STUDIES ON AMARANTH (*Amaranthus* spp.) CULTIVATION IN OKINAWA, JAPAN

（沖縄におけるアマランサスの栽培に関する研究）

THE DESSERTATION SUBMITTED TO THE GRADUATE SCHOOL

OF AGRICULTURAL SCIENCES, KAGOSHIMA UNIVERSITY

IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR

THE DEGREE OF DOCTOR OF PHYLOSOPHY

IN AGRICULTURE

MARCH 2016

BY

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REGISTRATION NUMBER: 3510710090

SCIENCE OF BIORESOURCE PRODUCTION

THE UNITED GRADUATE SCHOOL OF

AGRICULTURAL SCIENCES

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ENGLISH ABSTRACT

STUDIES ON AMARANTH (*Amaranthus* spp.) CULTIVATION IN OKINAWA, JAPAN

Amaranth (*Amaranthus* spp.) is popularly consumed as a vegetable in African and Asian countries, because this plant contains high protein, minerals, L-ascorbic acid and antioxidant properties. It is resistant to heat, drought and pest, and grows very fast under a variety of soils and agroclimatic conditions. Sun-radiation and air-temperature are high, and typhoon strikes several times in summer in Okinawa, which limit vegetable production. A series of experiments has been conducted to evaluate growth characteristics, yield and quality of some amaranth lines under seasonal variations, soil types and fertilizers to determine suitable lines, time, soil and fertilizer for amaranth cultivation as a vegetable in Okinawa.

Growth characteristics, yield and quality of seven amaranth lines Bangladesh B (BB), Bangladesh C (BC), Bangladesh Red (BR), India Bengal (IB), Vietnam (V), Taiwan (TW) and Biam Tricolor (BT) were evaluated to select suitable lines. The amaranth lines BB (stem vegetable) and BC (leaf vegetable) grew faster and had higher yield, minerals, crude protein and L-ascorbic acid than other lines.

Growth, yield and quality of amaranth lines BB and BC were evaluated in spring (April-June) and summer (August-September) seasons. Both amaranth lines required about 44 days in spring and 26 days in summer from seed sowing to harvest. Yield and L-ascorbic acid were higher when amaranths were cultivated in summer season.

Soil type (gray soil, pH 8.4; dark-red soil, pH 6.6; red soil; pH 5.4) and fertilizer (Cont (0 g m^{-2}), LN (nitrogen 50 g m^{-2}), HN (nitrogen, 100 g m^{-2}) and NPK (150 g m^{-2};

N:P:K=33.3:33.3:33.3)) regimes were evaluated on growth parameters, yield and quality of amaranth lines IB, TW, BB and BC. The amaranths grew faster, and had highest yield and minerals in gray soil than in other soils. The fertilizers LN and HN promoted yield in gray soil but not in other soils, and yield was highest with the fertilizer NPK in all soils.

Fertilizer regimes (Cont, N, P, K, NP, NK, PK and NPK; NPK at 0, 10, 20, 30, 40, 50, 60 and 80 g m^{-2} (N:P:K=33.3:33.3:33.3)) were evaluated on growth characteristics, yield and quality of amaranth lines BB and BC in gray soil, dark-red soil and red soil. The plants without N and P fertilizers did not grow well or died. Combined fertilizer NPK was the best for growth and yield of amaranths in all soils. Yield, some minerals and L-ascorbic acid were higher with the fertilizer NPK at 50-60 g m^{-2} in gray soil and 30-40 g m^{-2} in dark-red oil and red soil.

Overall results indicate that amaranth lines BB and BC are the best for yield, minerals, crude protein and L-ascorbic acid, which grow best in gray soil and summer season. Amaranth plants cannot grow well without N and P fertilizers. On the other hand, amaranth provides higher yield with N fertilizer in gray soil but not in other soils. Amaranth lines BB and BC provide higher yield and nutritional values with the combined fertilizer of NPK at 50-60 g m^{-2} in gray soil, and 30-40 g m^{-2} in dark-red soil and red soil in Okinawa.

JAPANESE ABSTRACT

沖縄におけるアマランサス栽培に関する研究

野菜アマランサス（*Amaranthus* spp.）は、高タンパク質でミネラル、アスコルビン酸に富み、抗酸化能を有する薬用効果を持つ野菜としてアジアやアフリカ地域で広く利用されている。耐暑性、耐乾性、耐病性や耐虫性を有し、多様な環境で生育する植物である。一方、沖縄では、強い日射、高温と台風が夏季の野菜生産を限定的なものとしてきた。そこで、沖縄の環境下に適した野菜アマランサスの系統、栽培時期、土壌および肥料の組合せを検討した。

まず、適性系統の選抜のためバングラデシュB（BB）、同C（BC）、同Red（BR）、インドベンガル（IB）、ベトナム（V）、タイワン（TW）およびバイアムトリカラー（BT）の7系統を用い、生育特性、収量および品質を検討した。その結果、BB（茎利用）とBC（葉利用）は他系統よりも生育が早く、収量が多く、ミネラル、粗タンパク質やアスコルビン酸含量が高いことが明らかになった。

次に、BB と BC の春作（4 月～6 月）と夏作（8 月～9 月）において、両系統とも収穫までに要する日数は、春作で 44 日、夏作で 26 日で、収量およびアスコルビン酸含量は、夏作で高くなった。

さらに、沖縄における３土壌（ジャーガル、pH8.4；島尻マージ、pH6.6；国頭マージ、pH5.4）において、IB、TW、BB と BC の生育パラメータ、収量、品質を比較したところ、ジャーガルでは、他の土壌より生育速度が早く、収量およびミネラル含量が高くなることがわかった。また、３土壌において BB に対する窒素単肥および混合肥料の効果を調べたところ、いずれの土壌においても混合肥料の施用により収量の増加を認め、特に島尻マージ、国頭マージでは欠かせない要素であった。

窒素、リン酸およびカリの各種組み合わせによる栽培試験を３土壌において行い、

生育特性等について調べたところ、いずれの土壌においても、窒素およびリン酸を施用しないと著しい生育不良を起こすことがわかった。収量、ミネラル含量およびアスコルビン酸含量を増加させるには、混合肥料（N:P:K＝33.3：33.3：33.3）をジャーガルにおいて 50-60g/㎡、島尻マージと国頭マージにおいては 30-40g/㎡の施用が有効であった。

以上の結果、沖縄の環境に適した野菜アマランサスとして、バングラデシュから収集した BB および BC を選抜し、さらに、沖縄の３土壌において、主に施肥量の観点から生育特性、収量、栽培時期、ミネラル含量、アスコルビン酸含量などを検討し栽培条件を明らかにした。

CHAPTER I

GERERAL INTRODUCTION

Amaranth (*Amaranthus* spp.), an annual plant of Amaranthaceae family (Bailey and Bailey 1978), is distributed widely from temperate zone to tropical zone (Iizuka 1987) in the world. Amaranth, a genus consisting of more than 60 species, is an important promising food crop for its resistance to heat, drought, diseases and pest, and high nutritional value (Sreelathakumary and Peter, 1993: Svirskis, 2003; Rastogi and Shukla, 2013). Some amaranth species are severe weeds in crop fields, which significantly reduce yield and quality of various crops (Holm et al., 1977; Guo and Al-Khatib, 2003). Many amaranth species are being cultivated as vegetable and grain in many countries, and popularly consumed as vegetable in Africa, Bangladesh, Caribbean, China, Greece, India, Nepal and South Pacific Islands (Prakash and Pal, 1991; Begum, 2000; Stalknecht and Schulz-Schaeffer, 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 and Campbell, 1982; Prakash and 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 100g of dry edible portion (Makus, 1984; Begum, 2000; Shukla et al., 2005; Shittu et al., 2006). *Amaranthus* species also contains various volatiles and polyphenols, and has antioxidant, antimalarial and antiviral properties, which prevent cancer, cardiovascular diseases, diabetes, etc. (Scalbert et al., 2005; Dasgupta and De, 2007; Khandaker et al., 2008; Shukla et al., 2010; Jiang et al., 2011). 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). Amaranth is also an important functional vegetable in tropical Asia (Iizuka and Nishiyama 2001; Shukla et al., 2006). Seeds of amaranth contain lysine, a kind of amino acids, and high protein, which give attention as the nutritional food (Iizuka 1987, Haruyama et al., 1990, Joshi and Rana 1991, Nemoto 1999, Katsuta et al.2001). Some amaranth species have colorful leaves, stems and inflorescences, which are used as the ornamental plants (Iizuka Nishiyama, 2001; Hoshikawa et al., 1989).Some amaranth species are used as animal feed.

Okinawa prefecture is situated in the subtropical area of the south-eastern part of Japan. The sun radiation intensity and air temperature are very high in summer season, especially from June to September. The average temperature in Okinawa ranges from 16.7 to 28.7 ℃ ((Fig. I. 1(A)). The higher temperature prevails from June to Okinawa, and it varies a little with the years. Average sun radiation is 7-23.4 11 $MJm^{-2}d^{-1}$, which is higher from June to September ((Fig. I. 1(B)). Average sun radiation varies significantly with the years, even with the months. Monthly average rainfall is 119 to 674 mm, which is high from May to August. Rainfall differs greatly with the years as well as months ((Fig. I. 2(A)). The average humidity is 61-85%, which is higher from May to August ((Fig. I. 2(B)). The vast differences in sun radiation, rainfall and humidity are found from May to September in Okinawa, which are not favorable for vegetable production in summer. In addition, typhoon strikes several times in summer seasons in Okinawa. These climatic factors limit the vegetable production in Okinawa. More than 80% of vegetables are imported to Okinawa in summer from main land Japan, and other neighborhood countries (Okinawa prefecture section of agriculture, forestry and fishery 2008, Kawamitsu et al., 2010). As amaranths is highly tolerant to heat and drought, and grows very fast in summer season (Rastogi and Shukla, 2013), it is thought that amaranth plant could be cultivated in Okinawa for supplying vegetable in summer season.

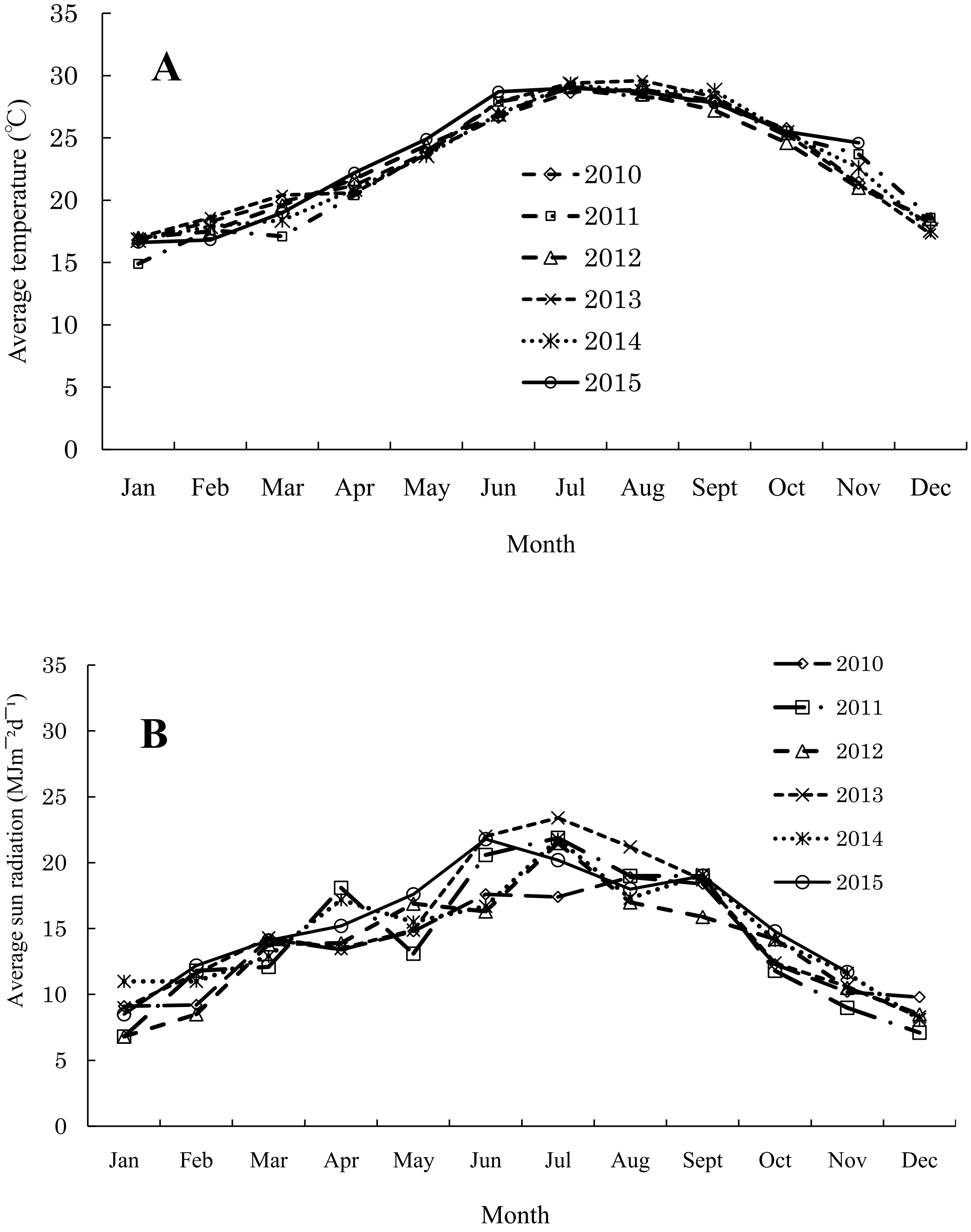


Fig. I. 1. Monthly temperature (A) and sun radiation (B) during experiments (2010-2015)

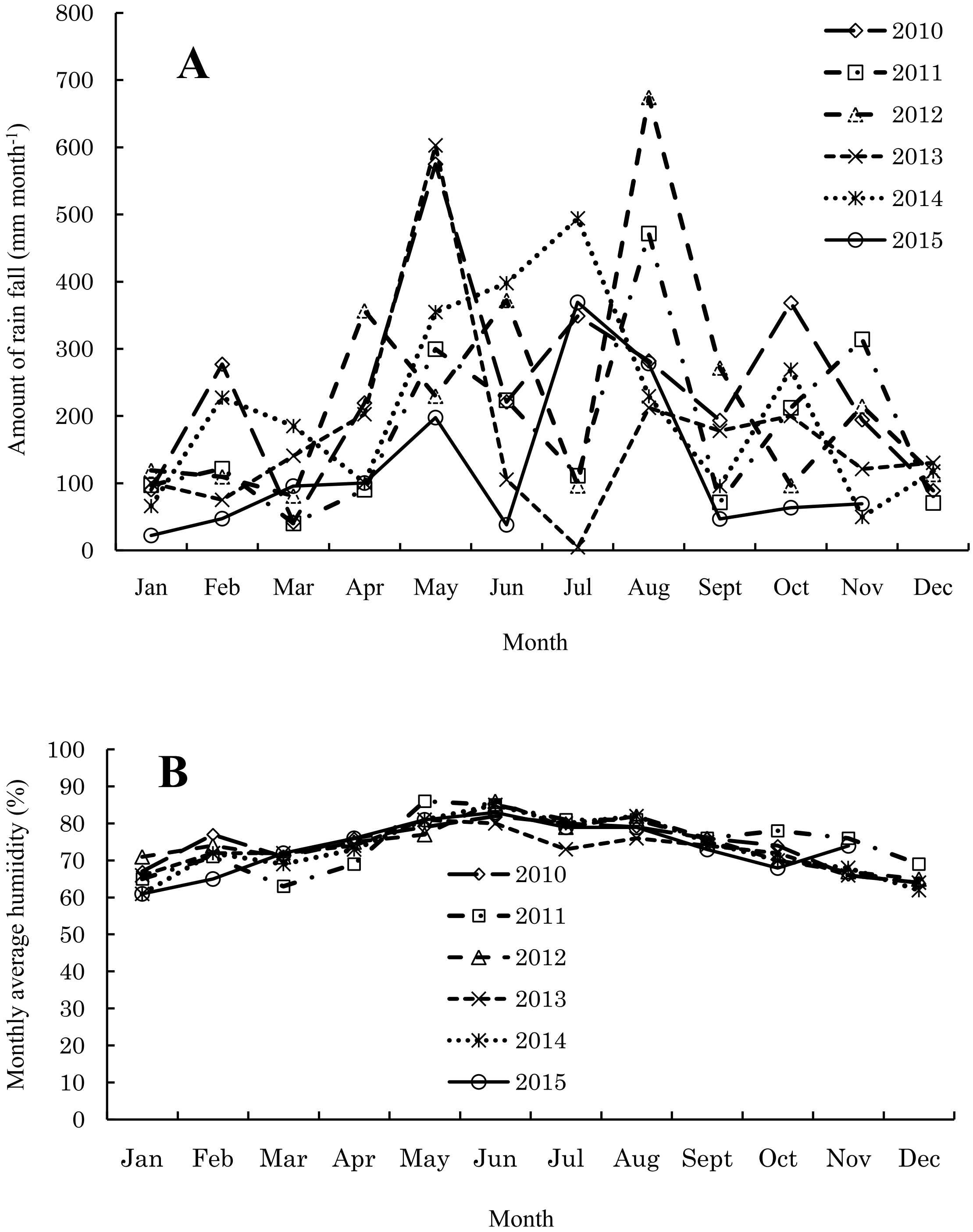


Fig. I. 2. Monthly rainfall (A) and humidity (B) during experiments(2010-2015)

Amaranthus grows very fast in tropical and subtropical areas under a variety of agroclimatic conditions (Begum, 2000; Makus, 1984; Singh and Whitehead, 1996). Research on the effect of climatic and edaphic factors on the growth patterns of a plant species over a year in a region is very much important to understand its seasonal distribution, cultivation technologies and management practices, which help to obtain a higher profit from the plant species (Anderson, 1998; Becker et al. 1998; Deen et al. 1998; Hossain, 1999; Katovich et al. 1998; Rushing et al. 1998). Determining proper cultivation time of a plant species is important for obtaining higher yield with better quality. In addition, proper planting time contributes to economic weed management, diseases management, fertilizer management and irrigation management in plant cultivation.

Growth, yield and quality of a plant species differ with soil types and soil nutrient status; and a plant species requires suitable soil for higher yield and better quality (Oya, 1972; Oya et al., 1977; Hossain and Ishimine, 2005; Akamine et al., 2007; Chowdhury et al.,2008; Hossain et al., 2011; Islam et al., 2011). Study on growth characteristics of a plant-species in local soils is important to develop management practices for higher yield with good quality (Hossain and Ishimine, 2005). *Amaranthus* is cultivated in many countries under a variety of soils in tropical and subtropical areas (Begum, 2000; Makus, 1984; Singh and 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 (Hossain and Ishimine, 2005; Okinawa Prefecture Agriculture, Forestry and Fisheries, 2008). Physical and chemical properties of the dark-red soil, red soil and gray soil are significantly different. Shittu et al. (2006) reported that balanced fertilizers in a specific soil provide higher yield and nutrient compositions of amaranth in Nigeria. It is thought that growth parameters, yield and quality of amaranth plants differ with soil types possessing different levels of minerals, pH, N and physical properties.

Studies on growth and development responses of a plant species to the nitrogen (N) fertilizer application at different rates are necessary for understanding the plant cultivation and management practices (Mazid 1993; Sunusi et al. 1999; Zakaria et al. 2000; Wadi et al. 2003). Growth, yield and quality of a plant species differ with soil nutrient status and fertilizer management (Oya, 1972; Oya et al., 1977; Hossain and Ishimine, 2005; Akamine et al., 2007; Chowdhury et al., 2008; Hossain et al., 2011; Islam et al., 2011). Soil fertility and crop productivity varies significantly with the amount and combination of Na, K, Ca, Mg, S. P, Fe, Al, pH and N in soil (Oya, 1972; Broadley et al. 2012a, 2012b; Hawkesford et al. 2012).

Different plant species response differently to fertilizer rates and combination and a plant species requires balanced fertilizers to maximize growth, yield and quality (Hossain et al., 2004; Akamine et al., 2007; Chowdhury et al., 2008; Hafsi et al., 2011). The major nutrients (N, P, K) individually or in combination maintain growth, yield and quality of plants (Mazid, 1993; Ivony et al., 1997; Nakano and Morita, 2009; Hafsi et al., 2011). Nitrogen influences chlorophyll formation, stomatal conductance and photosynthetic efficiency, which is responsible for 26-41% of crop yield (Maier et al., 1994; Ivony et al., 1997). 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). Different plant species response differently to fertilizer rates and combination, and a plant-species requires balanced fertilizer to maximize growth, yield and quality in a specific soil (Hossain et al., 2004; Akamine et al., 2007; Chowdhury et al., 2008). Crop productivity of a soil type differ significantly with the amount and combination of N, P and K fertilizer application (Hossain et al., 2011; Broadley et al. 2012a, 2012b; Hawkesford et al. 2012).

Amaranth(*Amaranthus* spp.) contains high nutritional values and grows very fast under a variety of soils and agroclimatic conditions; and it is resistant to heat, drought and pest. Sun-radiation and air-temperature are high, and typhoon strikes several times in summer in Okinawa, which limit vegetable production and causes to import 80% of vegetables required in summer. The growth characteristics and ecology of amaranth, and climatic and edaphic conditions of Okinawa indicate that amaranth plant could be cultivated as vegetable in Okinawa. Therefore, a series of experiments has been conducted to evaluate growth characteristics, yield and quality of some amaranth lines under seasonal variations, soil types and fertilizers to determine suitable lines/cultivars, time, soil and fertilizer for amaranth cultivation as vegetable in Okinawa.

CHAPTER II

Growth Characteristics, Yield and Quality of Some Vegetable Amaranths (*Amaranthus* spp.) Cultivated in Okinawa，Japan

Abstract: Growth characteristics, yield and quality of seven leafy-vegetable amaranth lines were evaluated in details for selecting suitable line(s) in Okinawa. The amaranth lines Bangladesh B (BB), Bangladesh C (BC), Bangladesh Red (BR), India Bengal line (IB), Vietnam line (V), Taiwan line (TW) and Biam Tricolor (BT, domestic line) were evaluated. The lines BB, BC, BR and IB grew faster and had higher plant height than other lines. Leaf nubmer and leaf area per plant were higest in the line BC followed by BB and BR. The lines BB, BC and BR had higher stem weight than leaf weight, whereas the lines V, TW and BT had higher leaf weight than stem weight. Total shoot weight was the highest in line BC followed by BB in all the experiments. Potassium (K) content was higher in the lines TW and BT. Calcium (Ca) was higher in the lines BB, BC and BR, and magnesium (Mg) was higher in the lines BB, BC, BR and TW. Total sodium (Na) and K per plant were the highest in line BC followed by BB, total Ca, Mg and crude protein were the highest in line BB followed by BC, and total iron (Fe) was the highest in the line BB followed by V and BC. Total L-ascorbic acid content was the highest in the line BB followed by TW, and the lines IB, V and BC had remarkable higher content of L-ascorbic acid. Considering growth characteristic, yield and quality parameters per plant, the amaranth lines BB and BC could be suitable for cultivation in Okinawa prefecture.

Introduction

Amaranth is an important promising food crop for its resistance to heat, drought, diseases and pest, and high nutritional value (Sreelathakumary and Peter, 1993: Svirskis, 2003; Rastogi and Shukla, 2013). Many amaranth species have been cultivated as vegetable and grain in many countries, and are popularly consumed as vegetable in African and Asian countries (Prakash and Pal, 1991; Begum, 2000; Stalknecht and Schulz-Schaeffer, 1993; Svirskis, 2003).

Amaranth species are different in plant height, stem color, leaf color, leaf size, stem diameter, stem- and leaf-softness, leaf-stem ratio, inflorescence color, inflorescence size, growth speed, grain color, grain size, nutrition values, etc., which are considerable parameters for quality vegetable (Iizuka and Nishiyama 2001). Amaranth grows very fast in tropical and subtropical areas, and is cultivated in many countries under a variety of soils and agroclimatic conditions (Begum, 2000; Makus, 1984; Singh and Whitehead, 1996).

The sun radiation intensity and air temperature are very high, and typhoon strikes several times in summer season, especially from June to September in Okinawa, which limit vegetable production. More than 80% of vegetables are imported to Okinawa in summer from main land Japan, and other neighborhood countries (Okinawa prefecture section of agriculture, forestry and fishery 2008, Kawamitsu et al., 2010). As amaranths is highly tolerant to heat and drought, and grows very fast in summer season (Rastogi and Shukla, 2013), it was thought that amaranth could be cultivated in Okinawa for supplying leafy vegetable in summer season. Many amaranth species and lines are cultivated in Asian and African countries as vegetables. But no suitable species, cultivar or strain of amaranth is available as vegetable in Okinawa. Present study has been conducted to evaluate growth, yield and quality performances of some amaranth strains/lines to select some suitable amaranth strains as vegetable in Okinawa.

Materials and Methods

1. Soil collection

Dark-red soil (Shimajiri mahji) was collected from the top 50 cm layer of a field at the Subtropical Field Science Center, University of the Ryukyus, Okinawa, Japan. Sodium (Na), K, Ca and Mg contents of the soil were 7.34, 1.29, 8.78 and 1.84 mg g^{-1}, respectively. The soil with pH 6.6 contained 0.08% N and 0.26% C. According to Hossain and Ishimine (2005), coarse sand, fine sand, silt, clay and apparent density were 2.93%, 7.33%, 23.94%, 57.24% and 0.87 g cm^{-3}, respectively in the dark-red soil.

2. Amaranth lines/strains collection

The *Amaranthus tricolor* lines IK (Indian Kishaka line, green leaf-amaranth), IB (India Bengal line, red leaf-amaranth), TW (Taiwan line, green leaf and stem), BB (Bangladesh B line, red stem), BC (Bangladesh C line, red leaf and stem), BR (Bangladesh red, red leaf and stem), V (Vietnam, green leaf and stem) and AP (Okinawa, *A. patulus, green*) were collected from the respective countries, and stored at the Subtropical Field Science Center of the University of the Ryukyus. The strain BT (Biam tricolor, green leaf and stem) was purchased from Takii Seed Co., Ltd.

1. House experiment 1 (spring sowing in 2010)

Several amaranth lines have been collected from different Asian countries and stored at the Subtropical Field Science Center, University of the Ryukyus, Okinawa, Japan. Growth characteristics and yield performance of 12 amaranth lines were evaluated to select some potential lines for the doctoral research work. Seven amaranth lines (IB, TW, BB, BC, BR, V and BT) were selected considering growth speed, growth characteristics, leaf size and color, stem diameter and color, leaf-stem ratio, total leaf weight, total stem weight and total shoot weight, etc. The amaranth lines IK and AP were found to be weedy type, which were not presented in this thesis.

