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Paddy Chlorophyll Concentrations in Drought Stress Condition and Endophytic Fungi Application

Syamsia¹, Abubakar Idhan², Noerfitryani³, Marhamah Nadir⁴, Reta⁵,
Muhammad Kadir⁶

¹Muhammadiyah University of Makassar, South Sulawesi, 90221 Indonesia
Email : [:syamsiatayibe@unismuh.ac.id](mailto:syamsiatayibe@unismuh.ac.id)

²Muhammadiyah University of Makassar, South Sulawesi, 90221 Indonesia
Email :

³ Muhammadiyah University of Makassar, South Sulawesi, 90221 Indonesia
Email :

⁴Hasanuddin University, Makassar, South Sulawesi, 90234 Indonesia
Email : marhamahnadir@unhas.ac.id

⁵Plantation Department, Politechnic of Agriculture and Fisheries, Pangkajene
and Kepulauan

Email :

⁶ Plantation Department, Politechnic of Agriculture and Fisheries,
Pangkajene and Kepulauan
Email: mkadir@gmail.com

Abstract - Drought is one of the major constraints in rainfed paddyfield production. Drought stress condition caused by long drought that often happens lately and it was major problem causing decreased paddy productivity. The research aimed to determine the chlorophyll concentration of paddy plants treated with endophytic fungi and drought. The research was conducted on farmer's paddy field in Galesong Sub-district of Gowa Regency from August to November 2017. The research consisted of two treatments that puddle height and fungi application. The puddle height treatment consists of two levels i.e. puddle 5 cm height (normal) and 1 cm (drought). Endophytic fungi treatment was applied to paddy seeds before planting. Leaf chlorophyll content was measured on 52 days after planting by using SPAD-502 Plus chlorophyll meter. Chlorophyll-a content, chlorophyll-b content and total chlorophyll content was measured by using extraction method in laboratory. The result showed that leaf chlorophyll concentration of paddy drought was low than the waterlogged conditions. Endophytic fungi application increased chlorophyll-a, chlorophyll-b concentration, total chlorophyll of paddy leaf on drought condition.

Keywords: chlorophyll a, chlorophyll b, total chlorophyll, SPAD-502 Plus

1. Introduction

Drought is major abiotic stress that greatly affects food crops worldwide. The agronomic and physiological associated with drought tolerance are good indicators for drought tolerance of genotype selection to reduce the impact of drought stress condition on harvest in breeding programs [1]. Drought is one of important factors of abiotic stress [2];[3]. The measurement of physiological character such as chlorophyll concentration is one



of approach to study effect of drought condition on growth and production for this parameter is closely related to rate of photosynthesis [1]);[4]. Drought stress affects the biochemical processes in plant cells [5].

High chlorophyll and carotenoid were associated with plant tolerance to stress [6];[7];[8]. Measurement of chlorophyll fluorescence is a tool that can detect photosynthesis interference caused by environmental stresses [9];[10]. Chlorophyll loss is a negative consequence of environmental stress; However, it has been considered an adaptive feature in plants growth under drought stress condition [11];[12].

Endophytic fungi increasing host tolerance in stress conditions [13]. Endophytic fungi also produce diverse of biochemical important metabolites [14]. [15] was reported that inoculated *Trichoderma hamatum* on cocoa plants with endophytic fungi resistant in drought conditions. There were six genera of endophytic fungi were isolated from healthy tissue in different part of paddy plants, they were *Fusarium*, *Aspergillus*, *Curvularia*, *Penicillium*, *Gilmaniella* and *A. foliicola* [16]. [17] also were reported that there were 27 endophytic fungi isolated from Kuruluthuda rice variety, the most isolated genera were *Acremonium*, *Arthrotrichum*, *Colletotrichum* and *Humicol*.

[4] reported that the difference in total chlorophyll concentration, chlorophyll a, and chlorophyll b on cultivar Serayu and IR 64 paddy leaves treated with PEG with PA 0; -0.5 and -0.1 MPa. [18] showed that total leaf chlorophyll on ginger plants decreased 8% due to drought for 7 days, while total leaf chlorophyll content in control increased 9% to day 7. In drought stress condition also significantly decreased the chlorophyll-a, chlorophyll-b and total chlorophyll content at the vegetative and flowering stages three cultivars of chickpea [19]. Thus this study to find out the total chlorophyll concentration, chlorophyll-a, chlorophyll-b and carotenoid of paddy leaf treated by endophytic fungi application in drought condition.

