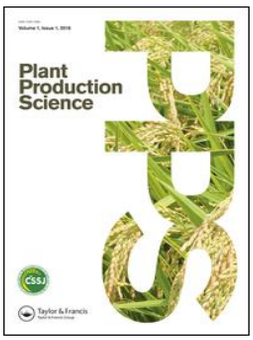
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Effects of Soil Types and Fertilizers on Growth, Yield, and Quality of Edible Amaranthus tricolor lines in Okinawa, Japan

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Effects of soil types and fertilizers on growth, yield, and quality of edible *Amaranthus tricolor lines* in Okinawa, Japan

Masanobu Ohshiro[a], Md. Amzad Hossain[b], Ichiro Nakamura[b], Hikaru Akamine[b], Masanobu Tamaki[b], Prasanta Chitta Bhowmik[c] and Akihiro Nose[d]

[a]The United Graduate School of Agricultural Sciences, Kagoshima University, Kagoshima, Japan; [b]Faculty of Agriculture, University of the Ryukyus, Nishihara City, Japan; [c]Department of Plant, Soil, and Insect Sciences, University of Massachusetts, Amherst, MA, USA; [d]Faculty of Agriculture, Saga University, Saga, Japan

ABSTRACT

Soil types and fertilizer regimes were evaluated on growth, yield, and quality of *Amaranthus tricolor* lines, IB (India Bengal), TW (Taiwan), BB (Bangladesh B), and BC (Bangladesh C) in developing management practices in Okinawa. Growth and yield of all amaranth lines were higher in gray soil (pH 8.4) than in dark red soil (pH 6.6) and red soil (pH 5.4). The combined NPK fertilizer resulted in highest growth parameters and yield of amaranths in all soils. Nitrogen fertilizer alone did not affect growth parameters and yield of amaranths in dark red and red soils. Growth parameters and yield increased similarly with the 30, 40, and 50 g m^{-2} of NPK fertilizer in BB line, and with the 20, 30, 40, and 50 g m^{-2} in BC line. Agronomic efficiency of NPK fertilizer at 50 g m^{-2} was not prominent on the amaranths, compared to the fertilizer at 40 g m^{-2}. Amaranth lines had higher Na in dark red and red soils, while K and Mg in gray soil, Ca in gray and red soils, and Fe in dark red soil. The NPK fertilizer resulted in higher Na, Ca, Mg, and P in BB line in glasshouse. These minerals in BB line were not clearly affected, but in BC line were lower with NPK fertilizer at 20–50 g m^{-2} in field. These studies indicate that gray soil is best for amaranth cultivation and combined NPK fertilizer at 20–40 g m^{-2} is effective in gray soil in Okinawa for higher yield and minerals of amaranth.

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Amaranthus; fertilizers; minerals; soil types; yield

Growth, yield, and quality of a plant species differ with soil types, soil nutrient status, and fertilizer management; and a plant species requires suitable soil for higher yield and better quality (Akamine et al., 2007; Chowdhury et al., 2008; Hossain & Ishimine, 2005; Hossain et al., 2011; Islam et al., 2011; Oya, 1972; Oya et al., 1977). Soil fertility and crop productivity differ significantly with the amount and combination of Na, K, Ca, Mg, S. P, Fe, Al, pH, and N in soil (Broadley et al., 2012a, 2012b; Hawkesford et al., 2012; Oya, 1972). Study on growth characteristics of a plant species in local soils is important to develop management practices for higher yield with good quality (Hossain & Ishimine, 2005).

Different plant species respond differently to fertilizer rates and combination and a plant species requires balanced fertilizers to maximize growth, yield, and quality (Akamine et al., 2007; Chowdhury et al., 2008; Hafsi et al., 2011; Hossain et al., 2004). The major nutrients (N, P, K) individually or in combination maintain growth, yield, and quality of plants (Hafsi et al., 2011; Ivonyi et al., 1997; Mazid, 1993; Nakano & Morita, 2009). Nitrogen influences chlorophyll formation, stomatal conductance, and photosynthetic efficiency, which is responsible for 26–41% of crop yield (Ivonyi et al., 1997; Maier et al., 1994). Potassium plays catalytic roles and regulates functions of various minerals in plants, and promotes N uptake efficiency of plants. Insufficient K causes shoot yellowing, poor growth, and low resistance to cold and drought of plant (Oya, 1972). Phosphorus promotes absorption of other nutrients and plant growth (Akamine et al., 2007).

Amaranthus, a genus consisting of more than 50 species, is an important promising food crop for its resistance to heat, drought, diseases and pest, and high nutritional value (Rastogi & Shukla, 2013; Sreelathakumary & Peter, 1993; Svirskis, 2003). *Amaranthus* species are severe weeds in crop fields, which significantly reduce yield and quality (Guo & Al-Khatib, 2003; Holm et al., 1977). Many *Amaranthus* species have been cultivated as vegetable and grain in many countries and are popularly consumed as vegetable in Africa, Bangladesh, Caribbean, China, Greece, India, Nepal, and South Pacific Islands (Begum, 2000; Prakash & Pal, 1991; Stalknecht & Schulz-Schaeffer,

CONTACT Md. Amzad Hossain amzad@agr.u-ryukyu.ac.jp

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1993; Svirskis, 2003). Vegetable amaranth is equal or superior in taste to spinach (*Spinacia oleracea*), which has higher carotenoids (90–200 mg kg^{-1}), protein (14–30%), and ascorbic acid (28 mg 100 g^{-1}) (Abbott & Campbell, 1982; Prakash & Pal, 1991). Some amaranth species contains 11.94 mg β-carotene, 43 mg vitamin C, 374 mg Ca, 5.0 g carbohydrate, 5.3 g protein, 0.1 g fat, and 43 kcal per 100 g of dry edible portion (Begum, 2000; Makus, 1984; Shittu et al., 2006; Shukla et al., 2005). *Amaranthus* species also contains various volatiles and polyphenols, and has antioxidant, antimalarial, and antiviral properties, which prevent cancer, cardiovascular diseases, diabetes, etc. (Dasgupta & De, 2007; Jiang et al., 2011; Khandaker et al., 2008; Scalbert et al., 2005; Shukla et al., 2010). It was reported that amaranth contains protein, ascorbic acid, and mineral nutrients of Ca, Fe, Mg, P, K, and Na, which are considered as the nutritional value in vegetables (USDA, 1984).