A glasshouse experiment was conducted from April 30 to July 8, 2010 at the Subtropical Field Science Center of the University of the Ryukyus. Outdoor environmental conditions, except rainfall, were maintained during the experiment in the house by keeping all the windows opened. Each plastic planter (planter-65E type, IRIS Ohyama, Japan) was filled with 7 kg of air-dried dark red soil and 6 kg of culture soil (Hanasaki monogateri, Biodo Ltd., Akimoto natural products). The dark-red soil of 7 kg and culture soil of 6 kg were mixed uniformly in each planter; and75 seeds of each amaranth line were placed/sown on soil surface and covered with a thin layer (<0.5mm) of the mixed soil. The plants were thinned to eight healthiest stands per planter at 2- to 3-leaf stage (14 days after seed sowing). Each amaranth line was cultivated in six planters (replications). The planters were arranged randomly in the house. Water was applied as required every day for proper seedling emergence and plant growth.

2. Experiment 2(summer sowing in 2010)

The amaranth lines BB and BC grew faster, and provided highest yield and quality in previous experiment 1. Therefore the amaranth lines BB and BC were cultivated in this experiment to evaluate their growth characteristics, yield and quality in summer season. This experiment was conducted from August 2 to September 21, 2010. Each amaranth line was cultivated in six planters. The planter, soil, culture soil, plantation procedure and management practices in this experiment were the same as those used in the previous experiment.

3. Experiment 3(spring sowing in 2011)

Weather condition changes somewhat with the years in a region or place, which may affect growth characteristics, yield and quality of the amaranth plants. Therefore, the amaranth lines

BB, BC and BR were cultivated from April 9 to May 25, 2011 to evaluate growth characteristics, yield performance and quality. Each amaranth line was cultivated in three planters (replications). This experiment was conducted in the same green house at the Subtropical Field Science Center, University of the Ryukyus, Okinawa Japan. The soil (dark-red soil), cultured soil (Hanasaki monogatheri), plastic planter, plantation procedures and management practices of this experiment were the same as those used in the previous experiments.

4. Data collection

Weather data during the experiments at the Subtropical Field Science Center of the Faculty of Agriculture, University of the Ryukyus (Naha city) were collected from the homepage 'http://www.jma-net.go.jp/Okinawa/'. In the experiment 1, plant height and leaf number were measured at a 10-day interval from24 days after seed sowing (DAS) to 54DAS. Five plants were harvested from each planter at 34 DAS. Stem diameter, internode length, largest leaf area, total leaf area, fresh and dry stem weight, fresh and dry leaf weight, and fresh and dry root weight of amaranth plants were measured. Stem diameter was measured at 5 cm height and internode length was measured at the 3rd internode from the soil surface. In the experiments 2 and 3, plant height and leaf number were measured at a 7- day interval from 15 DAS to 37 DAS. Other growth parameters were measured at 34 DAS according the experiment 1. Leaf shape, leaf color, stem type and root type were evaluated. The days from seed sowing to seedling emergence, panicle length and 1000-seed weight were evaluated.

3. 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 separately cut into species and dried separately at 80℃ for 48 hours using forced convection oven (DRLF23WA, Advantec) for dry weight measurement.

4. Determination of mineral, nitrogen, carbon, crude protein, L-ascorbic acid and total oxalic acid content of amaranth plants

Leaf and stem of amaranths were cut into pieces and dried at 60℃ for 48 hours using the same forced convection oven, and then leaf and stem were ground finely for chemical analysis. 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℃ for 24 hours, and the solution was then filtered sequentially with paper No. 2 and disposable syringe filter 0.45 µm (Advantec Co. Ltd.). The plant soultion was diluted as necessary by adding deionized water for determining mineral/nutrient elements. Mineral contents of amaranth were determined with Inductively Coupled Plasma Spectrometer (ICPS-8100, Shimadzu Co. Ltd.). Mineral contents g^{-1} of dry amaranth (edible shoot, leaf plus stem) were calculated. Percent of total C and N in the amaranths were determined with Gas Chromatograph (Soil GS-8A, Shimadzu Co. Ltd., NC-220F Juka analysis center) and Sumigraph (NC-90A, Shimadzu Co. Ltd.).Crude protein (CP) content was calculated by multiplying the protein conversion factor 6.25 to the amount of nitrogen. L-ascorbic acid content and the total oxalic acid content of amaranth lines were measured with the RQ flex/Agro check kit small reflective photometer (Kanto Chemical Co., Inc.).

5. 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 (Excel Statistics 2008, Corp. Social Information Service Co.).

Results

1. Weather conditions during cultivation

Monthly average air temperature, sun radiation and rainfall (precipitation) during the experiments in Naha area are shown in the Fig. II-1. Average air temperature ranges from 17.5 to 29.5 ℃, and high temperature prevails from June to September. Average precipitation was 100 to 600 mm. The rainfall was highest in May and August in 2010 and 2011, respectively. Average daily sun radiation was 15.5 to 20.00 $MJm^{-1}d^{-1}$. Rainfall and radiation differed significantly year to year, but temperature differed slightly (Fig. II-1, II-2).

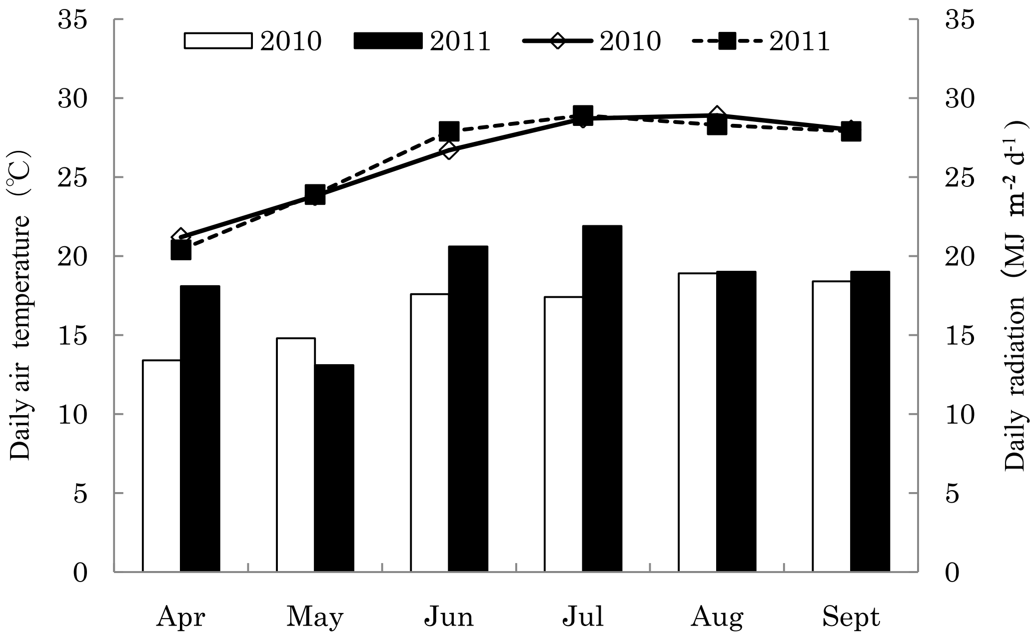


Fig. II-1. Monthly temperature and sun radiation during the experiments (2010-2-11)

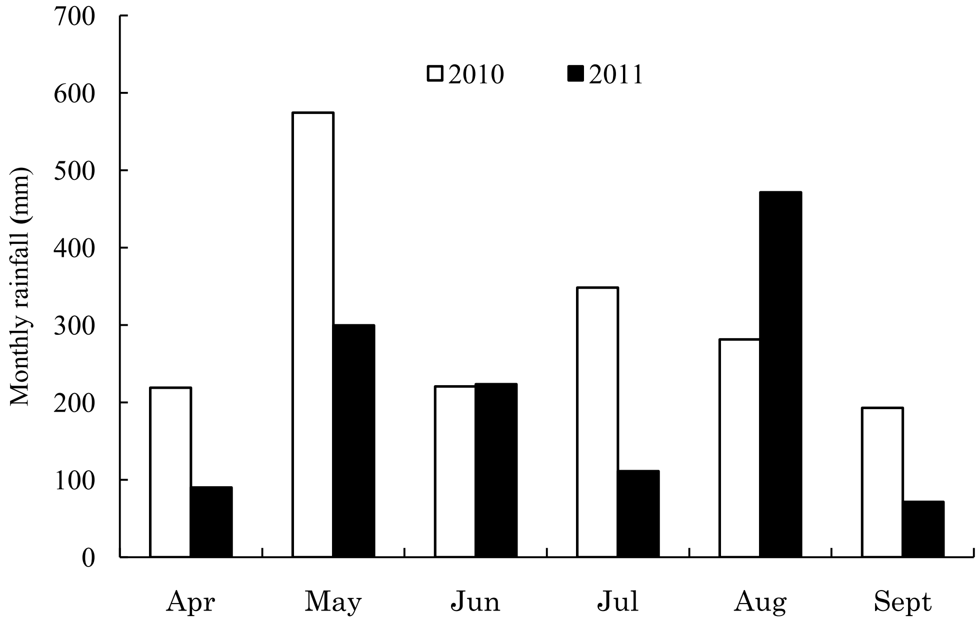


Fig. II-2. Monthly rainfall during the experiments

2. Growth characteristics of amaranth lines

Leaves of all amaranth lines were broad and big in size, except V line. The leaf of V line was comparatively narrow (Fig. II-3). Color of leaves differed greatly with the amaranth lines. Color of leaves and stems was red in the lines BB, BR and IB, reddish in the line BB, light green in the line TW, and deep green in other lines. Color of leaves and stems was deeper red in the line BC among the amaranth lines evaluated (Fig. II-3).

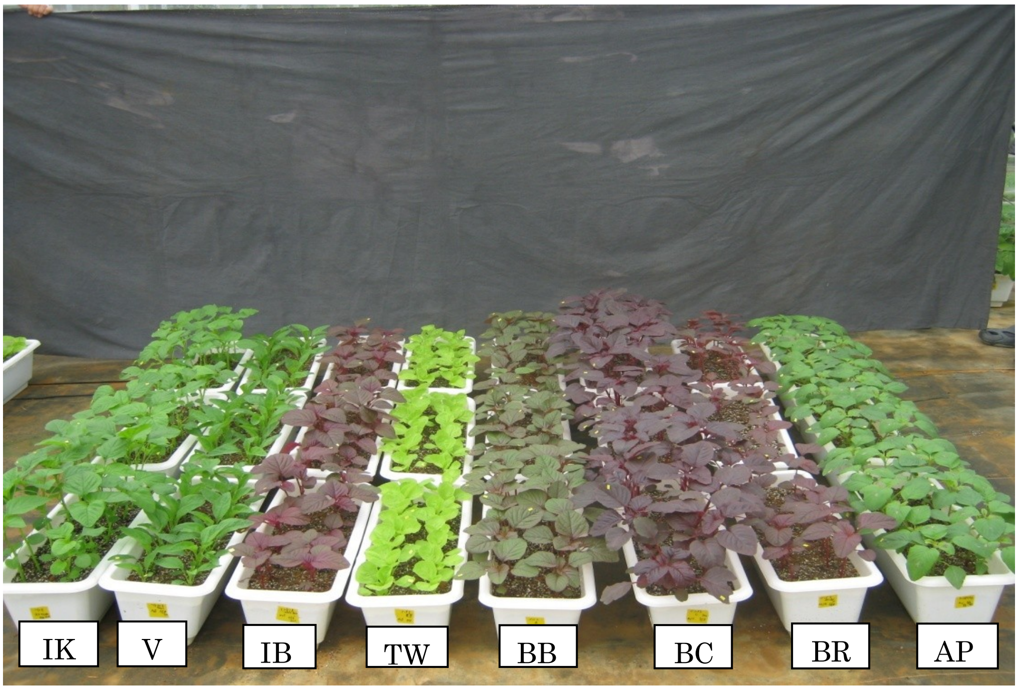
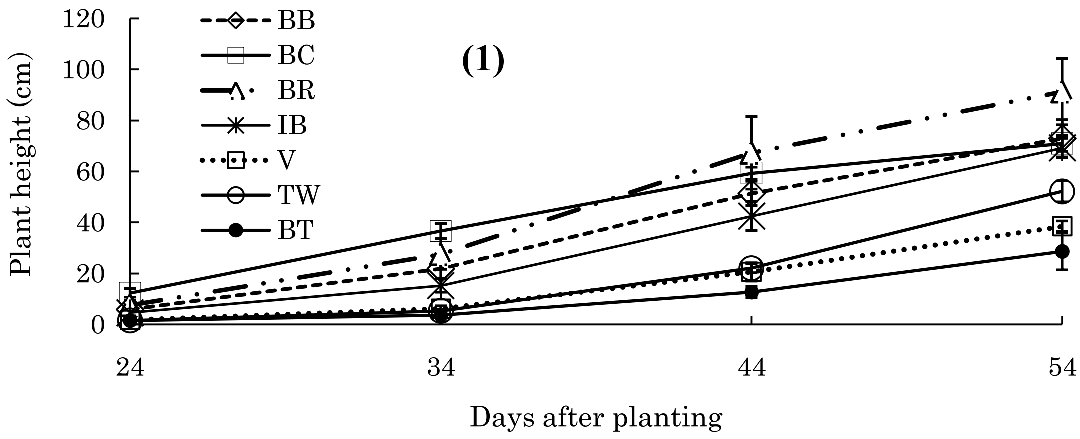
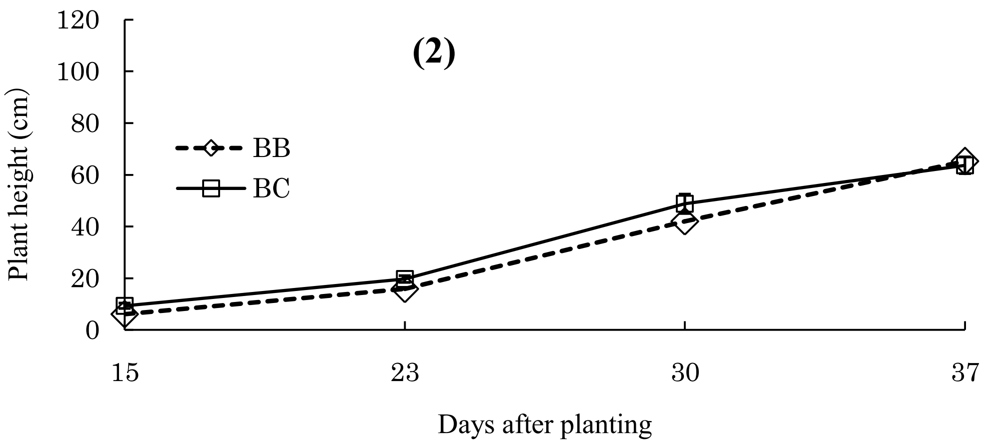


Fig. II-3. Growth of different amaranth lines at 30 day after seed sowing
IK: Indian Kishaka line; V: Vietnam line, IB: India Bengal line, TW: Taiwan line,
BB: Bangladesh B line, BC: Bangladesh C line, BR: Bangladesh red line,
AP: *Amaranthus parulus* Bert.

The amaranth lines BB, BC, BR and IB grew faster than other lines throughout the experimental period (Fig. II-4 (1)). The line BC elongated sharply up to 44 days after seed sowing (DAS), thereafter slowly. On the other hand, the other lines grew slowly until 34 DAS. Plant height was highest in BC followed by BB at 34 DAS, whereas the plant height was highest in the BB line at 54 DAS. In the experiment 2, the amaranth lines BB and BC grew slowly until 23 DAS, thereafter rapidly (Fig. II-4 (2)). Plant height of the line BC was higher than that of the line BB until 30 DAS, but similar at 37 DAS. Plant height of BB and BC was 42 and 49 cm, respectively at 30 DAS. All the amaranth lines grew slowly until 23 DAS, thereafter moderately (Fig. II-4 (3)). The line BC obtained the highest plant height among the amaranth lines evaluated. Plant height of BC was 32 cm, whereas the plant height of BB and BR was 22 cm. The plant height of the amaranth lines was almost similar at 37 DAS in the experiment 3.





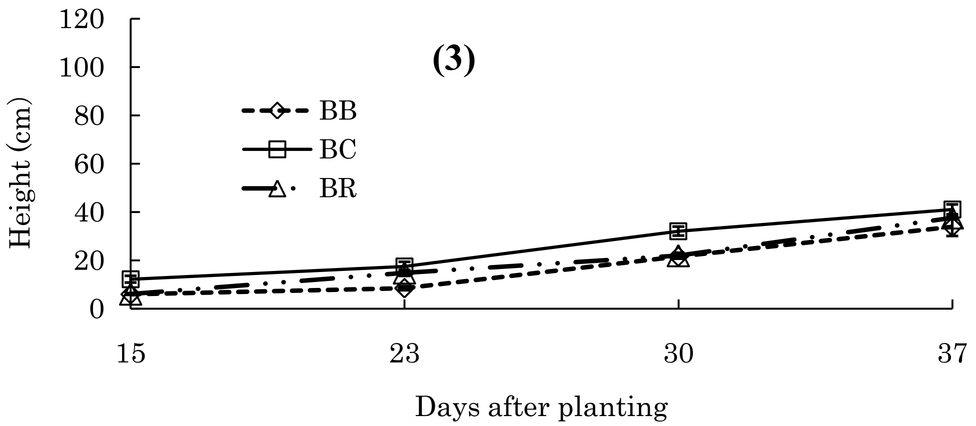


Fig. II-4. Plant height of different amaranth lines. **(1),** Planted on April 30, 2010; **(2),** Planted on August 2, 2010; **(3)**, Planted on April 9, 2011

Number of leaves was significantly highest in the amaranth BC line throughout the experiment (Fig. II-5 (1)) followed by BB line. Number of leaves per plant was 11 in the BC line and 10 in the BB line at 34 DAS. Number of leaves increased gradually in all amaranth lines until 54 DAS. The BT line resulted in the lowest number of leaves throughout the growth period. In the experiment 2, number of leaves per plant was the highest in amaranth BC line throughout the growth period when seeds were sown in August; the leaf number per plant was 14 and 12 in the amaranth line BC and BB, respectively at 30 DAS. In the experiment 3, the leaf number in the amaranth lines BB and BC was almost similar until 23 DAS, thereafter the leaf number was highest in the BC line; the leaf number was 12 and 9 in the line BC and BB, respectively at 30 DAS (Fig. II-5 (3)).

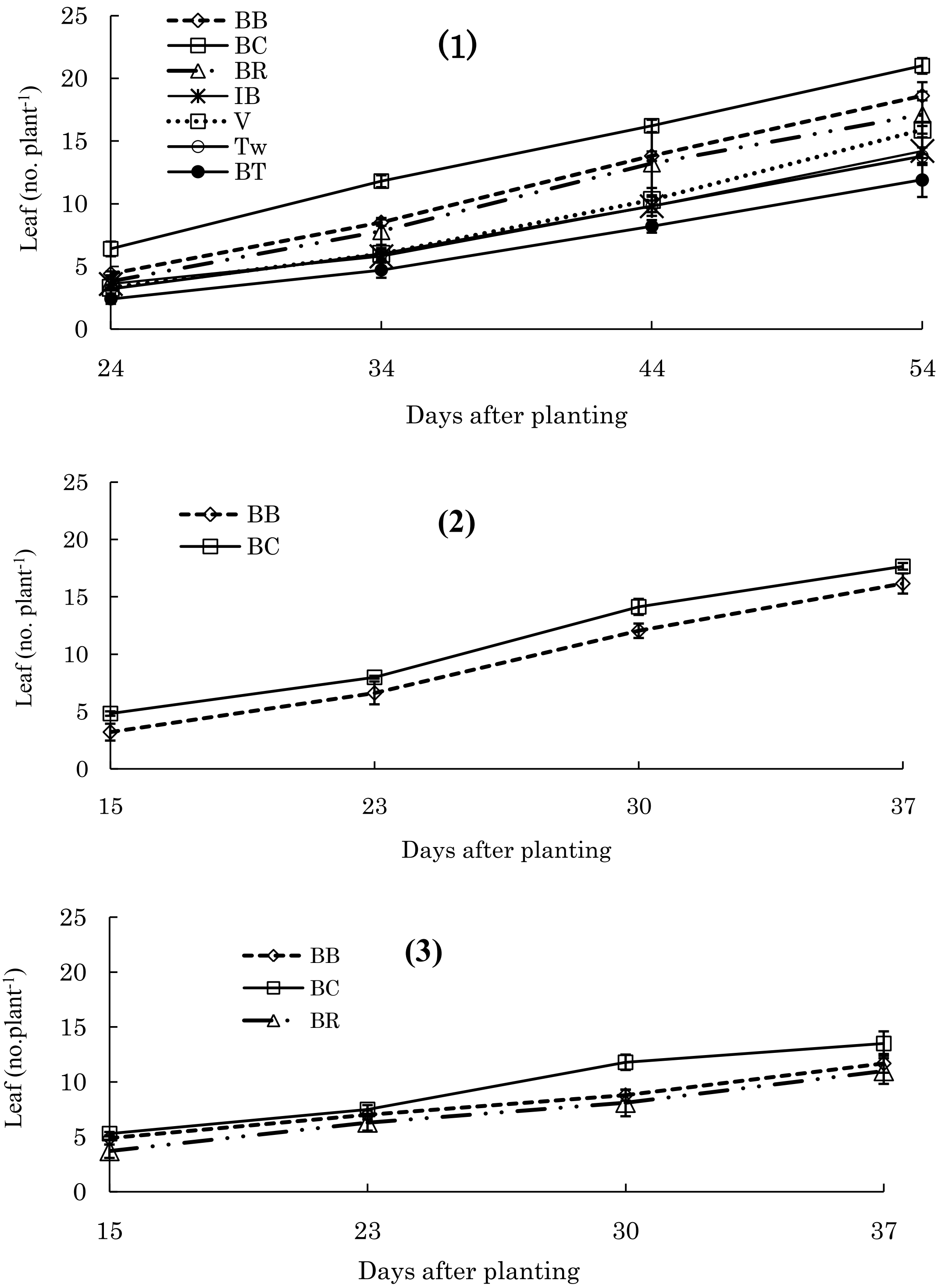


Fig. II-5. Number of leaves in different amaranth lines. **(1)**, Planted on April 30, 2010; **(2)**, Planted on August 2, 2010; **(3)**, Planted on April 9, 2011

At 34 DAS, shoot biomass (yield) was significantly highest in the amaranth line BC followed by BB, which was about 30% higher in the line BC than in the line BB (Fig. II-6). The lines BR, IB, V and TW had statistically similar yield. The yield was about 50% lower in the other lines, as compared to that in the BC line.

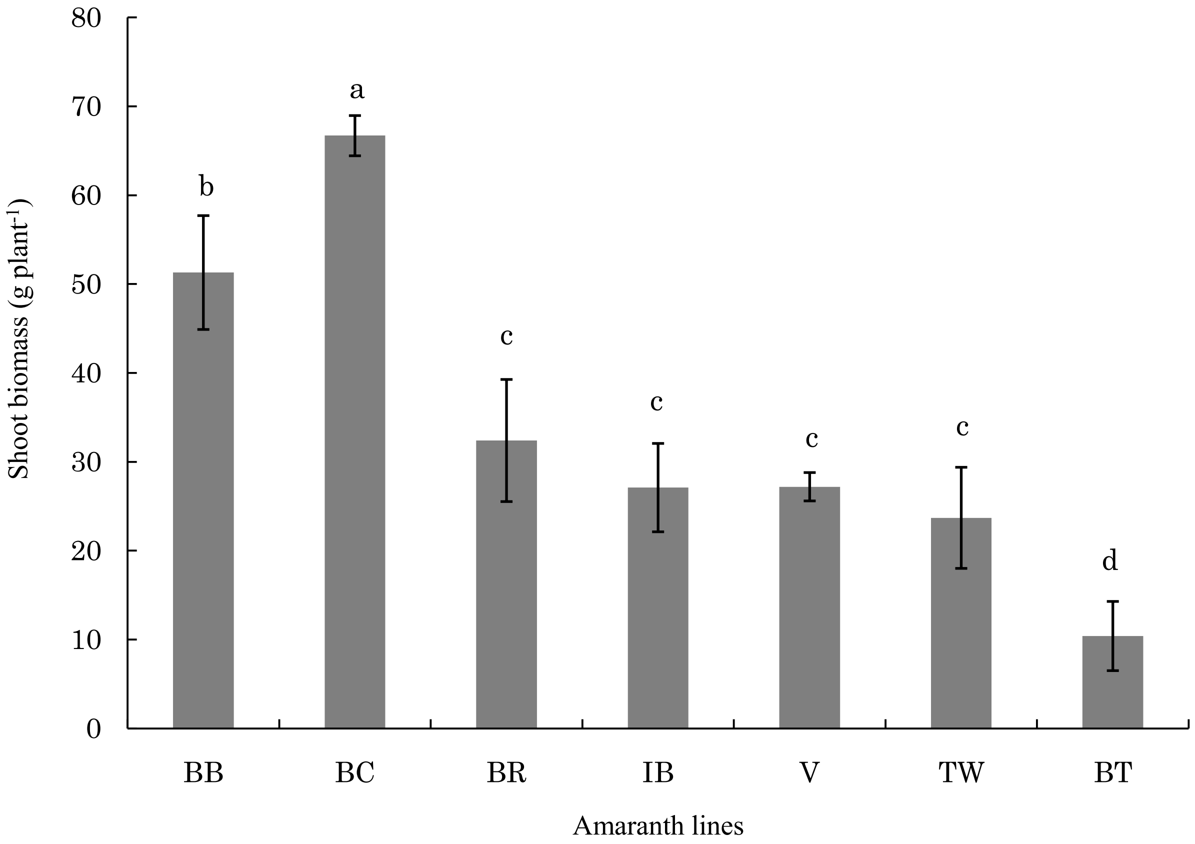


Fig. II-6. Shoot biomass (yield) of different amaranth lines at 34 day after seed sowing. Bars with the same letter are not significantly different at the 5% level, as determined by LSD test. Planting date: April 30, 2010.

The amaranth line BC had highest stem diameter followed by BB. The longest internode was recorded with the line BR followed by BB and BC. Leaf type of amaranth lines BB, BC, BR, IB and BT was ovate (Table II-1); and the amaranth lines V and TW had acute and orbicular type leaves, respectively. Red color leaves were found in the amaranth lines BB, BC, BR and IB, and green color leaves were found in the amaranth lines V, TW and BT. In early growth stage, especially up to 35 days after seed sowing, the stem of BC was not hollow type, but it was hollow type at mature stage. The other amaranth lines had parenchyma type stems. The amaranth lines BB, IB and V required about 49 days and other lines required 45 days to initiate inflorescences after seed sowing. Panicle length was highest in the line BB followed by BB, BR and IB. The 1000-seed weight was highest in the lines BC and BR followed by IB (Table II-1). All the amaranth lines developed tap root.

