EFFECT OF BIORICHAR AMENDMENT ON GROWTH, NUTRITIONAL PROPERTIES AND BIOCHEMICAL CHANGES OF BANANA (*Musa acuminata*) cv. BERANGAN ESTABLISHED IN AN ULTISOL SOIL AT VEGETATIVE STAGE

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ABSTRACT

Enrichment of soil fertility with organic amendment offers a new strategy for enhancing soil physical properties and improving soil fertility. Application of BioRichar can be a new alternative for adoption of organic banana cultivation in Malaysia. This study was conducted to determine optimum BioRichar rate for growth enhancement of banana cv. Berangan at vegetative stage. The BioRichar was mixed thoroughly with ultisol soil at 1.5, 3.0 and 4.5 t/ha, which were equivalent to 0.6, 1.2 and 1.8 kg/ polybag (size 16 x 16 inches), respectively. In the experiment, BioRichar at 3.0 t/ha and 4.5 t/ha improved plant growth characteristics significantly including plant height, pseudo-stem diameter, total leaf number and leaf area as compared to control. Application of 4.5 t/ha BioRichar changed total N, P, K, Ca and Mg significantly in leaves. Total N, P, K increased but Ca and Mg content decreased significantly, when BioRichar was applied at higher rate (4.5 t/ha). Meanwhile, proline and MDA contents in leaf tissue were higher in control as compared to BioRichar enriched plants. These findings suggested that optimum growth of banana cv. Berangan could be achieved with BioRichar at 4.5 t/ha applied at vegetative stages during acclimatization period prior to field transplanting.

Keywords: BioRichar, growth, nutrient content, proline, banana

INTRODUCTION

Banana (*Musa spp*.) is an important crop, and cultivated worldwide as a staple food by local farmers in many developing countries (Wang et al. 2012). In Malaysia, it is considered as the second most widely cultivated fruit with total plantation area of 29,000 ha and a total production of 294,000 MT in 2015 (Kayat et al. 2016). The major problems faced by banana growers are high cost of fertilizer and chemical residues which result in soil and water pollution. Consequently, it has been an issue to the researchers and concern to the farmers in adopting organic agriculture.

In Malaysia, 72% areas are covered by ultisol and oxisol. The soils are highly weathered, characterised by high accumulation of sesquioxides, more prone to leaching of plant nutrients, mostly Ca and Mg, due to high rainfall and lacking in organic matter (Anda et al. 2008, Anda et al. 2010). Furthermore, soil in Malaysia is also usually acidic in nature with pH 4.2 to 4.8. Acidic soil also prevents growth of the plant because the soil contains highly toxic heavy metals such as Al ion (Sharma 2003).

Soil amended with biochar based compost has been promoted as a sustainable practice that can improve the quality of agricultural land in impoverished and low fertility regions (Theeba et al. 2015). BioRichar is an organic biochar fertilizer developed through a composting process using a combination of rice husk and empty fruit bunch biochar. Selected high nutrient substrates are incorporated with biochar for effectiveness of the microbes, zeolite and plant enzymes. BioRichar as a fertilizer has advantages similar to biochar in improving yield production, shortening maturity period and sustaining good quality of fruits. BioRichar also reduced leaching of element by 10 to 15% compared to compost without BioRichar (Theeba et al. 2016). A study by Adila (2016) found that

application of BioRichar as amendment in planting media (25% BioRichar with 75% topsoil) resulted in 59.63% higher total leaf area than planting media without BioRichar. She also found that application of BioRichar alone as media exhibited 292.41% higher total number of fruit yield (*Solanum melongena* L.) as compared to media without BioRichar.

In addition, the commercial organic agriculture in Malaysia is relatively new and is considered a small industry. However, with the increasing awareness of the high chemical input in agriculture, there is an emerging market for alternative sources of food which is organically produced. In recent years, the global market for organic food has developed significantly, especially in North America, Europe and Japan (Yi et al. 2015). Hence, application of BioRichar as organic input can be a new alternative for adoption of organic banana cultivation in Malaysia. In view of the current problem, therefore, the study was undertaken to investigate into the usefulness of BioRichar as amendment in ultisol soil on growth performance, nutritional properties and biochemical changes of banana cv. Berangan and for its organic production.

MATERIALS AND METHODS

Experimental site and treatments

This study was conducted at experimental field 15, Faculty of Agriculture, Universiti Putra Malaysia (UPM), Serdang, Selangor (2°59'30.07"N 101°42'58.64"E). The plot was covered under 70% black netting. The mean daily temperature was 29 to 40°C with relative humidity ranged 60 to 65%. A total of 48 one-month old banana cv. Berangan plantlets obtained from MARDI were planted in polybags of 16×16 inches in size containing 9 kg ultisol which had been mixed thoroughly with BioRichar 1.5 t/ha, 3.0 t/ha and 4.5 t/ha which were equivalent to 0.6, 1.2, and 1.8 kg/polybag, respectively. The recommended amount of BioRichar was applied based on plant density in the field i.e. 2500 plants ha^{-1} with plant to plant distance of 2 m × 2 m. The plants were irrigated by using drip irrigation twice daily. The treatment was assigned by Randomized Completely Block Design (RCBD) with four replications.