Amaranthus grows very fast in tropical and subtropical areas, and is cultivated in many countries under a variety of soils and agroclimatic conditions during summer when vegetables are not available (Begum, 2000; Makus, 1984; Singh & Whitehead, 1996). In Okinawa, some *Amaranthus* species are found as weed in various crops and vegetables (personal survey) in the major soil types, dark red soil, red soil, and gray soil, and summer vegetables are very limited in supply during this period (Hossain & Ishimine, 2005; Okinawa Prefecture Agriculture, Forestry and Fisheries, 2008). We evaluated growth speed, yield per plant, total nutrient (minerals) per plant, and total L-ascorbic acid per plant of 12 amaranth lines cultivated under a management condition, and selected some high-yielding amaranth lines with high quality as summer vegetables in Okinawa (Ohshiro et al., in press). Shittu et al. (2006) reported that balanced fertilizers in a specific soil provide higher yield and nutrient compositions of amaranth in Nigeria, but no study has yet been conducted on the selected amaranth lines regarding these factors in Okinawa. It is thought that growth, yield, and quality of amaranth plants differ with chemical fertilizers and soil types possessing different levels of minerals, pH, and N. Therefore, the objectives of these studies were to (i) identify the best soil type, and (ii) evaluate rates of fertilizer combinations on growth, yield, and quality of four selected amaranth lines for developing management practices in Okinawa.

Materials and methods

Soil collection

Dark red soil (Shimajiri mahji) and gray soil (Jaagaru) were collected from the top 50-cm layer of fields at the Subtropical Field Science Center, University of the Ryukyus, and red soil (Kunigami mahji) from the same layer of a field in northern part of Okinawa, Japan. Chemical properties of the soils are presented in the Table 1. According to Hossain and Ishimine (2005), coarse sand, fine sand, silt, clay, and apparent density are 3.61%, 30.94%, 24.32%, 32.84%, and 0.90 g cm^{-3}, respectively, for the gray soil; 2.93%, 7.33%, 23.94%, 57.24%, and 0.87 g cm^{-3}, respectively, for the dark red soil; and 16.92%, 20.44%, 26.62%, 30.92%, and 0.92 g cm^{-3}, respectively, for the red soil.

Amaranth lines

The *Amaranthus tricolor* lines IB (India Bengal line, red leaf amaranth), TW (Taiwan line, green leaf amaranth), BB (Bangladesh B line, red stem amaranth), and BC (Bangladesh C line, red leaf amaranth) provided higher yield in our previous study (Ohshiro et al., in press) were evaluated in this study.

Glasshouse experiment: effects of soil types on amaranth 4 lines

A glasshouse experiment was conducted using gray soil, dark red soil, and red soil at the Subtropical Field Science Center of the University of the Ryukyus, from 10 July to 30 August 2011. Each planter (planter-65E type, IRIS Ohyama, Japan) was filled with 13 kg of air-dried soil; and seeds of the amaranth 4 lines were placed on soil surface and covered with a thin layer (<0.5 mm) of soil. The plants were thinned to eight healthiest stands per planter at 2- to 3-leaf stage. Each soil treatment consisted of three planters (replications). The planters were arranged randomly in the house. Water was applied as required every day. Fertilizer was not applied during the course of the experiment in order to determine the actual effect of three Okinawan soils on the amaranth lines.

Table 1. Mineral contents (mg kg^{-1}) and percent of total N and C in gray, dark red, and red soils in Okinawa, Japan.

Soil types	Na (mg kg^{-1})	K (mg kg^{-1})	Ca (mg kg^{-1})	Mg (mg kg^{-1})	Al (mg kg^{-1})	Fe (mg kg^{-1})	P (mg kg^{-1})	pH	N %	C %
Gray	3.59c	2.96a	24.30b	1.53b	0.04a	0.19b	0.06a	8.4	0.12a	1.80a
Dark red	7.34a	1.29b	8.78c	1.84a	0.00b	0.00b	0.00c	6.6	0.08b	0.26b
Red	4.51b	0.33c	29.50a	1.26c	0.04a	0.30a	0.01b	5.4	0.03c	0.11c

Data are means of three replications. Data with the same letters within each column are not significantly different at the 5% level, as determined by LSD test.

Glasshouse experiment: evaluation of fertilizer on amaranth BB line plants grown in different soil types

In experiment 1, we evaluated growth characteristics, yield, and quality of the amaranth 4 lines in gray soil, dark red soil, and red soil without fertilizer application. In this study, fertilizer regimes were evaluated on an amaranth line in the same three soils. The glasshouse experiment was conducted using the same soils and planter at the Subtropical Field Science Center of the University of the Ryukyus, from 25 September 2011 to 15 December 2011. Each planter was filled with 13 kg of air-dried soil. The experiment consisted of four treatments with three replications. The treatments were CONT (control, 0 g m^{-2}), LN (low nitrogen, 50 g N m^{-2} or 5 g N $planter^{-1}$), HN (high nitrogen, 100 g N m^{-2} or 10 g N $planter^{-1}$), and NPK (150 g NPK m^{-2} or 5 g N + 5 g P + 5 g K $planter^{-1}$). The planters were arranged randomly in the house. The fertilizers of N ($CO(NH_2)_2$), P_2O_5 ($CaH_4(PO_4)_2H_2O$), and K_2O (KCl) were mixed with the soil before seed sowing according to the experiment design. Seeds of amaranth BB line were sown according to previous experiment. The plants were thinned to 12 healthiest stands per planter at 2- to 3-leaf stage. Soil surface area of a planter was 0.1 m^{-2} (57.0 × 17.5 cm), and 100–150 plants are usually cultivated m^{-2}. Plant density and fertilizer rates per planter were calculated following the above facts. Water was applied as required.

Field experiment: effects of fertilizer rates on amaranth BB and BC lines grown in gray soil field

Experiments 1 and 2 showed that amaranth grew better in gray soil, and combined NPK fertilizer resulted in the highest yield in all soils. Therefore, field experiment was carried out to evaluate rates of combined NPK fertilizer in gray soil at the Subtropical Field Science Center of the University of the Ryukyus, from 19 April 2012 to 13 July 2012. The field was properly prepared, and plots of 1 × 1 m (1 m^2) were made using rope. Seeds of amaranth BB and BC lines were sown in five rows (20 cm row spacing) per plot according to previous experiments. The plants were thinned to 20 healthiest stands per row at 2- to 3-leaf stage. The experiment was a randomized block design, having five treatments with four replications. The fertilizer treatments were 0 g m^{-2} (control), 20, 30, 40, and 50 g m^{-2}. The fertilizers of N ($CO(NH_2)_2$), P_2O_5 ($CaH_4(PO_4)_2H_2O$), and K_2O (KCl) were applied 20 days after seed sowing at the ratio of N:P:K = 1:1:1. Water was applied as required for proper seed germination and plant growth.