Table II-1. Growth characteristics of some amaranth lines at 54 day after seed sowing

Lines	Stem Diameter (mm)	Internode Length (cm)	Leaf type	Leaf color	Stem type	Days to Inflorescenceinitiation (d)	Panicle length (cm)	1000 seed weight (g)	Root Type
BB	11.5b	8.1ab	Ovate	Red	Parenchyma	49a	15.1a	0.77c	Tap
BC	13.9a	7.2bc	Ovate	Red	Hollow	45b	11.4b	0.97a	Tap
BR	8.5c	9.8a	Ovate	Red	Parenchyma	45b	12.0b	0.97a	Tap
IB	8.1c	5.9c	Ovate	Red	Parenchyma	49a	11.6b	0.94b	Tap
V	9.2c	3.1d	acute	Green	Parenchyma	49a	9.3c	0.66d	Tap
TW	7.6c	2.5de	orbicular	Green	Parenchyma	45b	8.0c	0.63e	Tap
BT	5.0d	1.1e	Ovate	Green	Parenchyma	45b	8.5c	0.66d	Tap

Data with the same letter within each column are not significantly different at the 5% level, as determined by LSD test.

The largest leaf area was highest in the amaranth line BB followed by IB, TW and BR (Table II-2), whereas the total leaf area was highest in the line BC followed by BB. Dry leaf weight was statistically higher and similar in the lines BB, BC, V and TW, whereas dry stem weight was highest in the line BC followed by BB. Dry shoot weight was statisticaly highest in the line BC and BB. In the experiment 2, largest leasf area was higher in BB, but total leaf are was higher in BC. Dry leaf, stem and shoot weight was similir in the amaranth lines BB and BC. In the experiment 3, the largest leaf area was highest in the line BB followed by BR, whereas total leaf area was highest in the line BC followed by BB. Dry leaf weight was similar in the lines BB and BC (Table II-2). Stem weight and shoot weight were highest in the line BC followed by BB.

Table II-2. Largest leaf area, total leaf area, dry leaf weight, dry stem weight and dry shoot weight of some amaranth lines at 54 day after seed sowing

Lines	Largest leaf area (cm^2)	Total leaf area (cm^2 $plant^{-1}$)	Dry leaf weight (cm^2 $plant^{-1}$)	Dry stem weight (cm^2 $plant^{-1}$)	Dry shoot weight (cm^2 $plant^{-1}$)
Experiment 1					
BB	110.7a	617.4ab	2.37a	1.23b	3.60a
BC	84.7bc	673.5a	1.97ab	1.77a	3.74a
BR	88.4abc	475.1b	1.27cd	0.73c	2.00b
IB	100.2ab	445.4b	1.67bc	0.63cd	2.30b
V	73.7cd	418.5b	1.97ab	0.43cde	2.40b
TW	97.8ab	454.9b	2.00ab	0.30de	2.30b
BT	60.7d	253.4c	0.83d	0.13e	0.96c
Experiment 2					
BB	98.59a	558.16b	2.90a	1.80a	4.70a
BC	70.73b	639.07a	2.23a	2.52a	4.75a
Experiment 3					
BB	101.30a	572.50b	2.70a	1.02b	3.72b
BC	77.94b	625.89a	2.26a	2.22a	4.48a
BR	87.83b	432.09c	1.40b	0.45c	1.85c

Data with the same letter within each column for each experiment are not significantly different at the 5% level, as determined by LSD test.
Eperiment 1: Planted on April 30, 2010; Experiment 2: Planted on August 2, 2010
Experiment 3: Planted on April 9, 2011.

3. Mineral, N and C content in the amaranth lines

Mineral, N and C content in leaf and stem was shown in the Table II-3. Sodium content in leaves of the amaranth lines ranged from 2.28 to 3.72 mg g^{-1}, which was highest in the line BC followed by BT. Sodium content was lowest in the line BR (Table II-3). Potassium (K) content ranged from 52.06 to 66.73 mg g^{-1}, which was the lowest in the line BC. The lines V and BT obtained the highest K. Calcium content ranged from 15.12 to 21.22 mg g^{-1} in the amaranth lines, which was statistically higher and similar in the lines BB, BC and BR. Magnesium content ranged from 15.52 to 18.76 mgg^{-1}, which was similarly higher in the lines BB, BR and TW. The line BC obtained 16.76 mg g^{-1} Mg. Aluminum content was 0.16 to 0.41 mg g^{-1} in the amaranth lines. The lines BB, BC and BR obtained lowest Al (0.16-0.18 mg g^{-1}). Iron content was 0.14 to 0.19 mg g^{-1} in the amaranth lines, which was lower in the BB, BC, BR and IB (0.14-0.15 mg g^{-1}).

Phosphorous content in the amaranth lines was 3.05 to 5.17 mg g^{-1}, which was highest in the line BR and lowest in the line BC. The content of Mn in the amaranth lines ranged from 0.51 to 0.82 mg g^{-1}, which was similarly highest in the lines BB and BR. Nitrogen content in the amaranth lines ranged from 5.14 to 6.43%. The line BC had the lowest N (5.14%) and BR had the highest N (6.43%). The carbon content was 34.17 to 37.85% in the amaranth lines, which was highest in the BC (Table II-3).

Sodium content in the stem of amaranth lines ranged from 1.24 to 4.34 mg g^{-1}, which was highest in the line BC followed by the line V (Table II-3). The potassium content was 95.40 to 127.33 mg g^{-1} in the amaranth lines. The line BR had the highest K followed by BB. The line BC had 109.12 mg g^{-1} of K. The Ca content of amaranth lines was 4.26 to 6.24, which was similarly highest in the line V and BT. The amaranth line BB and BC obtained 4.48 and 4.76 mg $g^{-1}$of K, respectively. The Mg content was 2.94 to 6.36 mg g^{-1} in the amaranth lines, which was highest in the line BC, followed by V and TW. The range of Al in the amaranth stem was 0.10 to 0.32 mg g^{-1}. The lines BC and IB had the lowest and BT had the highest content of Al. The Fe content was 0.06 to 0.28 mg g^{-1} in the amaranth lines, which was the lowest in the line BC and highest in the line BT. The P content was 1.43 to 2.69 mg g^{-1} in the amaranth lines, which was lowest in the line BC and highest in the line BT. The Mn content was 0.11 to 0.22 mg g^{-1} in the amaranth lines evaluated, which was the lowest in BC and highest in the BB line. The N content in the stem of the amaranth line was 4.28 to 6.16%, which was the highest in the BB and lowest in the BC. The C content was 21.91 to 29.33% in the amaranth lines, which was highest in BT and lowest in the BB (Table II-3).

Table II-3. Mineral, total nitrogen and total carbon content in different amaranth lines at 34 day after seed sowing

Lines	Plant part	Na (mgg^{-1})	K (mgg^{-1})	Ca (mgg^{-1})	Mg (mgg^{-1})	Al (mgg^{-1})	Fe (mgg^{-1})	P (mgg^{-1})	Mn (mgg^{-1})	N (%)	C (%)
BB		2.45cd	56.60b	21.40a	17.20abc	0.18c	0.14b	4.03b	0.81a	5.37b	36.82b
BC		3.72a	52.06c	19.40ab	16.76bcd	0.16c	0.14b	3.05b	0.72b	5.14b	37.85a
BR		2.28d	55.53bc	21.22ab	18.12ab	0.17c	0.14b	5.17a	0.82a	6.43a	37.17b
IB	Leaf	2.91bc	57.80b	18.10bc	15.80cd	0.25bc	0.15b	3.82b	0.61b	5.27b	37.16b
V		3.08b	59.86b	17.76cd	15.52d	0.31ab	0.19a	3.78b	0.53c	5.34b	37.08b
TW		2.90c	64.60a	18.64bc	18.76a	0.25bc	0.16ab	3.48b	0.52c	5.26b	34.17c
BT		3.51ab	66.73a	15.12d	16.11cd	0.41a	0.18a	3.58b	0.51c	5.39b	36.54b
BB		2.34bc	115.32b	4.48cd	2.94d	0.13cd	0.09cd	2.30b	0.22a	6.18a	21.91d
BC		4.34a	109.12c	4.76c	6.38a	0.10d	0.06d	1.43e	0.11b	4.28c	26.13c
BR		1.24d	127.33a	4.40cd	3.14d	0.17c	0.11c	2.28b	0.21a	5.26b	25.62c
IB	Stem	1.7cd	109.40c	4.26d	3.04d	0.10d	0.06d	2.12c	0.12b	5.35b	25.14bc
V		2.72b	98.60de	6.24a	4.96b	0.17c	0.12c	2.69a	0.13b	4.91b	28.22ab
TW		1.94c	103.40d	5.98a	4.50b	0.23b	0.16b	1.97d	0.11b	4.95b	26.22bc
BT		1.62cd	95.40e	5.56b	3.90c	0.32a	0.28a	2.71a	0.21a	5.02b	29.33a

Data with the same letter within each column for leaf or stem are not significantly different at the 5% level, as determined by LSD test.
Planting date: April 30, 2010.

Sodium and K contents were 4.74-7.07 and 31-33.8 mg g^{-1}, respectively in the amaranth lines in the experiment 2 (summer cultivation). The Ca and Mg contents were 15.83-19.93 and 11.20-15.73 mg g^{-1}, respectively. The Na and K contents were higher in the line BC, whereas Ca and Mg contents were higher in the line BB (Table II-4). The content of Al, Fe, P and Zn was 0.88-0.99, 0.60-0.79, 12.53-12.97 and 0.52-0.67 mg g^{-1}, respectively, which were similar in the lines BB and BC. The Mn content was higher in the line BB (0.82 mg g^{-1}) than in BC (0.45 mg g^{-1}). The content of N in the line BB (3.10%) was higher than that in the line BC (2.40%). The C content was similar in both the lines BB and BC (37.32-37.45%).

In the experiment 3, the content of Na, K, Mg, Al, Fe, P, Mn and Zn was 2.19-8.70, 41.65-44.90, 19.21-22.61, 17.60-19.87, 0.55-1.05, 0.55-0.83, 10.63-18.20, 0.24-0.78 and 0.45-0.69 mg g^{-1}, respectively. The Na content was highest in the line BC (8.70 mg g^{-1}), followed by BR (5.55 mg g^{-1}), whereas K and Ca content was higher in the BB and BR than in BC. The content of Mg, Mn and Zn was higher in the line BR, and Al content was higher in the line BC. The P content was highest in the line BB followed by BR. The N content was 2.40-3.62%, and C content was 37.18-37.45% in the amaranth lines. The line BB had higher N followed by line BR. The C content was similar in all the amaranth lines.

Table II-4. Mineral, total nitrogen and total carbon content in leaves of some selected amaranth lines at 34 day after seed sowing

Lines	Na ($mg\ g^{-1}$)	K ($mg\ g^{-1}$)	Ca ($mg\ g^{-1}$)	Mg ($mg\ g^{-1}$)	Al ($mg\ g^{-1}$)	Fe ($mg\ g^{-1}$)	P ($mg\ g^{-1}$)	Mn ($mg\ g^{-1}$)	Zn ($mg\ g^{-1}$)	N (%)	C (%)
Experiment 2											
BB	4.74b	31.33b	19.93a	15.73a	0.99a	0.79a	12.97a	0.82a	0.67a	3.10a	37.32a
BC	7.07a	33.80a	15.83b	11.20b	0.88a	0.60a	12.53a	0.45b	0.52a	2.40b	37.45a
Experiment 3											
BB	2.19c	44.90a	22.61a	18.15b	0.55c	0.57b	18.20a	0.61b	0.45b	3.62a	37.22a
BC	8.70a	41.65b	19.21b	17.60b	1.05a	0.83a	10.63b	0.24c	0.51b	2.40c	37.45a
BR	5.55b	44.50a	21.31a	19.87a	0.87b	0.57b	14.75ab	0.78a	0.69a	3.29b	37.18a

T- test was performed to compare the data between the two treatments in the experiment 2. Data with the same letter within each column for each experiment are not significantly different at the 5% level, as determined by LSD test.
Experiment 2, planted on Augustl 2, 2010; Experiment 3, planted on April 9, 2010

Mineral and crud protein contents per plant of the amaranth lines were presented in the Table II-5. Amount of Na was 8.68, 16.00, 3.72, 5.72, 7.23, 6.38 and 3.12 mg plant^{-1} in the lines of BB, BC, BR, IB, V, TW and BT, respectively. The highest amount of Na was obtained from the line BC, followed by BB. The amount of K was 275.79, 295.79, 163.28, 165.17, 160.16, 160.05 and 67.78 mg plant^{-1} in the lines BB, BC, BR, IB, V, TW and BT, respectively. The amount of K was similarly highest in the lines BB and BC. The amount of Ca was 56.20, 46.64, 30.14, 32.88, 37.65, 39.05 and 13.27 mg plant^{-1} in the lines BB, BC, BR, IB, V, TW and BT, respectively, and the Ca amount was highest in the line BB (56.20 mg plant^{-1}) followed by BC. The Mg amount was 44.36, 44.30, 25.28, 28.28, 32.69, 38.85 and 13.88 mg plant^{-1} in the lines BB, BC, BR, IB, V, TW and BT, respectively, and the Mg amount was similarly highest in the lines BB and BC (44.30-44.36 mg plant^{-1}), and lowest in the line BT . The Al amount ranged from 0.68 to 0.37 mg plant^{-1}, which was highest in line V, followed by line BB. The amount of Fe was highest in the line BB and V (0.44-0.43) followed by BC (0.38 mg plant^{-1}), and the P amount was highest in the line BB (12.37 mg plant^{-1}) followed by V and BC (8.4708.60 mg plant^{-1}). The Mn was highest in line BB (2.22 mg plant^{-1}) followed by BC (1.55 mg plant^{-1}). The crude protein amount was highest in the line BB (1.27 mg plant^{-1}) followed by BC (1.10 mg plant^{-1}).

Table II-5. Mineral and crude protein content in different amaranth lines at 34 day after seed sowing.

Lines	Na (mg plant^{-1})	K (mg plant^{-1})	Ca (mg plant^{-1})	Mg (mg plant^{-1})	Al (mg plant^{-1})	Fe (mg plant^{-1})	P (mg plant^{-1})	Mn (mg plant^{-1})	C.P (mg plant^{-1})
BB	8.68b	275.79a	56.20a	44.36a	0.58b	0.44a	12.37a	2.22a	1.27a
BC	15.00a	295.79a	46.64b	44.30a	0.49c	0.38b	8.47b	1.55b	1.10b
BR	3.72e	163.28b	30.14d	25.28e	0.34d	0.26d	8.21bc	1.22b	0.75d
IB	5.72d	165.17b	32.88d	28.28d	0.47c	0.29c	7.71c	1.22b	0.76d
V	7.23c	160.16b	37.65c	32.69c	0.68a	0.43a	8.60b	1.11b	0.79c
TW	6.38d	160.05b	39.05c	38.85b	0.56b	0.37b	7.53c	1.11b	0.75d
BT	3.12f	67.78c	13.27e	13.88f	0.37d	0.19e	3.02d	0.44c	0.32e

Data with the same letter within each column are not significantly different at the 5% level, as determined by LSD test.
Planting date: April 30, 2010

Total L-ascorbic acid per 100 g of fresh amaranth was highest in the line IB followed by TW (Fig. II-7), and the lowest in the line BT. Total L-ascorbic acid per 100 g fresh amaranth was 66-85 mg in the amaranth lines. Total L-ascorbic acid per amaranth plant was 60-178 mg, which was highest in the line BB followed by TW, and the L-ascorbic acid in the amaranth line BC was 132 mg.

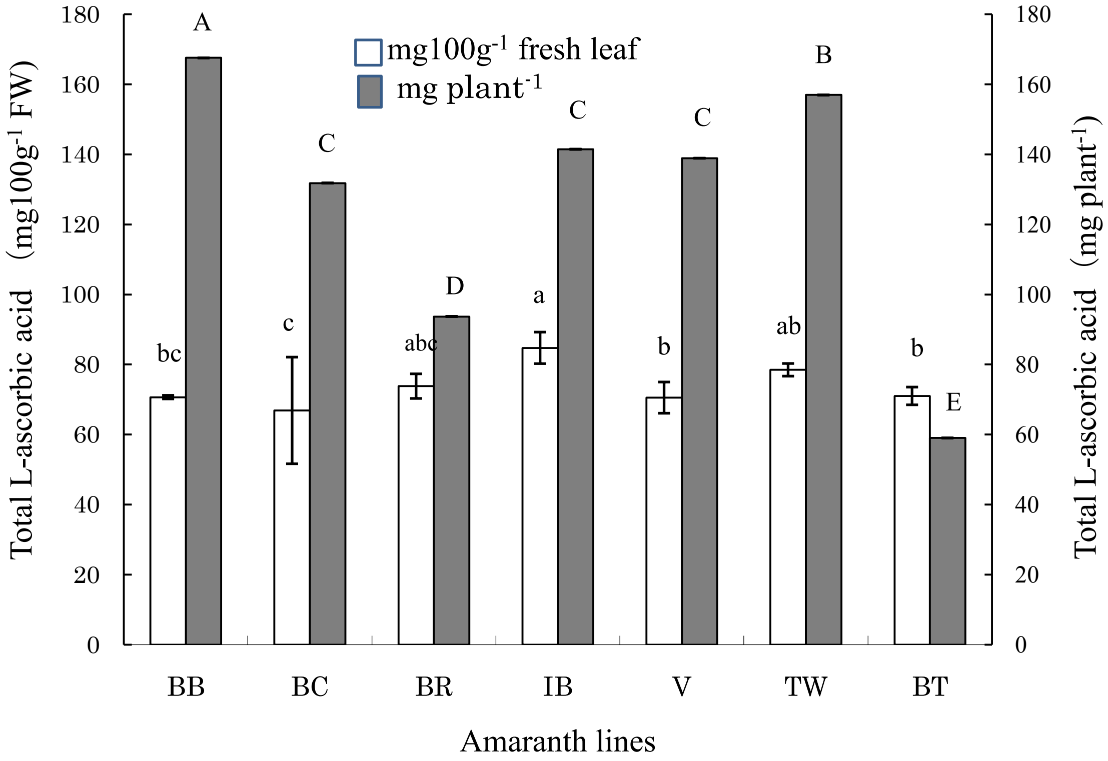


Fig. II-7. Ascorbic acid content in different amaranth lines at 33 day after seed sowing. Bars with the same letter are not significantly different at the 5% level, as determined by LSD test. FW indicates fresh weight.

Oxalic acid content in 100 g fresh amaranth was 210, 190, 244, 148, 180, 283 and 201 mg in th lines BB, BC, Br, IB, V, TW and BT, respicitively, which was highest in the line TW followed by BR (Fig. II- 8). The oxalic acid content in 100 g fresh amaranth was lowest in the line IB. Total oxalic acid per plant was highest in TW followed by BB (Fig. II-8).

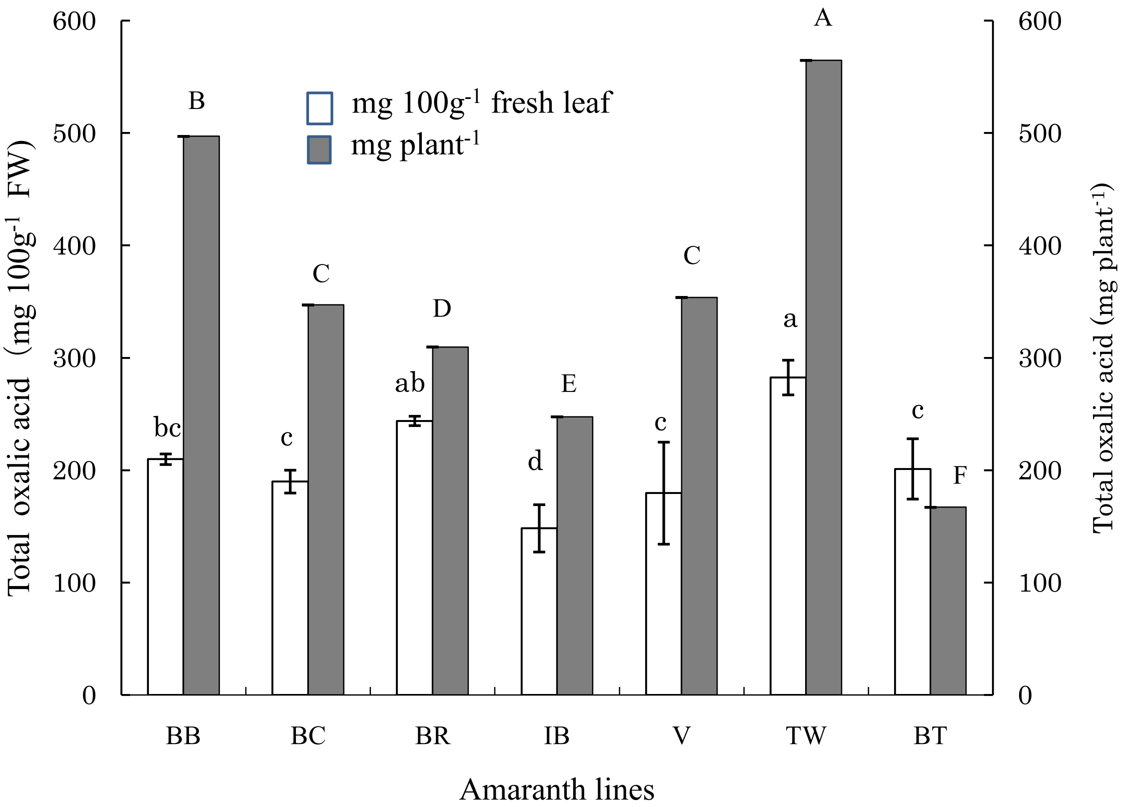


Fig. II-8. Oxalic acid content in different amaranthlines at 33 day after seed sowing. Bars with the same letter are not significantly different at the 5% level, as determined by LSD test. Planting date: April 30, 2010.

Discussion

1. Growth characteristics and yield of amaranth lines

All the amaranth lines evaluated in this study were visually different, and their growth characteristics were also different. The leaves were broad and big in size, and stems were soft in most of the amaranth lines, which were important parameters for the vegetable. Both the leaves and stems of all the amaranth lines were found to be good as vegetables. Other morphological characteristics and color of the amaranth lines were also considered for the quality vegetables. The appearances and color of the amaranth lines showed that all the amaranth lines could be used as vegetables. Similarly in Asian and African countries, both the weedy amaranths (collected from the crop fields) and cultivated amaranths are used as vegetables (Prakash and Pal, 1991; Begum, 2000; Stalknecht and Schulz-Schaeffer, 1993; Svirskis, 2003).

Amaranth is usually harvested as a vegetable when plant height is 25-40 cm; this size of amaranth is comparatively soft and delicious as vegetable, and all the leaves and stems are used. The amaranth lines BC and BB grew to 38 cm and 28 cm, respectively at 34 day after seed sowing (DAS) when cultivated in spring season (seed sowing in April). The amaranth lines BB, BC, BR, and IB grew 42-67 cm at 44 DAS. The amaranth lines BB and BC grew 42-49 cm at 30 DAS, when seeds were sown in summer season (August). In another experiment, the amaranth lines BB, BC and BR grew 34-41 cm at 37 DAS, when cultivated in spring (April seed sowing) season. All the experiments indicate that the amaranth lines BB, BC and BR grow very fast and the plants require 30 to 44 DAS from seed sowing to harvest in spring and summer season in Okinawa. These results indicate that growth of all amaranth lines was faster than other vegetables (Rastogi and Shukla, 2013).The growth of amaranth differed with the cultivation seasons due to the variations of temperature and sun radiation, which agreed the results in *Panicum repens* (Hossain et al. 2001).

The amaranth lines BB, BC and BR developed 8-14 leaves per plant within 34 DAS when seeds were sown from spring to summer season, which indicates that the leaf number of the amaranth lines was enough for quality leafy vegetable. The amaranth lines BC, BB and BR had higher stem diameter. Larger diameter of tender amaranth could result the softer vegetables. The amaranth lines BB, BC, BR and IB have red leaf, and the lines V, TW and BT have green leaves, which are attractive as vegetables. The amaranth lines/strains having hollow type stem are not considered as the quality stem vegetables. Amaranth plants require 45-49 days to initiate inflorescences and the amaranth with inflorescence are not usually used as vegetable. The plants grown within 44 days could be good for quality vegetables.

Leaf area, leaf weight and total shoot weight per plant were considerably hgher, which indicate that all the amarnth lines are good for quality leafy vegetable in Okinawa. However, total leaf area, leaf weight, stem weight and shoot weight were higher in the amranth lines BC and BB, which could be best for vegetable production in Okinawa.

2. Mineral, N and C content in the amaranth lines

The content of Na, K, Ca and Mg obtained in the amaranth lines evaluated in this study is considerably higher as compared to those in other vegetables, which indicate that all the amaranths are valuable as vegetable. Similarly other study reported that amaranth vegetables contain higher level of mineral (USDA, 1984). The minerals were higher in leaves than in the stem in all the amaranth lines. Mineral content differed more or less with the amaranth lines, and all the minerals were not similarly accumulated in the amaranth lines. The N content was 4.28-6.43% in the amaranth lines, which indicates that the amaranth lines contained higher level of protein value. Sodium, K, Ca and Mg contents were 4.74-7.07, 31-33.8, 15.83-19.93 and 11.20-15.73 mg g^{-1}, respectively in the amaranth lines cultivated in August-September. The content of N in the line BB (3.10%) was higher than in the line BC (2.40%). In another experiment, the content of Na, K Mg, Al, Fe and P was 2.19-8.70, 41.65-44.90, 19.21-22.61, 17.60-19.87, 0.55-1.05, 0.55-0.83 and 10.63-18.20 mg g^{-1}, respectively. All the minerals were considerably higher in the amaranth lines, which again indicates that all the amaranth lines could be good for nineral nutrition. Similarly other studies reported that amaranth plants contain several minerals, which are considered as the nutitional value ((Makus, 1984; Begum, 2000; Shukla et al., 2005; Shittu et al., 2006).