Characterization of ultisol soil and BioRichar

The ultisol soil was collected from Field 15, Faculty of Agriculture, UPM. The soil is considered as fine sandy loam and characterized by dark greyish brown. The structures are weak to moderate medium and fine sub-angular blocky and consistently friable to firm with depth. It was characterized by having an argillic region in B-horizon with high accumulation of clay (Shamshudin and Kapok 2010). BioRichar fertilizer was obtained from Greenearth International Sdn Bhd located at Banting, Selangor. Before starting the experiment, ultisol soil and BioRichar were analysed separately with three replications, respectively, to determine the pH, organic carbon content, nitrogen content, cation exchange capacity (CEC) and exchangeable cation (K^+, Ca^{2+}, Mg^{2+}). Similar data of media properties were taken at 12 weeks after transplanting (WAT). The amended soil with BioRichar was collected randomly at the depth of 5 to 10 cm from above the soil surface, air dried and sieved to 2 mm using sieve plate. The samples were kept in a vial bottle before analysis was run.

Measurement of soil physical and chemical properties

Measurement of soil physical characteristics included soil texture analysis, while soil chemical analysis included pH, carbon and nitrogen content, available phosphorus content, CEC and exchangeable cation.

The soil texture was determined with the pipette method (Teh and Jamal 2006). Soil texture classification was based on USDA scheme which classified clay soil as <2 µm, silt at 2 to 50 µm and sand at 50 to 2000 µm.

For determination of soil pH, 10 g mixed growing media was weighed and put into the plastic vial. Then, 25 mL of distilled water was poured into plastic vial containing growing media at the ratio of

1:2.5 (v/v). The plastic vial was shaken for 30 minutes and left overnight. After 12 hours, the pH was recorded using a digital Electrode pH meter (Model Lab CHEM-CP, Japan).

Carbon content was determined by dry combustion method using LECO CR-412 Carbon Analyser. One gram of soil sample was used for the analysis. Meanwhile, analysis of nitrogen content was determined by wet digestion method. A 0.25 g sample was put in the digestion tube, and subsequent 8 mL of sulphuric acid was added into the tube, mixed and heated for 1 hour at 180°C. Next, 5 mL of hydrogen peroxide was added into the digestion tube until the blackish colour turn to colourless. The sample was kept cool prior to transfer to 100 mL volumetric flask and distilled water was added to make up to the volume. The extracted samples were further analysed with auto analyser (AA) to determine total nitrogen.

Exchangeable cation and cation exchange capacity (CEC) were determined according to leaching method. An acid washed filter paper was inserted into the leaching tube until the thickness was about 1 cm. A 10 g air-dried (2 mm) sieved soil was put on the top of filter paper. A cut filter paper was put on top of the soil and extracted by leaching with 100 mL ammonium acetate solution at pH 7 for 5 hours. The leachate was collected in a 100 mL volumetric flask and the final leachate was diluted with distilled water to 100 mL. The diluted leachate sample was read for exchangeable cations (K^+, Ca^{2+}, Mg^{2+}) using the flame atomic absorption spectrophotometer (AAS). For determination of CEC, the previous soil sample was used and leached in the leaching tube with 100 mL of 80% ethanol. Then, 20 mL of 95% ethanol was used for final washing. The ethanol leachate was discarded. The same soil was leached with 100 mL of 0.1N potassium sulphate for 5 hours and the leachate was collected in a 100 mL volumetric flask and made up to volume of 100 mL using potassium sulphate solution. The diluted leachate was read for CEC reading using flame of AAS machine.

Measurement of vegetative growth

Once the banana plantlets were transferred into the polybags containing the amended media, they were kept to acclimatise for two weeks. Selective vegetative growth data were taken afterwards. The measurements for plant height, pseudo-stem, leaf number and leaf area were taken at weekly basis. Plant height was measured from the pseudo-stem base to the first internode of plant shoot emergence using a ruler. Pseudo-stem diameter was taken from 1 cm of media surface using vernier calliper. Leaf number increment was counted manually at weekly basis. The non-destructive method was performed for measurement of total leaf area and was calculated using the formula with correction factor of 0.755: leaf area = leaf length × maximum width × 0.755 (Robinson and Nel 1985). Meanwhile, total root length was measured at the end of the experimental period (12 WAT) using root scanner (Model: EPSON Flatbed Scanner 1680) and the measurements were expressed in centimetre.

Plant tissue nutritional properties

The samples of the third fully opened leaf were taken for nutritional property analysis during 12 weeks after transplanting (WAT). The samples were oven dried at 70°C for 3 days and were ground to medium size. The analysis was done using wet digestion method by weighing 250 mg ground sample into the digestion tube. Next, 5 mL of concentrated H_2SO_4 was added into the tube, rotated and allowed to stand overnight. Then, 2 mL of 50% H_2O_2 poured into the flask, rotated and left for heating process to complete for approximately 45 min. This step was repeated twice or until the digestion mixture was observed colourless. Colourless solution was then diluted with distilled water in plastic vial and made up to 100 mL for analysis. The extracted samples were analysed with Auto Analyzer (AA) for Nitrogen (N) and Phosphorus (P), while Atomic Absorption Spectrophotometer (AAS) was used for determination of Potassium (K), Calcium (Ca), Magnesium (Mg), Manganese (Mn), Zinc (Zn), Copper (Cu) and Iron (Fe).

