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Growth and development of moringa (*Moringa oleifera* L.) stem cuttings as affected by diameter magnitude, growth media, and indole-3-butyric acid

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Abstract. The acknowledged status of *Moringa oleifera* L. in sub-Saharan Africa, especially western Africa, has of recent accorded it the significance of being a good source of income to a large segment of many of its populace. Intensification of research into the realization of its full economic potential will be of utmost value to impoverished societies globally. One way to achieve this is the full exploration of all possible means that will facilitate its successful growth, propagation, and domestication. Even though it can be successfully raised through seeds, the high level outcrossing (64.3%) observed is a hindrance to realization of true to type trees. Vegetative propagation can be employed as an option to tackle the noted limitation, ease the cultivation process, and achieve the required realization of its economic potential. Our trial was carried out to study the influence of two growth media and three levels of indole 3-butyric acid (IBA) on root and shoot development in cuttings taken from a coppiced moringa tree existent in Universiti Putra Malaysia. Semi-hardwood cuttings of moringa, of between 20 and 30 mm diameter, cut into 25 cm length, were obtained, rinsed with a fungicide, then dipped, through their basal portion, inside varying levels (0, 1000, 2000, and 3000 ppm) of indole-3-butyric acid (IBA) for between 7 and 10 seconds. The treated cuttings were then transplanted into a polyethylene bags (23 cm × 36 cm), containing two growth media - a munchong series soil (M) and a combination of a munchong series soil thoroughly mixed with biochar (MB) in a 3:1 ratio sequence. The trial was conducted inside a shade house where the humidity of the experimental area was manipulated through a regular daily manual hand sprinkling. Plant height, percentage of primary branch produced, leaf area, and dry matter (DM) were found to be significantly (P<0.05) influenced by variation in stem diameter magnitude, while the diameter of the primary branch and spad chlorophyll content were found to be non-significantly (P>0.05) influenced. The MB growth media was observed to significantly affect the plant height, percentage root number, and root length as compared to the M growth media. For a successful vegetative propagation and subsequent domestication, the MB growth media coupled with a higher stem diameter size are recommended.
Keywords primary branch, munchong series, dry matter, biochar, plant hormone.

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Introduction

Moringa oleifera L. is a valuable tree enriched with health benefitting nutrients essential to human well-being. Its spread and establishment will, therefore, be beneficial to especially the many poverty stricken and impoverished nations in the developing world. Almost every part of 'moringa' tree has value as food. Young fruits, flowers and leaves (containing 5-10% protein) are consumed as vegetable (von Maydell 1990). Its leaves are a good source of vitamins A, B, and C, they are also a source of minerals, such as calcium and iron, and the short sulphur bearing amino acids – methionine and cysteine (arguably the most critical dietary ingredients for people lacking regular access to meat, milk, cheese, eggs, or fish (National Research Council 2006). The flowers can be eaten or used to make a tea, while the young, green pods are boiled and eaten like green beans (Janick & Paull 2008) and contain a high vitamin C content with a notable content of iron and copper (National Research Council 2006). Seeds from mature pods are browned in a skillet, mashed, and placed in boiling water; edible oil will float on the surface. The oil, which constitute about 38-42% of the seed, is clear, sweet, and odourless, and contains approximately 13% saturated fatty acids and 82% unsaturated fatty acids, of which there is 9.3% palmitic, 7.4% stearic, 8.6% behenic, and 65.7% oleic acids (Janick & Paull 2008). The pungent fleshy root is pulverised into a flaky condiment with a horseradish taste. It is eaten as a vegetable in East Africa (von Maydell 1990). This has given 'moringa' the widely used name "horseradish tree". The thick, soft roots are also pickled, through peeling, drying, grounding, and steeping in vinegar (National Research Council 2006) and are as well roasted and eaten (Miller & Morris 1988).

Manipulation of soil media to provide the best medium for the growth and development of crop plants is a common agricultural practice. Successful growth and development will require the provision of appropriate media, temperature, moisture, light, and air. Generally, soil serves as the primary medium of growing plants, but researchers nowadays commonly grow plants in greenhouses on growing media made up of both natural (soil) and artificial (soilless) ingredients mixed in various proportions with the aim of mimicking the growing condition that a natural soil is known to provide to the growing plant.