Total value of Na was 8.68, 16.00, 3.72, 5.72, 7.23, 6.38 and 3.12 mg $plant^{-1}$ in the lines of BB, BC, BR, IB, V, TW and BT, respectively. The amount of K was 275.79, 295.79, 163.28, 165.17, 160.16, 160.05 and 67.78 mg $plant^{-1}$ in the lines BB, BC, BR, IB, V, TW and BT, respectively. The amount of Ca was 56.20, 46.64, 30.14, 32.88, 37.65, 39.05 and 13.27 mg $plant^{-1}$ in the lines BB, BC, BR, IB, V, TW and BT, respectively, which was highest in the line BB (56.20 mg $plant^{-1}$) followed by BC. The Mg amount was 44.36, 44.30, 25.28, 28.28, 32.69, 38.85 and 13.88 mg $plant^{-1}$ in the lines BB, BC, BR, IB, V, TW and BT, respectively, which was similarly highest in the lines BB and BC (44.30-44.36 mg $plant^{-1}$). The amount of Fe was higher in the lines BB and V (0.44-0.43) followed by BC (0.38 mg $plant^{-1}$). The P amount was highest in the line BB (12.37 mg $plant^{-1}$) followed by V and BC (8.47, 08.60 mg $plant^{-1}$). The crude protein amount was highest in the line BB (1.27 mg $plant^{-1}$) followed by BC (1.10 mg $plant^{-1}$). The results show that different amaranth lines contain different levels of minerals. So it is very difficult to select an amaranth line with higher level of all minerals. Minerals and other nutritional values were also different in amaranth plants in other studies (USDA, 1984; Makus, 1984; Begum, 2000; Shukla et al., 2005; Shittu et al., 2006).

The mineral content accumulated in the amaranth lines was significantly higher as compared to that accumulated in cabbage, lettuce and Chinese cabbage, which indicate that all the amaranth lines provide sufficient mineral nutrition as vegetable (Kagawa, 2012). The line BB and BC provided remarkably higher minerals and crude protein, which could be considered as the quality amaranth lines for cultivation. Total L-ascorbic acid content differs with the amaranth lines. Total L-ascorbic acid in 100g fresh amaranth was 60-168 mg in the amaranth lines, which indicates that all the amaranth lines could provide L-ascorbic acid nutrition, which agreed the results in other studies (Abbott and Campbell, 1982; Prakash and Pal, 1991). Oxalic acid content in 100 g fresh amaranth was 210, 190, 244, 148, 180, 283 and 201 mg in th lines BB, BC, BR, IB, V, TW and BT, respicitively, which indicates that the concentration of oxalic acid in the amaranth lines is not harmful to human body when these amaranth lines are used as vegetable (USDA, 1984).

Conclusion

Among the eight lines, two lines were found to be similar in morphological characters and color therefore seven lines were evaluated in details. The amaranth lines IK and AP were found to be weedy type, which were not considered as the vegetables as compared to other lines. The color of leaves and stems of the line V was green; and this line had enough leaves and shorter stem, which could be used as vegetable. The line IB was red with broad leaf and soft stem, which could be considered as vegetables. The line TW was dwarf and green with soft stem and broad leaf, and the amaranth line BB was reddish with broad leaf and soft stem, which could be used as vegetable. The lines BC and BR were taller with broad leaves and soft stems, which could be used as vegetables.

The amaranth lines BB, BC, BR and IB required 34 days, and other lines required 44 days after seed sowing (DAS) to harvest when seeds were sown in April, whereas the lines BB and BC required 30 days when seeds were sown in August, 2010. On the other hand all the amaranth lines required 37 days to harvest when seeds were sown in April, 2011. The amaranth lines BB, BC and BR developed 8-12 leaves per plant within 34 DAS when seed were sown in April, whereas 12-14 leaves within 30 DAS when seeds were sown in August. All the experiments indicate that the amaranth lines BB, BC and BR develop enough leaf within 34 DAS, and had larger stem diameter, internode, total leaf area, leaf weight, stem weight and shoot, which contribute to better yield. Shoot biomass (yield) was highest in the line BC followed by BB. Mineral content differed somewhat with the amaranth lines however, all the amaranth lines contain higher Na, K, Ca and Mg, compared to those in other vegetables. The N content was 4.28-6.43% in the amaranth lines. The line BB and BC contained higher minerals and crude protein.

This study indicates that all the amaranth lines grew faster and resulted in sufficient yield within 44 DAS in spring and summer. The amaranth lines contain enough minerals and L-ascorbic acid. The amaranth lines BB and BC grew faster and provided highest yield with higher minerals and L-ascorbic acid, which could be the best cultivars in Okinawa, Japan.

CHAPTER III

Effects of Seed Sowing Time on Growth, Yield and Quality of Edible Amaranth *(Amaranthus* spp.) in Okinawa, Japan

Abstract: Growth, yield and quality of plants differ with cultivation time due to climatic variation in a year. Present study has been conducted to evaluate the effects of planting time on growth, yield and quality of amaranth plants for determining proper cultivation time in Okinawa. Amaranth lines BB (both leaves and stems of young plants are used, only stems of mature plants are used) and BC (both leaves and stems of young plants are used) grew faster when cultivated in August-September than the plants cultivated in April-June. The plant height, leaf number, largest leaf area, total leaf area, leaf weight, stem weight, and shoot (yield) weight of amaranth lines BB and BC were higher in August-September cultivation due to higher temperature prevailed as compared to that in April-June cultivation. The amaranth line BB contained higher Na, Al, Mn and TC, and lower K, Ca and Mg when cultivated from August to September in both the experiments in 2010 and 2013. The cultivation time did not clearly influence Na, K, Ca, Mg and Fe accumulation in the amaranth line BC. The TN and TC contents of the amaranth lines BB and BC did not differ with the cultivation time in 2010, but were higher when cultivated from August to September in 2013. The L-ascorbic acid in the amaranth plants was higher in the August-September cultivation. Growth parameters and yield of the amaranth lines BB and BC were higher, and seed sowing to harvest time is shorter in summer season than in spring season. Minerals, TN and TC contents of the amaranth lines differed somewhat with the cultivation time. Overall results suggested that summer season is better than spring season for better growth, yield and quality of amaranth in Okinawa, and amaranth plant could be harvested within 29 days after seed sowing in summer season.

Introduction

Research on the effect of climatic and edaphic factors on growth characteristic patterns of a plant species over a year in a region is very much important to understand its seasonal distribution, cultivation technologies and management practices, which help to obtain a higher profit from the plant species (Anderson, 1998; Becker et al. 1998; Deen et al. 1998; Hossain, 1999; Katovich et al. 1998; Rushing et al. 1998). Proper cultivation time of a plant species contributes to economic weed management, diseases management, fertilizer management and irrigation management in plant cultivation, which ultimately contributes to higher benefit from the crop production.

Amaranth is commercially cultivated widely in the Asian countries due to the prevailing subtropical climate. Demand of amaranth is increasing due to medicinal value, rapid growth, and resistance to diseases and drought (Rastogi and Shukla, 2013). The climatic factors limit the leafy vegetable production in Okinawa and more than 80% of vegetables are imported in summer season from main land Japan and other countries (Kawamitsu et al., 2010). The average temperature in Okinawa ranges from 16.7 to 28.7 ℃ (Fig. I. 1), which is high from May to September and low from October to April in Okinawa. Average sun radiation is 7-23.4 11 $MJm^{-2}d^{-1}$, which is high from May to September, and low from October to April. Monthly average rainfall is 119 to 674 mm, which is high from May to August. The average humidity is 61-85%, which is high from May to August (Fig. I. 2). The differences in air temperature, sun radiation, rainfall and humidity with the months may affect the growth, yield and quality of amaranth plants. In previous study (Ohshiro et al., 2015) the amaranth lines BB and BC were selected for higher yield and quality in Okinawa. However, farmers in Okinawa are not familiar with the amaranth cultivation time. Therefore, present study was undertaken to evaluate the effects of planting time (seed sowing month) on growth, yield and quality of amaranth plants to determine appropriate cultivation time in Okinawa.

Materials and Methods

1. Soil

Dark-red soil (Shimajiri mahji) was collected from the top 50 cm layer of a field at the Subtropical Field Science Center, University of the Ryukyus. The pH level of the soil was 6.6, and the soil contains 0.08% N and 0.27% C. The content of Na, K, Ca, Mg, Al, Fe and P was 7.34, 1.29, 8.78 and 1.84, respectively. According to Hossain and Ishimine (2005), coarse sand, fine sand, silt, clay and apparent density of the soil are 2.93%, 7.33%, 23.94%, 57.24% and 0.87 g cm^{-3}, respectively.

2. Amaranth lines

The *Amaranthus tricolor* lines BB (Bangladesh B line, red stem; leaves and stems of young plants are used, and only stems of mature plants are used as vegetable) and BC (Bangladesh C line, red leaf and stem; both leaves and stems of young plants are used) were evaluated in this experiment.

3. Experiment 1

A glasshouse experiment was conducted in 2010 at the Subtropical Field Science Center of the University of the Ryukyus using plastic planters (planter-65E type, IRIS Ohyama, Japan). Seeds of amaranth lines BB and BC were sown in different months (I) April 30 and (II) August 2. Each planter was filled with 7 kg of air-dried dark red soil (physical and chemical properties of the soil are presented in the previous chapter) and 6 kg of culture soil (Hanasaki monogateri, Biodo Ltd., Akimotonatural products), and the soils were mixed uniformly. In each planter, 75 seeds of each amaranth line were placed on soil surface and covered with a thin layer (<0.5mm) of the mixed soil. The plants were thinned to eight healthiest stands per planter at 2- to 3-leaf stage (14 days after seed sowing). Each amaranth line was cultivated in six planters (replications) for each seed sowing time. The planters were arranged randomly in the house, and water was applied to the plant as required every day.

4. Experiment 2

This experiment was conducted in 2013 at the Subtropical Field Science Center of the University of the Ryukyus using the same plastic planters. Seeds of amaranth lines BB and BC were sown in different months (I) April 5 and (II) August 18. Each planter was filled with 13 kg of air-dried dark red soil (physical and chemical properties of the soil are presented in the chapter II). Seventy five seeds of each amaranth line were placed on soil surface and covered with a thin layer (<0.5mm) of the soil. The plants were thinned to eight healthiest stands per planter at 2- to 3-leaf stage (14 days after seed sowing). Each amaranth line was cultivated in four planters (replications) for each seed sowing time. The planters were arranged randomly in the house, and water was applied to the plant as required every day.

5. Data collection

Plant height and leaf number were measured at a 7-day interval from15 days after seed sowing (DAS) to 36 DAS for both the experiments. Five plants were harvested from each planter at 34 DAS. Largest leaf area, total leaf area, dry leaf weight and dry stem weight of amaranth plants were measured.

6. Determination of leaf area, dry weight, mineral, nitrogen, carbon, crude protein and L-ascorbic acid in amaranth plants

Leaf area, dry weight, mineral, nitrogen, carbon, crude protein and L-ascorbic acid in amaranth plants were measured according to the previous studies (Chapter II).

7. Statistical analysis

Average data of each parameter 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 the treatment means.

Results

The amaranth line BB grew faster from 22 days after seed sowing when cultivated from August to September as compared to that cultivated from April to June. The plant height was 65 cm and 34 cm when seeds were sown in August and April, respectively (Fig. III-1(A)). The plant height of amaranth line BC was similar until 22 days after seed sowing for both the cultivation time, thereafter plant height was higher when cultivated from August to September (Fig. III-1 (B)).

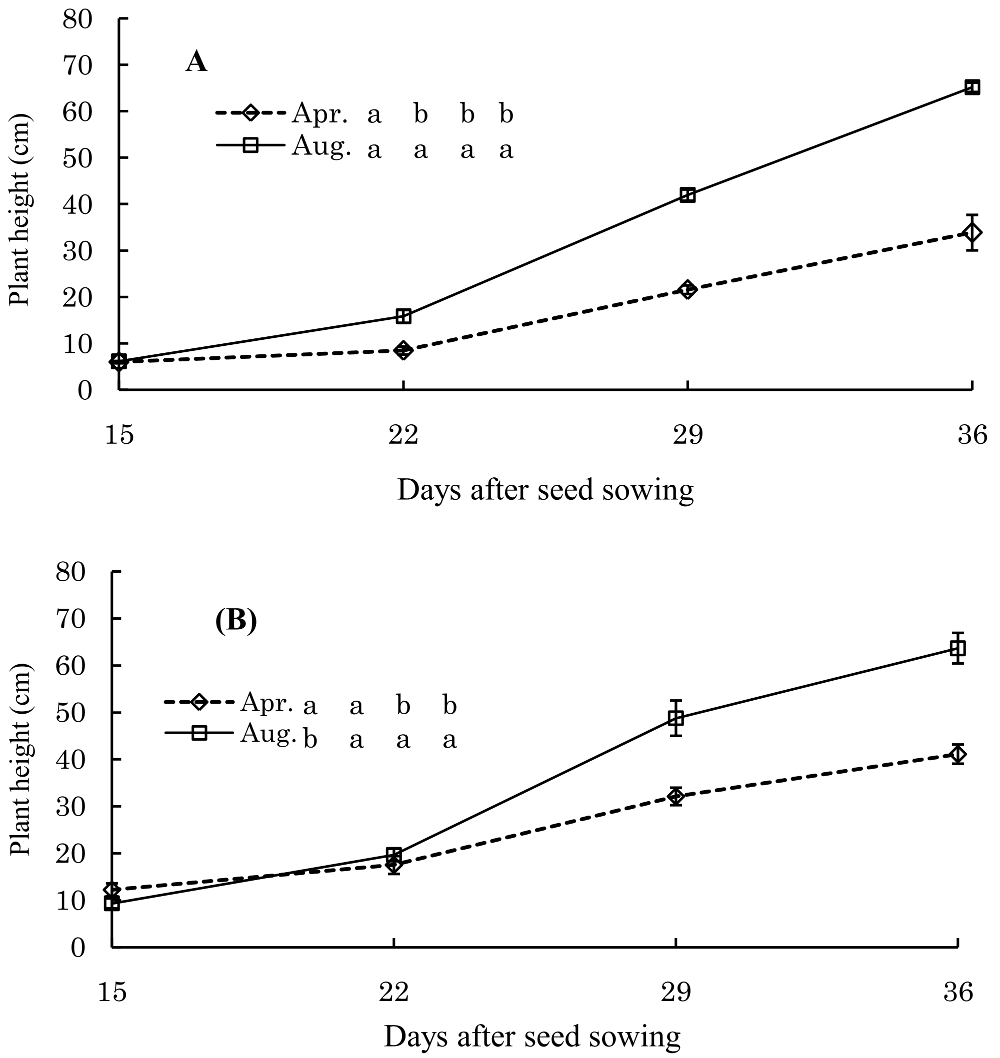


Fig. III-1. Differences in plant height of amaranth lines BB (A) and BC (B) cultivated in different months in 2010. Data with the same letter are not significantly different at the 5% level, as determined by*t-test*.

Leaf number of amaranth lines BB (A) and BC (B) was similar for both the cultivation time until 22 days after seed sowing, thereafter leaf number was higher when the amaranth plant was cultivated from August to September (Fig. III-2). Leaf number of amaranth line BB was 16 and 12 for the August and April seed sowing, respectively. Leaf number of amaranth line BC was 18 and 14 for the August and April seed sowing, respectively.

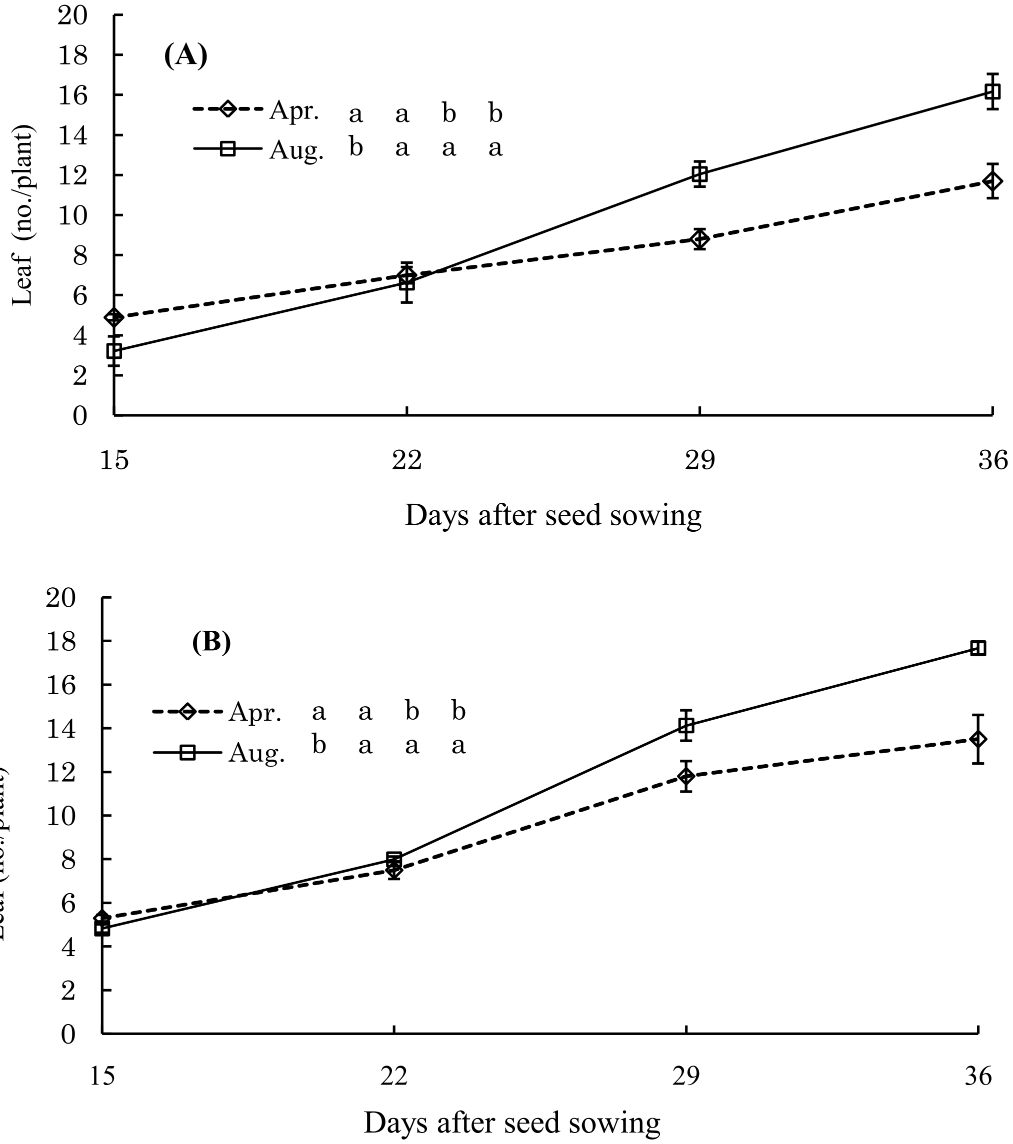


Fig. III-2. Differences in leaf number of amaranth lines BB (A) and BC (B) cultivated in different months in 2010. Data with the same letter are not significantly different at the 5% level, as determined by *t-test*.

Largest leaf area, total leaf area, dry leaf, dry stem and dry shoot were significantly higher in both the amaranth lines BB and BC when cultivated in August-September as compared to those cultivated in April–June (Table III-1). Shoot (yield) weight was higher by 28-33% in August-September cultivation than in April-June cultivation.

Table III-1. Effects of seed sowing date on growth parameters and yield (shoot) of amaranth lines BB and BC cultivated in 2010

Line	Seed sowing month	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	Apr.30	101.80b	571.30b	2.35b	1.22b	3.57b
	Aug.2	111.30a	614.60a	2.89a	1.81a	4.70a
BC	Apr.30	77.83b	624.87b	1.96b	1.75b	3.71b
	Aug.2	84.73a	639.37a	2.25a	2.51a	4.76a

The plants were harvested at 34 DAS. Data with the same letter within each column are not significantly different at the 5 % level, as determined by LSD test.

The Na, Al, Fe. Mn, Zn and TC contents of amaranth line BB were higher when cultivated from August to September, whereas K, Ca, Mg and TN were higher when cultivated from April to June (Table III-2). The Na, K, Ca, Mg, Al and Fe contents of amaranth line BC were higher when cultivated from April to June, whereas the P and Mn contents were higher when cultivated from August to September. The L-ascorbic acid of amaranth was higher in August-September cultivation. The Zn, TN and TC contents did not differ with the seed sowing time (Table III-2).

Table III-2. Effects of seed sowing time on mineral, total nitrogen and total carbon contents of amaranth lines BB and BC cultivated in 2010

Line	Seed sowing month	Na mgg^{-1}	K mgg^{-1}	Ca mgg^{-1}	Mg mgg^{-1}	Al mgg^{-1}	Fe mgg^{-1}	P mgg^{-1}	Mn mgg^{-1}	Zn mgg^{-1}	L-ascorbic acid mg per 100g	TN %	TC %
BB	Apr.30	2.19b	44.9a	22.61a	18.15a	0.55b	0.57b	18.20a	0.61b	0.45b	83.8b	3.62a	37.22b
	Aug.2	4.74a	31.33b	19.93b	15.73b	0.99a	0.79a	12.97b	0.82a	0.67a	85.6a	3.10b	37.45a
BC	Apr.30	8.70a	41.65a	19.21a	17.60a	10.50a	0.83a	10.63b	0.24b	0.51a	66.8b	2.40a	37.45a
	Aug.2	7.07b	33.80b	15.83b	11.20b	0.88b	0.60b	12.53a	0.45a	0.52a	75.5a	2.41a	37.46a

The plants were harvested at 34 DAS. Data with the same letter within each column are not significantly different at the 5 % level, as determined by LSD test.

Plant height of both the amaranth lines BB and BC was higher when cultivated in August-September than in April-May (Fig. III-3). The plant height of amaranth BB line was 13 and 25 cm in the April and August seed sowing time, respectively. Whereas the plant height of amaranth BC line was 22 cm and 31 cm in the April and August seed sowing time, respectively (Fig. III-3).

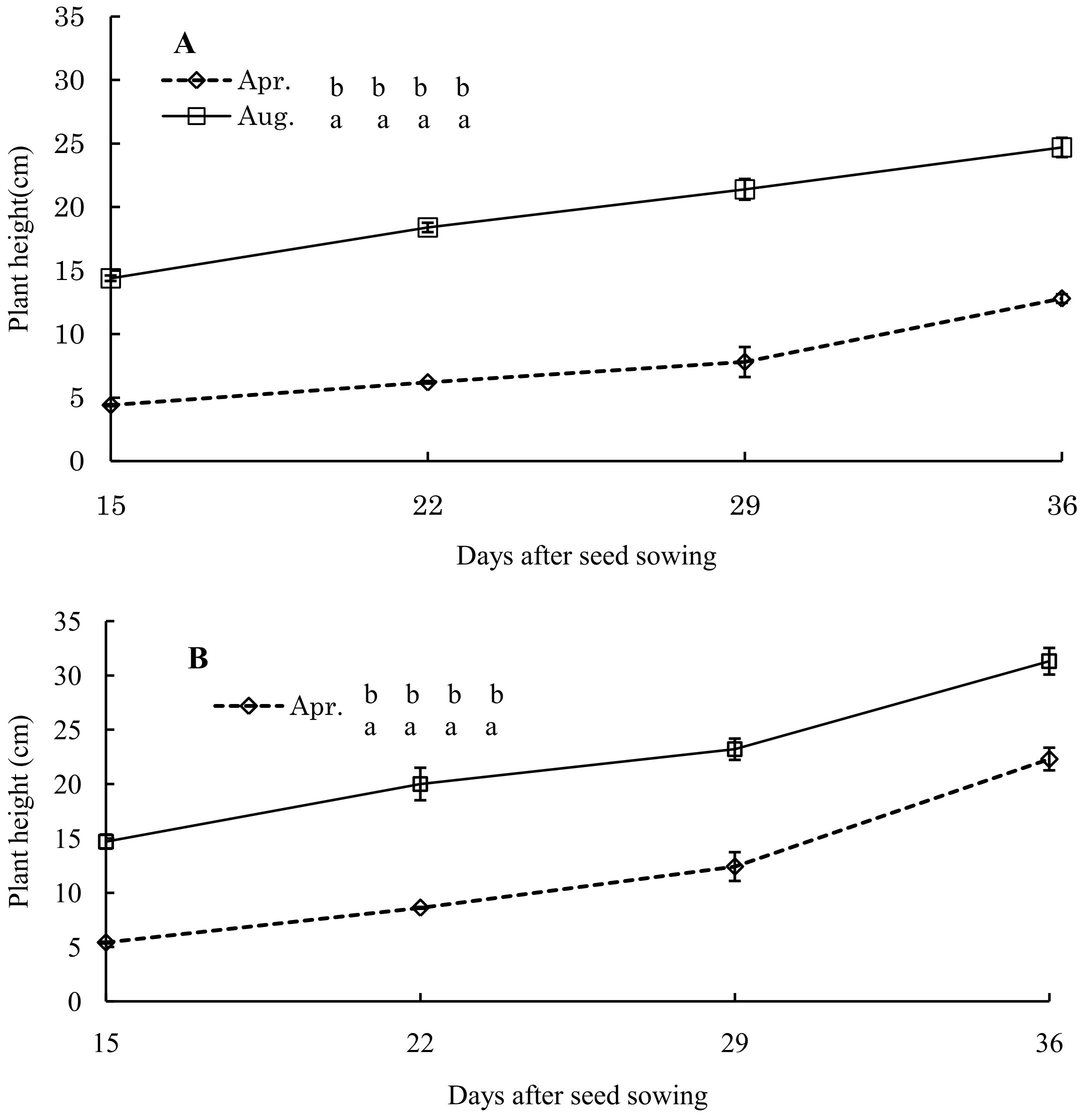


Fig. III-3. Differences in plant height of amaranth lines BB (A) and BC (B) cultivated in different months in 2103. Data with the same letter are not significantly different at the 5% level, as determined by *t-test*.

Leaf number of amaranth lines BB (A) and BC (B) was higher for the August-September cultivation in 2010. The experiment conducted in 2013 showed the same trend in leaf formation (Fig III-4). Leaf number of amaranth line BB was 13 and 7 for August and April seed sowing, respectively at 36 DAS in 2010. The leaf number of amaranth line BC was 14 and 10 for August and April seed sowing, respectively (Fig. III-4).