Data collection

Leaf amaranth is usually harvested at 20–35 cm in height; and both the leaf and stem are used as vegetable. Stem amaranth is usually harvested at young stage (20–35 cm) for using both leaf and stem, and at pre-flowering stage (semi mature plant) for using only stem. Plant height and leaf number were recorded up to 34 days after seed sowing (DAS) at 7-day interval in experiment 1. The plants were harvested at 34 DAS, and plant height, stem diameter, leaf number, leaf area, and fresh and dry weights of leaf, stem, and shoot were determined. In experiment 2, plant height and leaf number were measured at 28, 33, 38, and 43 DAS. Five plants were harvested from each planter at 34 DAS, and similar growth parameters were determined. In experiment 3, plant height and leaf number per plant were measured at 7-day interval from 25 to 43 DAS. Ten plants were harvested from the center row of each plot at 34 DAS, and similar growth parameters were determined.

Determination of leaf area and dry weights of amaranth plants

Leaf area was measured with an automation area meter (AAM-8, Hayashi Denkoh Co. Ltd.). Leaf and stem of amaranth plants were dried separately at 80 °C for 48 h using forced convection oven (DRLF23WA, Advantec) for dry weight measurement.

Determination of pH, mineral contents, nitrogen, and carbon of soil and nutrient status and L-ascorbic acid of amaranth plants

Soil samples were dried at room temperature of 25–28 °C for 5 days, and leaf and stem of amaranths were dried at 60 °C for 48 h using the same forced convection oven. Soil, leaf, and stem were ground finely for chemical analysis. Dried soil powder of 20 g was diluted with 50-ml distilled water, and the solution was shaken for 1 h using an electrical shaker at 170 rpm (Neo-Shaker, NSA-SNH, As One Corp. Ltd.). The solution was filtered with paper No. 2, and then centrifuged for 90 min using a centrifuge (Table Top Centrifuge 4000, Kubota Co. Ltd.) at 3500 rpm followed by filtering with the disposable syringe filter 0.45 µm (Advantec Co. Ltd.). Soil pH was determined with TOA pH meter (HM-20S, Toa Electronic Ltd.).

Soil sample of 10 g and distilled water of 50 ml were taken into a beaker, and the solution was shaken for 1 h using the same shaker at 170 rpm. The solution was filtered with paper No. 2, and then centrifuged for 90 min using the centrifuge at 3500 rpm followed by filtering with the disposable syringe filter 0.45 µm (Advantec Co. Ltd.). Plant (edible shoot, stem plus leaf) powder of 0.25 g was taken into a 50-ml beaker, and the beaker was filled with 0.5% nitric acid (HNO_3). For extracting elements, the beakers were kept into water bath adjusted to 80 °C for 24 h, and the solution was then filtered sequentially with paper No.

2 and disposable syringe filter 0.45 µm. The plant and soil solution was diluted as necessary by adding deionized water for determining mineral/nutrient elements. Mineral contents of soil and nutrients of amaranth were determined with Inductively Coupled Plasma Spectrometer (ICPS-8100, Shimadzu Co. Ltd.). Mineral contents per kilogram (kg) of soil and nutrient contents per gram (g) of dry amaranth (edible shoot, leaf plus stem) were calculated. Percent of total C and N in the soils and amaranths were determined with Gas Chromatograph (Soil GS-8A, Shimadzu Co. Ltd., NC-220F Juka analysis center) and Sumigraph (NC-90A, Shimadzu Co. Ltd.).

Statistical analysis

Average data for each replication were calculated, and then mean and standard deviation (SD) of the replications were determined using analysis of variance. Fisher's protected least significance difference (LSD) test at the 5% level was used to compare treatment means.

Results

Glasshouse experiment: effects of soil types on growth, yield, nitrogen, carbon, and nutrient contents of amaranth 4 lines

All *amaranth* plants grew faster and healthier with larger stem diameter and longer internode in gray soil than in dark red soil and red soil (Figure 1, numerical data not presented). Plant height, leaf number, leaf area, and dry shoot weight of the amaranth lines were 2–4 times higher in gray soil than in other soils (Figure 2).

The amaranth lines IB, TW, and BC contained highest Na when grown in red soil followed by dark red soil. The BB line contained higher Na in dark red and red soils (Table 2). Potassium content of the lines was 4–6 times higher in gray soil and Ca was higher in gray and red soils compared to those in other soils. All amaranth lines received highest Mg when grown in gray soil compared to that in other soils (Table 2). The lines TW and BC had almost similar Mg in dark red soil and red soil, whereas the lines IB and BB had higher Mg in red soil than in dark red soil. All the amaranth lines contained higher Al in gray and dark red soil compared to that in red soil (Table 2). All the lines contained highest Fe in dark red soil. Phosphorous content of IB line did not differ when grown in different soil types. The TW line had higher P in gray and dark red soils, while BB and BC lines accumulated higher P from dark red and gray soil (Table 2).

The amaranth lines IB, BB, and BC contained highest N (%) in dark red soil, while TW line had higher N in both dark red and red soils (Table 2). Percent of C in amaranth plants was lowest when cultivated in gray soil (Table 2).

