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Morphophysiology and the yield of two types of moringa (*Moringa oleifera* Lamk) cultivated in two different regions in Madura

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Abstract. Moringa (*Moringa oleifera* Lamk), green and red petiole, is tolerant plant to drought and widely grown in Madura Island. For that, this study aims to identify these Moringa morphophysiology and its yield. The Moringa plant were cultivated in two locations namely Bluto, a village with E5 climate type (wet month: 0-2, dry month: 10-12), and Guluk-guluk, a village with D3 climate type (wet month: 3-4, dry month: 4-6). The results show that higher humidity and air temperature were found in Bluto compared to Guluk-guluk. Such condition affected to the yield and morphophysiological character. The morphophysiology and yield of both Moringa plants in Bluto were lower than in Guluk-guluk. In September during dry season peak, and in February during rainy season peak, the morphophysiological characteristics and content of chlorophyll a, b, and total were lower in Bluto than in Guluk-guluk. Meanwhile, the quercetin content was higher in Bluto. Green petiole Moringa showed no different morphology and yield, but significantly different in physiology character than red petiole Moringa. Having more fertile of soil and more suitable climate with semi-intensive cultivation resulted higher and better plant quality in Bluto area though resulting in lower yields when compared to Moringa in Guluk-guluk.

1. Introduction

Moringa (*Moringa oleifera* Lamk) is a plant that having high tolerant to various environmental conditions. Therefore, this plant was easy to grow in extreme area either in high environmental temperatures or under the shade. Moringa reported to be tolerant to long dry seasons and grows well in areas which having annual rainfall ranged from 250 to 1,500 mm [1]. Moringa plants in Indonesia are found grow naturally in altitudes up to 1,000 m above sea level and able to grow well in hillsides. Commonly this plant is found to grow in grasslands or in river basins. As a non-cultivated plant, Moringa plant is known for its tolerant to drought and disease. To grow them well, Moringa plants require several conditions such as tropical to sub-tropical climates, altitudes of 0–2,000 m above sea level, temperatures of 25–35°C, rainfall of 250–2,000 mm per year, good irrigation when rainfall is less than 800 mm, sandy silt or sandy clay, and pH 5–9 [2].

Moringa plant is widely found and common in Indonesia, especially in rural areas of Madura, Java, Bali, West Nusa Tenggara, East Nusa Tenggara, Sulawesi, and in many other areas. It grows in almost



all areas in Madura Island with semi-intensive planting method in moors, rice fields, and home yards. Madura Island belongs to the category of tropical and dry regions in Indonesia. Sumenep Regency is one of the districts in Madura Island with the category of a dry region based on Oldeman climate classification. It has a D climate type such as Guluk-guluk district with D3 climate type and an E climate type such as Bluto with E5 climate type. Similar to the other regions in Indonesia, in Sumenep Regency the rainy season is within October-March, and the dry season is within April-September with the average rainfall of 1,479 mm. Moringa is a common plant for the community and is suitable to the land condition and agro-climate in Sumenep and generates the economy [3]. Its population in Sumenep Regency, especially in Bluto Subdistrict, consisted of around 12,800 trees and semi-intensively cultivated for long peppers (*Piper retrofractum* Vahl) to ascend and Moringa flour raw material. Meanwhile, in Guluk-guluk Subdistrict, it is planted in moorland and utilized only for land boundaries and long peppers (*Piper retrofractum* Vahl) to ascend [4]. Moringa plants are grown in Sumenep region which consist of 2 types of plants based on the color of the petiole, namely the green moringa and red moringa [5]. Until recently, there is no information on the morphophysiological character and yield of different types of moringa plants in the planting locations with such different environmental conditions. Therefore, it is essential to identify the growth of the plant based on its physiological character and yield from locations with different growing environmental conditions as well as the types of moringa.