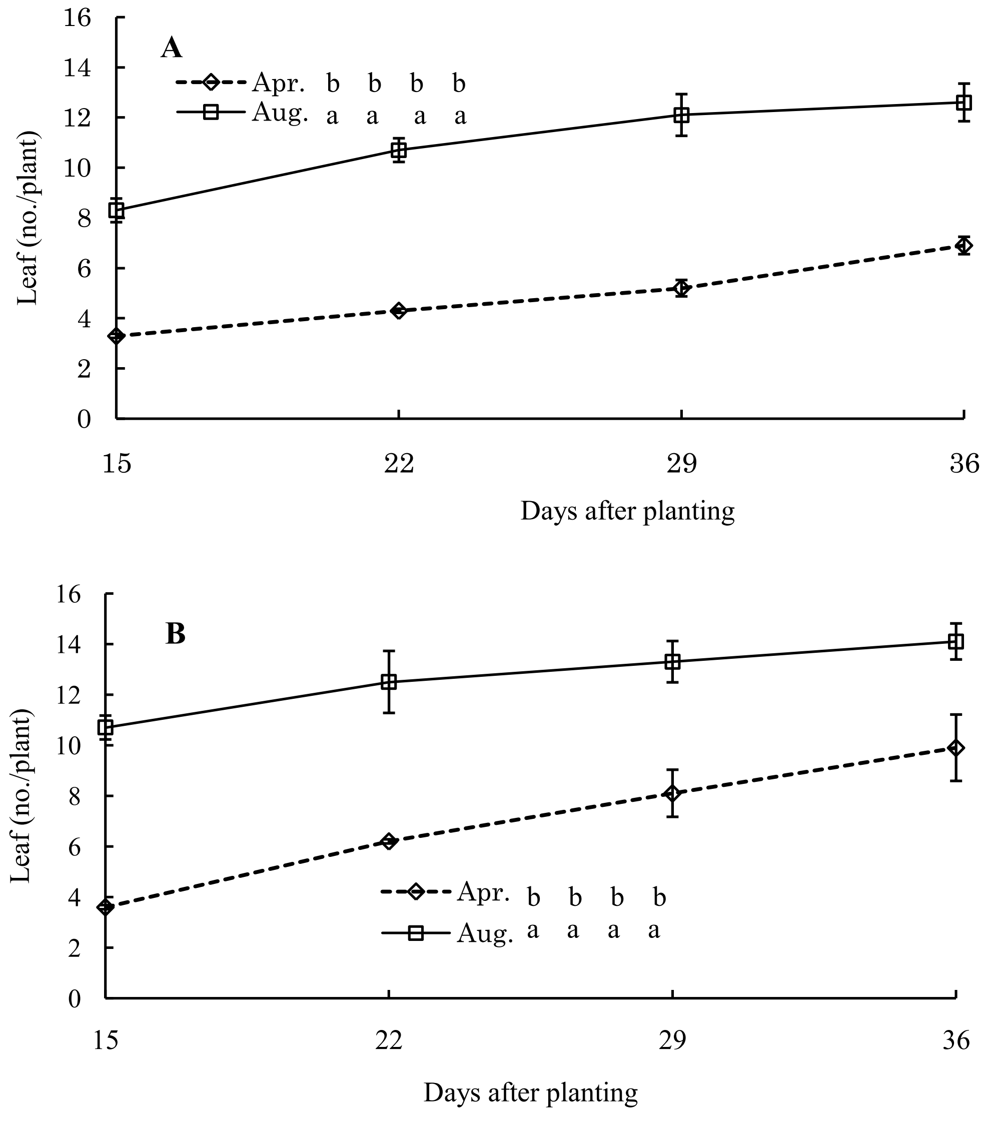


Fig. III-4. Differences in leaf number of amaranth lines BB (A) and BC (B) cultivated in different months in 2103. Data with the same letter are not significantly different at the 5% level, as determined by *t-test*.

In the experiment 2013, all the growth parameters and yield (shoot) of amaranth lines BB and BC were significantly higher when cultivated in August-September than the plants in April-May (Table III-3). Yield of the amaranth line BB was 2.0 times higher in the August-September cultivation than in the April-May cultivation. The yield of the amaranth line BC was 1.5 times higher in the August-September cultivation than in the April-May cultivation (Table III-3).

Table III-3. Effects of seed sowing time on growth parameters and yield (shoot) of amaranth lines BB and BC cultivated in 2013.

Line	Seed sowing month	Largest leaf area ($cm^2\ leaf^{-1}$)	Total leaf area ($cm^2 plant^{-1}$)	Dry leaf weight ($gplant^{-1}$)	Dry stem weight ($gplant^{-1}$)	Dry shoot weight ($gplant^{-1}$)
BB	Apr.5	35.14b	141.80b	0.60b	0.70b	1.30b
	Aug.18	41.30a	151.97a	1.60a	1.60a	3.20a
BC	Apr.5	31.42b	147.51b	1.77b	0.91b	2.68b
	Aug.18	34.89a	162.07a	2.35a	1.55a	3.89a

The plants were harvested at 34 DAS. Data with the same letter are not significantly different at the 5% level, as determined by *t-test*.

The content of Na, Al, P, Mn, TN and TC in the amaranth line BB was higher when cultivated in August-September. On the other hand, other minerals in the amaranth were higher when cultivated in April-May (Table III-4). The content of all the minerals except Al in the amaranth line BC was higher when cultivated in August-September, but the content of Al was higher when the plant was cultivated in April-May. The content of TN and TC was higher in August-September cultivation (Table III-4).

Table III-4.Effects of seed sowing date on mineral, total nitrogen and total carbon contents of amaranth lines BB and BC cultivated in 2013

Line	Seed sowing month	Na mg g^{-1}	K mg g^{-1}	Ca mg g^{-1}	Mg mg g^{-1}	Al mg g^{-1}	Fe mg g^{-1}	P mgg^{-1}	Mn mg g^{-1}	Zn mg g^{-1}	TN %	TC %
BB	Apr.5	1.12b	47.27a	13.33a	5.26a	0.53b	0.50a	2.80b	0.03b	0.13a	4.08b	34.68b
	Aug.18	1.34a	35.80b	3.64b	2.56b	0.56a	0.20b	7.66a	0.19a	0.11b	4.23a	35.02a
BC	Apr.5	1.63b	36.30b	12.40b	6.15b	0.35a	0.21b	2.87b	0.02b	0.08b	5.45b	35.30b
	Aug.18	3.07a	47.20a	16.10a	6.95a	0.12b	0.26a	7.81a	0.04a	0.11a	5.54a	39.70a

The plants were harvested at 34 DAS. Data with the same letter are not significantly different at the 5% level, as determined by *t-test*.

Discussion

The plant height and leaf number of amaranth lines BB and BC were not different with the seed sowing time until 22 days after seed sowing (DAS). It is thought that the temperature levels in April and August were suitable for early growth of amaranths which resulted in the similar plant height and leaf number or amaranth plant is able to grow in a large range of temperature in early stage. The plant height and leaf number were higher from 22 DAS in the August cultivation due to the higher temperature level as compared to that in April cultivation, which indicates that amaranth plants grow well in summer season in tropical and subtropical region than in spring season. Similar results were reported in other plant species (Deen et al., 1998; Hossain et al., 2001; Nada Y., 1980). All the growth parameters and yield of amaranth lines BB and BC were significantly higher at harvest time (36 DAS) when cultivated in August-September, due to higher temperature prevailed as compared to April-May. Similarly, other studies reported that tropical plants usually accumulate higher biomass in summer season than in spring or winter season (Becker and Fawcett, 1998; Hossain et al., 2001). All the amaranth lines provided enough leaves and yield within 29 days after seed sowing in August-September cultivation.

The Na, Al, Mn and TC contents of amaranth BB line were higher when cultivated from August to September in the experiments 2010 and 2013, which indicates that higher temperature prevailed in summer season was better for accumulation of the minerals and TC. The content of K, Ca and Mg in the amaranth line BB was higher in April-June cultivation in both the experiments 2010 and 2013, which indicates that these minerals require relatively lower temperature for proper accumulation. On the other hand, cultivation time did not show clear influences on the accumulation of Fe, P, Zn and TN in the amaranth line BB. In the amaranth line BC, the contents of Na, K, Ca, Mg and Fe were higher when cultivated from April to June than in the plants cultivated from August to September in 2010 experiment.

Whereas, the mineral contents in the plants were higher when cultivated from August to September than in the plants cultivated from April to June in 2013 experiment. It is assumed that temperature is not the only factor to influence mineral accumulation in the plants; some other unknown factors are involved. The TN and TC contents of the amaranth lines BB and BC did not differ with the cultivation time in 2010 but differed in 2013. The L-ascorbic acid in the amaranth plants was higher in August-September cultivation may due to the higher temperature. Becker and Fawcett (1998) reported that carbohydrate fluctuates in hemp dogbane (*Apocynum cannabinum)* with the seasonal variation. This study indicates that mineral accumulation patterns differ with the amaranth lines, but not clearly with the cultivation time. It is assumed temperature is not the only factor involves with the mineral accumulation in amaranth plant, light is probably another factor involves with the mineral accumulation, which need to be evaluated in future studies.

Conclusion

Amaranth lines BB and BC showed similar growth pattern in early growth stage when cultivated from April to June and August to September, but all the growth parameters and yield (shoot) were significantly higher at harvest when the plants were cultivated from August to September. This study indicates that amaranth plants can grow well in a large range of temperature in early growth stage but require higher temperature in moderate growth stage for higher yield.

The contents of Na, Al, Mn and TC in the amaranth line BB were higher when cultivated from August to September, but the contents of K, Ca and Mg were higher in April-June cultivation in both the experiments 2010 and 2013, which indicates that some minerals response differently with the different temperature levels. The amaranth line BC had higher content of Na, K, Ca, Mg and Fe when cultivated from April to June in 2010 experiment, but lower content of the minerals in 2013 experiment, which indicates that temperature is not the only factor involved with mineral accumulation in amaranth, light is probably another factor involved. L-ascorbic acid in the amaranth plants was higher in August-September cultivation may due to the higher temperature. The amaranth plants provided enough yield within 29 days after seed sowing in August-September cultivation, whereas needs longer time in April-May cultivation. Overall results of this study indicate that summer season is better than spring season for better growth, yield and quality of amaranth in Okinawa, Japan.

CHAPTER IV

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

Abstract: **S**oil types (gray soil, pH 8.4; dark-red soil, pH 6.6; red soil; pH 5.4) 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 cultivation practices in Okinawa. All the *Amaranthus* lines grew faster and better in gray soil than in other soils. Plant height, number of leaves, stem diameter, leaf area, and fresh and dry shoot of all lines were highest in gray soil. Dark-red soil and red soil provided almost similar growth parameters of the lines. The lines contained highest Na in gray soil followed by dark-red soil, except BC. All the lines had higher K in gray soil than in other soils. Calcium of the lines was similarly higher in gray soil and red soil, and Mg was highest in gray soil. All the lines had higher Al in gray and dark-red soil, and higher Fe in dark- red soil. Phosphorous did not differ with the soil types in IB, but TW had higher P in gray soil and dark-red soil, and BB had highest P in dark-red soil and BC had highest P in gray soil. All the lines contained Mn in dark-red soil. The line IB had similar Zn in all soils, TW had highest Zn in dark-red soil, and BB and BC had highest Zn in gray soil. The lines IB, BB and BC had highest N in dark-red soil, and TW had highest N in red soil. The overall results of study indicate that gray soil is better for *Amaranthus* cultivation in Okinawa for higher yield, K, Ca and Mg.

Introduction

Growth, yield and quality of a plant species differ with soil types and soil nutrient status; and a plant species requires suitable soil for higher yield and better quality (Oya, 1972; Oya et al., 1977; Hossain and Ishimine, 2005; Akamine et al., 2007; Chowdhury et al., 2008; Hossain et al., 2011; Islam et al., 2011). 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 (Oya, 1972; Broadley et al. 2012a, 2012b; Hawkesford et al. 2012). Study on growth characteristics of a plant species in local soils is important to develop management practices for higher yield with good quality (Hossain and Ishimine, 2005).

Many *Amaranthus* species have been cultivated in different types of soil as vegetable in many countries, and are popularly consumed as vegetable in Africa, Bangladesh, Caribbean, China, Greece, India, Nepal and South Pacific Islands (Makus, 1984; Prakash and Pal, 1991; Stalknecht and Schulz-Schaeffer, 1993;Singh and Whitehead, 1996; Begum, 2000; Svirskis, 2003). The soil types in Okinawa are different from the soil types in other countries or regions. In Okinawa, some *Amaranthus* species are found as weeds in various crops and vegetables in the dark-red soil, red soil and gray soil (Hossain and Ishimine, 2005; Okinawa Prefecture Agriculture, Forestry and Fisheries, 2008). Physical and chemical properties of the soils are different, which may affect growth, yield and quality of amaranth lines. Shittu et al. (2006) reported that a specific soil provide higher yield and nutrient compositions of amaranth in Nigeria. Growth, yield, minerals and L-ascorbic acid of 12 amaranth lines cultivated in a constant environment were evaluated; and some high yielding amaranth lines were selected as the quality summer vegetables in Okinawa (Ohshiro et al., 2015), but no study has yet been conducted on the selected amaranth lines regarding the soil factors in Okinawa. Therefore, the objectives of these studies were to identify the best soil type for higher growth, yield and quality of selected amaranth lines for developing cultivation practices in Okinawa.

Materials and Methods

1. 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 the northern part of Okinawa. Chemical properties of the soils are presented in the Table IV-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.

Table IV-1. Mineral contents and total N and C in gray, dark-red and red soils in Okinawa

Soil types	Na	K	Ca	Mg	Al	Fe	P	pH	N	C
	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}		%	%
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 3 replications. Data with the same letters within each column are not significantly different at the 5% level, as determined by LSD test.

2. 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) were evaluated in this study, considering the yield and quality of the lines evaluated in the previous study (Ohshiro et al., 2015).

3. 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 July10 to August 30, 2011. Each planter (planter-65E type, IRIS Ohyama, Japan) was filled with 13 kg of air-dried soil; and seeds of the four amaranth lines were placed on the soil surface and covered with a thin layer (<0.5mm) 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.

4. 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. 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.

5. Determination of leaf area and dry weights of amaranth plants

Leaf area and dry weight of amaranth plants were measured following the same procedures applied in the previous experiments.

6. Determination of pH, mineral contents, nitrogen and carbon of soil, and minerals, nitrogen, carbon and L-ascorbic acid of amaranth plants

The pH value, mineral contents, nitrogen and carbon of the soils, and minerals, nitrogen, carbon and L-ascorbic acid in the amaranth lines were determined following the same procedures applied in the previous experiments.

7. 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

1. Effects of soil types on growth, yield, nitrogen, carbon, and nutrient contents of amaranth 4 lines

The Fig. IV-1 shows the growth situation of the four amaranth lines cultivated in the different soils. The left four lines of planter are for the red soil, middle four planter lines are for the dark-red soil and right four planter lines are for the gray soil. Amaranth growth was very poor in the red soil (left 4 planter lines), comparatively better in the dark-red soil (middle 4 planter lines) and best in the gray soil (right 4 planter lines) at 34 day after seed sowing. 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 (data not presented).

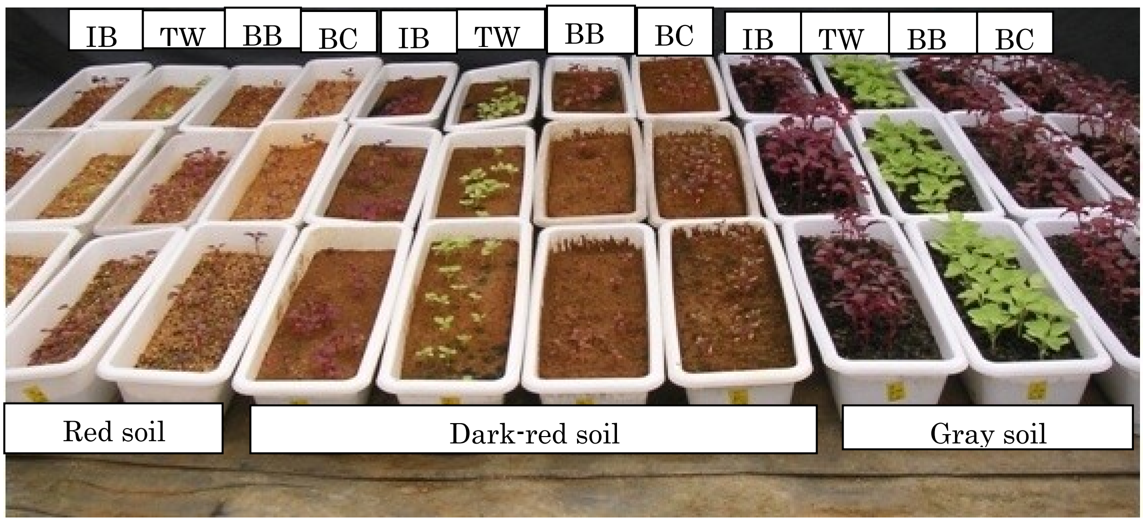


Fig. IV-1. Growth of four amaranth lines in red soil, dark-red soil and gray soil in Okinawa at 34 day after seed sowing (DAS)

Plant height of the amaranth line IB was about 15 cm in gray soil, whereas the plant height was about 4 cm in dark-red soil and red soil (Fig. IV-2). The plant height of amaranth line TW was 9 cm in gray soil and 3-4 cm in dark-red soil and red soil. The plant height of amaranth line BB was about 13 cm in gray soil and 5 cm in dark-red soil and red soil. The plant height of amaranth line BC was about 16 cm when cultivated in gray soil, and 7 and 5 cm in dark-red soil and red soil, respectively. The gray soil resulted in double plant height of all the amaranth lines than the other soils at 34 day after seed sowing.

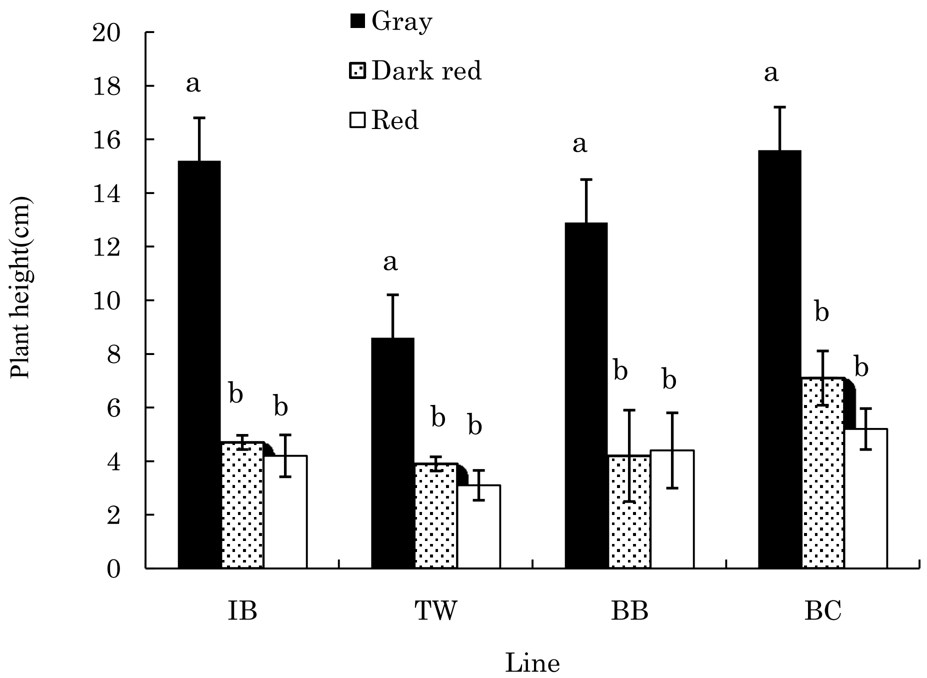


Fig. IV-2. Plant height of amaranth lines at 35day after seed sowing in different soils. Bars with the same letter are not significantly different with in each line at the 5% level, as determined by LSD test.

The leaf number of the amaranth line IB was highest in gray soil (6) followed by dark-red soil (4) and red soil (3). The leaf number of amaranth line TW was highest in gray soil (7), followed by dark-red soil (4) and red soil (3) (Fig. IV-3). The amaranth line BB provided 7 leaves when cultivated in gray soil, which was significantly higher than that in dark-red soil and red soil. The amaranth line BC developed highest leaves when cultivated in gray soil (9) followed by dark-red soil and red soil. All the lines indicate that leaves of all amaranth lines increased 30-40% when cultivated in gray soil, as compared to that in other soils.

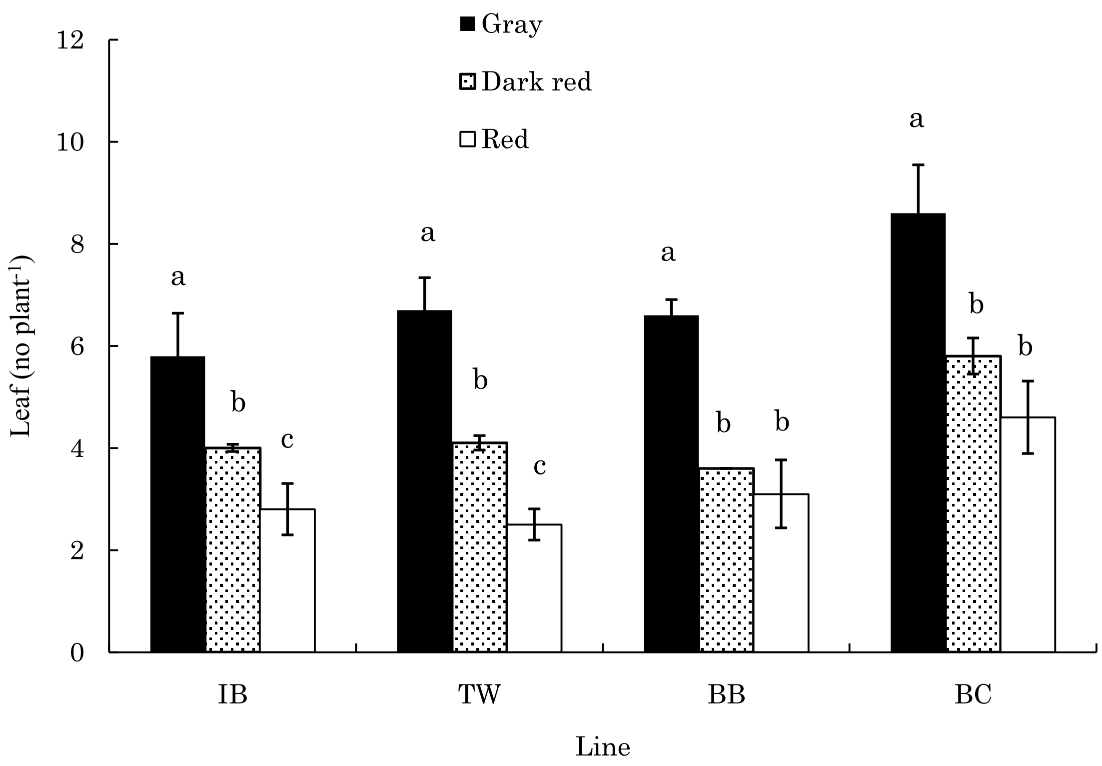


Fig. IV-3. Leaf number (B) of amaranth lines at 35day after seed sowing in different soils. Bars with the same letter are not significantly different within each line at the 5% level, as determined by LSD test.

Leaf area of the amaranth line was 34 cm^2 in gray soil and 9 cm^2 in dark-red soil and red soil (Fig. IV-4). The leaf area was more than three times higher in gray soil than in other soils. The leaf area of amaranth line TW was 47 cm^2 in gray soil, 9 cm^2 in dark-red soil and 7 cm^2 in red soil. The leaf area was 5-7 times higher in gray soil than in other soils. The leaf area of amaranth line BB was 48 cm^2 in gray soil, and 7 cm^2 in other soils. The leaf area was 7 times higher in grays soil than in other soils. The leaf area of amaranth line BC was 41 cm^2 in gray soil and 9 cm^2 in dark-red soil and 8 cm^2 in red soil. The line BC obtained about 4 times higher leaf area when cultivated in gray soil than in other soils. All the lines resulted in 4-7 times higher leaf area in gray soil than in other soils.

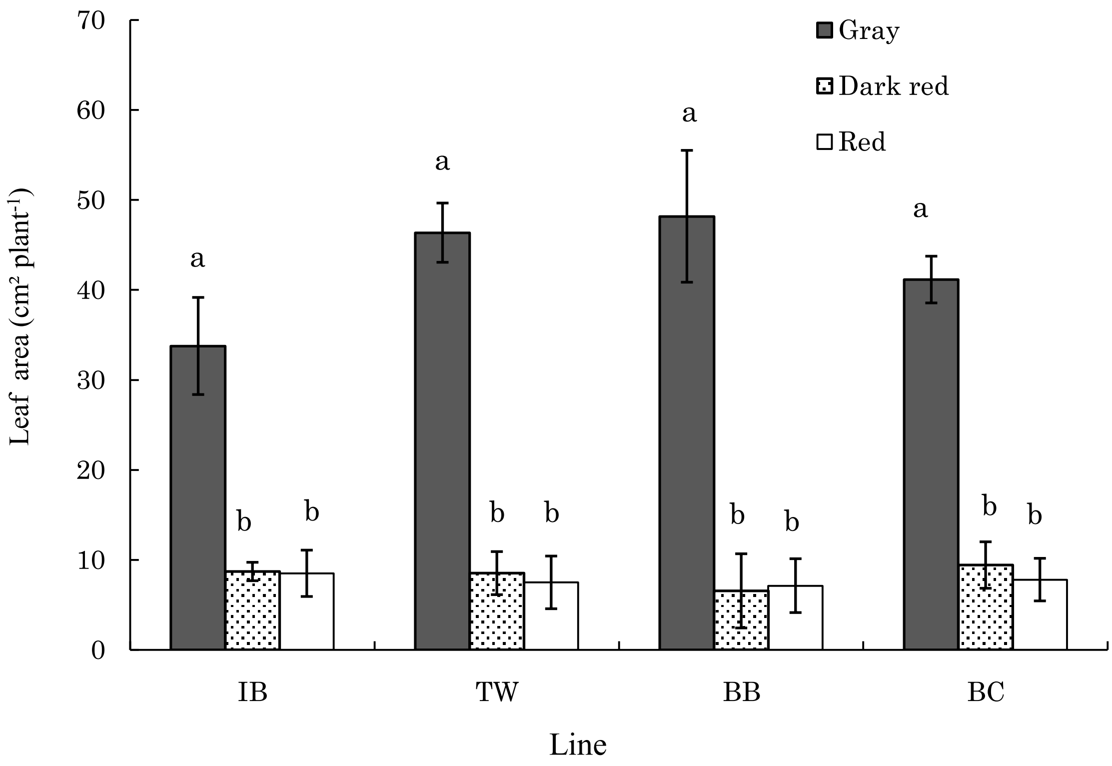


Fig. IV-4. Leaf area of amaranth lines at 35 day after seed sowing in different soils. Bars with the same letter are not significantly different within each line at the 5% level, as determined by LSD test.

Dry shoot (edible parts, yield) weight of amaranth line IB was 0.33 g per plant when cultivated in gray soil. The yield was only 0.08 g or less per plant in the dark-red soil and red soil (Fig. IV-5). The gray soil provided about 4 times higher yield of amaranth line IB than other soils. The amaranth line TW received 0.27 g shoot when cultivated in gray soil, which was about 3 times higher than that cultivated in other soils. The amaranth line BB obtained 0.42 g yield in gray soil, which was about 5 times higher than that in other soils. The gray soil resulted in 0.50 g dry shoot of amaranth line BC, which was about 5 times higher than that in other soils. All the amaranth lines obtained 3-5 times higher yield in gray soil than in other soils. All the lines showed similar yield in dark-red soil and red soil.

