**COVER PAGE**

1. **Physiological Character of Two Types of Moringa Plants (*Moringa oleifera* Lamk) at Different Planting Sites in Madura**

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1. **Acknowledgement**

The author is grateful to the Postgraduate Domestic Education Scholarship (BPPDN) from the Ministry of Research, Technology and Higher Education for financial support this study.

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**PHYSIOLOGICAL CHARACTER OF TWO TYPES MORINGA PLANTS (*Moringa oleifera* Lamk) AT DIFFERENT PLANTING SITES IN MADURA**

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**ABSTRACT**

Moringa plants grow in almost all areas of Madura Island with semi-intensive planting at the moor, rice fields, or home yards. Sumenep Regency is one of the districts in Madura Islan, which is categorized as a dry area based on the Oldeman climate classification. Moringa, which grows in Sumenep area based on the color of leaf stalks are categorized as green and red moringa. The study aimed to examine the differences in physiological characteristics of two types of moringa plants at different planting sites in Madura. The study was arranged in Split-Plot design with planting site as main plot consisting of Bluto with E5 climate type and Guluk-guluk with D3 climate type. Meanwhile, the subplot was the type of moringa consisting of moringa with green and red leaf stalks. The results showed that the different planting sites represented the differences in environmental elements which influenced the physiological characteristics of moringa plants. Based on the observation in September 2016, Moringa plants with green stalks planted in Guluk-guluk had high ANR content. The stomatal opening on moringa leaves with green stalks was larger than that on moringa plants with red stalks, whereas ANR content in green-stalked moringa was lower than in the red-stalked ones. Meanwhile, in February 2017, the red-stalked moringa plants planted at Bluto had the highest transpiration rate and proline content compared to the others.

**Keywords:** *Moringa oleifera*, type, site, physiology

**INTRODUCTION**

Moringa (*Moringa oleifera* Lamk) plants tolerant to various environmental conditions, thus easy to grow in extreme environmental condition. Moringa is resistant to long dry seasons, and it grows well in areas with annual rainfall ranging from 250 to 1500 mm (Chumark *et al*., 2008). Madura Island is included in the category of tropical and dry regions in Indonesia. Sumenep Regency is one of the districts in Madura Island which belongs to the category of dry regions based on the Oldeman climate classification meaning that it has D and E climate type as Bluto District has E5 climate type and Guluk-guluk District has D3 climate type (Sumenep District, 2014 )

Moringa plants grow in almost all areas of Madura Island with semi-intensive planting at the moor, paddy fields, and home yards. Moringa that grows the Sumenep area consists of 2 types based on the color of the leaf stalks, i.e., green-stalked and red-stalked moringa (Barselia *et al*., 2014). Moringa cultivation in several planting sites was reported to vary geographically or agro-ecologically in increasing nutrient composition in various organs of moringa plants. Khaerana *et al.* (2008) stated that plants experiencing drought stress tried to make physiological changes such as maintaining turgor pressure or osmotic adjustment as a form of adaptation. Plants have an adaptive mechanism to face biotic and abiotic stresses. This includes the mechanism of photosynthesis, osmoregulation, and antioxidant enzymes (Liu *et al*., 2007). Water shortages result in abnormal physiological and morphological processes causing the plant growth to be stalled or stopped. Wasonowati *et al.* (2019) explained that, in this study, the growth and yield of moringa trees were higher in Guluk-guluk compared to those in Bluto. Observations were performed in September 2016, during the peak of the dry season, and February 2017, during the peak rainy season. Chlorophyll a, b, and total contents were higher in moringa planted at Guluk-guluk, while quercetin content was higher in moringa planted at Bluto. There was no difference in growth, yield, and quality between the two types of moringa plants. Moringa planted at Bluto had better plant height growth, lower yields but higher quality than moringa planted at Guluk-guluk. This because Bluto has more fertile soil and the climate is more suitable for the growth of moringa plants. So far, there has not been much information about the physiological and biochemical properties of the green-stalked and red-stalked moringa plants planted at different growing environmental conditions in Madura. Therefore, this study was conducted to examine the physiological characteristics of different types of moringa plants at planting sites with different environmental conditions.

**MATERIALS AND METHOD**

The research was conducted in two locations, i.e., Bluto and Guluk-guluk Subdistrict, Sumenep Regency, Madura, East Java. Physiological character analysis was carried out at the Laboratory of Agroecotechnology Study Program, Faculty of Agriculture, Universitas Trunojoyo, Madura. Observations were performed in September 2016 and February 2017.

