.49$^{b}$ | 8.23$^{b}$ | 2.44$^{b}$ | 2.51$^{b}$ | 26.57$^{c}$ |
| $LSD_{\alpha = 0.05}$ | 2.29 | 1.47 | 1.25 | 1.11 | 4.07 |
| **Inoculation of AMF** | | | | | |
| 0.000 g/pot (c0) | 9.74$^{b}$ | 9.25$^{b}$ | 3.00$^{b}$ | 2.98$^{b}$ | 29.89$^{b}$ |
| 0.050 g/pot (c1) | 10.48$^{a}$ | 9.42$^{b}$ | 3.19a$^{b}$ | 3.64$^{ab}$ | 31.90$^{ab}$ |
| 0.100 g/pot (c2) | 11.36$^{a}$ | 9.78$^{ab}$ | 3.61$^{ab}$ | 3.79$^{ab}$ | 32.46$^{a}$ |
| 0.150 g/pot (c3) | 11.60$^{a}$ | 10.60$^{a}$ | 4.15$^{a}$ | 4.06$^{a}$ | 33.93$^{a}$ |
| $LSD_{\alpha = 0.05}$ | 1.16 | 0.80 | 1.06 | 0.91 | 2.12 |

Numbers followed by different letters in the column, were significantly different at the level of 95%, Fc: field capacity

**Stomatal opening and conductance:** Water treatment and application of AMF significantly affect stomatal aperture and stomatal conductance ($p<0.05$), but the interaction effect was not significant (Table 3). The provision of water treatment of 80-90% field capacity shows the widest stomatal openings or highest length and width, on the upper and lower epidermis of leaves. In addition, the treatment also resulted in the highest stomatal conductance compared to the provision of water treatment of 40-50 and 60-70% field capacity.

The AMF inoculation treatment 0.15 g/pot, shows wider average stomatal openings of the upper and lower leaf epidermis cells and higher stomatal conductance than other treatments i.e., AMF inoculation of 0.10 and 0.05 g/pot and control.

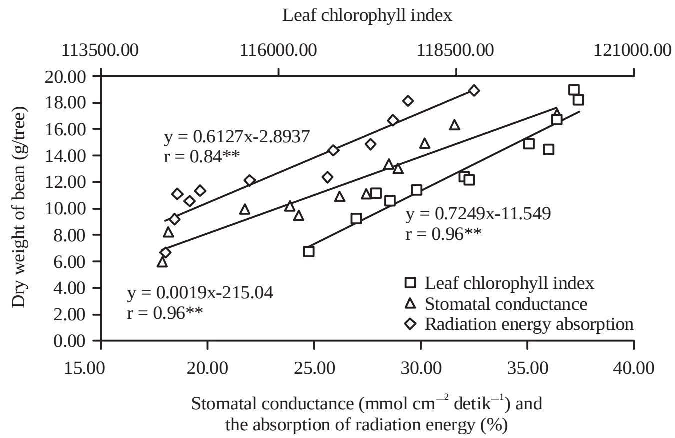


Fig. 3: Graph correlation between stomatal conductance, absorption of radiation energy and leaf chlorophyll index with dry weight of bean of soybean plant

Table 4: Average number of grains/plant, dry weight of 100 grains (wc 14%) and grain dry weight/plant (wc. 14%) of soybeans on the levels of water treatment and inoculation of AMF

| Treatments of water supply | Number of grain (grains/plant) | Dry weight of 100 grains wc. 14% (g) | Dry weight of grain wc. 14% (g/plant) |
|---|---|---|---|
| 80-90% Fc (k1) | 170.98$^a$ | 8.56$^a$ | 14.66$^a$ |
| 60-70% Fc(k2) | 148.82$^b$ | 6.90$^b$ | 10.39$^b$ |
| 40-50% Fc (k3) | 141.08$^b$ | 5.69$^c$ | 8.04$^b$ |
| $LSD_{\alpha = 0.05}$ | 17.58 | 0.90 | 2.44 |
| **Inoculation of AMF** | | | |
| 0.000 g/pot (c0) | 147.83 | 6.08$^b$ | 9.14$^b$ |
| 0.050 g/pot (c1) | 154.11 | 7.34$^a$ | 11.54$^{ab}$ |
| 0.100 g/pot (c2) | 158.94 | 7.73$^a$ | 12.41$^a$ |
| 0.150 g/pot (c3) | 165.10 | 7.92$^a$ | 13.33$^a$ |
| $LSD_{\alpha = 0.05}$ | Ns | 1.18 | 3.93 |