Currently, there is a renewed interest in biochar for incorporation as a growth media, with research findings uncovering its prospect of increasing the productivity of crop plants, while concurrently usefully effecting carbon sequestration into the cultivated soils (Woolf et al. 2010). So far, available research findings have shown that application of biochar to cultivated soils will not only influence its fertility through the improvement of nutrient exchange and retention, but will also alter a number of soil properties involving both biotic and abiotic interactions (Verheijen et al. 2014). Additionally, the utilization of the biochar has been reportedly linked to the improvement of seeds germination, restoration of grasslands, improvement of contaminated soils, improvement of forest management, suppression of plant diseases, interacting with soil fauna, impacting on applied pesticides, and promoting of soil mycorrhizal activity (Verheijen et al. 2014). Similarly, the biochar was also reportedly observed to very much lower the leaching of inorganic N (NH_4^+ and NO_3^-), as reported by Sika & Hardie (2014) after conducting a leaching experiment with sandy soils in their laboratory, observing how biochar decreased leaching of inorganic N by 12 to 96% for ammonium and nitrate ions, respectively.

The plant growth and development can be regulated or controlled by use of plant growth regulators (or plant hormones). The level of concentration of the applied hormone is manipulated during the cultivation process to achieve desired result. Indole-3-butyric acid (IBA) is a synthetic auxin employed to induce root formation (adventitious) on stem cuttings propagated from woody plants. Rooting hormone is in most instances required when dealing with more mature wood, and a rooting hormone, such as IBA at the rate of 2000-6000 ppm is commonly utilised (Kobayashi et al. 2007). Generally, higher concentrations may be required when dealing with cultivars that are difficult to root (Marzieh et al. 2012). Plant growth regulators are therefore expected to play a central role in enhancing their rooting ability. Indole butyric acid is an auxin that is regularly applied to promote the formation of roots from plant cuttings (Wiesman & Lavee 1994). The successful formation of roots from stem cuttings using IBA was linked to the establishment of a hormonal balance within the cuttings. A noticeable promotion of root callus growth from damaged ends of stem cuttings was observed to be promoted by rooting compounds (John 2004). It is recommended to employ the use of smallest amount of synthetic rooting compound; application of higher rates may hinder root growth and cause a delay in the rooting process. A delicate balance was noted to exist between the auxins that regulate root production and growth, and the cytokinins that regulate shoot development. It is the interaction between these two hormones that leads to the root callus formation. Where the hormonal balance is disrupted, there may be severe outcomes to the general plant growth pattern (Ahmed et al. 2010). One of the advantages derived from vegetative propagation as compared to sexual propagation is its production of uniform rootstocks and its ensurance of the maintenance of true-to-type high yielding existing progenies. In the world of agriculture, there are continually ongoing researches meant to generate true-to-type, disease free plants from stem cuttings, some of which are normally difficult to root under ordinary conditions. This trial was undertaken to study the effect of two levels of growth media, and various levels of stem diameter and IBA on the successful rooting ability and subsequent vegetative growth and development of moringa stem cuttings.

Materials and methods

Plant material

The research trial was undertaken under a nursery shade house condition within the Farm No. 2 of the Universiti Putra Malaysia (UPM), Serdang Selangor, 2°56'14.92"N, 101°42'40.87"E, and altitude of 40 meters above sea level. The trial was conducted between the months of January and April, 2015, with cuttings of the local accession, having the same length (20 cm) and diameter (25 to 30 mm). The cuttings were rinsed with a fungicide (furadan, 1 ml to 10 liters water), allowed to dry, and then dipped inside the IBA solutions of 0, 1000, 2000, and 3000 ppm for about to 7 to 10 seconds with the aim of moistening about 3-4 cm of the basal section. The IBA formulation was prepared by dissolving the pure IBA compound in 95% ethanol and topping up with various levels of distillated water to achieve the required concentration. They are then planted inside polyethylene bags (23 cm × 36 cm) filled with two types of growth media – munchong series soil (M) and munchong series mixed with biochar (MB) in a 3:1 ratio combination, with a control consisting of cuttings planted into the two set of growth media but with zero IBA application, making the total set of treatments applied to be 8, *viz*; M0 and MB0 (zero IBA application), M1 and MB1 (1000 ppm IBA application), M2 and MB2 (2000 ppm IBA application), M3 and MB3 (3000 ppm IBA application). All the experimental units were treated to a uniform application of 50 kg ha^{-1} NPK Blue (12-12-17+2+TE) broad spectrum fertiliser. The characteristics of Munchong series soil were a very fine, kaolinitic, iso-hyperthermic, red-yellow Tipik Tempalemoks that develops over fine grained sedimentary rocks (shale) and low grade metamorphic rocks (Paramananthan 2000).