The experiment was arranged in Split Plots Design. The main plot is the planting sites consisting of Bluto (E5 climate type, semi-intensive planting, planted moor and yards, smaller diameter) and Guluk-guluk (D3 climate type, non-intensive planting, planted in the moor and home yards, larger diameter), while the subplot is the type of moringa plants consisting of green-stalked and red-stalked moringa plants. Of the two factors, four treatment combinations were obtained for each plot replicated five times so that there were ten experimental plots with a size per plot of about 200 m2.

**Physiological characteristics of moringa plants**

**Observation on transpiration rate and period using cobalt chloride method.** Observations were made twice, i.e., in September 2016, during the dry season, and February 2017, during the rainy season. Transpiration rate was measured by using the time needed to change the cobalt chloride paper from blue to pink (Akunda and Kumar, 1981). Dry cobalt chloride paper will turn pink when exposed to moisture, including those from transpiration. The faster the transpiration rate, the shorter the time needed to change color. The measurement was carried out between 11 AM – 2 PM on the 2nd to 4th leaves which had opened fully and were exposed to direct sunlight. The paper was placed under 1-mm-thick hard transparent plastic with a size of 2 x 2 cm on the bottom surface of the leaf, pinned with a paper clip. The number of samples for each plant was three samples. The time taken to change the color of cobalt chloride paper from blue to pink was the same as the standard.

**Observation on stomata.** Observations were made twice, i.e., in September 2016, during the dry season, and February 2017, during the rainy season. The stomata components observed in this study included the stomatal density and width of the stomata openings. Observations were made on the bottom surface of the fully opened leaves. The stomata printing was attached to the object glass and observed using a microscope. Several stomata and the width of the stomata opening were observed with ocular equipped with optics-lab using 100x and magnification, respectively (Rahayuningsih, 2017).

**Proline content**. Analysis of the proline content was carried out twice, i.e., in September 2016, during the dry season, and February 2017, during the rainy season. Proline content was determined by the method developed by Bates *et al.* (1973). The leaves used were the leaves of the top of the canopy that had been inflated perfectly as many as 0.5 g. Leaves were pounded with a mortar and put in a solution of 3% sulfosalicylic acid as many as 10 ml then filtered with Whatman paper no. 2. Two ml of the filtrate was reacted with 2 ml of glacial acetic acid in a test tube at 100oC for 1 hour then put in beaker glass containing ice. The mixture was extracted with 4 ml of toluene then shaken out with a stirrer for 15-20 seconds. Red toluene containing proline at the top layer was sucked up with pipettes, and solution absorbance was read with a SHIMADZU 160A spectrophotometer at a wavelength of 520 nm.

Proline content = (64.3649 x absorbance + (- 5.2987)) x 0.347

= µ mol proline/gram

**Relative moisture content (RMC)**. The relative moisture content was observed twice, i.e. in September 2016, during the dry season, and February 2017, during the rainy season. RMC was measured by weighing the fresh weight of 5 compound leaf samples from each plant. After that, the samples were immediately immersed in distilled water for 24 hours to obtain turgid weight. The samples then were dried in an oven to get dry weight. Relative moisture content was calculated by the following formula (Islami and Utomo, 1995):

RMC = fresh weight - dry weight x 100%

turgid weight – dry weight

**Data analysis**

Observation data obtained were analyzed using analysis of variance by the split-plot design at 5% error level. If the results of the analysis of various treatments show a significant difference, the differences between treatments were determined using Duncan Multiple Range Test at 5% error level using the SAS 9.1 program.

**RESULTS AND DISCUSSION**

**The condition of research location**

The research was conducted in two sub-districts, i.e., Bluto and Guluk-guluk Subdistrict, Sumenep Regency which is a region that has a population of two types of moringa plants, namely moringa with green and red leaf stalks. The results of soil analysis in both locations showed that the actual water content in Bluto was 13 %, while in Guluk-guluk it was 18 %. Meanwhile, the pH in both locations was neutral. The N content in both locations was low, the P and K content in Bluto was in the moderate category and Guluk-guluk was in a low category. The micro climate observed included an average air temperature of 340C, air humidity of 61.94-63.39% (Bluto) and 57.74-59.50% (Guluk-guluk), and soil temperature of 30.76-310C (Bluto) and 29.61-30.020C (Guluk-guluk). From these two locations, Bluto is drier, but the soil fertility rate is slightly higher than that in Guluk-guluk. Meanwhile, the micro climate in both locations is almost the same.

