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The Growth of *Moringa* Seedling Originated from Various Sizes of Stem Cutting

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Abstract. The ddevelopment of an efficient technique for vegetative propagation would be a benefit for the nursery industry for the production of some clones with desirable characters. This study was conducted to develop a vegetative propagation protocol of *Moringa oleifera* using some different sizes of cutting started from April to July 2017. The factorial experiment was used, in which the first factor was stem cutting length (25cm, 50 cm, and 75 cm), and the second one was stem cutting diameter (3.0-4.0 cm, 4.1-5.0 cm, and 5.1-6.0 cm).This experiment was designed using Completely Randomized Design with five replications, and each replication contained10 seedlings. The results show that the seedling growth was affected by stem cut diameter and length alone during three months nursery period. To produce more valuable seedlings, the vegetative propagation using stem cutting ranging should use 50-75 cm in length and/or 3.0-6.0 cm in diameter.

Key words: cutting diameter, cutting length, propagation, survival, transplanting

1 Introduction

Moringa is a multi-potential plant because all parts of the plant, such leaves, seed, pod, flower, bark, and root are useful. The leaves can be used as high nutrition food to combat malnutrition [1], a herbal medicine [2]; [3], an antibacterial and fungal activities [4], an animal feedstock [5]. Moreover, its seed serves as a natural coagulant for turbid water [6]; [7]. It is also used as a bio-diesel production to provide the energy demands of the industry without competed the agricultural field product [8]; [9]; [10]. In Indonesia, especially in West Nusa Tenggara, the plant presents a traditionally important food commodity, as the leaves and fruits (tender pod) are locally used as a vegetable. It is generally planted in a house yard and hedge, but without using a good cultivation technique.

Nursery techniques or plant propagation to produce a high quality seedling is an important aspect to develop a perennial crop, including Moringa, since the tree becomes a famous plant as a source of nutritious and healthy food and other usages. As [11] states that germination and seedling resistance are the ability of a plant to continue to live, and it is a critical stage in the plant life cycle on a dry ecosystem. Seedling preparation also plays an important role in nursery stock production.

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Between generative and vegetative propagation methods, stem cutting is recommended for quicker establishment and production of many species on dry land [12]. Moringa, a heterozygous plant due to highly free cross-pollination [13] is suitable to propagate using stem cuttings because it will obtain a young plant that has identical characters to the mother plant and also uniformity in yield quantity and quality [14]. Therefore, preparing good seedlings with vegetative propagation techniques especially with efficient and effective cuttings is an important factor for the success of Moringa planting. A number of studies have reported that many shrub species can be propagated by leafy stem cutting easily [15], but not for Moringa. As [16] stated that the establishment and growth rate of stem cutting depend on age variation, stem position, and diameter. The cutting size, as physical factor, are the matter of concern as they affect the ability of cuttings to form root [17]. They are different for each plant [18]; [19]. The shorter the cuttings or the smaller the stem diameter, the lower the food reserve will be. On the technical aspect, however, the use of longer cutting requires more plant material, so then, the use of shorter cuttings would certainly be more profitable. Then [20] added that the behaviour of stem cuttings varies with age, genotypes, and physiological status of mother plant. As [21] reported that, the growth of Jatropha seedlings from cutting stems varied, depending on the size difference, the length or diameter of the stem cuttings material used. To get Jatropha seedling with a good adaptability, the propagation of plants vegetatively cold be done by using long stem cuttings ranging from 20-30 cm with a diameter of 2.5-3.0 cm or by stem cuttings diameter of 2.0-2.4 cm or 2.5-2.9 cm in length of 30 cm. And [22] reported that vegetative propagation of Moringa with higher stem cut diameter leads to a higher dry matter. This article discuss about the effect of stem cutting length and diameter on the growth of Moringa seedling.

2 Method

2.1. Experiment side condition and plant material

The experimental research was undertaken under a nursery shade condition located in Mataram, West Nusa Tenggara, Indonesia, at 8°34'47.19"S 116°05'47,91"E, and altitude of 16 meters above sea level. The experiment was conducted from May to July 2017, with the stem cutting of local Moringa accession, having the length (25 cm, 50 cm, and 75 cm) and the diameter (3–4 cm, 4,1–5 cm, and 5,1–6 cm). The stem cutting were rinsed with a fungicide then allowed to win dry. These stem cuttings are then planted inside black polyethylene bags (25 cm x 35 cm) filled with the mixture media of soil-manure-rice husk (1:1:1 v/v).