The plants were kept in a shade house, and manually watered daily. The cuttings were arranged in a completely randomized block design with four replications. Each replication consisted of 24 cuttings resulting in a total of 72 experimental units. The trial was run for about 3 month duration during which, data on growth parameters (plant height, spad chlorophyll content, and the diameter of primary branches produced [widest diameter selected for measurement] from the stem cuttings were gathered and compiled). After the 90 days duration, the cuttings were removed and data on number of roots per cutting, root length (longest root chosen), and total dry matter, TDM (consisting of the primary branches and leaves produced from the cuttings) were collated.

Based on the result obtained from the above trial, a second experiment with moringa cuttings of various stem diameter sizes – 25 to 35 mm (D1), 35 to 45 mm (D2), 45 to 55 mm (D3), and 55 to 65 mm (D4), was set-up and conducted for 2 and half months period (between April and June 2015) with the aim of recommending the best cutting diameter for the attainment of optimal growth and development of the moringa tree. The 4 set of selected diameters were planted in a 23 cm × 36 cm polyethylene bags and replicated 4 times giving a net total of 48 plants. The plants were treated to a uniform rate (50 kg ha^{-1}) NPK blue (12-12-17+2+TE). Data on plant height, number of branch produced, branch diameter, spad chlorophyll (through the use of Minolta SPAD 502 Chlorophyll Meter that makes an instantaneous and non-destructive readings on a plant based on the quantification of light intensity received), and total dry matter were collected and tabulated. The plant height (cm) was measured on monthly basis from the base of the stem to the tip of the highest branch leaf, the diameter of the primary branch (mm), was measured monthly from the base of the highest primary branch, at 1 cm from the base of attachment of the primary branch to the main stem, leaf area was collated at the point of termination of trial by passing, individually, the total leaves harvested per plant through the conveyor belt of the LICOR machine and recording the final reading, while the dry matter (DM) consisted of the primary branches and leaves at harvest after oven drying at 65ºC. The collected data were statistically analyzed with SAS though the use of both the *t*-test and ANOVA, and the means (for the ANOVA) were compared using Duncan's multiple rang tests.

Results

Effect of varied stem diameter sizes

The growth rate with respect to plant height (primary growth) was discovered to be significantly (*P <0.05,* or Type 1 error) affected by increment in stem diameter size. A stem cutting diameter increment from D1 to D4 was discovered to result in a significant height increment ranging from 59.5 to 71 cm (about 20% increase). However, even though the plant height increase linearly between D1 and D3 on one hand, and between D2 and D4 on the other hand (Table 1), the linear increase was noted to be non-significant (*P >0.05)*.

Response of number of primary branches (sub-division of the main stem or primary axis that diverges away from the main stem) that are produced depicts a similar pattern (Table 1) with the plant height, with treatments D1, D2, and D3 on one hand and treatments D2, D3, and D4 on the other hand all portraying a non-significant (*P >0.05)* variation, while noticeable significant variation was recorded between treatment D1 having a mean number of primary branches of 2.5 and D4 possessing a mean number of primary branches of 5.5 (a significant 120% variation).

Taking into account the primary branch diameter, it was noted that no significant variation (*P >0.05)* exist between treatments D1, D2, and D3, but a significant variation was noted between all of the aforementioned treatments and treatment D4 (a very high variation ranging from 133 to 140% when compared to D3 and D1, respectively).