2. Method

The research was conducted in the rainfed paddy field in Galesong Sub-district of Gowa Regency in August to November 2017. Irrigation paddy field using a water pump powered by a generator. Paddy seedlings IMPARI 20 that are 21 days after planting were treated with immersion of endophytic fungi and without immersion (control). Drought stress treatment performed after first fertilization, that is 14 days after planting until harvest by adjusting puddle is 1 cm height (drought) and puddle 5 cm height (normal). Analysis of chlorophyll content using Chlorophyll Meter Soil Plant Analysis Development (SPAD) 502 Plus which has been calibrated. The measure leaf were cleaned using tissue paper. To find out the chlorophyll content, green leaf were clipped with SPAD Chlorophyll Meter sensor. Value seen on the screen SPAD chlorophyll meter chlorophyll. Value of total leaf chlorophyll was calculated using the formula $Y = 0.0007x - 0.0059$, $Y =$ chlorophyll content and $x =$ value of the measurement results-502 Plus SPAD chlorophyll meter [20]; [21]. The concentration of chlorophyll a, chlorophyll b, carotenoid and total chlorophyll of paddy leaves was determined by leaf blade (lamina) sample for each treatment was taken 1 g, mashed with mortar and pestle, then extracted with 95% alcohol until all chlorophyll dissolved. Total chlorophyll concentrations, chlorophyll a and chlorophyll b were calculated by Wintermans & de Motts (1965) in Sasmitamihardja (1990) as follows: Total chlorophyll (mg / L) = 20.0 OD₆₄₉ + 6.1 OD₆₆₅ Chlorophyll a (mg / L) = 13.7 OD₆₆₅ - 5.76 OD₆₄₉ Chlorophyll b (mg / L) = 25.8 OD₆₄₉ - 7.7 OD₆₆₅ (OD = Optical density);[4].

3. Results and Discussion

Leaf greenishness level was measured using a chlorophyll meter SPAD 502 Plus. The principle of this tool is to record the greenishness level of leaves and relative total of chlorophyll molecules present in leaf in a single value based on the amount of light leaf transmitted (Konica Minolta, 1989). Result of measurement total chlorophyll of paddy leaf on drought treatment (puddle 1 cm height) is lower than normal treatment (puddle 5 cm height). While total chlorophyll content in treatment of endophytic fungi application showed that higher value both in drought treatment and normal treatment (Figure 1). The

results showed that increase in amount of chlorophyll with endophytic fungi treatment. Refer to [20], increased in the greenishness of leaf showed that increase of total chlorophyll in leaves. therefore, more of total chlorophyll in leaves, hence increasing the rate of photosynthesis. The leaves are yellow and old because of chlorophyll was lost, the power of photosynthesis has decreased (Ahmadi, 1985; [3]). Differences in physiological and biochemical responses in plants can be caused by drought condition in crops [4].

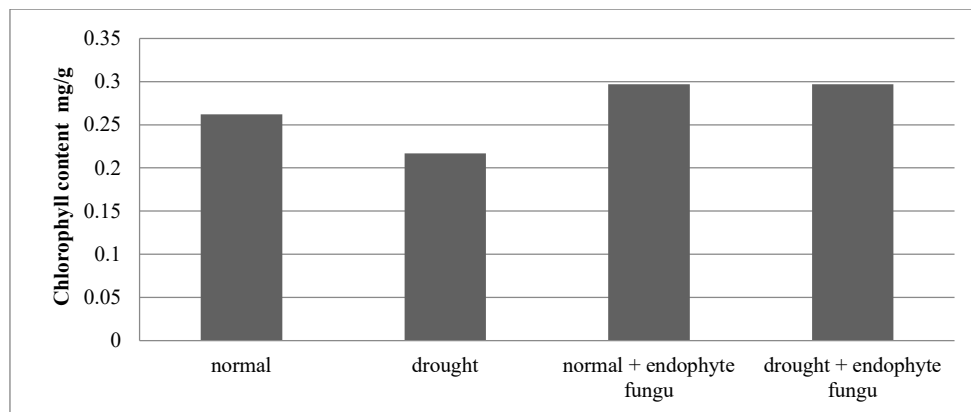


Figure 1. The measurement result using SPAD 502 Plus chlorophyll meter

Result analysis chlorophyll content of paddy leaf showed that the highest value on puddle treatment 5 cm and fungi application that is 0.069 (Figure 2). Chlorophyll on paddy leaves treated by drought (puddle 1 cm height) showed that the value was low that was 0.058. Treatment of endophytic fungi application increased chlorophyll content of paddy leaf both in drought treatment (puddle 1 cm height) as well as control (puddle 5 cm height). This suggests that paddy plants treated with endophytic fungi were more resistant to drought treatment. According to [8]; [3] states that the genotypes of wheat and corn resistant to oxidative stress have higher chlorophyll content than sensitive genotypes. Similarly also stated by [22]; [23], drought condition in Brazilian green dwarf coconut (*Cocos nucifera L. nana*) resulting in decreased leaf chlorophyll concentration per unit leaf area. Effect of drought stress on plant chlorophyll content depends on plant genotype and environmental conditions, some varieties reduced chlorophyll content and some varieties increased [24].