2. Methods

2.1. Experimental design

The study was conducted in Bluto and Guluk-guluk Subdistricts, Sumenep Regency, Madura, East Java from May 2016 to June 2017. The experiment was arranged in the split-plot design which were consisted of two factors: the planting location and the type of Moringa plant. The main plot was the planting locations: Bluto and Guluk-guluk. Meanwhile, the sub-plot was the type of Moringa leaf petiole: the green and red. Five plots were used as replication. The plot size of about 200 m².

2.2. Observation of physical and chemical soil

Physical properties of soil. Soil moisture: calculation by the gravimetric method at a depth of 30 cm. Soil texture: namely the level of soil fineness due to the composition of the content of sand, dust, and clay. Chemical properties of soil. pH (H₂O): measured by pH meter. C-organic: measured by the Walkey and Black method. N total (%): measured by the Kjeldhal method. CEC (1 mol L⁻¹ ammonium acetate) 0.1 N NH₄OAc pH7 with titration. P available: measured by the P-Olsen method. K with extract (1 mol L⁻¹ ammonium acetate) 0.1 N NH₄OAc pH7 with Flame Photometer. CN ratio: measured by comparing C-organic and N-total. Soil organic matter: measured by the Walkey and Black method with titration.

2.3. Observations of microclimate

Light intensity: measurement of sunlight with lux meter is carried out between 10 AM-14 PM at outside the canopy of Moringa plant. Air temperature (°C) was measured with an air thermometer outside the canopy. Soil temperature (°C) was measured by a soil thermometer in a place where plants grow 10 cm deep. Air humidity (%) was measured outside the canopy with hygrometer.

2.4. Moringa plant morphology variables

The morphology variables of Moringa plants were observed every forty days for plant height, stem diameter, and the number of branches. The plant height (m) was measured from the base of the stem above the soil surface to the tip of the plant by using Haga altimeter of a Universal brand. The stem diameter at breast height (cm) was obtained by wrapping the measuring tape to the plants at 1.3 m height with the tape position parallel to all directions to measure the stem perimeter or circumference

(perimeter = $2\pi r$). The number of branches was obtained by calculating the branches formed in the sample plant.

2.5. Plant physiological variables

Analysis of chlorophyll content of the leaf samples was carried out twice in September 2016 during dry season peak and in February 2017 during rainy season peak. This study applied a method developed by Comb (1985) known as Comb's method. Approximately 1 mg of Moringa leaves was pounded using mortar until pulverized, then 20 ml of 80% acetone was added. The mixture was then filtered using Whatman paper No. 1. The absorbent solution was read using SHIMADZU 160A type spectrophotometer at a wavelength of 645 μm and 663 μm . The contents of Chlorophyll a and b were calculated using the following formula:

Content of chlorophyll a = $0.0127 \times A_{663} - 0.00269 \times A_{645}$

Content of chlorophyll b = $0.0229 \times A_{645} - 0.00468 \times A_{663}$

Content of chlorophyll total = Content of chlorophyll a + b

Quercetin leaf analysis was carried out twice in September 2016 during dry season peak and February 2017 during rainy season peak. The quercetin content was calculated using Down method adapted by Arvounet-Grand, Vennat, and Legret [6]. Moringa powder was weighed 10 g and macerated in 20 ml methanol before the extract was concentrated. The standard curve was made by preparing a standard solution of flavonoids in methanol with a concentration of 10, 20, 30, 40 and 50 mgL⁻¹ as well as methanol as a blank solution. Then, the absorbance of each standard solution was measured by using SHIMADZU 160A type spectrophotometer at a wavelength of 415 nm before making the standard curve. The sample measurement was done by weighing 0.25 g of Moringa leaf extract plus 5 ml of AlCl₃ 2%. Then vortex was carried out for 20 seconds. The filtrate was taken and filled into the blank solution, which was 0.25 g extract in 5 ml of methanol in the cuvette. The absorbance of the sample was measured using a spectrophotometer at a wavelength of 415 nm, 10 minutes after the blank solution was measured. Next, the obtained absorbance was put into the standard curve equation. Quercetin = (Absorbance - 0.078) / 0.0184.