Determination of proline content

Proline content of the leaves was determined following the method of Bates et al. (1973). The leaf samples were taken during 12 WAT by selecting fully opened leaves (old leaves). Fresh leaves (0.5 g) were homogenized in 10 mL of 3% aqueous sulfosalicylic acid and the mixture was passed through Whatman no. 2 filter paper. Then 2 mL of the filtrate was reacted with 2 mL acid ninhydrin and 2 mL of glacial acetic acid in a test tube for 1 hour at 95°C and the reaction was then cooled in an ice bath. The reaction mixture was extracted with 4 mL toluene, stirred and mixed for 15 to 20 seconds. The chromophore (pink in colour) containing toluene was aspirated from the aqueous phase and the absorbance was read at 520 nm using a spectrophotometer. The proline concentration was determined from standard curve using L-Proline and expressed as proline per gram fresh weight. The calculation for proline content was made based on the following formula:

$$\text{Proline } (\mu\text{molg}^{-1}) = [(\mu\text{g proline / mL x mL toluene})/115.5\mu\text{g/ }\mu\text{mole})] / (\text{g sample } /5)$$
$$= \mu\text{moles proline / g of fresh weight material}$$

Determination of lipid peroxidase activity

The level of lipid peroxidation was measured in terms of Malondialdehyde (MDA) content, a product of lipid peroxidation following the method of Heath and Packer (1968). Fully opened fresh leaves (old leaves) during 12 WAT (1 g) were soaked with 5 mL of 0.1% trichloroacetic acid (TCA). The homogenate was centrifuged at 10,000 g for 5 min. Next, 4 mL of 20% TCA containing 0.5% thiobarbarbituric acid was added into every 1 mL of aliquot of the supernatant. The mixture was incubated in a boiling water bath at 95°C for 30 min then quickly cooled in an ice bath, and then warmed to room temperature. The resulting mixture was centrifuged at 10,000 x g for 15 minutes. The extinction was measured at 532 nm and 600 nm. After subtracting the non-specific absorbance (600 nm), the MDA concentration was determined by using molar extinction coefficient of 155, and the results were expressed as µmol MDA per gram fresh weight. The MDA content was calculated using the following formula:

$$\text{MDA content } (\mu\text{molg}^{-1}) = [(A_{532}-A_{600})/155] \times 10^3 \times \text{dilution factor (1)}$$

Statistical analysis

The results were analysed using statistical analysis system (SAS procedure version 9.4), and least significant difference (LSD) was computed at difference value $P < 0.05$ to differentiate the means. Regression analysis was performed from total leaf area result.

RESULTS AND DISCUSSION

Soil and BioRichar characteristics

Table 1 indicated the properties of the media (ultisol soil) and BioRichar. The soil used was acidic clay with low cation exchange capacity of 4.12 cmol(+)/kg with pH 5.20. The contents of organic C and N were 1.85% and 0.18%, respectively. The available nutrients of the soil were found to be very low. In contrast, BioRichar had pH 7.23 and high content of available K^+, Ca^{2+} and Mg^{2+}. Cation exchange capacity of BioRichar was also high at 12.92 cmol(+)/kg.

Table 1. Properties of ultisol soil and BioRichar

Properties	Soil	BioRichar
Sand (%)	44.00	-
Silt (%)	9.91	-
Clay (%)	46.08	-
pH (1:2.5 water)	5.20	7.23
Cation exchange capacity (1 M NH_4OAc, pH 7) (cmol(+)/kg)	4.12	12.92
Organic C (Dry Combustion) (%)	1.85	12.39
N content (Dry Combustion) (%)	0.18	1.32
Exchangeable K^+ (1 M NH_4OAc, pH 7) (mg/kg)	22.20	151.60
Exchangeable Ca^{2+} (1 M NH_4OAc, pH 7) (mg/kg)	8.14	10.04
Exchangeable Mg^{2+} (1 M NH_4OAc, pH 7) (mg/kg)	1.51	12.21

Effect of unamended and amended BioRichar on the soil

The effects of BioRichar application on pH, organic carbon, total nitrogen and available phosphorus are given in Table 2. The statistical analysis revealed a significant ($P < 0.05$) increase in soil pH during commencement of the experiment after BioRichar was applied followed by a decrease at the end of the experiment. The reason for the increase in soil pH could be because of high surface area and porous nature of BioRichar that increases the cation exchange capacity of the soil. Increases in soil pH are likely to affect electrical conductivity (EC), cation exchange capacity (CEC) and increase alkaline metal (Mg^{2+}, Ca^{2+} and K^+) oxides. Likewise, it reduces soluble forms of aluminium, which are suggested as the most significant biochar factor affecting P solubility (De Luca et al. 2009). The presence of biochar in the soil can also provide a physical niche for growing hyphae and bacteria which can alter the soil pH (Warnock et al. 2007).