Glasshouse experiment: Evaluation of fertilizer on growth, yield, nitrogen, carbon, and nutrient contents of amaranth BB line plants grown in different soil types

Plant height increased with all fertilizer treatments in gray soil, but only with the NPK combination in dark red soil and red soil (Figures 3 and 4A). Plant height was 2–7 times higher in dark red soil and red soil when grown with combined NPK fertilizer as compared with other fertilizer treatments. Stem diameter increased with the LN and NPK treatments in gray soil, but only with the combined NPK fertilizer in other soils (Figure 4B). Leaf number increased similarly with LN, HN, and NPK treatments in gray soil. Highest number of leaves was noted with the NPK combination in dark red soil and red soil. Leaf number was 2–5 times higher with the NPK combination than with the control plant in dark red and red soils (Figure 4C). Total leaf area per plant was higher for all the fertilizer rates in gray soil, which was highest for the NPK followed by LN. The

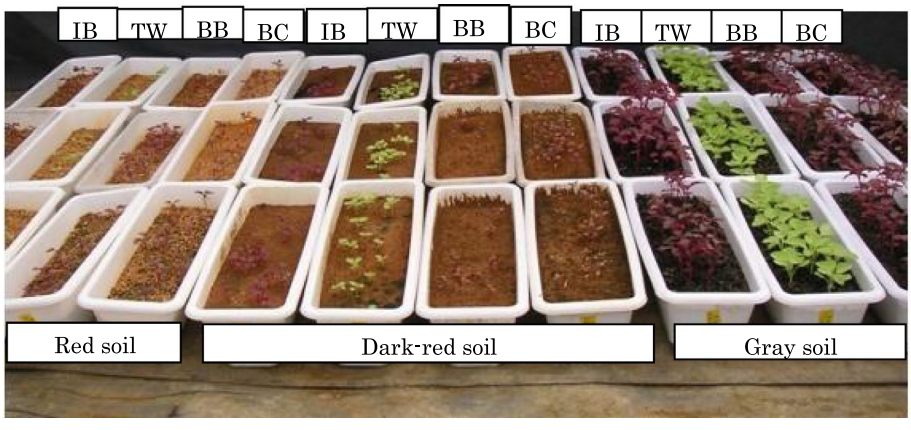


Figure 1. Growth of amaranth 4 lines in red soil, dark red soil, and gray soil in Okinawa at 34 days after seed sowing (DAS).

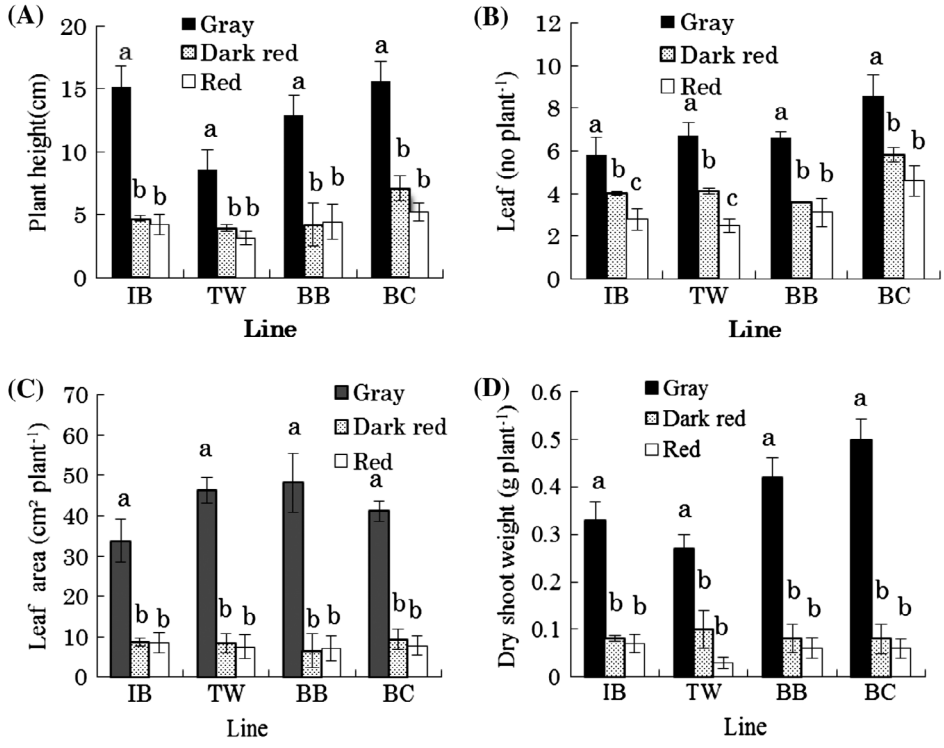


Figure 2. Plant height (A), leaf number (B), leaf area (C), and dry shoot weight (D) of 35-day-old plants of amaranth lines grown in different soils. Bars with the same letter are not significantly different within each line at the 5% level, as determined by LSD test.

Table 2. Various nutrient contents (mg g DW^{-1}) and percent of total N and C in edible shoot of amaranth plants of 4 lines cultivated in gray, dark red, and red soils in Okinawa.

		Na	K	Ca	Mg	Al	Fe	P		
Line	Soil	mg g DW^{-1}	mg g DW^{-1}	mg g DW^{-1}	mg g DW^{-1}	mg g DW^{-1}	mg g DW^{-1}	mg g DW^{-1}	N %	C %
IB	Gray	6.68b	223a	132a	56a	7.95a	2.27b	11.6a	1.47b	37.2c
	Dark red	7.48b	51b	86b	31c	4.58b	4.69a	13.2a	1.76a	39.1a
	Red	16.05a	51b	116a	43b	2.16c	1.07c	11.2a	1.45b	38.1b
TW	Gray	8.13b	263a	148a	56a	3.39a	1.20b	15.8a	1.90b	36.6c
	Dark red	11.80b	68b	108b	42b	3.66a	4.34a	18.0a	2.14a	37.8a
	Red	21.75a	69b	141a	43b	1.83b	1.34b	12.9b	2.18a	37.1b
BB	Gray	8.84b	262a	169a	64a	11.50a	2.02b	14.0b	1.56b	36.3b
	Dark red	17.93a	65b	107b	36c	8.11b	5.75a	18.2a	2.31a	36.9a
	Red	15.53a	73b	147a	44b	1.71c	1.08b	13.3b	1.76b	37.2a
BC	Gray	9.16c	205a	107a	62a	11.80a	1.57b	12.8a	1.29b	37.2c
	Dark red	12.80b	29b	55b	35b	10.57a	5.88a	10.9b	1.53a	37.9b
	Red	20.80a	33b	93a	31b	2.78b	3.03b	10.7b	1.30b	38.2a

Notes: IB, India Bengal line; TW, Taiwan line; BB, Bangladesh B line; BC, Bangladesh C line. Data with the same letter within each column for each line are not significantly different at the 5% level, as determined by LSD test. DW represents dry weight.

leaf area with NPK treatment was larger about 7 times in dark red soil and 10 times in red soil, as compared to that with other treatments (Figure 4D).