2.6. The yield component

Observation of yield component variables consisted of total fresh leaf weight, total dry leaf weight, fresh weight of leaves for the air-dried, air-dried leaf weight, and powder leaves weight. The total fresh leaf weight (g) is the weight of fresh Moringa leaves and petiole from two secondary branches of sample plants. The total dry leaf weight (g) is the weight of Moringa leaves and stems dried in oven at 80°C for 24 hours. The fresh weight of leaves for air-dried (g) is the weight of fresh Moringa leaves without petiole from one secondary branch sample plant. The air-dried leaf weight (g) is the weight of Moringa leaves without petiole dried at 30-35°C room temperature for 3 days. The powder leaves weight (g) is the weight of powder produced from blended air-dried leaves.

2.7. Data analysis

All data were analyzed using the analysis of variance based on the split-plot design at $\alpha = 5\%$, followed with the Duncan's Multiple Range Test (DMRT) The statistical analysis was calculated under the help of SAS 9.1 program.

3. Result and discussion

3.1. Field condition

The study location was in two sub-districts: Bluto and Guluk-guluk Subdistricts. The criterias of these locations were presented in **Table 1**.

Table 1. The criteria for both Moringa planting locations.

Criteria	Bluto	Guluk-guluk
Position	7°7'8"S' and 113°45'57"E	7°7'8"S' and 113°41'57"E
Climate	E5 (Wet Month: 0 – 2), (Dry Month: 10-12)	D3 (Wet Month: 3 – 4), (Dry Month: 4-6)
Height	100 m above the sea level	300 m above the sea level
Cultivation system	semi-intensive	not intensive
Planting location	moor and yard	border side and yard

The results of soil analysis on both locations shows that the actual water contents in Bluto and Guluk-guluk were 0.13 g.g⁻¹ and 0.18 g.g⁻¹, respectively. This indicated that Bluto was drier compared to Guluk-guluk. The soil pH was neutral in both locations. Low N content but with moderate P and K content were observed in Bluto, but N, P, K values were low in Guluk-guluk.

Table 2. The results of soil analysis for Bluto and Guluk-guluk Subdistricts

Code	WC Actual (g.g ⁻¹)	pH H ₂ O	C-org %	N Tot %	CN	OM %	P Olsen mg.kg ⁻¹	K me/100g	CEC
Bluto	0.13	7.34 (N)	1.502(L)	0.19(L)	7.6(L)	2.6(VL)	11.79(L)	0.45(H)	31.16
Guluk-guluk	0.18	7.24(N)	0.704(L)	0.11(L)	6.4(L)	1.2(VL)	8.33(L)	0.19(H)	27.01

Soil samples were analyzed at the Soil Laboratory, Brawijaya University. (L) ; low, (VL) : very low, (H) : high

The microclimate observation along with light intensity average in outside of canopy in Bluto was lower than in Guluk-guluk. The air temperature in Bluto was similar to that in Guluk-guluk. Air humidity in Bluto was higher than in Guluk-guluk. Soil temperatures in Bluto was higher than in Guluk-guluk (**Table 3**).

Table 3. The microclimate for Bluto and Guluk-guluk Districts.

Parameter	Bluto		Average	Guluk-guluk		Average
	Green	Red		Green	Red	
Light intensity (over canopy) (Lux)	33277	36755	35016±1738	38220	44575	41397±3177
Air temperature (°C)	34.52	34.27	34.39±0.13	34.11	34.30	34.20±0.09
Air Humidity (%)	63.39	61.94	62.67±0.73	57.74	59.50	58.62±0.88
Soil temperature (°C)	31.00	30.76	30.88±0.12	29.61	30.02	29.82±0.21

3.2. The moringa plant morphologies

In June 2016 to May 2017, planting location had a significant effect on plant growth while the type of Moringa did not show any significant effect. The plant height was higher in Bluto than in Guluk-guluk, with no significant difference between green and red types. The stem diameter was larger in Guluk-guluk than that of in Bluto, with no significant difference between green and red type of Moringa plant (**Table 4**).