2.2. Seedling maintenance

The seedling were kept in a simple shade house. Shading was done during the first month of seedling growth, and thereafter, no shading was applied. Seedling were manually watered daily and at four weeks after planting, the seedling were given fertilizer of 10 g Phonska.polybag^{-1}.

2.3. Experiment design and statistical analysis

The stem cuttings were arranged in a Completely Randomized Design with two factorials trial with five replications. Each replication consisted of five serial unit cuttings (for destructive observation) resulting in a total of 225 experimental units. The experiment was run for about three months, and the parameters observed were shoot length and number, number of leaves. The shoot diameter were collected every two weeks, and the number of root, root length, shoot fresh and dry weight, and root fresh and dry weight were collected monthly. The cuttings were monitored daily for flushing (bud burst). The number of days to flushing for each treatment was then determined by taking the average for the five replications. The number of fully opened leaves presented in each cutting was counted and expressed as number of leave.cutting^{-1}. The root and shoot fresh weight were calculated and expressed in g.cutting^{-1}. The root length of the seedling was measured from collar region to the root tip, expressed in centimeter. For root and shoot dry weight, they were placed in paper bag, dried in a hot air oven maintained at 85 ± 2^{O}C for 24 h (for root) and 48 h (for shoot), cooled in a desiccators, weighed and expressed as g.cutting^{-1}.

The collected data were statistically analysed using SAS through the use of ANOVA, and the means were compared using Duncan's Multiple Range Tests.

3 Result and Discussion

The growth of Moringa seedling studied showed significantly difference through the effect of stem cutting diameter and also length magnitude, but no significantly difference of their interaction at p= 0.05 during three months nursery cultivation. The growth of seedling varied within each of stem cutting size either length or diameter of stem cutting.

The increase of stem cutting length from 25 cm to 75 cm resulted in a significant of the first day of sprouting, and shoot length during a three-month observation period. Meanwhile, the stem cutting diameter affected significantly on shoot length started from 56 to 84 day after planting (Table 1). From Table 1, it seems that shoot sprouting from stem cutting length of 75 cm was earlier (7.0 days after planting) than that of stem cutting length of 25 cm (11.44 days after planting) and 50 cm (10.22 days after planting). The shoot length from the length of 75 cm was longer than the length of 25 and 50 cm. There is no significant difference in shoot length between the length of 50 and 25 cm. On the stem cutting diameter, the stem diameter of 4.1-5.0 cm was longer than the shoot diameter of 3.0-4.0 cm and 5.1-6.0 cm. As showed by stem cutting length, shoot length of seedling between d1 and d3 was not significantly different. Table 2 showed that the stem cutting length increment from p1 (25 cm) to p3 (75 cm) resulted in a significant number of shoot of seedling increment ranging from 1.16 to 6.13 shoots at the seedling old of 84 days after planting. The increase in stem cutting diameter from d1 (3.0-4.0 cm) to d3 (5.1-6.0 cm); however, it resulted a significant decrease in the shoot number ranging from 3.94 to 3.02.

There was an increase in the number of leaves as the length and diameter of the stem cutting increase (Table 3). The longest and the biggest cutting showed the highest number in leaves number. There was a significant difference between the three lengths of cutting treatment, and the highest number of leaves was showed by the length of stem cuttings of 75 cm (p3) of 47.24 leaves. Similarly, it occurred in the stem cutting diameter. The highest number of leaves was found in seedlings from stem cuttings with diameter of 5.1-6.0 cm (d3).

In Table 4 appeared that the diameter of shoot stem significantly different among three treatments of stem cutting lengths. The diameter of shoot of stem cutting length of 50 cm (p2) and 75 cm (p3) were found 0.86 cm and 0.98 cm, the three stem cutting diameter (d1, d2, and d3) was not significantly different.