The SPAD chlorophyll content measurement (that works on the principle of measuring leaf greenness based on received optical responses from crop exposure to light) showed a non-significant linear decrease (of about 13%) in the chlorophyll content value from D1 until D4 (Table 1), research need to be further undertaken to determine whether there is a hidden concept of, 'the higher the dry matter content the lower the chlorophyll value', as observed from this trial.

The leaf area, with its notable primary role of regulating photosynthesis, was significantly affected by treatment levels. Data obtained from measurement undertaken with a LICOR machine (LI 3100 Area Meter, USA) through destructive sampling at harvest, revealed the occurrence of a significant difference (*P <0.05)* between the treatment levels D1 and D2, D1 and D4, and D3 and D4, a non-significant difference was, however, recorded between treatment levels D2 and D3 (Table 1).

The dry matter (DM) accumulation recorded was based on the total primary branches harvested and their associated leaves. There is usually a direct relationship existing between the sum total of a plant's total leaf area and the

Table 1 Effect of varied stem diameter sizes on some selected growth and yield parameter components of *Moringa oleifera* L.

Treatment (mm)	Plant height (cm)	Number of primary branch	Diameter of primary branch (mm)	Spad chlorophyll content	Leaf area	Dry matter (DM) of leaves/primary branch (g)
D1	59.5^{b}	2.5^{b}	9.61^{b}	35.30^{a}	118.50^{c}	18.95^{b}
D2	63^{ab}	4.0^{ab}	10.97^{b}	32.55^{a}	286.50^{b}	26.80^{b}
D3	68^{ab}	3.5^{ab}	10.11^{b}	34.75^{a}	290.50^{b}	29.45^{b}
D4	71^{a}	5.5^{a}	13.46^{a}	30.80^{a}	440.00^{a}	56.45^{a}

Note. Means sharing similar letter in a column for different levels of the same nutrient are statistically non-significant according to Duncan's Multiple Range Test (DMRT) at α=0.05; values are the means of five replicates ± standard error.

corresponding DM generated – the higher the leaf area, in most instances, the higher the DM and vice-versa. Recorded data obtained from this trial affirms this relationship, with treatment level D4 that possess a significantly high leaf area value recording an equally significantly higher DM, and treatment level D1 that recorded the lowest leaf area value portraying the lowest DM content (Table 1).

Effect of growth media and IBA levels

The response of the moringa stem cuttings to IBA applications obtained from the 2 growth media - soil munchong series (M) and soil munchong series mixed with Biochar (MB), portrayed non-significant (*P >0.05)* variation on nearly all the agronomic parameters assessed with respect to the IBA levels applied as compared with the control (zero IBA application). Such growth parameters like number of primary branch, spad chlorophyll content, plant height, root number, and root length were all discovered to be non-significantly affected by the different IBA rates applied (Table 2) – in fact in the case of root length grown under the M media, the control was found to display a higher mean root length (27.25 cm) compared to the applied IBA treated experimental units. The only exception, where a significant response was observed with respect to the IBA application is diameter of primary branch with cuttings grown in the M media, where treatment level M0 (the control) significantly recorded a higher primary branch diameter (9.24 mm) when compared to the M3 (3000 ppm IBA application), which is the highest applied IBA level (Table 2). With regards to the spad chlorophyll values recorded at 3 periods within the day, even though not statistically significant, assessment of the values showed that a higher chlorophyll content value was obtained from the afternoon (13:00-14:00) recorded values as compared to the morning and evening recorded values.

While the IBA application rates were generally observed to have no significant influence on the moringa cuttings growth and development, when comparism was made with respect to growth media (M and MB) a different outcome was noted. Results from t-test comparison of the M and MB media with respect to

Table 2 Effect of indole-3-butyric acid levels and growth media (soil munchong series [M] and munchong series combined with biochar [MB]) on *Moringa oleifera* L. stem cuttings growth