Dry leaf weight and dry shoot weight increased with all the fertilizer treatments, which were highest for the NPK followed by LN treatment in gray soil (Figure 4E and F). Dry leaf weight and dry shoot weight increased with only NPK in dark red and red soils (Figure 4E and F). Both the dry leaf weight and dry shoot weight increased to about three times with the combined NPK fertilizer application as compared with control treatment. Dry leaf weight increased to about 16 times in dark red soil and 20 times in red soil, and dry shoot weight increased to about 25 times in dark red soil and 30 times in red soil with the application of combined NPK fertilizer as compared with other treatments (Figure 4E and F).

Sodium, Ca, and P content in edible shoot of amaranth increased with NPK combination in gray soil, and the treatments both LN and HN did not show clear effect on mineral accumulation in the plants (Table 3). In general, K, Al, Fe, Mn, and Zn contents in plants were not influenced by the fertilizer treatments. However, Mg, N, and C content

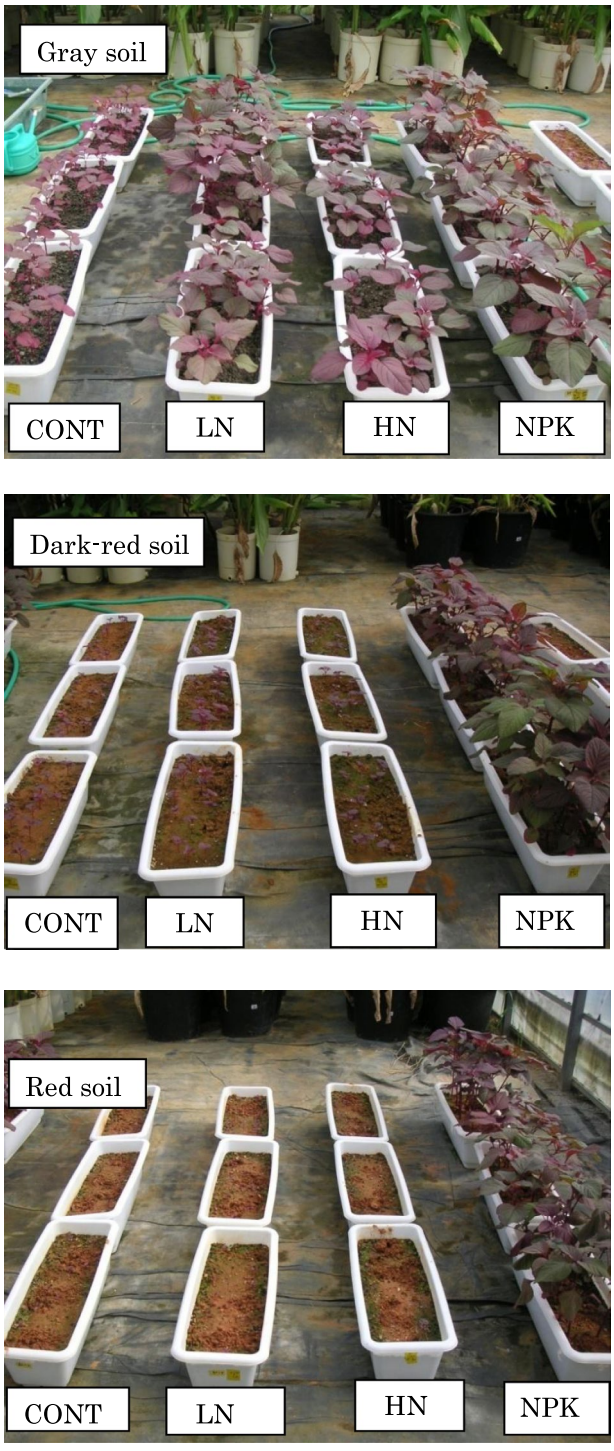


Figure 3. Effects of nitrogen rates and NPK combined fertilizer {CONT (control, 0 g m^{-2}), LN (low nitrogen, 50 g N m^{-2} or 5 g N $planter^{-1}$), HN (high nitrogen, 100 g N m^{-2} or 10 g N $planter^{-1}$), and NPK (150 g NPK m^{-2} or 5 g N + 5 g P + 5 g K $planter^{-1}$)} on growth of 35-day-old amaranth BB line plants grown in gray soil, dark red soil, and red soil.

in amaranth plants increased with all fertilizer treatments, and percent of total N was highest with NPK treatment (Table 3). The amaranth plants cultivated in dark red soil and red soil with the control, LN and HN treatments were not analyzed because of insufficient samples.

Field experiment: effects of fertilizer rates on growth, yield, mineral, nitrogen, and carbon contents of amaranth BB and BC lines grown in gray soil field

In general, plants were similar in height with all fertilizer rates at 38 days after seed sowing (DAS), whereas the plants were taller at 43 DAS with the 50 g m^{-2} followed by 40 and 30 g m^{-2} in BB line and with the 50 g m^{-2} followed by 40 g m^{-2} in BC line. Leaf number per plant increased with the fertilizer 40–50 g m^{-2} in BB line but not in BC (data not presented).

All growth and yield parameters of BB line increased with increasing fertilizer rates (Table 4). Stem diameter, internode length, largest leaf area, and total leaf area were higher in plants with the fertilizer of 30, 40, and 50 g m^{-2}, and these treatments had similar parameters. The fertilizer rates of 30, 40, and 50 g m^{-2} provided higher dry shoot weight, and these rates had similar value (Table 4). The growth parameters and yield of amaranth BC line increased almost similarly with all fertilizer rates, as compared to those with the control plant (Table 4).

The contents of all minerals except K in edible shoot of amaranth BB and BC lines were the same or somewhat lower with the fertilizer rates, as compared with the control plant (Table 5). Potassium content of the plants increased significantly or non-significantly with all the fertilizer rates. Percent of total N increased with the fertilizer rates in both the BB and BC lines. Carbon content was not clearly influenced with the fertilizer rates (Table 5).