Table 1. Effect of stem cutting length and diameter on the first day of sprouting and shoot length during a three-month observation period

Stem cutting size	The first day of sprouting (dap)	Shoot length (cm)					
		14 dap	28 dap	42 dap	56 dap	70 dap	84 dap
Length (cm)							
25	11.44 a	0.63 b	1.22 b	2.72 b	6.43 c	16.47 b	21.15 b
50	10.22 a	0.64 b	3.22 b	9.99 b	21.32 b	16.72 b	26.12 b
75	7.00 b	1.65 a	7.78 a	19.30 a	38.01 a	48.28 a	65.48 a
	1.48	0.82	3.31	8.88	14.77	21.80	25.00
Diameter (cm)							
3.0-4.0	9.00	1.04	4.31	9.55	18.89 b	21.77 b	34.07 b
4.1-5.0	9.33	1.25	5.09	8.62	30.36 a	39.27 a	48.70 a
5.1-6.0	10.33	0.63	2.80	7.86	16.49 b	20.43 b	29.96 b
	ns	ns	ns	ns	9.22	10.32	11.77

Explanation: Means within the same column followed by the same letters are not significantly different according to Duncan's Multiple Range Test at 5%. ns = non significantly dap = day after planting

Table 2. Effect of various stem cutting lengths and diameters on the number of shoot during a three-month seedling period

Stem cutting size	Number of shoot					
	14 dap	28 dap	42 dap	56 dap	70 dap	84 dap
Length						
p_1 (25 cm)	0.32 c	0.49 c	0.64 c	0.81 c	1.03 c	1.16 c
p_2 (50 cm)	2.20 b	2.48 b	2.77 b	2.96 b	3.21 b	3.44 b
p_3 (75 cm)	4.86 a	5.43 a	5.60 a	5.74 a	6.02 a	6.13 a
	0.87	0.66	0.61	0.59	0.60	0.57
Diameter						
d_1 (3.0-4.0 cm)	2.84	3.11	3.31 a	3.47 a	3.75 a	3.94 a
d_2 (4.1-5.0 cm)	2.39	2.77	3.03 ab	3.27 ab	3.54 ab	3.68 a
d_3 (5.1-6.0 cm)	2.16	2.52	2.67 b	2.76 b	2.97 b	3.02 b
	ns	ns	0.61	0.59	0.60	0.57

Explanation: Means within the same column followed by the same letters are not significantly different according to Duncan's Multiple Range Test at 5%. ns = non significantly dap = day after planting

Table 3. Effect of various stem cutting lengths and diameters on number of leaves during a three-month seedling period

Stem cutting size	Number of leaves					
	14 dap	28 dap	42 dap	56 dap	70 dap	84 dap
Length						
p_1 (25 cm)	0.34 b	0.75 b	1.35 c	1.74 c	2.55 c	4.03 c
p_2 (50 cm)	0.66 b	4.78 b	11.05 b	12.95 b	16.26 b	20.13 b
p_3 (75 cm)	2.18 a	17.41 a	27.40 a	36.25 a	41.80 a	47.24 a
	0.91	5.83	7.32	8.06	6.88	7.74
Diameter						
d_1 (3.0-4.0 cm)	1.22	9.07	11.00	13.56	15.86 b	18.51 b
d_2 (4.1-5.0 cm)	1.12	8.13	15.60	17.27	21.75 ab	26.03 ab
d_3 (5.1-6.0 cm)	0.83	5.73	13.20	20.10	22.99 a	26.96 a
	ns	ns	ns	ns	6.88	7.74

Explanation: Means within the same column followed by the same letters are not significantly different according to Duncan's Multiple Range Test at 5%. ns = non significantly dap = day after planting

Table 4. Effect of various stem cutting lengths and diameters on the number of leaves during a three-month seedling period

Stem cutting size	Diameter of shoot stem (cm)				
	28 dap	42 dap	56 dap	70 dap	84 dap
Length					
p_1 (25 cm)	0.22 b	0.31 b	0.38 b	0.46 b	0.54 b
p_2 (50 cm)	0.41 a	0.59 a	0.56 a	0.68 a	0.86 a
p_3 (75 cm)	0.47 a	0.68 a	0.59 a	0.70 a	0.98 a
	0.11	0.24	0.17	0.16	0.20
Diameter					
d_1 (3.0-4.0 cm)	0.34	0.42	0.48	0.60	0.69
d_2 (4.1-5.0 cm)	0.38	0.59	0.55	0.65	0.78
d_3 (5.1-6.0 cm)	0.37	0.46	0.51	0.59	0.74
	ns	ns	ns	ns	ns

Explanation: Means within the same column followed by the same letters are not significantly different according to Duncan's Multiple Range Test at 5%. ns = non significantly dap = day after planting

Table 5. Effect of various stem cutting lengths and diameters on the number of root and length of root during a three-month seedling period.