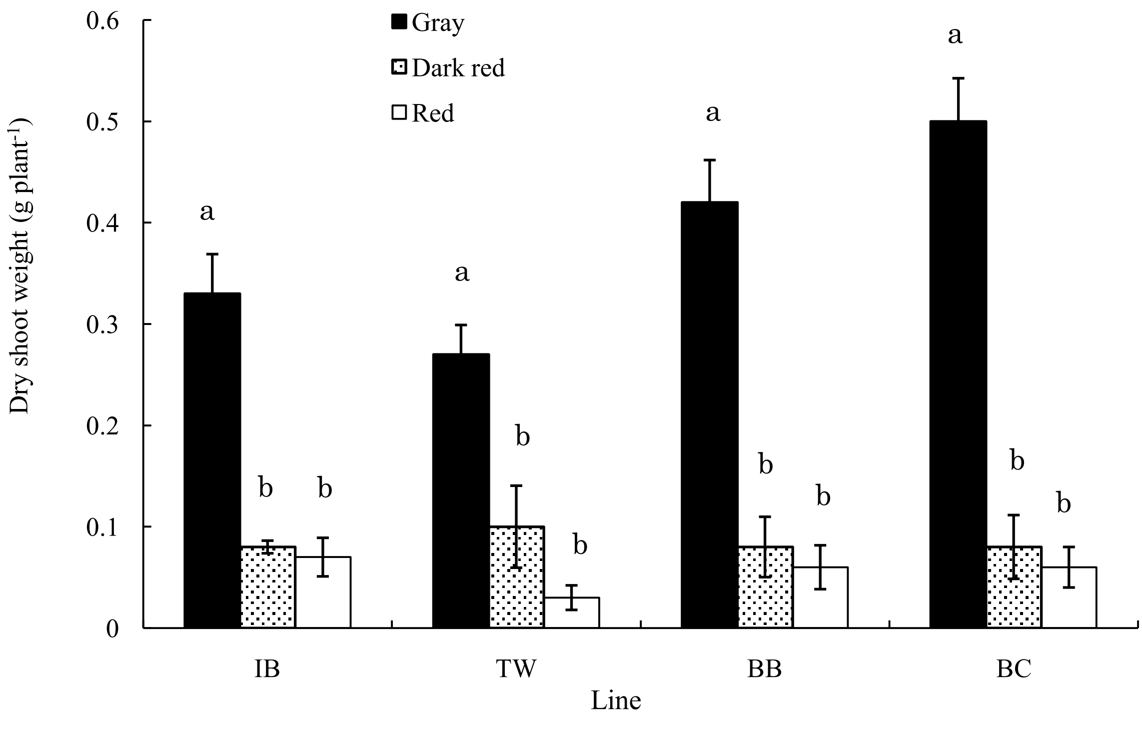


Fig. IV-5. Dry shoot weight of amaranth lines at 35 day after seed sowing in different soils. Bars with the same letter are not significantly different within each line at the 5% level, as determined by LSD test.

The range of Na content was 8.68-16.05, 8.13-21.75, 8.8-17.93 and 9.16-20.80 mg g^{-1} in the amaranth line IB, TW, BB and BC, respectively. 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 IV-2). The range of K content was 51-223, 68-263, 65-262 and 29-205 mg g^{-1} in the amaranth line IB, TW, BB and BC, respectively. The K content of the lines was 4-6 times higher in gray soil, as compared to that in other soils (Table IV-2). The range of Ca content was 86-132, 108-148, 107-167 and 55-107 mg g^{-1} in the amaranth line IB, TW, BB and BC, respectively. The Ca content was higher in gray and red soils compared to those in other soil. The range of Mg content was 31-56, 42-56, 36-64 and 31-62 mg g^{-1} in the amaranth line IB, TW, BB and BC, respectively. All amaranth lines received highest Mg when grown in gray soil compared to that in other soils (Table IV-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. The range of Al content was 2.16-7.95, 1.83-3.66, 1.71-11.50 and 2.78-11.80 mg g^{-1} in the amaranth line IB, TW, BB and BC, respectively. All the amaranth lines contained higher Al in gray soil and dark-red soil compared to that in red soil (Table IV-2). The range of Al content was 2.16-7.95, 1.83-3.66, 1.71-11.50 and 2.78-11.80 mg g^{-1} in the amaranth line IB, TW, BB and BC, respectively. The range of Fe content was 1.07-4.69, 1.34-4.34, 1.08-5.75 and 1.57-5.88 mg g^{-1} in the amaranth line IB, TW, BB and BC, respectively. All the lines contained highest Fe in dark-red soil. The range of P content was 11.2-13.2, 12.9-18.0, 13.3-18.2 and 10.7-12.8 mg g^{-1} in the amaranth line IB, TW, BB and BC, respectively. Phosphorous content of IB line did not differ with the 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 IV-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 IV-2). Percent C in amaranth plants was lowest when cultivated in gray soil (Table IV-2).

Table IV-2. Minerals and total N and C contents inedible shoot of four amaranth lines cultivated in gray soil, dark-red soil and red soil in Okinawa

Line	Soil	Na	K	Ca	Mg	Al	Fe	P	N	C
		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}	%	%
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

Note : 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.

Discussion

1. Effects of soil types on growth and yield of four amaranth 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 IV-1 and Fig. IV-2). Similarly, several studies reported that N is more significant than other nutrients for vegetative growth of plants (Sarker et al., 2001; Akamine et al., 2007; Chowdhury et al., 2008; Hossain et al., 2011, 2012). In addition, higher K, P, N and pH, and lower Na probably made suitable combination in gray soil for better growth of amaranths, as compared to those in other soils (Table 1 and Fig. 2). Other studies reported similar effects of balanced nutrients for higher biomass production in various crops (Oya, 1972; Akamine et al., 2007; Mazid, 1993; Hao and Papadopoulos, 2004; Hossain et al., 2012). 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 was reported earlier (Hossain and Ishimine, 2005; Shittu et al., 2006).

Gray soil had optimum moisture than other soils (hand feeling), which may contributed to greater vegetative growth and shoot biomass of the plant. On the other hand, water-logging 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 and 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 and Ishimine, 2005), which may not be texturally favorable for nutrient absorption and plant growth. Similarly, Donald and Katherine (1999) reported that nutrient availability, absorption and plant growth differ significantly with the physical, chemical and biological factors of soil.

2. Effects of soil types on nitrogen, carbon and nutrient contents of four amaranth lines

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 (TablesIV-1 andIV-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*) (Johnson et al., 2003; Hossain and Ishimine, 2005; Chowdhury et al. 2008; Omirou et al., 2009; Hossain et al., 2011). 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, Results of this study agreed the result in another study (Donaldand 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 and Ishimine, 2005; Hossain et al., 2012).

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.

The contents (%) of Na, K and Ca in amaranth plants were higher when cultivated in the soils with higher level of available minerals Na, K and Ca, but other minerals 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 mineral accumulations in the amaranth plants. These results indicate that a specific level of minerals and pH in soil is required for maximizing mineral accumulation in amaranth plants. This study suggests that gray soil is better for amaranth cultivation in Okinawa.

CHAPTER V

Effects of N Fertilizer Levels and Combined NPK Fertilizer on Growth, Yield and Quality of Edible *Amaranthus tricolor* line BB in Different Soils in Okinawa

Abstract: A glasshouse experiment was conducted using gray soil (pH 8.4, N 0.124%), dark–red soil (pH 6.6, N 0.076%) and red soil (pH 5.4, N 0.028%) at the Subtropical Field Science Center of the University of the Ryukyus, from September 25 to December 15, 2011. Fertilizer treatments were Cont (0 g m^{-2}), LN (nitrogen 50 g m^{-2}), HN (nitrogen, 100 g m^{-2}) and NPK (150 g m^{-2}; 50 g N, 50 g P, 50 g K) for each soil types. Plant height was highest for NPK application in all soils, and the plants with LN and HN resulted in a significantly higher height compared to control in only gray soil, but not in other soils. Leaf number was similarly higher for LN, HN and NPK than for control in gray soil, whereas it was highest for NPK in other soils. Leaf area per leaf and per plant was highest for the NPK in all soil types. Fresh and dry weight of amaranth was highest for the NPK treatment in all soils. The fertilizer LN and HN increased leaf area, and fresh and dry weight of amaranth compared to control in gray soil, but not in other soils. Sodium content in leaf was similar with all treatments, but K increased with NPK in all soils. Calcium was highest with HN in gray soil, LN and NPK in dark-red soil, and LN and HN in red soil. Magnesium was same with all treatments in dark-red soil, but higher with NPK in gray soil and with LN and HN in red soil. Nitrogen did not differ with the treatments in gray soil, but was higher with HN in dark-red soil and with LN in red soil. This study indicates that amaranth plant responses differently with different fertilizers in different soils may due to different levels of nutrients and pH in the soils. The plant requires combined N, P and K in all soils for proper growth and yield, and it could be cultivated in all soils in Okinawa by applying appropriate rate of N, P and K fertilizer.

Introduction

Studies on growth and development responses of a plant species to the nitrogen (N) fertilizer at different rates (doses) are important for understanding the plant's cultivation and management practices (Mazid 1993; Sunusi et al. 1999; Zakaria et al. 2000; Wadi et al. 2003). Growth, yield and quality of a plant species differ with soil nutrient status and fertilizer management (Oya, 1972; Oya et al., 1977; Hossain and Ishimine, 2005; Akamine et al., 2007; Chowdhury et al., 2008; Hossain et al., 2011; Islam et al., 2011). 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 (Oya, 1972; Broadley et al. 2012a, 2012b; Hawkesford et al. 2012).

Different plant species response differently to fertilizer rates and combination and a plant-species requires balanced fertilizers to maximize growth, yield and quality (Hossain et al., 2004; Akamine et al., 2007; Chowdhury et al., 2008; Hafsi et al., 2011). The major nutrients (N, P, K) individually or in combination maintain growth, yield and quality of plants (Mazid, 1993; Ivony et al., 1997; Nakano and Morita, 2009; Hafsi et al., 2011). Nitrogen influences chlorophyll formation, stomatal conductance and photosynthetic efficiency, which is responsible for 26-41% of crop yield (Maier et al., 1994; Ivony et al., 1997). 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).

Amaranth plant contains protein, ascorbic acid and mineral nutrients of Ca, Fe, Mg, P, K and Na, which are considered as the nutritional value in vegetables (Sreelathakumary and Peter, 1993: Svirskis, 2003; Rastogi and Shukla, 2013; USDA, 1984). It is thought that growth, yield and quality parameters of amaranth differ with the fertilizers in different soils.

Shittu et al. (2006) reported that balanced fertilizers in a specific soil provide higher yield and nutrient compositions of amaranth in Nigeria. Growth, yield, minerals and L-ascorbic acid of 8 amaranth lines were evaluated under a management condition, and selected some high yielding amaranth lines with high quality as summer vegetables in Okinawa (Ohshiro et al., 2015). In the chapter III, growth, yield and quality of amaranth BB, BC and BR differed with the major soil types of dark-red soil, red soil and gray soil in Okinawa. It is thought that growth, yield and quality of amaranth plants differ with N fertilizer levels and combined NPK fertilizer in different soil types possessing different levels of minerals, pH and N. Therefore, the objectives of this study was to evaluate N fertilizer rates and combined NPK on growth, yield and quality of four amaranth lines for developing management practices in Okinawa.

Materials and Methods

1. Soil collection

Dark-red soil (Shimajiri mahji), gray soil (Jaagaru) and red soil (Kunigami mahji) were used in this study. All the soils were collected from the same places of the soils used in the previous chapters. Chemical and physical properties of the soils are presented in the chapter III (Table V-1).

2. Amaranth line

The *Amaranthus tricolor*line BB (Bangladesh B line, red stem-amaranth) was evaluated in this study.

3. Evaluation of N fertilizer levels and combined NPK fertilizer on amaranth grown in different soil types

In previous chapters, we evaluated growth characteristics, yield and quality of the amaranth four lines in gray soil, dark-red soil and red soil without fertilizer application. In this study, N fertilizer levels and combined NPK fertilizer were evaluated on an amaranth line in the same three soils. The glasshouse experiment was conducted at the Subtropical Field Science Center of the University of the Ryukyus, from September 25, 2011 to December 15, 2011. Each planter was filled with 13 kg of air-dried soil. The experiment consisted of four treatments with three replications for each soil type. 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 $0.1m^{-2}$ (57.0cm×17.5cm), 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.

4. Data collection

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 measured at 28, 33, 38 and 43 DAS. Five plants were harvested from each planter at 34 DAS, and all the growth parameters were determined according to previous experiments described in chapters III and IV.

5. Determination of leaf area and dry weights of amaranth plants

Leaf area and dry weight of leaves and stems were measured according to the previous experiments.

6. Determination of mineral contents, nitrogen content, carbon content and L-ascorbic acid of amaranth plants

Mineral, nitrogen, carbon and L-ascorbic acid contents of amaranth plants were determined according to previous experiment (Chapter II).

7. 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

1. Evaluation of N fertilizer levels and combined NPK fertilizer on growth, yield, nitrogen, carbon, and nutrient contents of amaranth grown in different soil types

The plant grown with the fertilizer LN, HN and NPK in the gray soil were healthier than the plant grown without fertilizer (Fig. V-1). All the plants with the fertilizer were found to be soft with attractive color.

Fig. V-1. Effects of N rates and combined NPK fertilizer on growth of amaranth line BB at 35 day after seed sowing in gray soil. CONT (control, 0 gm^{-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 plant grown without fertilizer and with the fertilizer LN and HN in dark-red soil were very poor, and the appearances of the plants grown in these treatments were not found to be good for vegetable (Fig. V-2). Whereas, the plant grown with the fertilizer NPK was very good appearance for vegetable, and the leaves and stems of this plant was found to be soft.

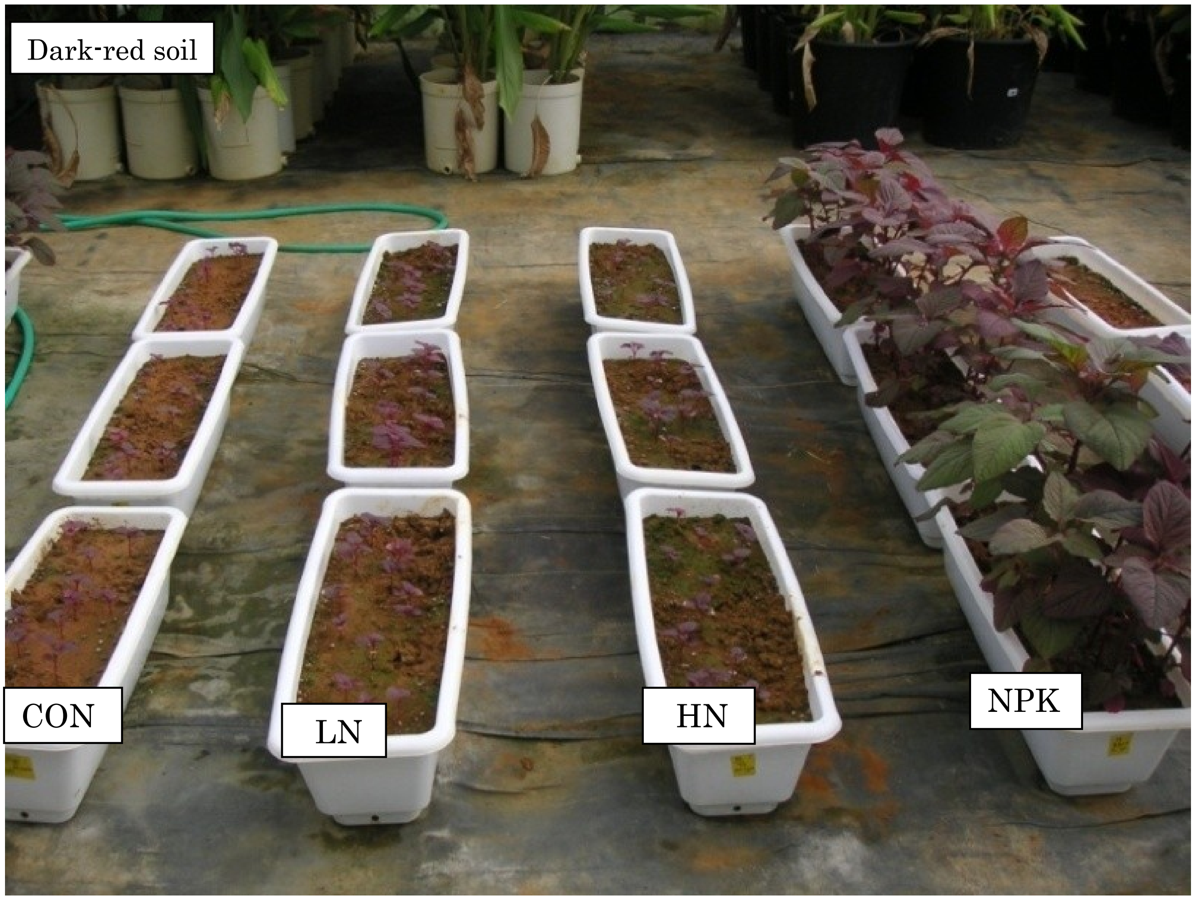


Fig. V-2. Effects of N rates and combined NPK fertilizer on growth of amaranth line BB at 35 day after seed sowing in dark-red soil. CONT (control, 0 gm^{-2}), LN (low nitrogen, 50 g N m^{-2} or 5 g N planter^{-1}), HN (high nitrogen, 100 g N m^{-2} or 10 g N planter^{-1}) and NPK (150 g NPK m^{-2} or 5 g N + 5 g P + 5 g K planter^{-1}).

The plant did not grow well when cultivated without fertilizer and with the fertilizer LN and HN in red soil, and the appearances of the plants showed that this plant could not be used as vegetable (Fig. V-3). Whereas, the plant grown with the fertilizer NPK was very good for vegetable, and the leaves and stems of this plant were found to be soft.

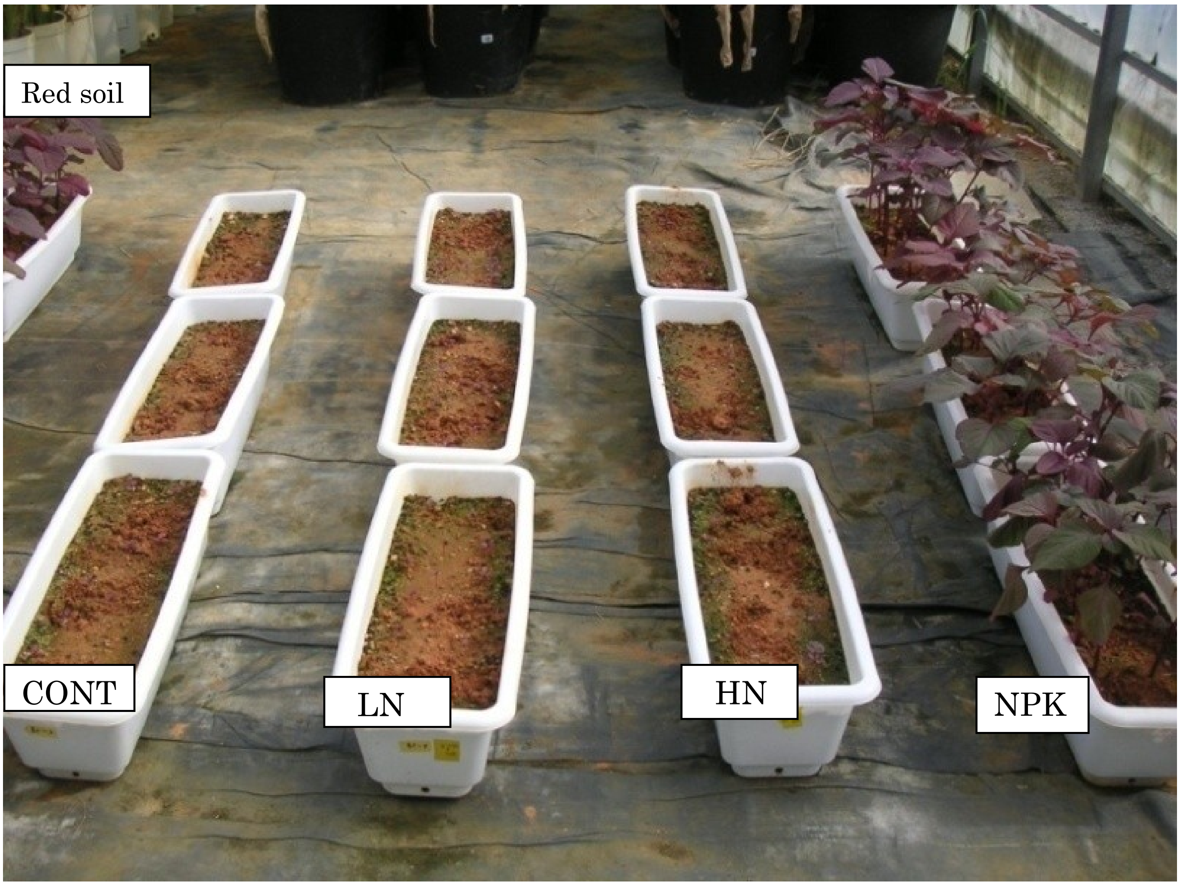


Fig. V-3. Effects of N rates and combined NPK fertilizer on growth of amaranth line BB in red soil. CONT (control, 0 gm^{-2}), LN (low nitrogen, 50 g N m^{-2} or 5 g N $planter^{-1}$), HN (high nitrogen, 100 g N m^{-2} or 10 g N $planter^{-1}$) and NPK (150 g NPK m^{-2} or 5 g N + 5 g P + 5 g K $planter^{-1}$)

Plant height increased with all the fertilizer treatments in gray soil (Fig. V-4). The plant height of the amaranth grown with the NPK was 66 cm. The plants grown with the HN and LN were 51 and 54 cm, respectively. The plant height increased by 106%, 29% and 22% with the NPK, HN and LN fertilizer, respectively. The plant height of amaranth grown with the NPK in dark-red soil was 60 cm, whereas the plant height was 14, 19, and 11 cm when grown with HN, LN and without fertilizer, respectively. The plant height was 3-6 times higher with the NPK than with the other treatments. The plant height of amaranth grown with the NPK in red soil was 59 cm, whereas the plant height was 6, 5 and 4 cm when grown with HN, LN and without fertilizer, respectively. The plant height was 10-15 times higher with the NPK fertilizer than with the other treatments.

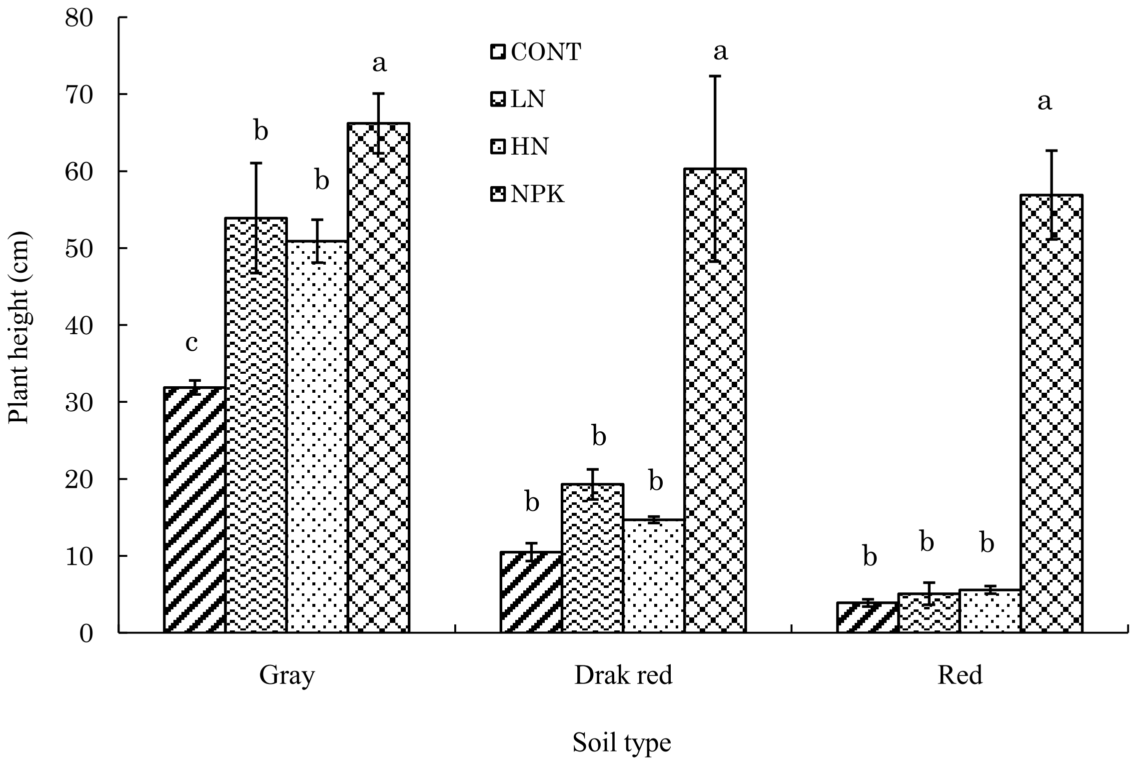


Fig. V-4. Effects of N rates and combined NPK fertilizer on plant height of amaranth line BB in different soils. Bars with the same letter within each soil type are not significantly different at the 5%level, as determined by LSD test.

Leaf number increased similarly with the LN, HN and NPK treatments in gray soil. Highest number of leaves was noted with the combined NPK 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 (Fig. V-5).