The decrease in pH at the end of the experiment could be explained by the BioRichar cation content. The combination of the cations and the carbonate in the soil will form slightly soluble carbonates and restrict the hydrolysis of carbonates, while decreasing the content of hydroxyl in the soil. Thus, the soil pH was decreased to some extent after the addition of BioRichar. Another reason could be because of acidic rain since the experiment was conducted under 70% black netting shade and eventual oxidation and decomposition of BioRichar and organic matter in the soils can form the acidic materials that will partly neutralise soil alkalinity.

Application of BioRichar significantly ($P < 0.05$) increased the mean values of organic C and total N (Table 2). The highest values of organic C and total N were observed in soils amended with 4.5 t/ha BioRichar. The increase in organic C and total N could have resulted from the presence of high amount of carbon and nitrogen in rice husk and empty fruit bunch biochar that became the main ingredient in BioRichar. Available phosphorus was also significantly ($P < 0.05$) increased in BioRichar amended soil as indicated in Table 2, i.e. by 900%, 2900% and 5566% at 1.5, 3.0 and 4.5 t/ha, respectively, compared to the control treatment. High P content could be from the compost which had been mixed together with biochar during BioRichar production.

Effect of unamended and amended BioRichar on exchangeable bases

The effects of BioRichar additions on exchangeable bases are presented in Table 2. The analysis of variance showed that the application of BioRichar at higher rate increased the exchangeable bases (Ca^{2+} and Mg^{2+}) in the BioRichar amended soil. Exchangeable bases of Ca^{2+} and Mg^{2+} were higher for 4.5 t/ha compared to other treatments. However there was no significant difference of K^+ for all the

treatments. These results were in accordance with the study by Lehman et al. (2003), Rondon et al. (2007) and Chan et al. (2008) who reported the highest exchangeable bases in biochar amended soil.

Table 2. Effect of BioRichar application on organic carbon (C), total nitrogen (N), available phosphorus (P), and exchangeable bases; potassium (K^+), calcium (Ca^{2+}) and magnesium (Mg^{2+})

BioRichar treatment (t/ha)	pH					Exchangeable bases (%)		
	Initial Exp.	Final Exp.	C (%)	N (%)	P (%)	K^+	Ca^{2+}	Mg^{2+}
0	5.15c	5.04c	1.98c	0.18c	0.003c	0.12a	0.09b	0.01b
1.5	7.37a	6.18b	4.10b	0.36b	0.03c	0.05a	0.12b	0.03b
3.0	6.82b	5.90b	3.98b	0.39b	0.09b	0.19a	0.11b	0.04b
4.5	7.40a	6.88a	5.59a	0.50a	0.17a	0.23a	0.19a	0.09a
LSD ($P < 0.05$)	0.17***	0.43***	0.68*	0.06*	0.05***	NS	0.05*	0.03*

Means followed by the same letters within a column are not significantly different at ($P > 0.05$) by least significant difference (LSD) with n=16. * and *** indicate significant difference at $P < 0.05$ and 0.001, respectively, and NS= not significant.

Plant height and pseudo-stem diameter

The effect of different rates of BioRichar on plant height increment for 12 weeks of planting was shown in Figure 1. All the treatments showed increased plant height consistently with time. However, among all treatments, 3.0 t/ha and 4.5 t/ha resulted in the fastest growth rate and higher in plant height with the values 40.50 cm and 43.25 cm at 12 weeks of planting, respectively. In contrast, the plant without enrichment of BioRichar was 48.56% shorter than the plant enriched with 4.5 t/ha BioRichar. BioRichar application at 4.5 t/ha seemed more prominent to increase plant height among all the treatments with an increased difference at 6.35% compared to 3.0 t/ha. The growth enhancement of the plant was shown in Figure 2. The plant at 4.5 t/ha was observed to have darker green leaves compared to other treatments indicating that the plant obtained enough nutrients. Darker leaf colour was also associated with good nitrogen absorption and high chlorophyll production. Significant increase in plant height as a consequence of BioRichar addition could also be resulted from improved pH, EC and soil fertility leading to better nutrient absorption as reported by Lehmann et al. (2003), Liang et al. (2006) and Solomon et al. (2007). Lehman et al. (2003) also suggested that biochar incorporation could subsequently promote soil alkalisation which can increase nitrification and uptake of nitrogen. High nitrogen uptake will lead to vigorous vegetative leaf and pseudo-stem growth.

Figure 3 (A) showed that there was a significant increase in pseudo-stem diameter growth for treated BioRichar plants compared to control. Bar chart indicated the increment in pseudo-stem diameter by increasing the BioRichar up to 3.0 t/ha during 12 WAT. Among the treatments, 3.0 t/ha BioRichar showed the highest mean value of 6.25 cm, while control treatment had the lowest mean value of 3.75 cm. Reduction of growth in pseudo-stem diameter for control treatment was induced by nutrient deficiency and low soil fertility. Drew et al. (2012) noted that incorporation of 5% biochar resulted in a higher midday stem water potential in *Quercus rubra* ($P = 0.066$), and significantly greater stem biomass in *Acer rubrum* compared to control plants (0% biochar, $P < 0.05$). However, addition up to 4.5 t/ha was observed to decrease pseudo-stem diameter to 9.92% compared to 3.0 t/ha.