Stem cutting size	Number of root			Length of root (cm)		
	28 dap	56 dap	84 dap	28 dap	56 dap	84 dap
Length						
p_1 (25 cm)	0.62 b	1.50 c	4.11 c	0.38 b	4.66 b	9.09 b
p_2 (50 cm)	3.33 ab	10.11 b	17.22 b	0.81 b	5.66 ab	10.39 b
p_3 (75 cm)	9.11 a	21.11 a	32.78 a	2.36 a	7.09 a	14.66 a
	5.81	7.92	10.08	0.63	1.82	2.57
Diameter						
d_1 (3.0-4.0 cm)	4.52	9.67	18.11	1.01 b	6.15 ab	12.54 a
d_2 (4.1-5.0 cm)	4.66	13.67	17.33	1.74 a	6.57 a	11.82 ab
d_3 (5.1-6.0 cm)	3.88	9.39	18.67	0.80 b	4.69 b	9.79 b
	ns	ns	ns	0.63	1.82	2.57

Explanation: Means within the same column followed by the same letters are not significantly different according to Duncan's Multiple Range Test at 5%. ns = non significantly dap = day after planting.

Table 6. Effect of variousstem cutting lengths and diameters on the root number and length during a three-months seedling period

Stem cutting size	Fresh weight of shoot (g)			Dry weight of shoot (g)		
	28 dap	56 dap	84 dap	28 dap	56 dap	84 dap
Length						
p_1 (25 cm)	2.61	9.57 b	20.85 c	0.13	0.91 b	2.66 b
p_2 (50 cm)	5.27	85.68 a	186.44 b	0.47	13.83 a	34.79 a
p_3 (75 cm)	5.89	95.90 a	309.56 a	0.80	14.01 a	51.22 a
	ns	63.85	122.13	ns	10.66	23.26
Diameter						
d_1 (3.0-4.0 cm)	4.55	58.23	138.63	0.61	8.42	22.96
d_2 (4.1-5.0 cm)	5.96	70.99	182.16	0.50	10.93	34.82
d_3 (5.1-6.0 cm)	3.26	61.93	196.07	0.27	9.40	30.90
DMRT 5 %	ns	ns	ns	ns	ns	ns

Explanation: Means within the same column followed by the same letters are not significantly different according to Duncan's Multiple Range Test at 5%. ns = non significantly dap = day after planting

The number of root was affected by stem cutting length, but not by stem cutting diameter. Meanwhile, the length of root was affected by those two of cutting size treatment (Table 5). The highest number and the length of root were found in stem cutting length of 75 cm (p3), i.e.32.78 and 14.66 respectively, while the number of root between the three stem cutting diameter treatments (d1, d2, and d3) was not significantly different, but in the length of root.

There was significantly different in fresh and dry weight of shoot among stem cutting lengths. However, there was no significant difference between shoot fresh and dry weight among three stem cutting diameters studied (Table 6). This phenomenon also occurs in fresh weight and dry weight of root (Table 7). Based on the components of seedling crowns elaborated in Table 1, Table 2, Table 3, Table 4, and Table 6, it can be explained that good seedling growth was showed by stem cutting length of 50 cm and 70 cm, and also stem cutting diameter of 3.0-4.0 cm and 4.1-5.0 cm, as well as on the root growth components elaborated in Table 5 and Table 7. Physiologically, the greater the size of the diameter and length describes the more mature the cutting tissue is. Therefore, the results of this study in line with [23] that the large mature trees cut are particularly difficult to propagate. Maturation in woody plants is an on-going process that results in developmental changes involving lower growth potential [24] and also reduced regenerative competence [25].