During dry season, October to November 2016, red type of Moringa in Guluk-guluk resulting highest number of secondary branches, which was significantly different from the green type of Moringa in Guluk-guluk and the green and red type planted in Bluto. While during rainy season, January to May 2017, red type of Moringa also showing highest number of secondary branches. Moreover, type of Moringa in Guluk-guluk significantly different than the green and red type planted in Bluto (**Table 5**).

Table 4. Effect of different planting locations and petiole types on the height and stem diameter of Moringa.

Variables	Treatments	2016					2017			
		June	August	Sept	Oct	Nov	Jan	Feb	April	May
Plant Height (m)	Planting location									
	Bluto	4.53 a	4.98 a	5.57 a	5.93 a	6.36 a	6.74 a	7.08 a	7.36 a	7.62 a
	Guluk-guluk	3.36 b	3.60 b	4.05 b	4.37 b	4.73 b	5.13 b	5.41 b	5.64 b	5.88 b
	Moringa type									
	Green	3.98 a	4.41 a	4.89 a	5.25 a	5.66 a	6.03 a	6.38 a	6.68 a	6.89 a
	Red	3.91 a	4.18 a	4.73 a	5.05 a	5.43 a	5.84 a	6.11 a	6.32 a	6.60 a
Stem Diameter (cm)	CV (%)	20.81	20.31	13.12	11.78	9.15	9.24	8.19	7.94	7.68
		(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
	Planting location									
	Bluto	10.70 b	11.37 b	12.13 b	12.57 b	13.04 b	13.69 b	14.31 b	15.02 b	15.75 b
	Guluk-guluk	15.38 a	15.80 a	16.25 a	16.73 a	17.17 a	17.61 a	18.19 a	18.64 a	19.35 a
	Moringa type									
	Green	12.45 a	12.77 a	13.23 a	13.65 a	14.08 a	14.62 a	15.22 a	15.89 a	16.61 a
	Red	13.64 a	14.42 a	15.12 a	15.65 a	16.13 a	16.68 a	17.23 a	17.76 a	18.49 a
	CV (%)	22.01	20.77	20.72	19.51	19.66	20.16	19.47	19.41	18.70
		(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)

The average data with the same letter in the same column per variable was not significantly different from the Duncan test at the 5% level. (-) : no interaction between planting locations and petiole types

Table 5. Effect of different planting locations and petiole types on the secondary branches of Moringa

Treatment	Observation					
	2016			2017		
	October	November	January	February	April	May
Bluto, green	6.40 b	7.20 b	8.00 b	9.00 b	9.90 b	10.90 bc
Bluto, red	5.20 b	6.20 b	7.20 b	8.30 b	9.30 b	10.10 c
Guluk-guluk, green	6.70 b	7.70 ab	8.40 ab	9.30 b	10.60 ab	12.10 ab
Guluk-guluk, red	8.50 a	9.20 a	10.30 a	11.60 a	12.70 a	13.60 a
CV (%)	18.87	14.38	12.51	12.16	11.01	7.66
	(+)	(+)	(+)	(+)	(+)	(+)

The average data with the same letter in the same column was not significantly different from the Duncan test at the 5% level. (+) : interaction between planting locations and petiole types

3.3. Physiological character

In the dry season peak (September 2016), planting location and type of Moringa showed a significant difference in its physiological character. Red type in Guluk-guluk gave highest contents of chlorophyll a, chlorophyll b, and chlorophyll total among Moringa type in Bluto and Guluk-guluk. Type of Moringa did not give any significant differences of the quercetin content. However, the quercetin content was higher in Bluto than that of in Guluk-guluk. In the rainy season peak (February 2017), planting location and type of Moringa did not significant difference in contents of chlorophyll a, chlorophyll b, and chlorophyll total. The quercetin content was higher in Bluto with red type of Moringa than that of in among Moringa type in Bluto and Guluk-guluk (**Table 6**).