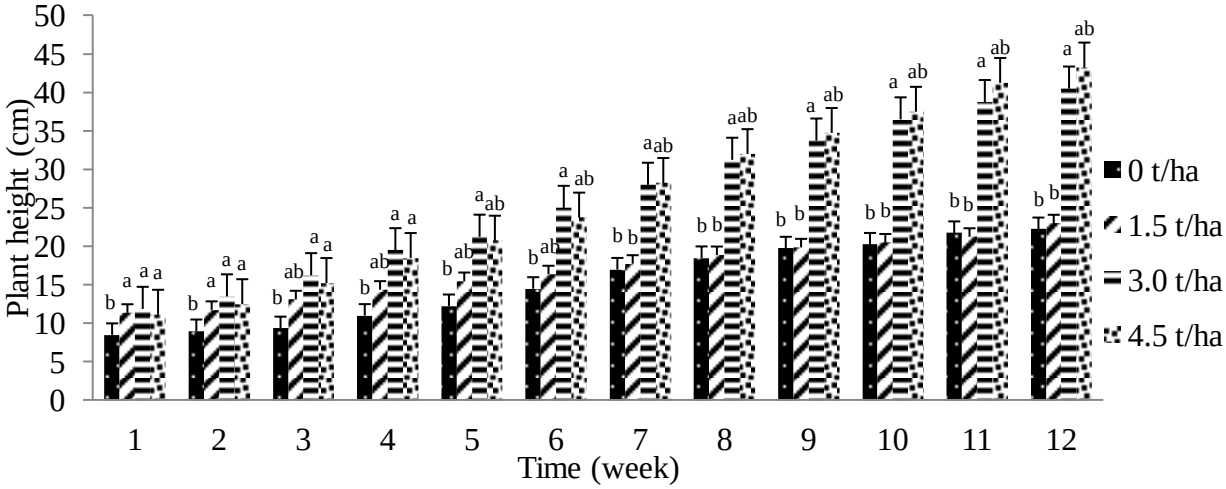

Figure 1. The plant height as affected by four different rates of BioRichar . Mean values with the same letter within the same period are not significantly different at (P>0.05) using LSD.

Figure 2. Growth enhancement by different rates of BioRichar

Total leaf number, total leaf area and total root length

Comparison of total leaf number, total leaf area, and total root length between control and BioRichar treatment during 12 weeks after transplanting (WAT) is illustrated in Figure 3.The mean number of leaves was higher with BioRichar addition up to 4.5 t/ha compared to other treatments. BioRichar at 4.5 t/ha resulted in better total leaf number at 22.73% compared to control. The reduced number of leaves in control treatment indicated that the plant was stunted by limiting supply of soil nutrient. According to Turner et al. (2007), the period for normal leaf emergence for banana to receive adequate nutrient was 7 days. During the whole production period, 30 to 50 leaves will be produced.

The total leaf area of banana also showed synergistic interaction with the increasing rates of BioRichar as in Figure 3 (C). An increasing trend was observed among the treatments, and BioRichar rate at 4.5 t/ha showed the highest mean value of 5516.8 cm^2 compared to the control (2682.3 cm^2). This indicated that BioRichar enriched plant was 55.81% higher in leaf area compared to non-BioRichar enriched plant. Leaf area growth determines light interception, and is an important parameter in determining plant productivity (Gifford et al. 1984, Koester et al. 2014). Meanwhile, according to Smart (1974) and William (1987), leaf area measurement is an important variable for most eco-

physiological studies in relation to crop growth performance, photosynthesis, water or nutrient use efficiency and yield potential. Higher leaf area during vegetative stages is a good indicator for optimum growth performance. Previously, Wardlaw (1972) stated that leaf area and the number of functional leaves in banana are closely related to the size, quality and rate of fruit development. In banana, during vegetative stages, the number of leaves needs to be pruned and maintained at 10 to 12 leaves, while flowering stages need 8 to 9 leaves and by two months before fruit harvesting, 5 to 6 leaves are needed to achieve optimum growth and high yield production. In different studies on BioRichar application, Adila (2016) reported significant variations ($P < 0.05$) for the total leaf area as influenced by BioRichar and fertiliser treatments in eggplant. It was found that plant grown in planting media of 25% BioRichar and 75% top soil resulted in 59.63% higher total leaf area than in planting media without BioRichar.