Table 7. Effect of various stem cutting lengths and diameters on fresh weight and dry weight of root during a three-month seedling period

Stem cutting	Fresh weight of root (g)			Dry weight of root (g)		
size	28 dap	56 dap	84 dap	28 dap	56 dap	84 dap
Length						
p_1 (25 cm)	0.18	0.46 b	0.87 b	0.08	0.12 b	0.19 b
p_2 (50 cm)	2.01	4.89 a	17.99 ab	0.66	0.68 ab	2.64 ab
p_3 (75 cm)	1.87	7.77 a	43.78 a	0.39	1.20 a	6.56 a
	ns	4.33	35.61	ns	0.85	4.85
Diameter						
d_1 (3.0-4.0 cm)	1.18	5.62	22.60	0.32	0.99	2.96
d_2 (4.1-5.0 cm)	1.94	4.23	15.28	0.70	0.54	2.55
d_3 (5.1-6.0 cm)	0.94	3.29	24.76	0.12	0.47	3.87
	ns	ns	ns	ns	ns	ns

Explanation: Means within the same column followed by the same letters are not significantly different according to Duncan's Multiple Range Test at 5%. ns = non significantly dap = day after planting

Table 8.Change on fresh weight of stem cutting stock during seedling growth and development during a three-month seedling period

Stem cutting	Fresh weight of stem cutting (g)			
size	dbp	28 dap	56 dap	84 dap
Length				
p_1 (25 cm)	360.80 c	323.40 c	292.30 c	357.90 c
p_2 (50 cm)	713.90 b	651.00 b	688.80 b	709.10 b
p_3 (75 cm)	1078.60 a	1014.80 a	1034.20 a	1075.20 a
	40.63	106.75	188.41	156.30
Diameter				
d_1 (3.0-4.0 cm)	471.80 c	392.90 c	428.60 c	444.80 c
d_2 (4.1-5.0 cm)	662.90 b	639.20 b	646.80 b	659.20 b
d_3 (5.1-6.0 cm)	1001.60 a	926.20 a	942.10 a	983.00 a
	40.63	106.75	188.41	156.30

Explanation: Means within the same column followed by the same letters are not significantly different according to Duncan's Multiple Range Test at 5%. ns = non significantly dbp = day before planting dap = day after planting

In this study, stem cuttings used were leafless stems cutting. The benefits in using large cutting have also been reported for multi-node cuttings of leafless cutting. As [26] stated that it was common experience that leafless cuttings produced rarely root and their further growth entirely dependent on the leaf for photosynthate. Then, the supply and redistribution of carbohydrates within cuttings can sometimes limit their capacity to root. Generally, the longer a cutting takes to root, the fewer roots develop.

Due to the cutting size difference in length and diameter tested in this experiment, the initial weight of stock cuttings is significantly different. The initial fresh weight of the lowest cuttings was showed by the cutting length of 25 cm, followed by the cuttings of 50 cm, and 75 cm (Table 8). It also happened in the stem cutting diameter. The fresh fresh weight of cutting was chaned (Table 8) while the growth and develop. The fresh weight decreased in the first month of seedling growth and development, and then gradually increased in the following months although the increase did not reach the initial fresh weight yet. This phenomenon indicates that the growth and development of the stem cuttings require the energy obtained from the material stored (dry matter-photosynthate) in the stem cuttings.

Table 9. Seedling shoot-root ratio and percentage of seedling produced

Stem cutting size	Shoot-root ratio			Seedling produced (%)
	28 dap	56 dap	84 dap	
Length				
p_1 (25 cm)	1.63	07.58 a	13.12 b	19.67 c
p_2 (50 cm)	0.71	18.33 c	11.15 ab	85.33 a
p_3 (75 cm)	2.05	11.67 b	07.77 a	71.22 b
	ns	5,66	4.87	11.42
Diameter				
d_1 (3.0-4.0 cm)	1.91	08.51 a	07.74 a	84.56 a
d_2 (4.1-5.0 cm)	0.74	20.19 b	10.64 b	83.13 a
d_3 (5.1-6.0 cm)	2.25	19.82 b	07.98 a	70.33 b
	ns	6.61	3.53	10.35