3.4. Yield in the form leaves

In June 2017, the planting location give a significant difference in the yield of Moringa plants in the form of leaves. The total fresh weight and total dry weight of leaves differ significantly in Guluk-guluk compared to Bluto, while the type of green Moringa was not significantly different from that of red. The fresh weight of air-dried leaves, air-dried dry weight, and weight of Moringa leaf powder was differ significantly in Guluk-guluk compared to Bluto, while the type of red Moringa was not significantly different from the green type (**Table 7**).

Table 6. Effect of different planting locations and petiole types on the content of chlorophyll a, b, total and quercetin of *Moringa oleifera*

Time	Variable	Planting location	Type of Moringa		Average
			Green	Red	
September 2016 (Dry season)	chlorophyll a (mg.g ⁻¹)	Bluto	0.33 ab	0.25 b	0.29
		Guluk-guluk	0.31 b	0.42 a	0.36
		Average	0.32	0.33	
		CV (%)	20.64		(+)
	chlorophyll b (mg.g ⁻¹)	Bluto	2.22 b	1.74 b	1.98
		Guluk-guluk	2.24 b	3.25 a	2.75
		Average	2.23	2.49	
		CV (%)	25.43		(+)
	chlorophyll total (mg.g ⁻¹)	Bluto	2.55 b	1.99 b	2.27
		Guluk-guluk	2.55 b	3.67 a	3.11
		Average	2.55	2.83	
		CV (%)	24.56		(+)
	Quercetin (mg.L ⁻¹)	Bluto	8.66	11.59	10.13 a
		Guluk-guluk	2.61	4.46	3.53 b
		Average	5.64 p	8.03 p	
		CV (%)	40.42		(-)
February 2017 (Rainy season)	chlorophyll a (mg.g ⁻¹)	Bluto	0.354	0.387	0.37 a
		Guluk-guluk	0.475	0.455	0.47 a
		Average	0.41 p	0.42 p	
		CV (%)	18.83		(-)
	chlorophyll b (mg.g ⁻¹)	Bluto	3.10	3.21	3.16 a
		Guluk-guluk	3.65	4.07	3.86 a
		Average	3.38 p	3.64 p	
		CV (%)	20.50		(-)
	chlorophyll total (mg.g ⁻¹)	Bluto	3.46	3.59	3.53 a
		Guluk-guluk	4.12	4.53	4.33 a
		Average	3.79 p	4.06 p	
		CV (%)	20.20		(-)
	Quercetin (mg.L ⁻¹)	Bluto	0.72 b	2.17 a	1.45
		Guluk-guluk	0.68 b	0.53 b	0.61
		Average	0.70	1.35	
		CV (%)	15.54		(+)

The average data with the same letter in the same column and row per variable was not significantly different from the Duncan test at the 5% level. (+) / (-) :interaction / no interaction between planting locations and petiole types

In this study, the soil analysis (**Table 1**) at the two research locations showed a low level of soil fertility. Soil quality in Bluto and Guluk-guluk were as limiting factors of plant growth due to the low mineral nutrients and organic matter in the soil [7]. Soil fertility were influenced by soil chemical properties, which included pH, C organic, CEC, and soil nutrient content. Soil pH had an important role in maintaining nutrient balance and soil fertility [8]. The best condition for plants is when the soil pH was neutral where almost all nutrients were available to support plants optimal production. The higher the CEC value the higher the soil fertility level will be, due to the number of cations available and the increased its exchange. The altitude position of Guluk-guluk District is higher (± 300 m above sea level) than Bluto District (± 100 m above sea level). The differences in plantation location were followed by difference in environmental elements (air temperature, relative air humidity, etc) during the life of plant (**Table 3**).