Discussion

Glasshouse experiment: effects of soil types on growth, yield, nitrogen, carbon, and nutrient contents of amaranth 4 lines

Higher N content in gray soil resulted in higher growth parameters and healthiest shoot of the amaranth lines compared to that in other soils (Table 1 and Figure 2). Similarly, several studies reported that N is more significant than other nutrients for vegetative growth of plants (Akamine et al., 2007; Chowdhury et al., 2008; Hossain et al., 2011, 2012; Sarker et al., 2002). In addition, higher K, P, N, and pH, and lower Na probably made better combination in gray soil for better growth of amaranths, as compared to those in other soils (Table 1 and Figure 2). Other studies reported similar effects of balanced nutrients for higher biomass production in various crops (Akamine et al., 2007; Hao & Papadopoulos, 2004; Hossain et al.,

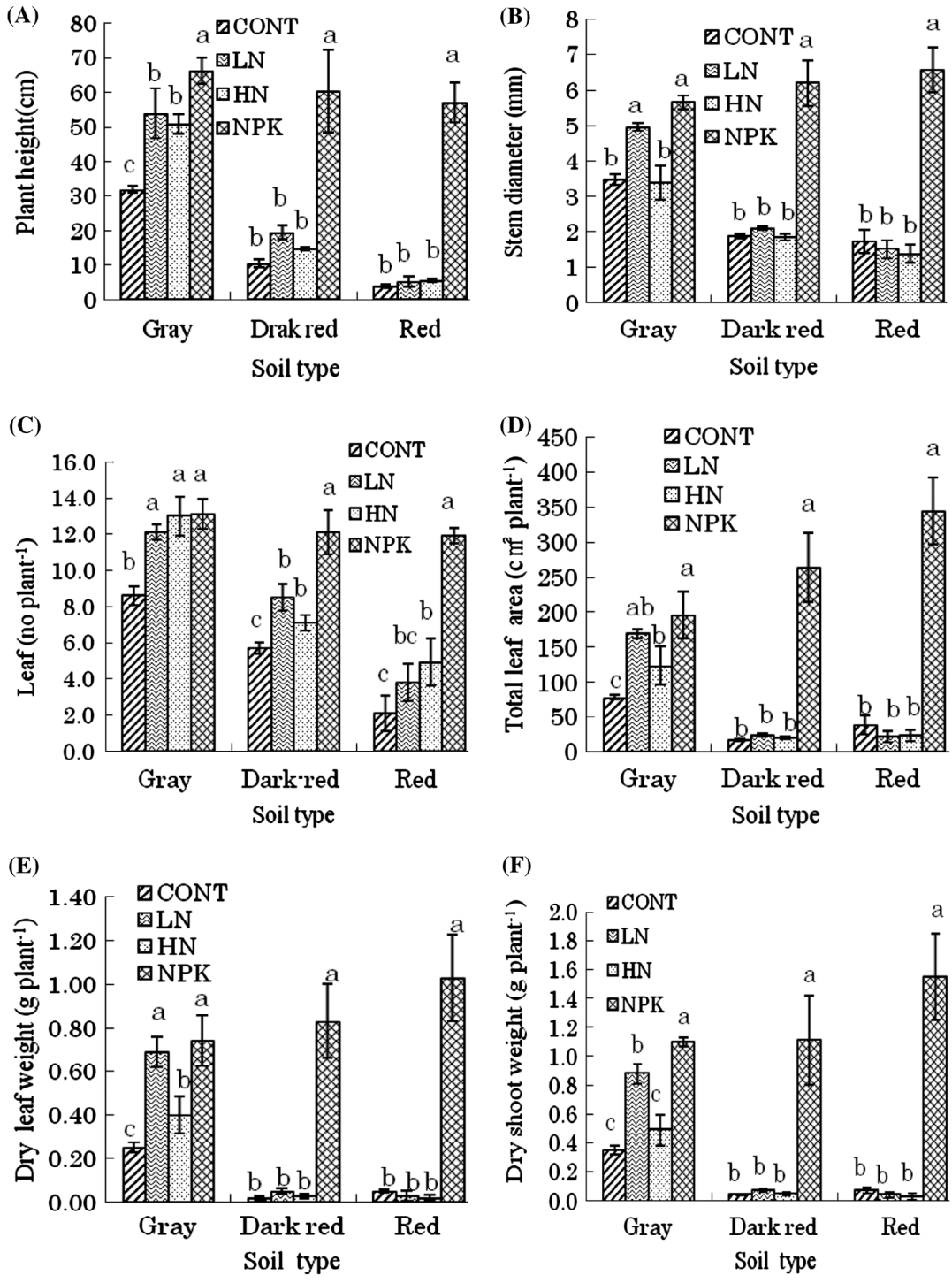


Figure 4. Effects of various rates of fertilizer {CONT (control, 0 g m^{-2}), LN (low nitrogen, 50 g N m^{-2} or 5 g N $planter^{-1}$), HN (high nitrogen, 100 g N m^{-2} or 10 g N $planter^{-1}$), and NPK (150 g NPK m^{-2} or 5 g N + 5 g P + 5 g K $planter^{-1}$)} on plant height (A), stem diameter (B), leaf number (C), total leaf area (D), dry leaf weight (E), and dry shoot weight (F) of amaranth BB line plants grown in gray soil, dark red soil and red soil. Bars with the same letter within each soil type are not significantly different at the 5% level, as determined by LSD test.

2012; Mazid, 1993; Oya, 1972). The pH 8.4 in gray soil was probably better for amaranth growth than in dark red soil (pH 6.6) and red soil (pH 5.4). Yield and quality of *Curcuma longa*, *Amaranthus cruentus,* and *Celosia argentea* were affected with field conditions, soil types, and soil chemical properties as reported earlier (Hossain & Ishimine, 2005; Shittu et al., 2006).

Gray soil had optimum moisture than other soils (hand feeling), which contributed to greater vegetative growth and shoot biomass of the plant. On the other

Table 3. Effects of fertilizers on nutrient contents (mg g DW^{-1}) and percent of total N and C in edible shoot of amaranth BB line grown in gray, dark red, and red soils.