Explanation: Means within the same column followed by the same letters are not significantly different according to Duncan's Multiple Range Test at 5%. ns = non significantly dap = day after planting

This study found the fact that the release of dry material (stored material) was more essential to occur for stem cuttings length of 75 cm and stem cutting diameter of 5.1-6.0 cm. Associated with the length of cuttings, this is because more axillary buds that exist and require energy to grow (break the dormancy). While related to the diameter of stem cuttings, this is because the large diameter indicates that the cuttings are more mature than the smaller size. The term more mature means that their tissues have differentiated cell which requires more energy (dry matter) for the occurrence of a process, namely re-differentiation as a trigger for axillary bud growth and development in which they are firstly dormant.

As [18] stated that the age of plant from which stem cuttings were taken as well as the stem position on plant determined rooting and shoot growth and survival percentage. And [27] also stated that the size of cuttings such as the length and diameter of the cuttings should be considered in vegetative propagation of this plant, since the size of the cuttings is related to the presence of food reserves, which are generally carbohydrates. The shorter the size of the cuttings or the smaller the stem diameter, the lower the food reserve material will be. The potential reserves of food owned by each cuttings will determine the growth and development of seedlings. The effect of the length and diameter of the cuttings has a similar pattern, which has more significant effect on the canopy component than the root component.

This study also found that the length of stem cuttings did not significantly affect the first time of root to grow (the data is not described in detail), that is about 9.2-11.6 days after planting, but the roots in this experiment grow and develop after being led by the growth and development of shoots, which was 5.8-6.2 days after planting. There was a significant effect of length of stem cuttings on root length, root number, and also seedling produced (Table 5). The findings on *Moringa* vegetative propagation reported here are in accordance with those of several other studies. The longer the stem cuttings, the better the root growth in each of these plants will be. The effect of the length of stem cuttings was related to the amount of carbohydrate accumulation and the greater amount of it in cuttings will support better rooting than that with less carbohydrate reserves [18] and [26].

The better development of roots will certainly be able to balance and simultaneously support the better growth and development of a seedling canopy as well. The balance of canopy tothe root growth was reflected by the value of the shoot-root ratio (Table 9). As [28] reported in the rubber seedlings, that the lower the value of the shoot-root ratio, the more resistant the rubber seedlings to the stress at time of post trans-planting in the field will be. The increase in the root surface per unit of root dry weight, water supply with dissolved nutrients will be better so that newly transferable young plants could pass the period of the stress. On the other hand, the small ratio means the smaller canopy so that the transpiration was also smaller than that of the seedlings with the larger ratio.

The manifestation of the roots and shoots growth and development is on the magnitude of the percentages of successful stem cuttings to produce good quality seedlings and their adaptability after transplanting to the field. As [29] arguing that the frequent failure to form young plants occurs in large-diameter cuttings due to root formation difficulties, as a result of block by the Sclerenchyma tissue. The effectiveness of different cutting size, such as length and diameter of stem to induce rooting, was different due to the unlike nutritional or carbohydrate storage status of the stock cutting. The minimal amount of carbohydrate storage was needed of stock cutting for survival and to trigger physiological activity and to emerge roots and then shoot. The result of this study emphasizes the fact that, a new independent seedling produced by vegetative propagations through stem cutting is a clone which can express morphological phenotypes of the donor tree. It was in agreement to [18] and [23]. The rooting ability varied among seedlings derivate from various size of stem cutting, and attributable mainly to the physiological condition of the stock cutting, such as the age and size of stem. Based on the explanation above, it could be said that, the cutting length of 75 cm was the best cuttings size for vegetative propagation. To produce more number of seedlings, however, the length of stem cuttings of 50 cm could still be used as a propagation material of *Moringa* plant. Meanwhile, the diameter of the stem did not significantly affected on the growth of seedlings. Therefore, in order to propagate *Moringa* seedlings vegetatively, it could use stem cuttings with the length of 50-75 cm in some diameters (3.0-6.0 cm).

4Conclusion
The growth of Moringa seedlings originated from stem cuttings varied, depending on the stem length and diameter. To produce better performance seedlings, the vegetative propagation throughout stem cuttings ranging from 50-75 cm in length and/or 3.0-5.0 cm in diameter could be used.

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