Treatment	Number of primary branch	Diameter of primary branch	Spad chlorophyll content			Plant height (cm)	Root number	Root length (cm)
			9-10 am	1-2 pm	5-6 pm			
M0	1.5^{a}	9.24^{a}	31.40^{a}	33.30^{a}	32.45^{a}	88.00^{a}	27.00^{a}	27.25^{a}
M1	3.0^{a}	8.13^{ab}	29.70^{a}	30.10^{a}	30.30^{a}	116.00^{a}	32.00^{a}	23.00^{a}
M2	2.5^{a}	11.05^{a}	35.60^{a}	35.75^{a}	34.35^{a}	82.00^{a}	29.00^{a}	25.30^{a}
M3	2.0^{a}	6.18^{b}	34.60^{a}	38.20^{a}	35.00^{a}	100.00^{a}	37.00^{a}	22.00^{a}
MB0	3.00^{a}	8.10^{a}	31.56^{a}	33.10^{a}	32.13^{a}	102.00^{a}	36.66^{a}	28.83^{a}
MB1	3.32^{a}	9.32^{a}	33.60^{a}	36.00^{a}	31.82^{a}	126.00^{a}	35.67^{a}	27.22^{a}
MB2	2.00^{a}	10.47^{a}	28.72^{a}	30.42^{a}	27.72^{a}	128.68^{a}	40.32^{a}	29.82^{a}
MB3	3.00^{a}	9.47^{a}	32.80^{a}	34.60^{a}	31.92^{a}	115.33^{a}	36.32^{a}	30.42^{a}

Note. Means sharing similar letter in a column for different levels of the same nutrient are statistically non-significant according to Duncan's Multiple Range Test (DMRT) at α=0.05; values are the means of five replicates ± standard error.

number of primary branch formed showed the pooled equal variance type 1 error probability value to be greater than 0.05 *(P>0.05)* with mean values of 2.33 and 2.83 for the M and MB media, respectively, signifying a non-significant variation (Table 3). Similar trend of a non-significant *(P>0.05)* was observed with respect to the primary branch diameter between the M media mean value (7.89 mm) and MB media mean value (9.33 mm).

The plant height was found to be significantly (*P*<0.05) influenced (34.7% increase) by the MB media with mean values of 118 cm as compared to the M media mean value of 87.58 cm. Root number was similarly discovered to be significantly affected (21.14% increase) by the MB media with mean value of 37.25 compared to the M media mean value of 30.75 (Table 3). The MB media similarly influenced the root length (11.03% significant increase) as compared to the M media. However, variation in the growth media was discovered not to affect or influence the SPAD chlorophyll content values taken at various timings of the day, but the recorded afternoon values were discovered to be higher than the morning and evening values.

Discussion

The growth and development of all plants is controlled by the interaction of two sets of internal factors – nutrition and hormone. The determination of the role played by growth hormones, growth media, and diameter magnitude is an essential step towards a successful conduct of the vegetative propagation. Diameter assessment is an important parameter for the general management of plantation crops as it determines the proportions of branch distribution and their subsequent leaf dry matter and fruit development. It is also noted to be an important conservation and biodiversity tool in view of the fact that larger diameter trees tend to harbour a larger amount of species (Fries et al. 1997). From the results obtained from our trial, increased diameter was seen to significantly increase leaf area and dry matter generation. The plant stem diameter increase occurs as a result of secondary growth generated by cell division of the vascular cambium (a lateral meristem) between the xylem and phloem tissues. It is particularly this capacity of seasonal girth increment that differentiates arboreals from annuals. Light is noted to be one of the main factors influencing the structure and morphology of the tree crown (Doruska & Burkhart 1994). Based on this, it can be deduced that branches of trees react to improved conditions by intensifying branch growth due to the better light conditions in the canopy leading to the intensification of photosynthesis and increased assimilates generation. This is confirmed by our trial generation of highest dry matter values with the highest diameter treatment (Table 1).