Numbers followed by different letters in the column, were significantly different at the level of 95%. Fc: Field capacity, WC: Water content

Correlation analysis showed that the intensity of light radiation is positively correlated with the density of stomata (Fig. 2a), the average length and width of stomatal aperture and stomatal conductance (Fig. 2b). The higher the intensity of radiation, the denser the stomata, follows the equation y = 0.0057x-317.03, r = 0.81** on the upper epidermis, y = 0.0118x-810.85, r = 0.87** on the lower epidermis, the greater the stomatal openings follows the equation y = 0.0002x-5.2733, r = 0.89** on stomatal aperture length, y = 2E-05x+0.8453, r = 0.77* and the higher the value of stomatal conductance by following equation y = 0.0004x-5.9386, r = 0.83**.

**Crop production:** Statistical analysis showed that the rates of water treatment and inoculation dose of AMF had significant effects on production parameters. The provision of water treatment 80-90% field capacity shows the biggest average number of grains per plant, highest dry weight of 100 grains and grain dry weight per plant [water content (wc) of 14%] and significantly different at $p<0.05$ compared to the provision of water treatment of 60-70% field capacity and 40-50% field capacity (Table 4).

Correlation analysis showed that the average index of leaf chlorophyll, stomatal conductance, radiation energy absorption are positively correlated linearly with the dry weight of seed per plant. The seed dry weight increased with the leaf chlorophyll index follows the equation y = 0.7249x-11.549, r = 0.96, stomatal conductance follows the equation y = 0.0019x-215.04, r = 0.96 and the absorption of radiation energy following the equation y = 0.6127x-2.8937, r = 0.94 (Fig. 3).

## DISCUSSION

Plant response to drought stress involving various interchangeability of anatomical and physiological processes, even the possibility of tissue damage will occur. The results showed that the level of acceptance of the intensity of the radiation and the radiation energy absorption index decreased chlorophyll leaves the plant soybeans based on the level of irrigation (Table 1). Anatomical changes caused by drought stress in higher plants are a good indicator and was relatively easy and can be directly observed[11]. Soybean is a crop within legume group which was relatively have faster growth[19] and more vulnerable to drought stress[20]. A decrease in the level of provision of water to the soybeans crop will result in a decrease in content of total chlorophyll of leaves significantly[21,22] and cause damage to thylakoid membranes[20], which have an impact on the level of radiation reception intensity and absorption of radiation energy. Leaf chlorophyll was one of primary pigments of light absorber contained in chloroplasts[23]. The chloroplast, the primary site for photosynthesis, are extremely sensitive to the environment such as drought, extreme temperatures, light intensity, flooding, salinity, UV radiation and plays a major role in the modulation of the stress response[24]. In this recent study, the decline in chlorophyll content under limited water may be due to the reduced synthesis of complex pigment chlorophyll principal encoded gene taxis[25] or destruction of chiral macro pigment protein complexes (CHCIIs chlorophyll 'a' or 'b') by an aggregate of light that protect the photosynthetic apparatus[14].

The results showed that the higher the dose of AMF inoculation, more influence on the level of acceptance of radiation intensity, radiation energy absorption and leaf chlorophyll index (Table 1). This was because the AMF infected plants can improve the efficiency of water absorption and uptake of N, $P_2O_5$ and $K_2O$. Cavagnaro *et al*.[26] reported that symbiotic plants with AMF can improve the uptake of water and nutrients, especially N, P and K through AMF ability to increase the surface area of absorption by the external hyphae development. This will result in increased leaves water potential and leaf chlorophyll formation which will improve the level of acceptance of radiation intensity hence, the absorption of energy radiation.

Solar light energy conversion in photosynthesis contributed greatly to the growth and development of plants and is a key process to plant productivity[27]. However, environmental stress such as water shortage and unfavorable temperatures, much impede the process of photosynthesis by altering the ultra structure of organelles and concentration of various pigments and metabolites resulting in stomatal regulation.