Soil type	Treatment	Na (mg g DW^{-1})	K (mg g DW^{-1})	Ca (mg g DW^{-1})	Mg (mg g DW^{-1})	Al (mg g DW^{-1})	Fe (mg g DW^{-1})	P (mg g DW^{-1})	Mn (mg g DW^{-1})	Zn (mg g DW^{-1})	N %	C %
Gray	Cont	9.63b	128a	107b	65b	3.10a	0.04a	4.4b	1.90a	0.80ab	2.33c	36.0b
	LN	9.11b	160a	129ab	74ab	3.20a	0.07a	5.0b	1.89a	0.88a	4.66b	37.7a
	HN	9.08b	172a	111b	80a	3.06a	0.08a	5.0b	1.90a	0.66b	4.72b	37.4a
	NPK	11.30a	126a	147a	79a	3.41a	0.07a	6.8a	1.87a	0.75ab	5.25a	37.4a
Dark red												
	NPK	11.60	89	75	31	3.38	0.32	7.6	0.25	1.25	5.14	38.5
Red												
	NPK	15.90	56	175	58	1.81	0.26	7.4	1.68	0.81	4.78	38.4

Notes: Data with the same letter within each column are not significantly different at the 5% level, as determined by LSD test. Data were not recorded for the control, LN and HN in Dar-red soil and Red soil due to poor growth of plants (sample not enough). DW represents dry weight.

Table 4. Effects of NPK combined fertilizer rates on growth parameters and yield of BB and BC amaranth lines grown in gray soil field.

Line	Fertilizer level (g m^{-2})	Stem diameter (mm)	Internode length (cm)	Largest leaf area (cm^2 $leaf^{-1}$)	Total leaf area (cm^2 $plant^{-1}$)	Dry leaf weight (g $plant^{-1}$)	Dry stem weight (g $plant^{-1}$)	Dry shoot weight (g $plant^{-1}$)
BB	0	3.7d	0.9c	23c	83c	0.2d	0.2c	0.4c
	20	5.0cd	1.5bc	34bc	107bc	0.4cd	1.4bc	1.8bc
	30	6.0abc	2.2ab	44ab	163ab	0.6bc	3.0ab	3.6ab
	40	5.8bc	2.4a	47ab	165ab	0.7ab	4.1a	4.8a
	50	7.3a	2.4a	56a	204a	0.9a	4.5a	5.4a
BC	0	3.1b	0.7b	14b	45b	0.2b	0.4b	0.6b
	20	5.4a	1.6a	28a	105a	0.5a	1.3a	1.8a
	30	5.6a	2.0a	31a	109a	0.5a	1.6a	2.1a
	40	5.2a	1.8a	31a	114a	0.4a	1.5a	1.9a
	50	5.5a	1.8a	32a	127a	0.5a	1.6a	2.1a

Note: Data with the same letter within each column for each line are not significantly.
Different at the 5% level, as determined by LSD test.

Table 5. Effects of NPK combined fertilizer rates on nutrient status (mg g DW^{-1}) and percent of total N and C in edible shoot of amaranth BB and BC lines grown in gray soil field.

Line	Fertilizer level (g m^{-2})	Na (mg g DW^{-1})	K (mg g DW^{-1})	Ca (mg g DW^{-1})	Mg (mg g DW^{-1})	Al (mg g DW^{-1})	Fe (mg g DW^{-1})	P (mg g DW^{-1})	Mn (mg g DW^{-1})	Zn (mg g DW^{-1})	N %	C %
BB	0	9.36a	221b	105a	42a	8.56a	7.60a	20.7ab	0.24a	0.76a	3.11b	34.0a
	20	8.56ab	248b	95b	33b	4.12b	3.72b	18.2bc	0.16a	0.72a	3.66ab	34.9a
	30	8.52ab	259b	94b	42a	3.96b	3.48b	17.6c	0.16a	0.76a	4.08a	34.7a
	40	8.04b	245b	91b	35b	3.84b	3.44b	17.4c	0.16a	1.04a	4.04a	34.6a
	50	7.68b	338a	100ab	31b	2.92b	1.88c	21.4a	0.16a	0.88a	3.57ab	34.7a
BC	0	13.76a	231b	108a	60a	7.60a	3.24a	29.8a	0.16a	0.80a	4.27c	35.1a
	20	12.52bc	236b	91b	49b	2.88b	1.56b	22.2b	0.12a	0.64a	4.79b	35.3a
	30	11.60c	253a	66c	47b	2.20b	0.96c	20.2b	012a	0.60a	4.75b	34.9a
	40	12.96b	253a	91b	49b	2.76b	1.44bc	21.0b	0.12a	0.60a	4.78b	35.3a
	50	12.84b	233b	91b	46b	2.64b	1.24bc	19.0b	0.12a	0.64a	5.45a	34.2a

Note: Data with the same letter within each column for each line are not significantly different at the 5% level, as determined by LSD test. DW represents dry weight.

hand, waterlogging was observed for a while in red soil after water application and the soil was compact when dried, which probably affected soil aeration, soil microbial activities, and nutrient absorption, and resulted in a lowest shoot biomass. The amount and ratio of coarse sand, fine sand, silt, and clay in gray soil are found to be better than that in other soils (Hossain & Ishimine, 2005), which maybe resulted in favorable environment for better nutrient absorption and growth of the amaranths. Red soil contains lowest clay (30.9%) and highest coarse sand (16.9%), and dark red soil contains highest clay (57.2%) and lowest coarse sand (2.9%) and fine sand (2.9) (Hossain & Ishimine, 2005), which may not be texturally favorable for nutrient absorption and plant growth. Similarly, Donald & Katherine (1999) reported that nutrient availability, absorption, and plant growth differ significantly with the physical, chemical, and biological factors of soil.

Some amaranth lines accumulated higher level of minerals with higher level of respective available mineral nutrients in the soils, but some other lines did not show similar trends (Tables 1 and 2), indicating that higher content of available soil minerals are not the common phenomena to increase mineral contents in all amaranth lines, and a certain level and combination of available soil minerals may be required to increase minerals in a plant species or variety, which agreed with the results in redflower ragleaf (*Crassocephalum crepidioides*), turmeric (*Curcuma longa*), red amaranth (*Amaranthus* sp.), and broccoli (*Brassica oleracea*) (Chowdhury et al., 2008; Johnson et al., 2003; Hossain & Ishimine, 2005; Hossain et al., 2011; Omirou et al., 2009). Gray soil, dark red soil, and red soil had different levels of pH which was probably another factor to influence mineral availability in the soils and affected mineral accumulation in amaranths, this result agreed with the result in another study (Donald & Katherine, 1999). Some studies reported that yield and quality of crops are positively or negatively correlated with soil chemical properties (Miyazawa et al., 2004; Shittu et al., 2006).

Gray soil contained higher total N but resulted in lower percent of N in the amaranth lines because shoot biomass was 2–4 times higher in gray soil than that in other soils. Similarly, N content (%) in *L. usitatissimum*, *C. longa,* and *C. crepidiodies* decreased with the increasing shoot biomass (Flénet et al., 2006; Hossain & Ishimine, 2005; Hossain et al., 2012).