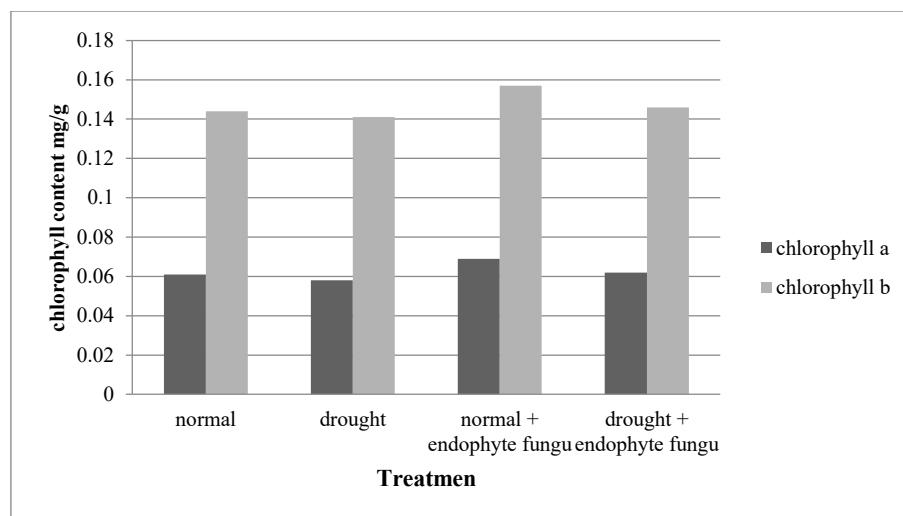


Figure 2. The content of chlorophyll a and b of paddy leaf on drought treatment and endophytic fungi application

Chlorophyll-b content showed that higher value than chlorophyll-a content in all treatments. Value of chlorophyll-b content on paddy leaf is lowest on drought treatment that is 0.141 compared with other treatments. According to [3] synthesis of chlorophyll is very important, after heavy rains total of chlorophyll increased, but in dry time its value decreased. Water stress condition also reduced chlorophyll content compared to well irrigated plants. While in low water stress chlorophyll-a, chlorophyll-b and total chlorophyll content increased by increasing stress pressure [25]. Chlorophyll-a and chlorophyll-b attributes for accumulation of solutes in cell sap through passive accumulation resulting from reduced cell size (Morgan, 1984); [26] which significantly did osmotic adjustment. [13] were reported that endophytic-infected plants were significantly higher chlorophyll content than non-inoculated endophytic fungi in stressful conditions. *Trichoderma harzianum* endophytic fungi inoculated in tomatoe increasing root, shoots growth and chlorophyll pigments compared with control treatment uninoculated of endophytic fungi, in addition plant resistant in drought stress condition [27].

Amount of chlorophyll content in plant will decrease in drought stress condition, endophytic fungi application affect to plant growth especially roots growth better for water absorb and water retention than without endophytic fungi application. Endophytic fungi significantly increased amount of chlorophyll in drought treatment as well as in control treatment. Refer to [23] showed that amount of chlorophyll a and chlorophyll b content and total chlorophyll in leaf was increased under drought stress condition. [28]. There was a symbiotic relationship between endophytic fungi and plant growth, endophytic fungi produce secondary metabolites and have a positive effect on plant growth under field conditions, they were indicated that *Penicillium citrinum* endophytic fungi produce secondary metabolites that increase in physiological characteristics such as chlorophyll content, especially chlorophyll b content, carotenoid content, photosynthesis rate and tolerance to under high-light and drought condition.

4. Conclusion

Paddy leaf chlorophyll concentration decreased during drought condition. Decrease of total chlorophyll content showed that response of paddy plants to drought treatment. Endophytic fungi application increased chlorophyll-a, chlorophyll-b concentration, total chlorophyll of paddy leaf on drought stress condition.

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