With continual increment in tree height, the tree become exposed to more chances of breakage from wind flow, however this is avoided by a corresponding increase in the tree diameter. It is reported that for every doubling

Table 3 Mean values for effect of growth media – soil munchong series (M) and soil munchong series with biochar (MB) as per *t*-test analysis on *Moringa oleifera* L. stem cuttings growth components

Variable	M media	MB media	*P*-value
Primary branch number	2.33	2.83	0.2567
Diameter of primary branch (mm)	7.89	9.33	0.0847
Plant height (cm)	87.58	118	0.0089
Root number	30.75	37.25	0.0058
Root length (cm)	26.19	29.08	0.0079

of tree height, the diameter or girth increases 3-8 times to make it reasonably safe from wind breakage (Thomas 2014). The stem's internal structure is enclosed in an epidermis with a thin, grayish bark. The tree stem is essentially a dead heartwood surrounded by living sapwood, enclosed in a layer of living tissue called the cambium. Formation of new tracheids occurs beneath the inner bark, the old cells die and the tree becomes bigger in diameter (Suzuki & Grady 2004).

The plant growth regulators facilitated the generation of true-to-type, disease free, and high value nursery plants, and were normally employed for utilization on plants that are observed to be hard to root under ordinary conditions; their utilization will therefore be expected to play a central role in enhancing their rooting ability. Plant organs, notably the leaves and buds, produce hormones (auxins) that travel down the plant's stem along with nutrients in the phloem and concentrate in areas requiring rapid cell growth. Indole butyric acid is an auxin that is regularly applied to promote the formation of roots from plant cuttings. In this trial, the utilization of the MB media resulted in a significant root growth and shoot establishment (as portrayed by percentage root number, root length, and plant height). However, the various applied rates of the IBA were found not to have any significant influence on the moringa agronomic growth parameters, with some 2 recorded cases of the control (zero IBA rate) outperforming the IBA rooted cuttings. Even though it is an established fact that employing the utilization of an external hormone is a beneficial process in vegetative production as it stimulates the production of increased root number and a corresponding root elongation (Baul et al. 2008, Majeed et al. 2009). But our trial with the moringa stem cuttings revealed the IBA with having no significant influence on nearly all the parameters assessed, with the control outperforming the IBA treated stem cuttings in some instance. This development could be linked to the understanding that the moringa tree has been found to be enriched with secondary metabolites (Mooza et al. 2014), this could probably have played a role in enhancing the rooting process resulting in the non-significant influence of the IBA. Related to this are other stem cuttings research trial conducted with the IBA with the aim of enhancing the rooting production and development but discovering, based on results obtained, the IBA having a non-significant influence (Ofori et al. 1996, Oni & Ojo 2002, Akinyele 2010).

The MB media was discovered to significantly result in the highest mean number of roots per cutting (37.25), as compared to the M media recorded value (30.75). This could be attributed to improve aeration that created a conducive rooting environment with increased respiration at the basal end of the cutting stimulating thereby improved rooting. Ventura et al. (2015) in their trial with a Haplic Calcisol mixed with a 10 ton ha^{-1} biochar, observed an increase in the rhizosphere of the apple orchard of their study with a correspondingly near doubling in the rooting length, within the biochar-mixed soil compared to the control. Similarly, Prendergast-Miller et al. (2014) working with barley grown in sandy loam mixed with10 ton ha^{-1} observed a larger rhizosphere region in the biochar mixed soil compared to the control. The improvement in the root number and length of the MB growth media could also be linked to the improvement of water holding capacity within the rhizosphere perimeter. Ulyett et al. (2014) experimenting with two soil types (Cambisol and Luvisol) mixed with biochar, reported a significant increase of up to 1.3% in the available water capacity.

Conclusions

Evaluating the stem diameter is an important step to successful attainment of true-to-type trees. Our trial findings observed higher diameter leading to a higher generation of dry matter yield, as it apparently defines the proportions of branch distribution and their subsequent leaf dry matter accumulation. The MB growth media was observed to be the best medium of growth for the moringa stem cuttings, with higher root numbers, root length, and plant height obtained from its medium, which apparently has good drainage, good aeration, and a good water absorbance. While IBA (an auxin) was, without doubt, generally considered to be an effective and suitable root initiation hormone, but based on our trial results, conclusion can be deduced that it is not required for the rooting process of the moringa stem cuttings. The moringa cuttings control were observed to develop a more enhanced root system and vegetative parameters better than the IBA applied treatments. More research is recommended to determine the proper cutting length that will give optimum vegetative growth.

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