Glasshouse experiment: evaluation of fertilizer on growth, yield, nitrogen, carbon, and nutrient contents of amaranth BB line plants grown in different soil types

All the growth parameters and yield (shoot) of amaranth plants increased with all the fertilizer treatments, which

were highest for the NPK followed by LN in gray soil, but only with the NPK in dark red and red soils (Figures 3 and 4). These results indicate that fertilizer requirements for optimum growth of amaranths differ with the soil types, and combined application of NPK is best for the growth of amaranth in all soil types, which agreed with the study on *C. crepidiodies* and turmeric plants (Akamine et al., 2007; Hossain et al., 2012). The treatment of HN resulted in lower shoot dry weight than LN, indicating that excessive level of N fertilizer without P and K was not balanced for plant to uptake necessary nutrients or proper photosynthetic function. Similarly, other studies reported that a plant species increases biomass with the increasing N application up to a certain level, thereafter decreases (Hossain et al., 2004; Nori et al., 2012; Olanite et al., 2010).

All growth parameters and shoot biomass of amaranth in the dark red and red soils were not affected by the N fertilizer applied alone (Figure 4). It is possible that K and P contents in the dark red and red soils were not enough to regulate the function of N for promoting growth of the amaranth plant. It is also apparent that addition of just N fertilizer to the dark red and red soils created unbalanced nutrient combination which was not effective for the plant growth, optimum levels of P and K are also essential for photosynthesis (Donald & Katherine, 1999). Similar results of fertilizer effect were reported on redflower ragleaf (Hossain et al., 2012).

Sodium, Ca, and P contents, and percent of total N were highest in amaranths with NPK treatment in gray soil, indicating that the three fertilizer elements together influenced the plants effectively to accumulate higher minerals and N. The treatments LN and HN did not show clear effect on Na, Ca, and P accumulation in the plants, indicating that N without P and K was not effective for the mineral accumulation, and a specific combination of fertilizer elements is required for absorbing optimum nutrients from soil. Similar results with turmeric plants were reported by Akamine et al. (2007).

Field experiment: effects of fertilizer rates on growth, yield, mineral, nitrogen, and carbon contents of amaranth BB and BC lines grown in gray soil field

The fertilizer treatments of 30, 40, and 50 g m^{-2} resulted in a similarly higher growth parameters and dry shoot weight in BB line (Table 4), and agronomic efficiencies of the fertilizer treatments {(total yield in treated plant – total yield in control plant)/total fertilizer applied in treated plant} were 0.11 g g^{-1} fertilizer, 0.11 g g^{-1} fertilizer and 0.10 g g^{-1} fertilizer, respectively. Dry shoot was lower in the fertilizer rate of 30 g m^{-2} than those of 40 and 50 g m^{-2}. Dry shoot weight was similar with the fertilizer rates of 40 and 50 g m^{-2}, but agronomic efficiency tended to decrease with the 50 g m^{-2}, which indicate that the fertilizer rate of 40 g m^{-2} is better for BB cultivation. The growth parameters and yield of BC amaranth line increased almost similarly with all fertilizer levels (Table 4), but agronomic efficiency tented to decrease with the 40 and 50 g m^{-2}, indicating that the fertilizer rate of 20–30 g m^{-2} is better for BC cultivation in gray soil. Similar trend in agronomic efficiency of fertilizer was found in *Panicum repens*, *Sorghum almum,* and *Allium sativum* (Hossain et al., 2004; Nori et al., 2012; Olanite et al., 2010).

Mineral accumulation in both amaranth BB and BC lines were not clearly influenced with the fertilizer rates (Table 5). Similar result in some amaranth lines was reported by Miah et al. (2013). Percent of total N increased with the fertilizers in both the BB and BC lines, which agreed with the results in *S. almum* reported by Olanite et al. (2010).

Conclusion

The gray soil provided 2–4 times higher growth parameters and yield of all the amaranth lines than other soils. The amount and ratio of coarse sand, fine sand, silt, and clay in gray soil were better and this soil contained optimum soil moisture as compared to those in other soils, which maybe resulted in favorable environment for better nutrient absorption and growth of the amaranths. In addition, higher N, K, P, and pH, and lower Na in gray soil were favorable for higher growth parameters and yield of amaranths, as compared to those in dark red soil and red soil.

Nitrogen applied alone promoted growth and yield of amaranth plant in gray soil, but not in other soils, indicating that K and P contents in the dark red and red soils were not enough to regulate the function of N for promoting growth and yield of the plant. Combined application of N, P, and K fertilizers resulted in the highest growth parameters and yield of amaranths in all the three Okinawan soil types. The BB amaranth line obtained similarly higher growth parameters and yield with the combined fertilizer (NPK) rates of 30, 40, and 50 g m^{-2}, whereas the BC amaranth line obtained similarly higher growth parameters and yield with the 20, 30, 40, and 50 g m^{-2}. The BB amaranth line produced about two times higher yield which required higher rate of fertilizer than BC amaranth line, indicating that fertilizer requirement or rate differs with the plant size (plant biomass). Agronomic efficiency of the fertilizers decreased when applied at 50 g m^{-2} in both the BB and BC amaranth lines, indicating that the fertilizer rate of 20–40 g m^{-2} is better for cultivation of the amaranth lines.

The contents (%) of Na, K, and Ca nutrients in amaranth plants were higher when cultivated in the soils with higher level of available minerals Na, K, and Ca, but other nutrients in the amaranth plants did not show similar trends

in the soils with the respective minerals. Soil pH levels did not show any clear effect on nutrient accumulations in the amaranth plants. These results indicate that a specific level of minerals and pH in soil is required for maximizing nutrient accumulation in amaranth plants. Combined application of N, P, and K fertilizers resulted in higher level of Na, Ca, and Mg in the amaranth plants in all soils. The contents of nutrients in amaranth plants were almost similar with all the fertilizer (NPK) rates.

This study suggests that gray soil is better and combined fertilizers of N, P, and K are effective in all the major soil types in Okinawa for amaranth cultivation. Combined fertilizer (NPK) rate of 20–40 g m^{-2} is effective for higher yield and nutrients (minerals) of amaranth in gray soil in Okinawa.

Disclosure statement

No potential conflict of interest was reported by the authors.

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