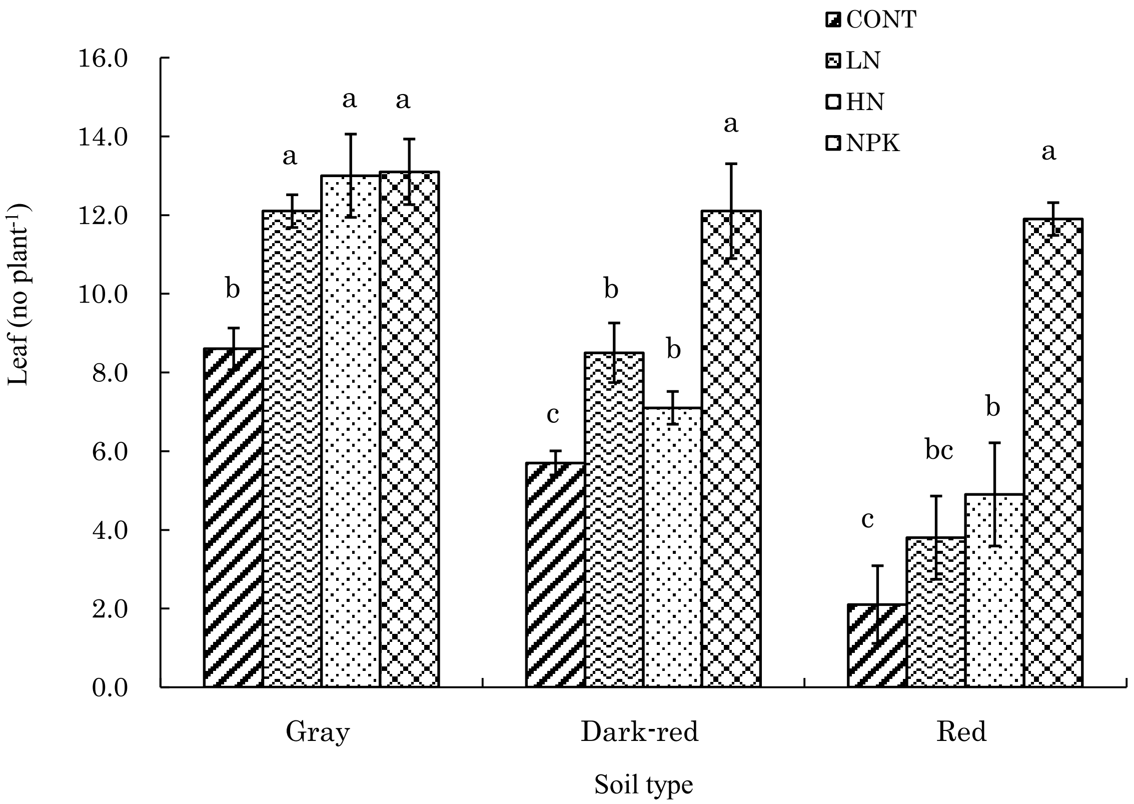


Fig. V-5. Effects of N rates and combined NPK fertilizer on leaf number of amaranth line BB in different soils. Bars with the same letter within each soil type are not significantly different at the 5%level, as determined by LSD test.

Stem diameter increased with the LN and NPK treatments in gray soil, which was highest with the NPK followed by LN, and the treatments CONT and HN resulted in the similar diameter (Fig. V-6). The stem diameter increased significantly with the NPK fertilizer in dark-red soil and red soil. The fertilizer NPK resulted in 3 times higher diameter of the amaranth plants in dark-red soil and 5 times in red soil (Fig. V-6). The diameter was highest in red soil followed by dark-red soil, when amaranth plant was cultivated with the fertilizer NPK.

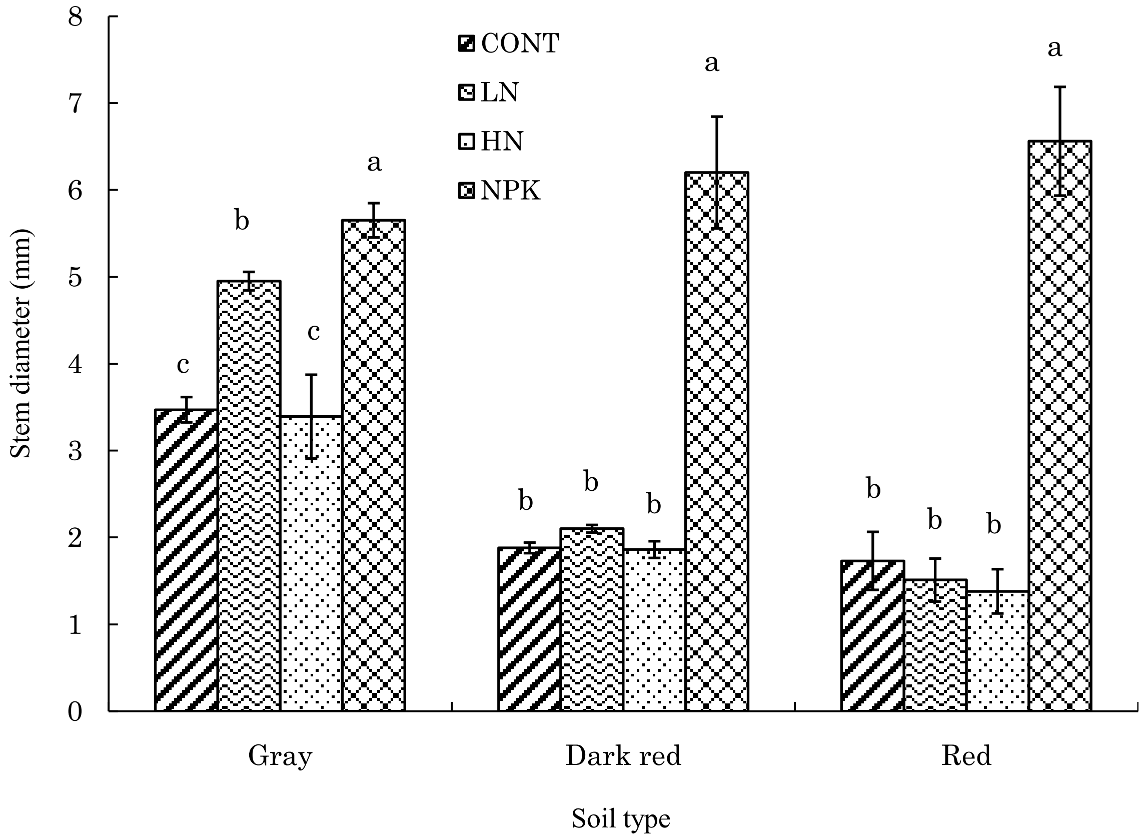


Fig. V-6. Effects of N rates and combined NPK fertilizer on stem diameter of amaranth line BB in different soils. Bars with the same letter within each soil type are not significantly different at the 5%level, as determined by LSD test.

Total leaf area per plant increased by 58-151% with the fertilizer application when amaranth was cultivated in the gray soil, and the leaf area was highest with the application of fertilizer NPK (196 cm^2) followed by LN (169 cm^2) and HN (123 cm^2) (Fig. V-7). Total leaf area per plant increased with the combined fertilizer NPK in dark-red soil and red soil. Leaf area was too small when amaranth was cultivated without fertilizer and with the fertilizer LN and HN. The leaf area with NPK treatment was about 7 times higher in dark-red soil and 10 times in red soil, as compared to that with other treatments. Total leaf area per plant was highest in red soil followed by dark-red soil when combined fertilizer of NPK was applied.

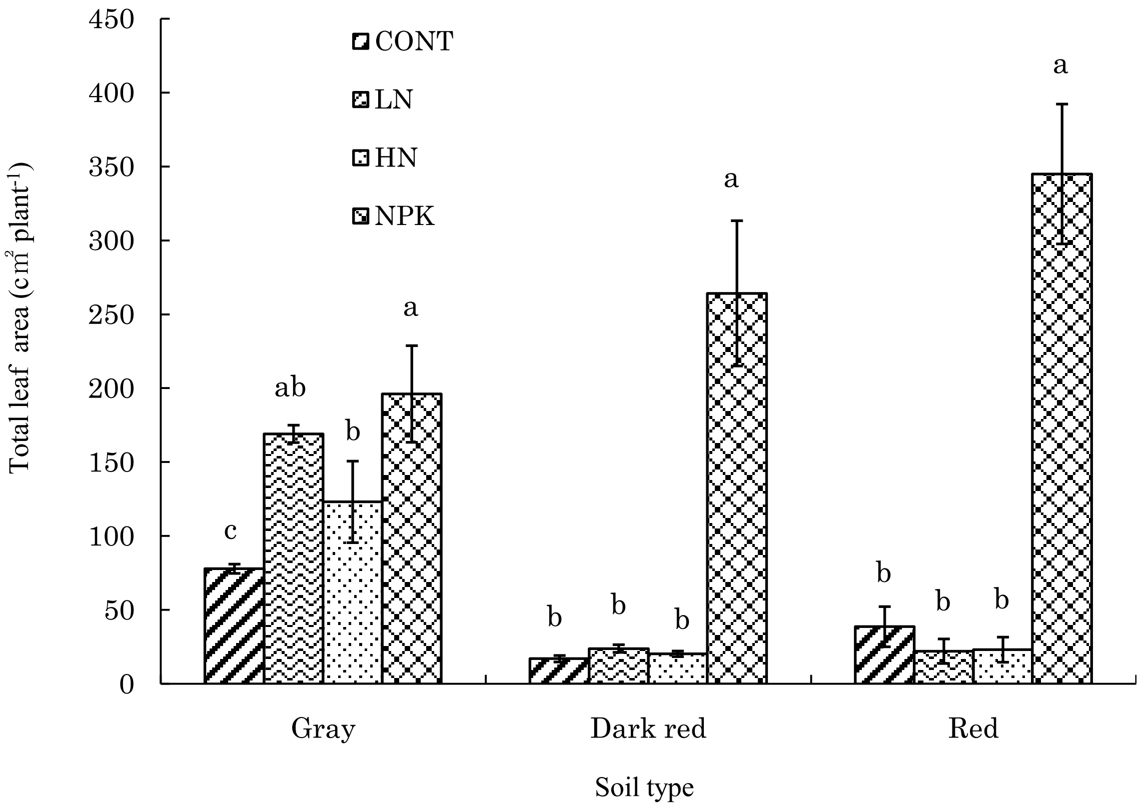


Fig. V-7. Effects of N rates and combined NPK fertilizer on total leaf area of amaranth line BB in different soils. Bars with the same letter within each soil type are not significantly different at the 5%level, as determined by LSD test.

Dry leaf weight increased significantly with all the fertilizers when amaranth plant was cultivated in gray soil (Fig. V-8). The dry leaf weight was highest with the NPK followed by LN and HN, and was statistically similar with the LN and NPK. The dry leaf weight was more than double with the LN and NPK, as compared to that cultivated without fertilizer. Dry leaf increased only with the combined fertilizer NPK when amaranth plants were cultivated in dark-red soil and red soil (Fig. V-8). The dry shoot increased 17-28 times with the NPK, as compared to that with the LN and HN fertilizer applied in dark-red soil. The treatments LN and HN did not show any positive effect on dry leaf weight. Dry leaf of amaranth increased 21-34 times with NPK fertilizer compared to that with the other treatments when cultivated in red soil. The dry leaf weight was highest in red soil followed by dark-red soil when cultivated with fertilizer NPK (Fig. V-8).

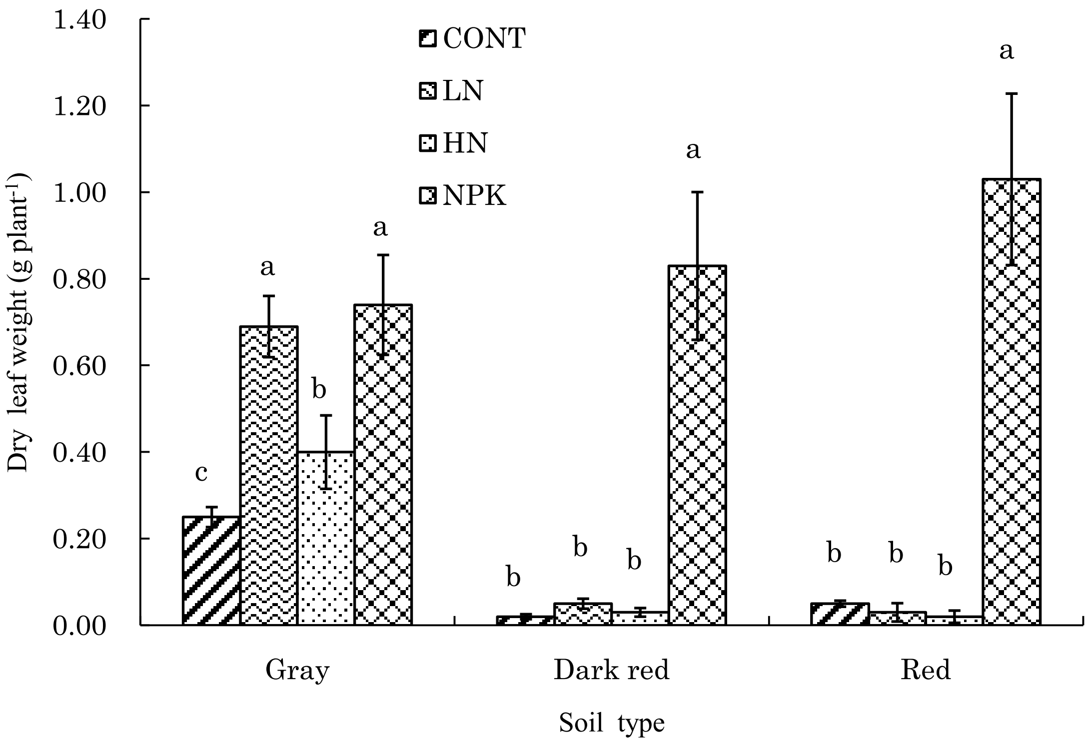


Fig. V-8. Effects of N rates and combined NPK fertilizer on dry leaf weight of amaranth line BB in different soils. Bars with the same letter within each soil type are not significantly different at the 5%level, as determined by LSD test.

Dry shoot weight increased with the LN and combined NPK fertilizer applied in gray soil, and the fertilizer HN did not show significant effect on dry shoot production (Fig. V-9). The dry shoot weight was highest with the NPK (1.10 g $plant^{-1}$) followed by LN (0.88 g $plant^{-1}$). Dry shoot weight of amaranth increased significantly with the NPK in dark-red soil and red soil. The fertilizer treatments LN and HN did not show positive effect on shoot production in the dark-red soil and red soil. The dry shoot weight increased 28 times with the NPK in dark-red soil, and 22 times in red soil (Fig. V-9). The dry shoot weight was the highest in red soil followed by dark-red soil when amaranth was cultivated with the fertilizer NPK (Fig. V-9)

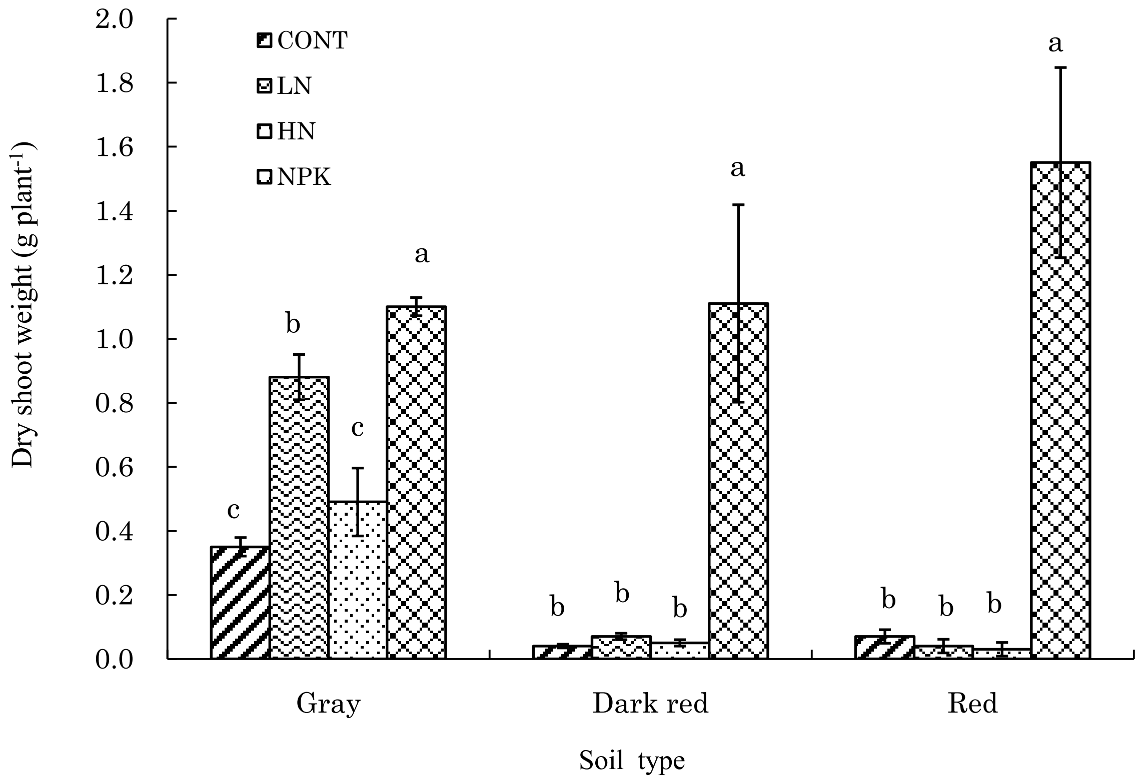


Fig. V-9. Effects of N rates and combined NPK fertilizer on dry shoot weight of amaranth line BB in gray soil, dark-red soil and red soil. Bars with the same letter within each soil type are not significantly different at the 5% level, as determined by LSD test.

Sodium, Ca and P content in edible shoot of amaranth increased with NPK fertilizer in gray soil, and the treatments both LN and HN did not show clear effect on mineral accumulation in the plants (Table V-1). In general, K, Al, Fe, Mn and Zn contents in plants were not influenced by the fertilizer treatments. However, Mg, N and C contents in amaranth plants increased with all fertilizer treatments, and total N was highest with NPK treatment (Table V-1). 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.

Table V-1. Effects of fertilizers on nutrient contents and percent of total N and C in edible shoot of amaranth line BB grown in gray soil, dark-red soil and red soil

		Na	K	Ca	Mg	Al	Fe	P	Mn	Zn	N	C
Soil type	Treatment	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}	mg g DW^{-1}	mg g DW^{-1}	%	%
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

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 Dark-red soil and Red soil due to poor growth of plants (sample not enough). DW represents dry weight.

Discussion

1. Effects of N fertilizer levels and combined NPK fertilizer on growth and yield, and mineral, nitrogen and carbon contents of amaranth 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 (150 gm^{-2}) followed by LN (50 gm^{-2}) in gray soil, but only with the NPK fertilizer in dark-red and red soils (Fig.V-3 andV-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 the results reported for *C. crepidiodies* and turmeric plants (Akamine et al., 2007; Hossain et al., 2012). The treatment HN (100 gm^{-2}) resulted in lower growth parameters and 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, proper photosynthetic function or other normal physiological function in the plant. Similarly, other studies reported that a plant species increases biomass with the increasing N application up to a certain level, thereafter the biomass decreases with the increasing rate of fertilizer (Hossain et al., 2004; Olanite et al., 2010; Nori et al., 2012).

All growth parameters and shoot biomass of amaranth in the dark-red and red soils were not affected by the N fertilizer applied alone (Fig. V-2~9). It is possible that K and P content 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 and other normal function (Donald and Katherine, 1999). Similar effects of fertilizer were reported on redflower ragleaf (Hossain et al., 2012).

Sodium, Ca and P content, and total N were highest in amaranths cultivated with NPK fertilizer in gray soil, indicating that the three fertilizer elements together influenced the plants effectively to accumulate higher minerals and N. It is also thought that the plants were healthier with the fertilizer NPK which resulted in the higher mineral accumulation. The fertilizer 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 the soil. Similar results with turmeric plants were reported by Akamine et al. (2007).

Conclusion

Nitrogen applied alone promoted growth and yield of amaranth plant in gray soil, but not in other soils, indicating that K and P content 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 growth parameters and yield of amaranth were highest in red soil followed by dark-red soil and gray soil when cultivated with the NPK fertilizer. It is assumed that the nutrients existed in the dark-red soil or red soil and the applied fertilizer NPK created a favorable nutrient balance for the better growth and development of amaranth. This result indicates that the ratio and amount of N, P and K fertilizer applied to the plants were best for the red soil among the three soil types. In addition, it is assumed that there are some positive or negative interaction between the applied fertilizers and existing fertilizers in the soil, which were the causes of different effects of the same fertilizer in different soils. However, this study indicates that combined fertilizer of NPK is required for amaranth cultivation in dark-red soil, red soil and gray soil in Okinawa.

CHAPTER VI

Effects of NPK Fertilizer Rates on Amaranth Cultivation in Gray Soil

Abstract: Present study has been conducted to evaluate rates of combined fertilizer NPK (0, 20, 30, 40, 50 60 and 80 g m^{-2}) on growth, yield and quality of amaranth lines BB and BC in gray soil (pH 8.4) in Okinawa. The fertilizer of 30, 40 and 50 g m^{-2} resulted in a similarly higher growth parameters and yield (dry shoot) in BB line, but agronomic efficiency tended to decrease with the 50 g m^{-2}, which indicate that the fertilizer of 30-40 g m^{-2} is better for BB cultivation. In the experiment 2012, the growth parameters and yield of amaranth line BC increased almost similarly with all fertilizer rates, but agronomic efficiency tented to decrease with the 40-50 g m^{-2}, indicating that the fertilizer rate of 20-30 g m^{-2} is better for BC cultivation in gray soil. In the experiment 2015, the amaranth line BC resulted in the highest growth parameters and yield with the fertilizer 60 g m^{-2}. The SPAD value was highest with the 60 g m^{-2}, which contributed to higher photosynthesis and resulted in higher plant growth and yield. The yield of amaranth line BC cultivated in 2015 was higher, which required higher rate of fertilizer than that amaranth cultivated in 2012. Mineral accumulation in both amaranth BB and BC lines were not clearly influenced with the fertilizer NPK rates in 2012 and 2015. Total N (%) increased with the fertilizers in both the BB and BC lines, whereas total C did not clearly influenced with the fertilizer rates. The overall results of this study indicate that the requiring rate of fertilizer NPK differ with the plant size and cultivars/lines; and the NPK fertilizer at 50-60 gm^{-2} is required for higher yield and quality of the amaranth in gray soil in Okinawa, Japan.

Introduction

Different plant species response differently to different fertilizer rates, and a plant species requires an optimum amount of fertilizers to maximize growth, yield and quality in a soil type (Hossain et al., 2004; Akamine et al., 2007; Chowdhury et al., 2008; Hafsi et al., 2011). Amaranth is a valuable vegetable because of high content of carotenoids, carbohydrate, protein, fat, β-carotene and ascorbic acid in the plant (Abbott and Campbell, 1982; Makus, 1984; Prakash and Pal, 1991; Begum, 2000; Shukla et al., 2005; Shittu et al., 2006).

In the chapter V, N fertilizer levels (0, 50 and 100 g m^{-2}) and combined fertilizer NPK (150 m^{-2}) were evaluated on the growth, yield and quality of amaranth in gray soil, and found that combined fertilizer NPK was best for amaranth cultivation. It is thought that growth, yield and quality of amaranth plants differ with the rates of combined NPK fertilizer in gray soil. In the previous study the fertilizer NPK rate of 150 g m^{-2} was evaluated on the growth, yield and quality of amaranth. It is important to determine the optimum rate of the fertilizer NPK for obtaining maximum yield and quality in the gray soil. Present study has been conducted to evaluate rates of combined fertilizer NPK on growth, yield and quality of amaranth in gray soil in Okinawa for determining effective fertilizer rate.

Materials and Methods

Field experiment 2012

The field experiment was conducted in gray soil at the Subtropical Field Science Center of the University of the Ryukyus, from April 19 to July 13, 2012. The content of Na, K, Ca, Mg, Al, Fe and P in the gray soil was 2.59, 2.96, 24.30, 1.53, 0.04 and 0.06 mg kg^{-1} soil, respectively. The pH value of the soil was 8.4, and the content of N and C was 0.12% and 1.80%, respectively. The field was properly prepared, and plots of 1m×1m ($1m^2$) were separated by using rope. Seeds of amaranth BB and BC lines (selected from the previous experiment, Ohshiro et al., 2015) were sown in 5 rows (20 cm row spacing) per plot, and the seeds were covered with a thin layer of soil (about 1 cm). 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 g m^{-2}, 30 g m^{-2}, 40 g m^{-2} and50 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.

Field experiment 2015

The field experiment was conducted to evaluate rates of combined NPK fertilizer in gray soil at the Subtropical Field Science Center of the University of the Ryukyus, from May 22 to July 23, 2015. The field was properly prepared, and plots of 1m×1m ($1m^2$) were separated by using rope. Seeds of amaranth BC line (selected from the previous experiment, Ohshiro et al., 2015) were sown in 5 rows (20 cm row spacing) per plot, and the seeds were covered with a thin layer of soil (about 1 cm). 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 g m^{-2}, 40 g m^{-2}, 60 g m^{-2} and 80 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 15 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.

2. Data collection

In the experiment 2012, plant height, leaf number, stem diameter, internode length, largest leaf area, total leaf area, fresh and dry leaf weight, and fresh and dry shot shoot weight were measured at 43 day after seed sowing. In the experiment 2015, the same parameters were recorded at 33 day after seed sowing. The temperature level in 2015 was higher than in 2012, which resulted in faster growth of amaranth, and the size of amaranth in the experiment 2015 was enough to harvest at 33 day after seed sowing.

3. Determination of leaf area, dry weight, mineral contents, nitrogen and carbon and L-ascorbic acid of amaranth plants

Leaf area, dry weight, mineral contents, nitrogen, carbon and L-ascorbic acid of amaranth plants were measured following the procedures applied in the previous experiments (Chapter III and IV).

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

Experiment 2012

1. Effects of NPK fertilizer rates on growth and yield of amaranth BB and BC lines

Growth of amaranth lines BB and BC cultivated using different levels of fertilizer NPK in the gray soil is shown in the Fig. VI-1. Amaranth in deep red color is the line BC and the amaranth in light red color is line BB. It is observed that growth differs due to different levels of fertilizer in each amaranth line (Fig. VI-1).

Fig. VI-1. Amaranth cultivation in gray soil field under different rates of fertilizer NPK in 2012

Plant height of amaranth line BB increased significantly with the 30, 40 and 50 g m^{-2} at 38 day after seed sowing (DAS), but increased with all fertilizer rates at 43 DAS. The plant increased with the increasing fertilizer rates (Fig. VI-2). Plant height of the line BC increased significantly with the fertilizer rate of 50 g m^{-2}, but non-significantly with other fertilizer rates at 43 DAS (Fig. VI-3).