Stomatal response or sensitivity to environmental conditions through changes in the characteristics of guard cells, affecting the mechanisms of opening and closing of the stomata. Results of the research conducted showed that the water supply 80-90% of field capacity and inoculation of AMF extended the stomatal aperture through the length and width of the stomatal aperture (Table 3). The availability of adequate water will lead to increased uptake of water on host plants so as to allow an increase in the moisture leaves and differences in water vapor pressure in the guard cells and epidermal cells/mesophyll leaf with atmospheric water vapor pressure so that the stomata open more. Previous study have reported that the water vapor pressure differences between the cells of the leaf epidermis and mesophyll leaf with water vapor in the air pressure at high temperature conditions will result in an open stomata[12]. In general, the movement of stomata in response to differences in water vapor pressure, the level of acceptance of the intensity of light, temperature and $CO_2$ concentration, except the stomata of plants that follow Crassulacean acid metabolism[23]. The regression analysis shows that inoculation AMF positively correlated linearly to the density of the upper epidermis stomata (Fig. 2a) as well as length and width of stomata openings below the epidermis (Fig. 2b). The AMF inoculation influence on leaf stomatal aperture of soybeans due to the improvement in the level of water absorption on the AMF infected plants and increased content of leaf N, P and K especially when water were limited. The mechanism of stomatal movement influences by environmental conditions in addition to the characteristics of $K^+$ transport in the guard cells and mesophyll cells of leaves[23]. Thus, the rate of absorption and the concentration of $K^+$ in the leaf tissue affect stomatal aperture width. This might explain the increase of stomata aperture in infected plants subjected to improvement in the efficiency of $K_2O$ and increased concentration of $K^+$ in the leaves, especially in the treatment of low levels of water provision. This also indicates that regulation of stomata is influenced by the movement of ions in guard cells and mesophyll cells of leaves that contribute to an increase in osmotic pressure in the guard cells and the cells in the leaf mesophyll accompanist[27]. McAinsh and Pittman[22] reported that $K^+$ transport by anion malate and succinate to guard cells and the cells of the epidermis and mesophyll accompanist leaves will cause increased flow of water so as turgor pressure increased and stomatal guard cells open. Malic acid ion metabolism in the cell cytosol keeper made a major contribution to the increase in osmotic potential protoplasm guard cells[18].

The density of stomata on the upper and lower epidermis of leaves positively correlated to light intensity (Fig. 2a). This was because the level of water provision treatment led to differences in leaf relative water content at different levels of water supply. In addition, the influence of the AMF dose cause differences in the level of water and nutrient uptake efficiency, especially N, P and K on the conditions of limited water that affect the ability of the plant to retain water. Water availability has very strong influence on the density of leaf stomata[28]. Kouwenberg *et al*.[5] reported that higher leaves temperature and humidity increased stomatal density. Differences in nutrient content of leaves and leaf relative water content will lead to differences in temperature and leaves humidity. Correlation analysis shows that the effects of temperature and humidity on the number of stomata on the upper leaf epidermis were denser than on the lower leaf epidermis.

Water use efficiency has a very close relationship to the stomatal behavior by setting the loss of water and transpiration efficiency under drought stress[29]. Soybeans plants experiencing water deficit during the 12 days will decline drastically in stomatal conductance[30]. Under conditions of limited soil water, the balance between the increase in water uptake by the roots and decrease water loss by stomata control was essential to maintain the growth and productivity of plants. Results of the study indicate that the lower water supply levels and the higher the dose inoculation AMF, the better its effect on stomatal aperture size. The size of the stomata opening was set by the turgor pressure and volume of the guard cells associated with the transport of ions and water through the channel proteins across the plasma membrane and the vacuole[31]. Drought stressed plants will result in increased production of abscisic acid (ABA) in leaves that contribute to the mechanism of opening and closing of stomata[23]. The results in the last 5 years research of Arve *et al*.[8] reported that ABA synthesized in the roots and also in the mesophyll cells, vascular tissue and leaf stomata determines the level of stomata and conductance of stomata in plants that experienced water deficit. Thus, the level of water supply and dosage of AMF inoculation will cause the improvements of size of the stomatal opening as a consequences of repaired turgidness of leaf cells by the role of the water supply and the role of AMF in maintaining the relative water content in leaves.