**Physiological activities of moringa plants**

**Stomata**. Data in Table 1 show that there is no interaction effect between the planting site and the type of moringa on the stomatal density and opening during observations in September 2016 and February 2017. According to observations in September 2016, during the dry season peak, moringa plants in Bluto had lower stomatal density than in Guluk- guluk. Meanwhile, there was no significant difference between the types of moringa on the stomatal density. The width of the stomata opening of moringa plants in Bluto was not significantly different from those in Guluk-guluk, but there was a significant difference observed between the types of moringa. According to observations in February 2017, during the peak of the rainy season, there was no significant difference in the stomatal density and opening of moringa plants either between the types or the planting sites.

The stomatal density of moringa plants in Guluk-guluk was higher than that of plants in Bluto since the actual moisture content in Guluk-guluk was higher than in Bluto. Stomatal density is an indicator of the number of stomata found in a particular leaf area. The high stomatal density indicates that the number of stomata able to carry out transpiration is high. The width of the green-stalked moringa stomata opening was wider than that of the red ones. This is presumably because the green-stalked moringa plant is more resistant to drought than the red one. Stomata is an important part of plants functioning as the entrance and exit of CO2 and water that will be used in the process of photosynthesis and also as a control for transpiration rate through the mechanism of stomata opening and closure (Kramer and Boyer, 1995). The amount of water that can be transmitted by plants through the stomata is influenced by the stomatal density and the width of the stomata opening.

**Table 1**. Stomatal density and opening of different types of moringa plants planted at

different planting sites

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | Observation time | | | | |
| September 2016 | | February 2017 | | |
| Treatment | Stomatal density  (mm2)-1 | The width of stomata opening  (µm) | Stomatal density  (mm2)-1 | The width of stomata opening  (µm) | |
| Planting site |  |  |  | |  |
| Bluto | 191.01 b | 2.77 a | 252.87 a | | 4.18 a |
| Guluk-guluk | 229.41 a | 2.89 a | 257.66 a | | 4.32 a |
| Stalk color |  |  |  | |  |
| Green | 210.91 p | 3.04 p | 269.34 p | | 4.19 p |
| Red | 209.52 p | 2.61 q | 241.18 p | | 4.31 p |
| CV (%) | 15.23 | 11.71 | 15.68 | | 12.23 |
|  | (-) | (-) | (-) | | (-) |

Remarks: means followed by the same letters in the same column are not significantly different according to Duncan’s multiple range test at 5%. (-) : no interaction between treatment

**Photosynthesis rate measured with Bromthymol Blue (BTB).** Data in Table 2 show that there is no interaction effect between the planting sites and the type of moringa plants on the photosynthesis rate measured with the BTB method in September 2016, during the peak of the dry season, and in February 2017, during the peak rainy season.

**Table 2.** Photosynthesis rate of different types of moringa plants planted in Bluto

and Guluk-guluk

|  |  |  |
| --- | --- | --- |
| Treatment | Photosynthesis rate (mgCO2dm-2h-1) | |
| Observation time | |
| September 2016 | February 2017 |
| Planting sites |  |  |
| Bluto | 30.22 a | 2.169 a |
| Guluk-guluk | 41.36 a | 3.978 a |
| Stalk color |  |  |
| Green | 42.96 p | 3.412 p |
| Red | 28.63 p | 2.735 p |
| CV (%) | 78.08 | 80.85 |
|  | (-) | (-) |

Remarks: means followed by the same letters in the same column are not significantly different according to Duncan’s multiple range test at 5%. (-) : no interaction between treatment

Observations in September 2016 and February 2017 showed that the planting sites and types of moringa did not affect the rate of photosynthesis. Photosynthesis rate is related to chlorophyll content. Photosynthesis is a metabolic process in which plants synthesize organic compounds from organic raw materials in the presence of sunlight. Photosynthesis in plants is influenced by sunlight which includes radiation and the intensity of sunlight.

**Period and rate of transpiration measured with cobalt chloride.** Data in Table 3 show that there was no interaction effect between planting sites and moringa type on the period and rate of transpiration measured with cobalt chloride paper in September 2016 and there was interaction effect observed in February 2017. In September 2016, the period and rate of transpiration of moringa left grown in Bluto were not different from in Guluk-guluk. Likewise, there was no significant difference on the period and rate of transpiration between the types moringa. On the contrary, observed in February 2017, the period and rate of transpiration of red-stalked moringa leave grown in Bluto had a higher value than those of the green-stalked ones. Meanwhile, the period and rate of transpiration of green-stalked moringa leaves grown in Guluk-guluk had a lower value than those of the red-stalked

This is because the environmental condition in Bluto is drier and the red-stalked moringa is less resistant to drought conditions. Thus the plants respond to water shortages by reducing the transpiration rate to save water. Lack of water in the leaves will cause plant cells to lose turgor. The mechanism that can slow down the rate of transpiration or reduce the impact of the loss of water is by closing the stomata and reducing the surface area of the leaves by rolling leaves (Fischer and Fukai 2003). Limited water conditions with lower transpiration rate or equal to the rate of water absorption by the roots resulted in uninhibited plant growth. The transpiration rate is controlled by the opening of the stomata. Under conditions of water shortage, the stomata will close, and gas exchange decreases. The ability to suppress water loss through transpiration is one of the important factors in the tolerance of plants to lack of water.