Effect of BioRichar applications on root length was demonstrated in Figure 3 (D). BioRichar rates at 1.5, 3.0 and 4.5 t/ha significantly ($P < 0.05$) increased total root length by 52.88, 234.34 and 239.89% compared to the control. Application of BioRichar at higher rate gave benefits in terms of the root morphological traits and development to alleviate plant nutrient and water deficiency. It is believed that the good development of root with high rate of BioRichar was affected by the soil texture and soil pH. The pH measured in 3.0 t/ha and 4.5 t/ha were comparatively higher than control. Brennan et al. (2014) also reported an increment in root length in biochar amended soil. BioRichar amendment had a general beneficial effect on plant resources allocation below ground and root establishment in a poor fertile soil, but this is not true for all parameters. The types of biochar source used in the BioRichar (rice husk and empty fruit bunch) could also become the main factors that determine the effectiveness for root growth. Xiang et al. (2017) in their studies on the effect of biochar application towards root traits noted that the source of biochar and the process involved during biochar production, which includes the time and pyrolysis temperature, could affect the regulation of root development. Previously, Lehmann et al. (2011) also discussed that different sources of materials used for biochar production or pyrolysis condition resulted in varied structure, pH, nutrient content and phenolic content.

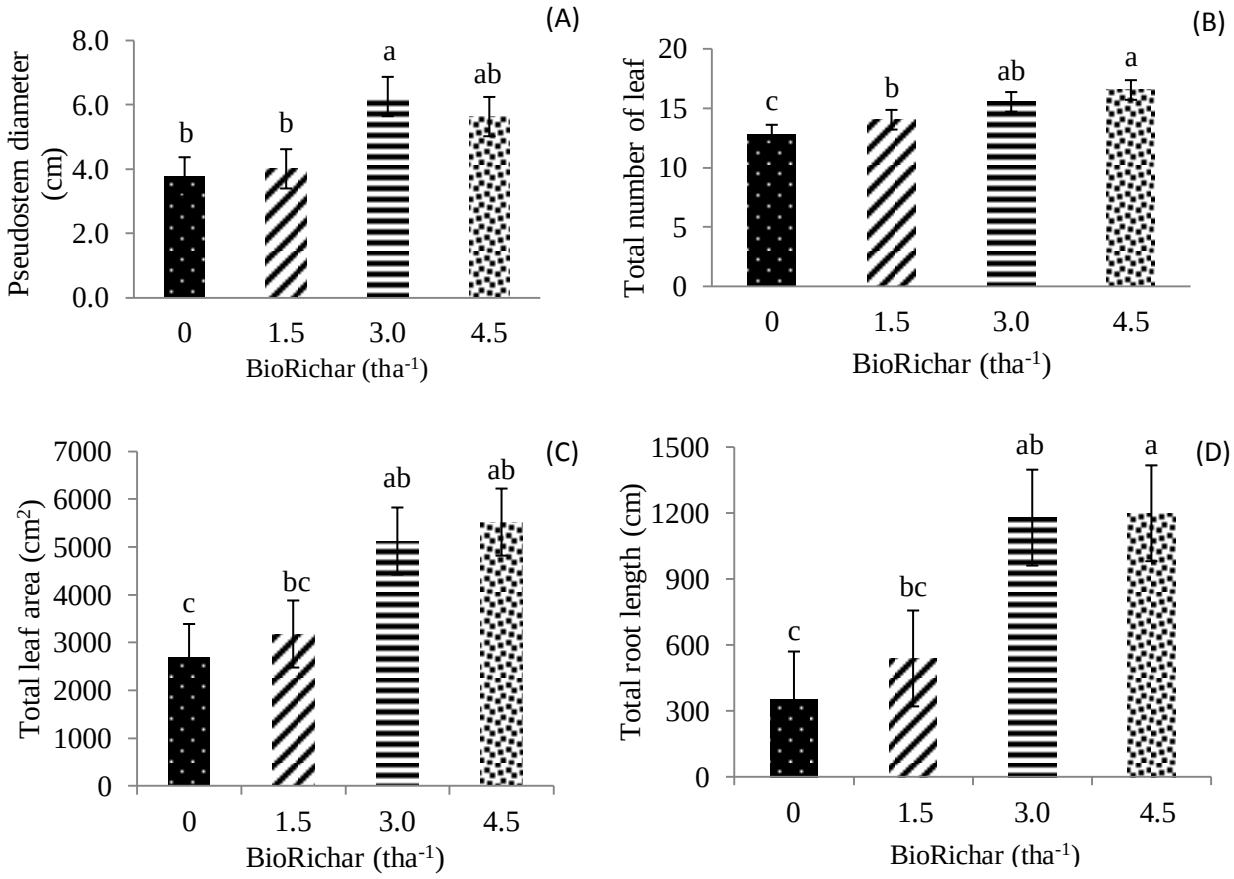

Figure 3. Effect of different BioRichar rates on banana cv. Berangan: (A) Pseudo-stem diameter, (B) Total number of leaf, (C) Total leaf area, (D) and Total root length during 12 weeks after transplanting (WAT). Mean values with the same letter are not significantly different at ($P > 0.05$) using LSD.