Stomatal control is the main physiological factors to optimize the use of water in drought conditions. In general, stressed plants suffer direct impact on the decrease in leaf water potential and stomatal conductance[32]. Results of the study conducted by Pan *et al*.[27] reported that soybeans experiencing drought stress resulted in a decrease in stomatal conductance reached 42% compared with plants grown under conditions of normal water availability. In general, drought stress affects the balance between stomatal conductance and internal leaf photosynthetic capacity. Songsri *et al*.[29] reported that the highest stomatal conductance of soybeans plants under conditions of field capacity and experience loss based on the level of ground water availability. Stomatal conductance plant soybeans undergo a drastic reduction in the level of water availability decreases based on the level of ground water availability 1/3 field capacity. Thus, the level of provision of water and inoculation dose AMF especially in plants that lack of water pressure will allow the plant to maintain the relative water content and plant capacity to enable ongoing process of internal leaf photosynthetic activity and other metabolic processes.

The results showed that the average weight of 100 seeds and planting seeds dry weight decreased in time with the decline of water supply. The lower the level of water supplies the lower the 100 seed weight and seed production (Table 4). Increased chlorophyll content of leaves chlorophyll index was based on the increase dose of AMF application. Symbiosis of AMF with plants have an important role in enhancing the solubility of nutrient and nutrient uptake, especially N, P and K as well as the absorption of water through its ability to increase the surface area of absorption[26]. Improved water absorption and N content in the leaf on the soybean plant particularly on the low water supply levels will reduce the activity of the enzyme khlorofilase[33]. Plants that suffer from water shortages will increase chlorophyllase enzyme activity resulting in a degradation of chlorophyll[34] and accelerate leaf senescence[35].

The AMF inoculation treatment on soybean plants can improve the level of acceptance of radiation energy and the percentage of energy absorption of light at wavelengths of radiation PAR 411.49 and 554.09 nm. The level of absorption of the radiation energy was positively correlated linearly with the percentage of good light absorption of radiation at a wavelength of 411.19 nm and at a wavelength of 554.09 nm (Table 1). Increased energy acceptance levels of radiation and energy absorption of radiation in the treatment of inoculation AMF caused by an increase in the amount of chlorophyll of leaves and leaf water potential. Jenabiyan *et al*.[36] reported that increased leaf chlorophyll content of crop soybeans through measurement with (SPAD) bioscience content chlorophyll meter followed by an increase in the intensity of solar radiation up to 8000 lux at the NYC area with wavelengths between 400-700 nm.

Stomata in soybeans plants are amphistomatic because stomata in plants soybeans found in the upper and lower epidermis of the leaves. Inoculation AMF in soybeans plants showed more number of stomata, wider stomatal openings based on the length and width of stomata openings in upper and lower epidermis of the leaves (Fig. 2a). The increased numbers of stomata of the soybeans are indirect influence of inoculation AMF. The AMF infection in the host plant is directly involved in the repaired of nutrients and water absorption.

Symbiosis of AMF and host plants will form the structure of hyphae that directly improve the efficiency of absorption and water use of the host plants[37], increasing the absorption of N, P and K and micro nutrients given especially Zn, Cu and Fe[38]. Zhu *et al.*[39] reported that mycorrhizae can increase the rate of photosynthesis of host plants. Thus the inoculation of AMF will increase the growth rate of plant leaf and indirectly will increase the number of stomata.

Results of the recent study implied the advantages of mycorrhiza use on crop particularly in dry land. However, it should be beared in mind that the study has limitation as it was conducted in a screen house and further test in the filed needs to be undertaken.

## CONCLUSION

Application of mycorrhiza fungi, as much as 0.150 g/pot, to stressed soybean can helped the plants in water uptake and retard reduction in leaves chlorophyll index, stomatal density and stomatal opening and conductance, hence, a normal photosynthesis during drought condition can be maintained. The impact of these conditions will result in better growth and crop production as a consequence of the increased capacity of the plant in utilizing resources.

Water shortage reduced soybean leaves capability in absorbing the photosynthetic active radiation (PAR) by altering the photosynthetic components of the leaves such as decreased leaf chlorophyll index, stomatal density and stomatal opening and conductance.

## SIGNIFICANCE STATEMENTS

The study gives the physiological adaptation mechanism of soybean plant and its interaction with mycorrhizae when water is scarce. Study on response of plants to application of mycorrhizal fungi in against drought conditions have been conducted widely, however the recent study explored the relationship of these responses to plant physiological properties related to photosynthetic process, one of important metabolisms of the plant. Therefore, the significant finding of this study could add to the knowledge regarding fungi-plant response to drought in term of its photosynthetic apparatus. Hence, this study contributes in the improvement of soybean crop production in Indonesia.

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