**Table 3.** Period and rate of transpiration of different types of moringa plants planted at

different sites

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Observation time | Variable |  | Type of moringa plants | | Mean |
|  |  | Planting site | Green-stalked | Red-stalked |  |
| September | Period of | Bluto | 177.80 | 180.60 | 179.20 a |
| 2016 | transpiration | Guluk-guluk | 162.00 | 148.40 | 155.20 a |
| Dry season | (second.cm-2) | Mean | 169.90 p | 164.50 p |  |
|  |  | CV (%) | 19.33 |  | ( - ) |
|  | Rate of | Bluto | 20.50 | 20.38 | 20.44 a |
|  | transpiration | Guluk-guluk | 24.00 | 26.41 | 25.21 a |
|  | (g.dm-2second-1) | Mean | 36.81 p | 36.82 p |  |
|  |  | CV (%) | 20.33 |  | ( - ) |
| February | Period of | Bluto | 93.80 bc | 120.60 a | 107.20 |
| 2017 | transpiration | Guluk-guluk | 108.00 ab | 83.60 c | 95.80 |
| Rainy season | (second.cm-2) | Mean | 100.90 | 102.10 |  |
|  |  | CV (%) | 15.48 |  | ( + ) |
|  | Rate of | Bluto | 38.97 ab | 30.34 c | 34.66 |
|  | transpiration | Guluk-guluk | 34.66 bc | 43.30 a | 38.98 |
|  | (g.dm-2second-1) | Mean | 36.81 | 36.82 |  |
|  |  | CV (%) | 11.62 |  | (+) |

Remarks: means followed by the same letters are not significantly different according to Duncan’s multiple range test at 5%. (+) : there is an interaction effect between treatment. (-) : no interaction between treatment

**Proline.** Data in Table 4 show that there was no interaction effect observed in September 2016, whereas there was an interaction effect between the planting sites and the types of moringa on the proline content of moringa leaves observed in February 2017. In September 2016, the proline content of red-stalked moringa leaves planted either in Bluto or Guluk-guluk was not significantly different from that of the green-stalked ones. Meanwhile, in February 2017, red-stalked moringa leaves planted in Bluto had the highest proline content. Conversely, red-stalked moringa leaves planted in Guluk-guluk had the lowest proline content.

This is because, in September 2016, the planting sites experienced drought so that the proline content tended to be higher than in February 2017. Plants experiencing drought stress will accumulate compounds that play a role in osmotic adjustment. Proline is a free amino acid that is formed and accumulated in the leaves in greater number if the plant experiences drought stress. Proline also plays an important role in avoiding dehydration by increasing cell solute levels and also maintaining high water levels. At the same time, proline accumulation plays a role in tolerating dehydration by protecting proteins and membrane structures (Verslues *et al.,* 2006).

Proline is a metabolite that can be used as an indicator of drought resistance (Lakitan, 1993). Plants that are in drought conditions will inhibit proline oxidation so that the concentration in plants increases. In line with the opinion of Maestri *et al.* (1995), who stated that the proline content in the roots would increase if the plant experiences drought stress. The increased amount of proline is considered to be an indication of tolerance to drought stress becaus of proline functions as conservator compound of N and osmoregulator or as a protector of certain enzymes. Cells, tissues, or plants over proline production are considered to have better drought tolerance properties (Hamim *et al.,* 1996).

**Table 4**. Proline content of moringa leaves of different moringa types planted at different planting sites

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Proline (µ mol proline.gram-1) | | | Mean |
| Observation time | Planting sites | µ mol proline/g | |  |
|  |  | Green | Red |  |
| September 2016 (Dry season) | Bluto | 35.87 | 34.06 | 35.14 a |
|  | Guluk-guluk | 42.57 | 31.69 | 37.27 a |
|  | Mean | 39.36 p | 33.05 p |  |
|  | CV (%) | 17.84 |  | (-) |
| February 2017 (Rainy season) | Bluto | 5.71 ab | 8.12 a | 6.24 |
|  | Guluk-guluk | 6.76 ab | 4.56 b | 5.66 |
|  | Mean | 6.24 | 6.34 |  |
|  | CV (%) | 27.66 |  | (+) |

Remarks: means followed by the same letters are not significantly different according to Duncan’s multiple range test at 5%. (+) : there is interaction effect between treatment. (-) : no interaction between treatment

**Relative moisture content.** Data in Table 5 show that there was no interaction effect between the planting sites and the types of moringa plants on the relative moisture content of moringa leaves observed in September 2016 and February 2017. Single factor either the planting sites or the types of moringa did not give significant effect on the relative moisture content of moringa leaves.