Foliar nutritional properties

Application of BioRichar resulted in nutritional alterations in banana leaves (Table 3). Contents of N and K were found significantly ($P < 0.05$) higher with the application of 3.0 and 4.5 t/ha BioRichar compared to control. Ca and Mg contents, too, were significantly ($P < 0.05$) different for all of the treatments. Higher contents of Ca and Mg were exhibited in banana plant tissue grown in 3.0 t/ha with no significant difference with control plant. Lower contents of Ca and Mg in leaf tissue were found in plant grown in media with BioRichar of 1.5 t/ha and 4.5 t/ha compared to 3.0 t/ha and control plant. Arunachalam et al. (1976) showed that adequacy level of nutrients in banana leaf ranged from 3.18 to 3.43% for N, 0.46 to 0.54% for P, 3.36 to 3.76% for K, 2.3 to 2.4% for Ca and 0.25 to 0.28% for Mg. It was observed that N uptake by plants from BioRichar amended soils was still low, but it did not affect its growth. From the result, it was observed that the nutrient concentrations for P, K and Mg were at optimum levels in both BioRichar enriched and non-enriched. However, when compared to adequate standard level, it was found that Ca content in leaf was very low for all treatments. The symptom of nutrient deficiency was clearly shown by plant grown in non-enriched BioRichar and 1.5 t/ha as observed in Figure 2. The old leaves were observed to exhibit yellowing, chlorosis and necrosis that spread along the leaf base. The leaves were crumpled in appearance while the midrib bent leaving the leaves hanging on the pseudo-stem. Meanwhile, the non-enriched BioRichar plant showed prominent Ca deficiency by exhibiting general dwarfing (stunted growth), reduced leaf emission and reduced leaf length. The contents of micronutrient for Manganese (Mn) and Ferum (Fe) were found higher in 3.0 and 4.5 t/ha, respectively. However, there was no significant difference for the contents of Zinc (Zn) and Copper (Cu) for all the treatments.

Table 3. Effect of different rates of BioRichar on plant nutrition

Treatment (t/ha)	Macro nutrient (%)					Micro nutrient (mg/L)			
	N	P	K	Ca	Mg	Mn	Zn	Cu	Fe
0	0.72c	0.51a	11.33b	0.73a	0.31a	1.35a	0.13a	0.02a	1.31ab
1.5	0.72c	0.44b	14.62b	0.46b	0.20c	0.92b	0.14a	0.02a	1.58a
3.0	1.15b	0.49a	19.61a	0.77a	0.32a	1.44a	0.11a	0.02a	1.16b
4.5	1.28a	0.49a	19.92a	0.37b	0.25b	0.86b	0.12a	0.02a	1.47a
$LSD_{P<0.05}$	0.09^{***}	0.02^{***}	3.47^{***}	0.08^{***}	0.01^{***}	0.364^{*}	NS	NS	0.272^{*}

Means followed by the same letters within a column are not significantly different at ($P > 0.05$) by Least Significant Difference (LSD) with n=16. * and *** differ significantly at $P < 0.05$, and 0.001, respectively and NS = not significant.

Proline content and lipid peroxidation

High proline accumulation during stress was observed as an adaptive mechanism by which it served as a store of N and respiratory substrates to facilitate post stress recovery (Dix et al. 1986). Possible functions of proline are cytoplasmic osmoregulation, prevention of enzyme inactivation and stabilisation of biopolymers. Banana plant responded to different rates of BioRichar by a substantial decrease in leaf proline concentration with increasing BioRichar rate. Figure 4 (A) showed variations in proline accumulation ranging between 34.2 and 38.1 µmol/g during 12 weeks after transplanting (WAT). Control plant had higher proline content by 9.43% compared to BioRichar enriched plant. This might be due to stress of the plant over nutrient deficiency and osmotic stress. A study by Hafeez et al. (2017) also found similar results of decreased proline content in *Glycine max* L. by application of biochar at the rate of 20 t/ha. Zhang et al. (2006) discussed that accumulation of proline content usually occurred with decreased water supply to the plants. Plants exhibited stress due to water stress and nutrient stress are more frequently found to have higher proline accumulation in the leaf tissues. Based on this result, it is believed that application of BioRichar in the ultisol soil ensured water availability, provided proper porosity and supplied the plants with good nutrients thus reduced the proline accumulation. Thus, under this condition, BioRichar amendments have provided the plant with enough water due to its physical property of high water holding capacity characteristics. This feature reduced the development of stress and concurrently no increase in the proline content in BioRichar enriched plant was recorded compared to control plant with no BioRichar.

Malondialdehyde (MDA), a product of lipid peroxidation, has been considered as an indicator of oxidative damage (Neto et al. 2006). Figure 4 (B) showed the response of plant towards different rates of BioRichar treatments on MDA content. It was observed that lipid peroxidation was low at 4.5 t/ha BioRichar and the highest for control treatment. However, there was no difference in lipid peroxidation in 3.0 t/ha and control treatment. BioRichar reduced lipid peroxidation and hence reduced oxidative damage in banana plant. Wang et al. (2012) suggested that oxidative damage was minimised with the increase of enzymatic and metabolic responses. This result showed that proline accumulation increased as lipid peroxidation increased. Therefore, it is suggested that increased accumulation of proline helps to protect membranes from oxidation as a response to stress.