Table 7. Effect of different planting locations and petiole types on the total leaf fresh weight, total leaf dry weight, leaf fresh weight, air-dried leaf weight, and powder weight of *Moringa oleifera*

Time	Variable	Planting location	Type of Moringa		Average
			Green	Red	
Juni 2017	Total leaf fresh weight (g. plant ⁻¹)	Bluto	368.9	355.1	362.0 b
		Guluk-guluk	1247.0	1224.0	1235.5 a
		Average	807.9 p	789.5 p	
		CV (%)	10.69		(-)
	Total leaf dry weight (oven dry) (g. plant ⁻¹)	Bluto	75.5	71.6	73.6 b
		Guluk-guluk	231.3	241.1	236.3 a
		Average	153.4 p	156.4 p	
		CV (%)	31.00		(-)
	Leaf fresh weight for air-dried (g. plant ⁻¹)	Bluto	112.5	128.9	120.7 b
		Guluk-guluk	379.6	395.0	387.3 a
		Average	246.0 p	261.9 p	
		CV (%)	30.30		(-)
	Air-dried leaf weight (g. plant ⁻¹)	Bluto	29.44	29.42	29.43 b
		Guluk-guluk	65.42	69.10	67.26 a
		Average	47.43 p	49.26 p	
		CV (%)	35.67		(-)
	Leaf powder weight (g. plant ⁻¹)	Bluto	26.97	26.92	26.94 b
		Guluk-guluk	63.37	67.24	95.57 a
		Average	45.17 p	47.08 p	
		CV (%)	37.35		(-)

The average data with the same letter in the same column and row per variable is not significantly different from the Duncan test at the 5% level. (-) : no interaction between planting locations and petiole types.

Moringa plant in Bluto was high, but the diameter and number of branches were higher in Guluk-guluk. Those character were due to the different of cultivation method applied in Bluto. Moringa plants were semi-intensive cultivation by carrying out routine pruning, which was utilized for Moringa powder raw material and animal feed. On the other hand, pruning was not applied intensively in Guluk-guluk. Pruning has the effect to production and quality of the leaves. The leaf production in Guluk-guluk was higher in number than that of in Bluto due to the greater numbers of secondary branches than in Bluto. Moringa leaves in Bluto either observed in September during dry season peak or in February during the rainy season, showed higher quercetin content in red type Moringa. Chlorophyll a, b, and total during dry season peak for the red petiole type were highest in Guluk-guluk and lowest in Bluto.

In the rainy season peak, the content of chlorophyll a, b, and total in Guluk-guluk was not significantly different than in Bluto as well as red petiole type when compared to the green type. This was due to the environmental conditions of planting field that experienced by drought as one of external factors affected plant growth. Biochemical changes can be in the form of accumulated organic compounds that function to maintain osmolytes balance in the plant. Physiological characters were associated with drought stress resistance including photosynthesis enhance due to higher chlorophyll content [9]. Plants that are resistant to water and heat stress have higher chlorophyll content [10]. Moringa with green leaf petiole showing growth, yield, and quality that were not different to red petiole. In Bluto, Moringa plants showed higher plant, lower yields but higher quality than in Guluk-guluk. This because Bluto had more fertile soil, more suitable climate for Moringa plant growth in addition to the semi-intensive cultivation system where applied in Bluto, such as watering, fertilizing, and pruning system. Pruning the moringa plants branches resulted in lesser amount of leaves than in Guluk-guluk. Based on the previous results [11], Moringa gives a positive response to nitrogen application and better harvest frequency for leaf growth and quality. All growth characteristics and

nutrient composition of leaves are affected by increased nitrogen and harvest frequency. Based on these studies, it can be recommended that to obtain better growth and quality of Moringa leaves, nitrogen fertilizer and harvest frequency should be applied 4 weeks after the initial harvest at 12 MST. According to the previous study [5], Moringa type that grows in the Sumenep region is based on the color of the leaf petiole, namely green moringa and red moringa. Such classification is based on the branch color and chemical content. Green moringa is preferred for flour industrial scale due to its growth better, wider leaf, higher nutrient content and higher growth power compared to the red type.

4. Conclusions

Based on this study it is concluded that difference of climate type, altitude, and cultivation method affected the morphophysiological character and yield of Moringa plants. Moreover, Green petiole Moringa showed no different morphology and yield but significantly different in physiology character and quality compared to red petiole. Moringa plants in Bluto shows higher growth, lower leaf yields but higher quality than in Guluk-guluk due to Bluto area has more fertile soil and suitable climate, accompanied with application of semi-intensive cultivation methods.

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