**Table 5**. The relative moisture content of different types of moringa leaves planted at different planting sites

|  |  |  |
| --- | --- | --- |
| Treatment | Relative moisture content (%) | |
| Observation time | |
| September 2016 (Kemarau) | Pebruari 2017 (Penghujan) |
| Planting sites |  |  |
| Bluto | 48 a | 54 a |
| Guluk-guluk | 49 a | 39 a |
| Stalk color |  |  |
| Green | 49 p | 49 p |
| Red | 48 p | 44 p |
| CV (%) | 14.01 | 18.09 |
|  | (-) | (-) |

Remarks: means followed by the same letters in the same column are not significantly different according to Duncan’s multiple range test at 5%. (-) : no interaction between treatment

High relative moisture content is a mechanism of plant resistance to drought and the high relative water content is the result of excessive osmotic regulation or a reduction in the elasticity of cell wall tissue. Khalili *et al*. (2011) reported that water content was relatively influenced by season and irrigation, so that drought stress can significantly reduce the value of moisture content.

**Nitrate Reductase Activity (NRA).** Data in Table 6 show that there was an interaction effect between the planting sites and the types of moringa on the NRA content of moringa leaves observed in September 2016, and there was no interaction observed in February 2017. In September 2016, green-stalked moringa leaves planted in Guluk-guluk had the highest NRA content compared to other treatments. Meanwhile, the other treatments had no significant effects on NRA content. In February 2017, moringa leaves planted either in Bluto and Guluk-guluk had NRA content values which were not significantly different. Whereas, the content of NRA of green-stalked moringa leaves was lower than that of red-stalked ones.

**Table 6**. NRA content of different types of moringa leaves planted at different sites

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  |  | Nitrate Reductase Activity (µmol NO2-g-1h-1) | | |
| Observation time | Planting sites | Moringa types | | Mean |
|  |  | Green | Red |  |
| September 2016 | Bluto | 1.37 b | 1.44 b | 1.40 |
| Dry season | Guluk-guluk | 2.12 a | 1.51 b | 1.82 |
|  | Mean | 1.75 | 1.47 |  |
|  | CV (%) | 16.77 |  | (+) |
| February 2017 | Bluto | 0.92 | 2.05 | 1.88 a |
| Rainy season | Guluk-guluk | 1.09 | 2.49 | 1.79 a |
|  | Mean | 1.01 q | 2.27 p |  |
|  | CV (%) | 18.09 |  | (-) |

Remarks: means followed by the same letters are not significantly different according to Duncan’s multiple range test at 5%. (+): there is an interaction effect between treatment. (-) : no interaction between treatment

This is because the NRA content decreases in plants experiencing water stress. The reduction in NRA content can be related to a decrease in nitrate translocation in the xylem. The decrease of NRA in plants occurs during drought. Low NRA content causes a small amount of nitrite to be converted into ammonium. If the ammonium produced is small, it will automatically decrease the levels of amino acids formed. The decrease affects the level of protein produced (Salisbury, 1992) so that it also affects the production, which can ultimately reduce crop yields. Brandao and Sodek (2009) stated that reduced nutrient uptake of nitrogen from the soil caused a decrease in the movement of nitrate to leaves, which resulted in low nitrate reductase activity.

**CONCLUSION**

Red-stalked moringa plants planted in Guluk-guluk exhibited an increase in the content of chlorophyll a, b and total chlorophyll, while green-stalked moringa plants planted in Guluk-guluk showed higher Nitrate Reductase Activity (in the dry season). Red-stalked moringa plants planted in Bluto had the longest transpiration period, higher proline content and higher quercetin content (in the rainy season).

Moringa plants planted in Guluk-guluk had higher stomatal density than those planted in Bluto. The green-stalked moringa plants performed wider stomata opening compared to the red-stalked ones, while NRA content on green-stalked moringa plants was lower than that on the red-stalked ones.

**Acknowledgement**

The author is grateful to the Postgraduate Domestic Education Scholarship (BPPDN) from the Ministry of Research, Technology and Higher Education for financial support this study.

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