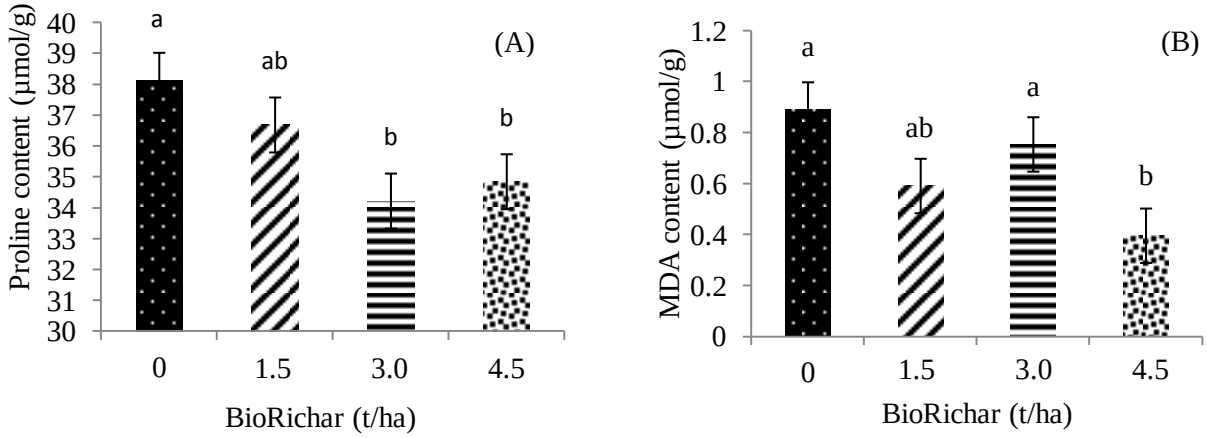

Figure 4. (A) Proline content and (B) MDA content of leaves as affected by four different rates of BioRichar. Mean values with the same letter within chart are not significantly different at ($P > 0.05$) using LSD.

Regression analysis for total leaf area

Leaf area determination is an important parameter for studies of crop growth performance, light interception, photosynthesis efficiency and evapotranspiration. Regression analysis was performed to establish relationship between effects of different rates of BioRichar on leaf area during 6 and 12 weeks after transplanting (WAT) as shown in Figure 5. It was clear from the trend and regression equation that the increasing rate of BioRichar significantly ($R^2 = 0.9881$; $P < 0.001$ and $R^2 = 0.9234$; $P<0.01$) increased the total leaf area at 6 WAT and 12 WAT, respectively. The increase in the leaf area could be explained by the improvement in various mineralogical properties of media by addition of BioRichar. Addition of BioRichar has improved soil porosity and water holding capacity which eventually improved soil aeration and root health and facilitated the plant growth (produced high leaf number and leaf area). Application of BioRichar at the highest rate of 4.5 t/ha seemed more superior than the other treatments in causing the growth of the leaves. Higher total leaf area during vegetative stage indicated that the plant has grown well.

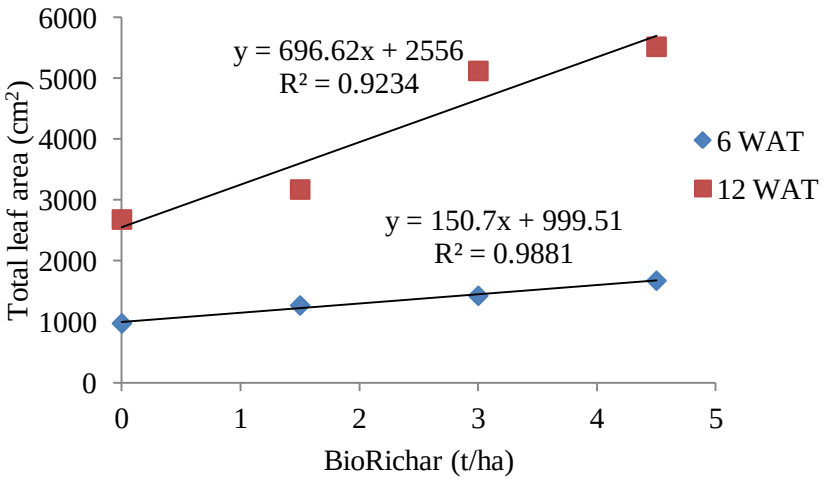

Figure 5. Regression analysis for total leaf area was performed during 6 and 12 weeks after transplanting (WAT)

CONCLUSION

The amendment of ultisol soil with BioRichar improved growth and nutritional properties in banana plant. The study revealed that an addition of BioRichar at 3.0 and 4.5 t/ha promoted growth and increased resistance of plant over nutrient stress. Addition of BioRichar had increased the pH and cation exchange capacity of low fertile soil. Enriched BioRichar soil also had improved nutrient concentration of the leaf at adequate level. However, N uptake by plants in BioRichar amended soils was still low, and therefore it was required to supplement BioRichar amended soils with N fertiliser to improve field growth of the crop. Nevertheless, application of 4.5 t/ha BioRichar was adequate for optimum growth at nursery level (vegetative stages up to 3 months) during acclimatisation period prior to field transplanting. Since the study had focused on the growth of banana at vegetative stage only, further work is required to investigate into the effect of supplementing BioRichar for banana planting in the field condition up to fruiting stage.

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