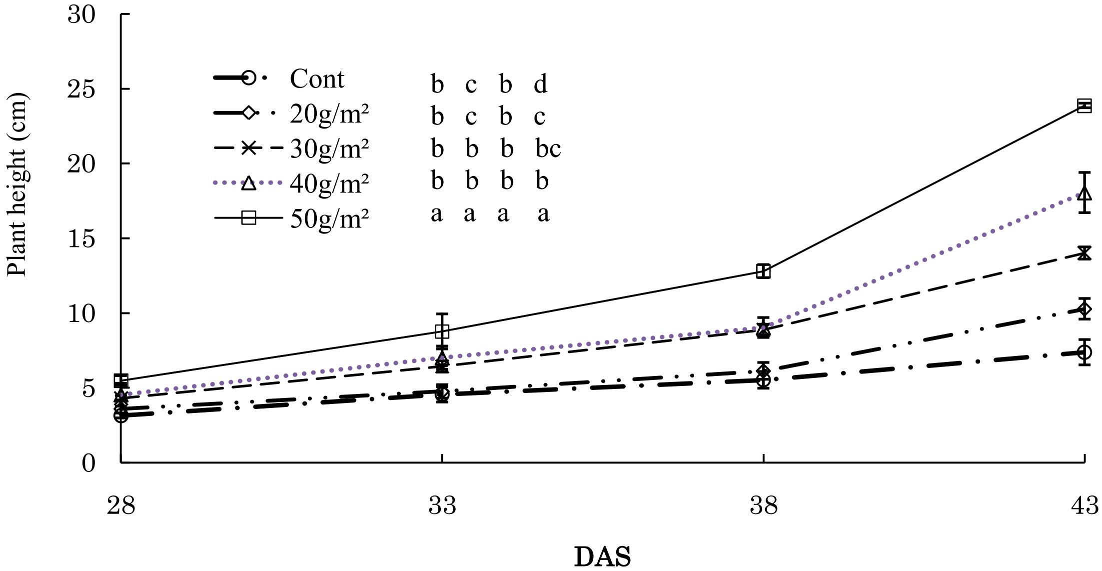


Fig. VI-2. Effects of fertilizer NPK rates on plant height of amaranth line BB

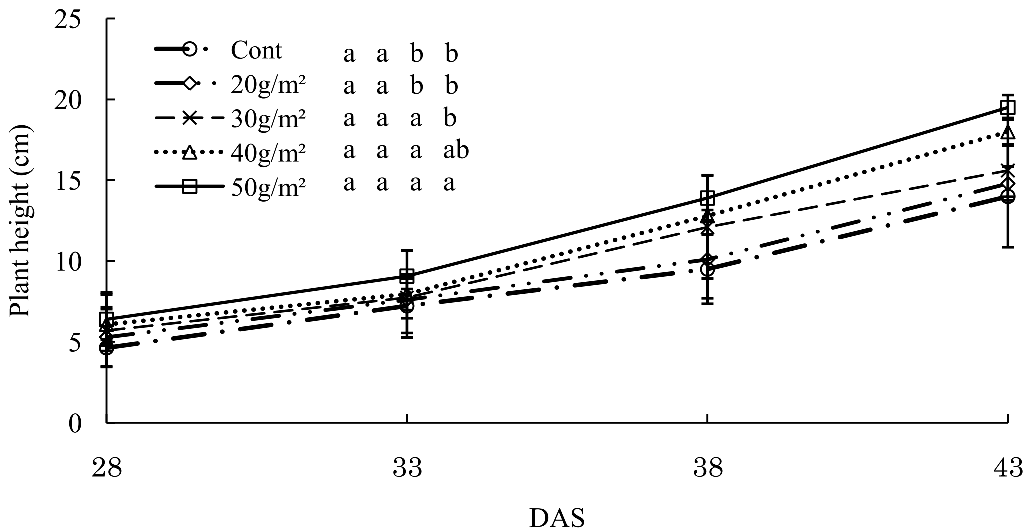


Fig. VI-3. Effects of fertilizer NPK rates on plant height of amaranth line BC

Leaf number per plant increased with the fertilizer rates of 30-50 g m^{-2} in BB line but not in BC at 43 DAS (Fig. VI-4, VI-5).

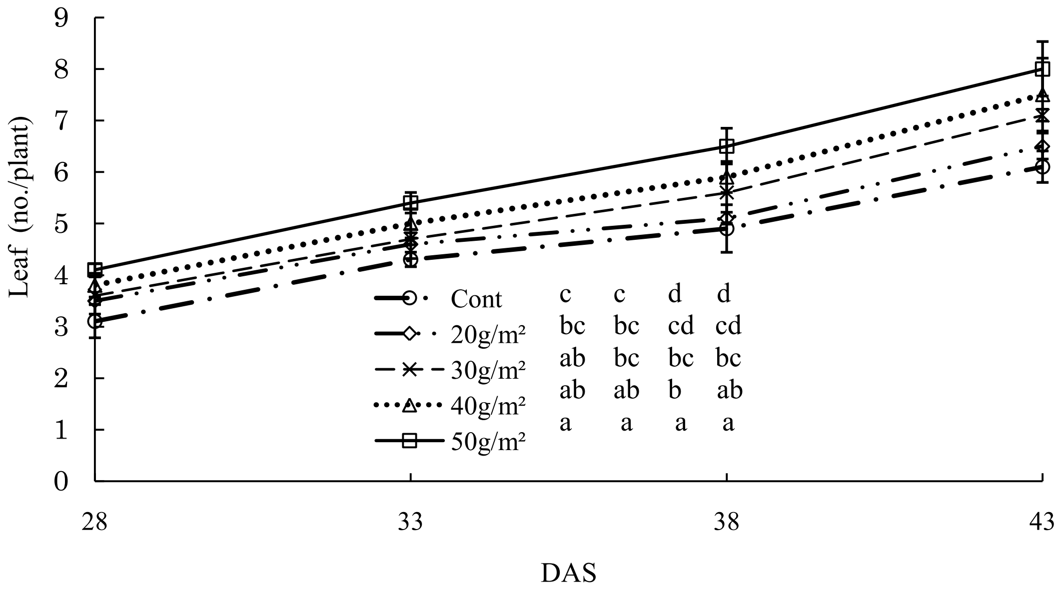


Fig. VI-4. Effects of fertilizer NPK rates on leaf number of amaranth line BB

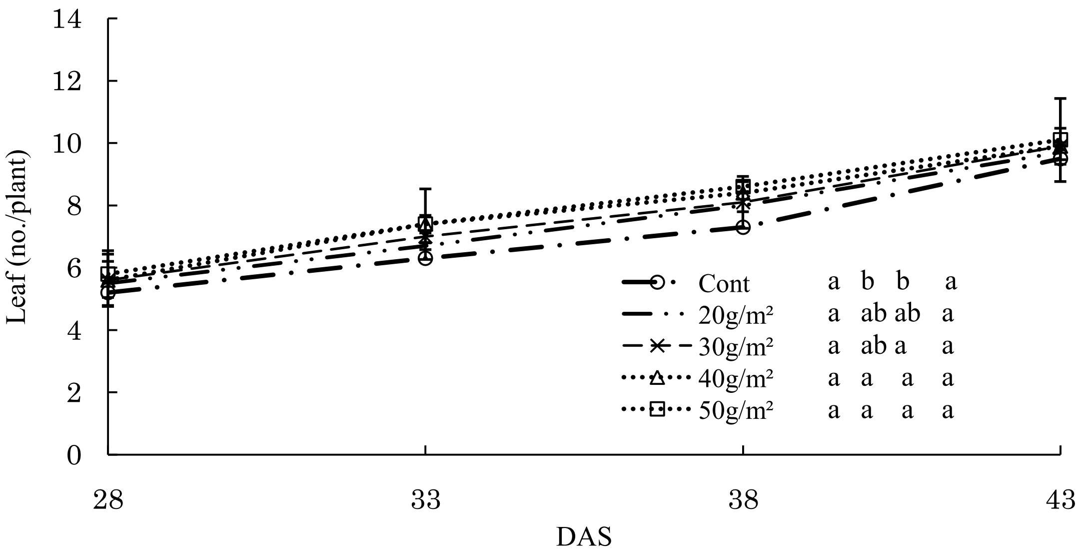


Fig. VI-5. Effects of fertilizer NPK rates on leaf number of amaranth line BC

All growth parameters and yield of BB line increased with the increasing fertilizer rates (Table VI-1). 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 growth parameters. The fertilizer rates of 30, 40 and 50 g m^{-2} provided similarly higher dry shoot weight, and these rates had similar value (Table VI-1). The growth parameters and yield of amaranth BC line increased almost similarly with all the fertilizer rates, as compared to those with the control plant (Table VI-1).

Table VI-1. Effects of combined NPK fertilizer rates on growth parameters and yield of amaranth linesBB and BC grown in gray soil field

Line	Fertilizer level (gm^{-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.

2. **Effects of fertilizer NPK rates on mineral, nitrogen and carbon contents of amaranth lines BB and BC**

The contents of all minerals except K in edible shoot of amaranth lines BB and BC were the same or somewhat lower with the fertilizer rates, as compared with the control plant (Table VI-2). 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 VI-2).

Table VI-2. Effects of combined NPK fertilizer rates on nutrient status and total N and C in edible shoot of amaranth lines BB and BC grown in gray soil field

Line	Fertilizer level gm^{-2}	Na	K	Ca	Mg	Al	Fe	P	Mn	Zn	N	C
		$mg\ g^{-1}$	$mg\ g^{-1}$	$mg\ g^{-1}$	$mg\ g^{-1}$	$mg\ g^{-1}$	$mg\ g^{-1}$	$mg\ g^{-1}$	$mg\ g^{-1}$	$mg\ g^{-1}$	%	%
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.

Experiment 2015

3. Effects of NPK fertilizer rates on growth and yield of amaranth line BC

The amaranth line BC cultivated in gray soil in 2015 is shown in the Fig. VI-6. The growth of amaranth in the field is different due to different rates of fertilizer NPK.

Fig. VI-6. Amaranth cultivation in field under different rates of fertilizer NPK in 2015

Plant height was not different with the fertilizer application until 22 days after seed sowing, thereafter the plant height increased with the increasing fertilizer rates (Fig. VI-7). The plant height was highest with the fertilizer NPK at 60 g m^{-2}. The plant growth was almost similar with the fertilizer rates of 40 g m^{-2} and 80 gm^{-2}.

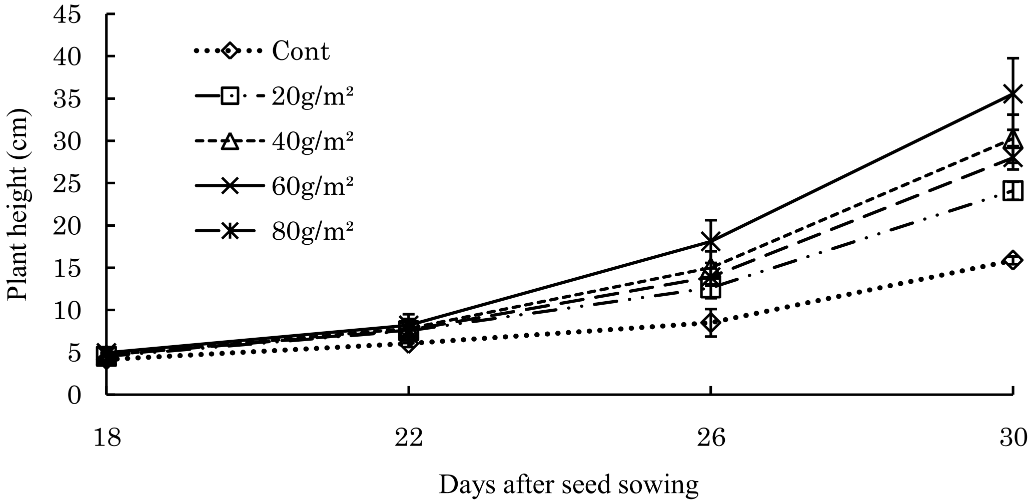


Fig. VI-7. Effects of fertilizer NPK rates on plant height of amaranth line BC in 2015

Leaf number increased with the increasing fertilizer rates of NPK, which was highest for the 60 g m^{-2}. The fertilizer NPK applied at 40 g m^{-2} and 80 g m^{-2} resulted in the similar number of leaves (Fig. VI-8).

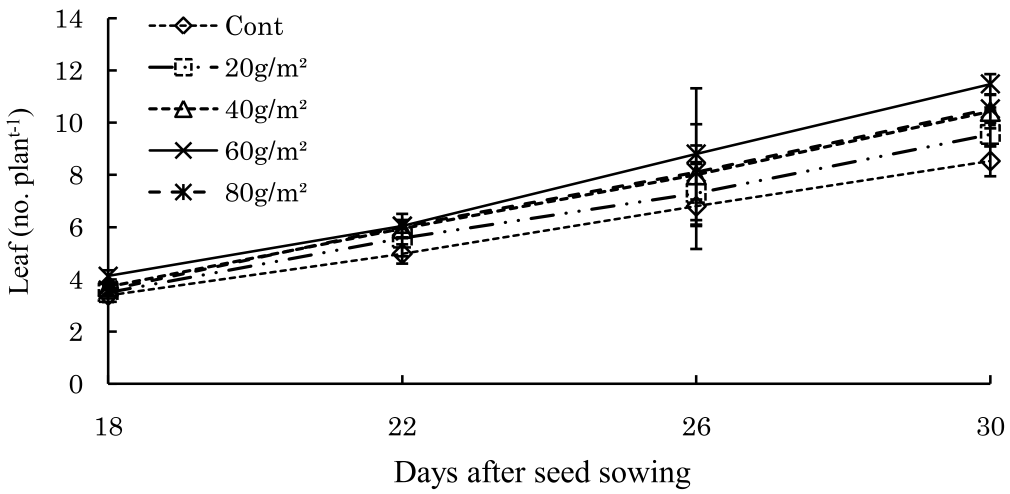


Fig. VI-8. Effects of fertilizer NPK rates on leaf number of amaranth line BC in 2015

The SPAD value of amaranth leaf increased with the increasing fertilizer levels (Fig. VI-9), and the SPAD value was similar with the fertilizer NPK rates of 60 and 80 g m^{-2}.

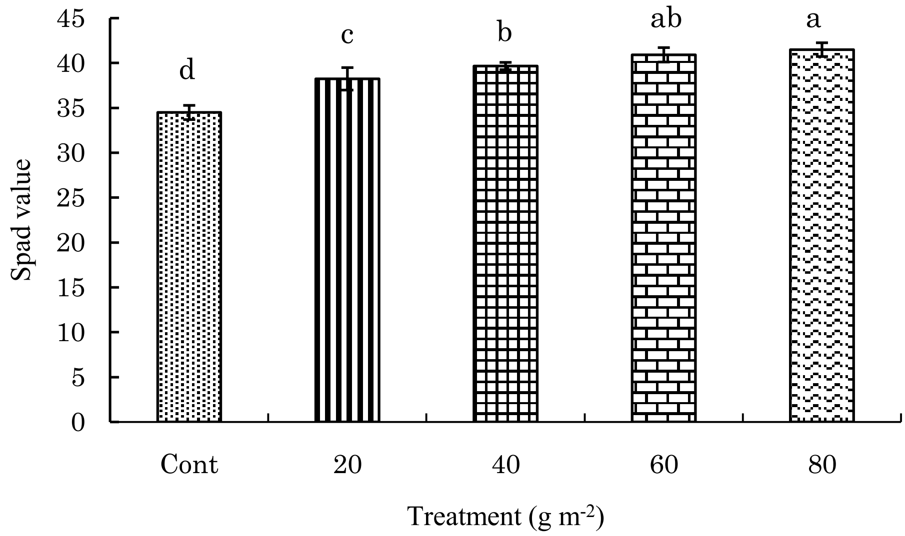


Fig. VI-9. Effect of fertilizer NPK rates on SPAD value of amaranth leaf in 2015

The stem diameter increased with all the fertilizer rates applied, and the diameter was the same with the fertilizer rates of 40, 60 and 80 g m^{-2} (Table VI-3). The internode length also increased with the fertilizer, which was highest for the 60 g m^{-2}, followed by 80 g m^{-2} and 40 g m^{-2}. The largest leaf area and total leaf area increased similarly with all the fertilizer rates. However, largest leaf area and total leaf area were highest with the fertilizer of 60 g m^{-2}. Fresh and dry leaf weight increased with all the fertilizer rates. The fresh leaf weight was similar with the fertilizer rates of 40, 60 and 80 g m^{-2}, and dry leaf weight was similar with all the fertilizer rates (Table VI-3). Fresh stem weight was highest with the 60 g m^{-2} followed by 80 and 40 g m^{-2}., whereas dry stem weight increased similarly with the fertilizer rates of 40, 60 and 80 g m^{-2} (Table VI-3). Fresh and dry shoot weight was highest with the fertilizer rates of 60 g m^{-2} followed by 80 and 40 g m^{-2}.

Table VI-3. Effects of fertilizer NPK rates on growth parameters and yield of amaranth line BC in gray soil in 2015

Treatment (g/m^2)	Stem diameter (mm)	Internode length (cm)	largest leaf area (cm²)	Total leaf area (cm²)	Fresh leaf weight (g $plant^{-1}$)	Dry leaf weight (g $plant^{-1}$)	Fresh stem weight (g $plant^{-1}$)	Dry stem weight (g $plant^{-1}$)	Fresh shoot weight (g $plant^{-1}$)	Dry shoot weight (g $plant^{-1}$)
Cont	5.91c	1.93d	32.19b	153.53b	6.45c	0.85b	5.58d	0.30c	12.03d	1.15c
20	8.57b	2.69c	43.17a	274.76a	12.16b	1.37a	14.46c	0.66b	26.62c	2.03b
40	10.33a	2.94bc	44.97a	282.96a	15.24ab	1.58a	22.33b	0.93a	37.57b	2.51ab
60	11.04a	3.89a	50.67a	341.29a	18.06a	1.69a	31.82a	1.15a	49.88a	2.84a
80	10.03ab	3.26bc	43.29a	307.59a	17.59a	1.69a	23.10b	0.91ab	40.69ab	2.60ab

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

4. Effects of fertilizer NPK rates on mineral, nitrogen and carbon contents of amaranth BC line

In the experiment 2015, Na, K, Ca, Mg and P content in amaranth line BC did not differ with the fertilizer rates, The Al content deceased similarly with the fertilizer rates (Table VI-4). The Fe content increased with the fertilizer NPK at 60 g m^{-2}. Other fertilizer rates did not show positive effects on Fe accumulation. The N content increased with all the fertilizer rates, and was highest with the fertilizer rates of 60 and 80 g m^{-2}. The C content decreased with the increased fertilizer rates (Table VI-4)

Table VI-4. Effects of fertilizer NPK rates on mineral, N and C content of amaranth line BC in gray soil in 2015

Treatment (g/m^2)	Na mg/g	K mg/g	Ca mg/g	Mg mg/g	Al mg/g	Fe mg/g	P mg/g	N %	C %
Cont	6.2a	106.7a	34.0a	19.3a	0.35a	0.17bc	17.70a	3.77d	35.19a
20	5.9a	126.4a	33.0a	20.8a	0.14b	0.16bc	15.70a	5.22c	32.4b
40	6.5a	137.3a	32.8a	21.0a	0.13b	0.18b	17.90a	5.65b	31.44c
60	6.5a	137.2a	34.2a	19.9a	0.12b	0.28a	16.71a	5.74a	31.17d
80	6.1a	133.3a	34.3a	19.9a	0.19b	0.12c	18.70a	5.79a	31.28cd

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

Discussion

1. Effects of fertilizer rates on growth and yield of amaranth lines BB and BC

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 VI-1), and agronomic efficiencies of the fertilizer treatments {(total yield of fertilizer treated plants – total yield of control plants)/total fertilizer applied in treated plant} were 0.11 g g^{-1}, 0.11 g g^{-1} and 0.10 g g^{-1}, 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.

In the experiment 2012, the growth parameters and yield of amaranth line BC increased almost similarly with all fertilizer levels (Fig. VI-3, Table VI-2), 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; Olanite et al., 2010; Nori et al., 2012). In the experiment 2015, the amaranth line BC resulted in the increasing plant height and leaf number with the increasing rate of fertilizer NPK until 60 g m^{-2}, thereafter plant height and leaf number decreased with the increasing fertilizer rate of 80 g m^{-2} (Fig. VI-7, VI-8). The SPAD value of amaranth leaf increased with the increasing fertilizer levels up to 60 g m^{-2} (Fig. 9), which contributed to higher photosynthesis and resulted in higher plant growth. Stem diameter, internode length, largest leaf area, total leaf area, and fresh and dry weight of leaf, stem and shoot increased with the increasing fertilizer rates up to 60 g m^{-2} (Table VI-3). All the growth parameters and yield tended to decrease when the amaranth line BC was cultivated with the fertilizer rate of 80 g m^{-2}. The experiment

2015 showed that fertilizer NPK of 60 g m^{-2} is required for cultivation of amaranth line BC for higher yield in gray soil. The shoot (yield) weight of amaranth line BC was higher in 2015 which required higher rate of fertilizer NPK, compared to that cultivated in 2012.

2. Effects of fertilizer NPK rates on mineral, nitrogen and carbon contents of amaranth line BB and BC

In the experiments 2012 and 2015, mineral accumulation in both amaranth BB and BC lines were not clearly influenced with the fertilizer NPK rates (Table VI-2, VI-4). Similar result was reported in some amaranth lines 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 *Sorghum almum* reported by Olanite et al. (2010). Total C was not clearly influenced with the fertilizer rates.

Conclusion

In the experiment 2012, the amaranth line BB obtained similarly higher growth parameters and yield with the combined fertilizer NPK rates of 30, 40 and 50 g m^{-2}, whereas the amaranth line BC obtained similarly higher growth parameters and yield with the 20, 30, 40 and 50 g m^{-2}. The amaranth line BB obtained about 2 times higher yield which required higher rate of fertilizer than amaranth line BC, indicating that fertilizer requirement differs with the plant size (biomass) and cultivars. Agronomic efficiency of the fertilizers decreased when applied at 50 g m^{-2} in both the amaranth lines BB and BC, indicating that the fertilizer rate of 20-40 g m^{-2} is better for cultivation of the amaranths in gray soil.

In the experiment 2012, the growth parameters and yield of the amaranth line BC increased similarly with the fertilizer rates of 30-50 g m^{-2}, whereas the growth parameters and yield were highest with the fertilizer rate of 60 g m^{-2} in the experiment 2015. The shoot (yield) weight of amaranth line BC cultivated in 2015 was higher than that cultivated in 2012, which required higher rate of fertilizer NPK in 2015.

In the experiment 2012, all the minerals except K in the amaranth lines BB and BC did not differ with the fertilizer rates; K increased with all the fertilizer rates. In the experiment 2015, all the minerals except Al and Fe in the amaranth line BC did not differ with the fertilizer rates; Al content deceased with all the fertilizer rates, but Fe content increased with the fertilizer rate of 60 g m^{-2}. Total N in both the amaranth lines BB and BC increased with all the fertilizer rates in both the experiments 2012 and 2015.

The overall results of this study indicate that the requirement of fertilizer NPK differs with the plant size and cultivars/lines. All the minerals (except Al) and total N in the amaranth lines were same or somewhat higher with all the fertilizer rates. Considering growth parameters, yield, minerals and total N, the fertilizer rates of 50-60 gm^{-2} is required for cultivation of the amaranth lines BB and BC in gray soil in Okinawa, Japan.

CHAPTER VII

Effects of N, P and K Fertilizers Applied Alone and In Combination on Edible Amaranth *(Amaranthus* spp.) in Dark-red Soil, Okinawa

Abstract: Fertilizer regimes were evaluated on growth, yield and quality of amaranth (*Amaranthus* spp.) lines BB (red stem-amaranth) and BC (red leaf-amaranth) in dark-red soil (pH 6.6). The fertilizer P and K applied alone did not promote growth parameters and yield of amaranths, whereas N alone promoted growth parameters and yield. The fertilizer K promoted the function of N slightly but P did significantly in the growth of amaranth. All the growth parameters were highest with the combined fertilizer of NPK followed by NP in both experiments. Leaf area and leaf weight increased by 2-11 and 2-9 times, respectively with the NPK or NP, compared to those with the other fertilizer treatments. Yield (dry shoot) was highest with the NPK fertilizer followed by NP. All the growth parameters and yield of the amaranth lines increased with the increasing fertilizer rate, which were highest with the 40 g m^{-2} followed by 30 g m^{-2}. The Na content in amaranth was highest with NP fertilizer, whereas K, Ca and Mg contents were not clearly influenced with the fertilizers. The P content in amaranth increased with the P, NPK, NP and PK fertilizers, and total N content increased with the N, NK, NPK and NP fertilizers. The Na, K, Ca, Mg and P content increased with all the fertilizer NPK rates in BB line, whereas, Na, K and P content tented to increase with the fertilizer rate of 20-40 gm^{-2}, and Ca and Mg content were not clearly influenced with the fertilizer rates in BC line. Total N content was increased or same with the increasing fertilizer rate. L-ascorbic acid content of amaranth increased with all the fertilizer rates, which was highest with 40 g m^{-2}. This study indicates that the fertilizer N and P are more effective than K, combined fertilizer NPK is necessary, and the fertilizer NPK at 30-40 g m^{-2} is required for higher yield and quality of the amaranths in dark-red soil in Okinawa.

Introduction

Growth, yield and quality of a plant species differ with soil types and soil nutrient status; and a plant species requires specific and optimum fertilizers in a soil type for higher yield and quality (Hossain et al., 2004; Hossain and Ishimine, 2005; Akamine et al., 2007; Chowdhury et al., 2008; Hossain et al., 2011).The major nutrients (N, P and K) individually or in combination maintain growth, yield and quality of plants (Mazid, 1993; Ivony et al., 1997; Nakano and Morita, 2009; Barbara et al.,2011). Nitrogen is effective in chlorophyll formation and influences stomatal conductance and photosynthetic efficiency, which is responsible to 26-41% of crop yield (Maier et al., 1994; Ivony et al., 1997). Potassium regulates activities of minerals, 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).

Shittu et al. (2006) reported that balanced fertilizers in a specific soil provide higher yield and nutrient compositions of amaranth in Nigeria. Dark-red soil, red soil and gray soil are the major soils in Okinawa (Hossain and Ishimine, 2005). Previous study has been conducted on fertilizer management in gray soil, but no study has yet been conducted on dark-red soil in Okinawa. Therefore, the objectives of these studies were to i) identify the effect of different fertilizer elements and (ii) evaluate rates of fertilizer combinations on growth, yield and quality of two edible amaranth lines for developing management practices in dark-red soil in Okinawa.

Materials and Methods

1. Soil collection

Dark–red soil (Shimajiri mahji) was collected from the top 50 cm layer of a field at the Subtropical Field Science center, University of the Ryukyus, Okinawa, Japan. The soil pH was 6.2, and the soil contained 0.09% total N and 0.31% total C. Sodium (Na), K, Ca, Mg, Al, Fe, P and Mn contents in soil were 1.24, 2.28, 18.88, 2.85, 0.05, 0.23, 0.23 and 0.07 mg kg^{-1}, respectively. Coarse sand, fine sand, silt, clay and apparent density are 2.93%, 7.33%, 23.94%, 57.24% and 0.87 g cm^{-3}, respectively (Hossain and Ishimine, 2005).

2. Amaranth lines

The *Amaranthus tricolor* line BC (Bangladesh C line, red leaf-amaranth) selected for higher yield in previous study (Ohshiro et al., 2015) was evaluated in this study.

3. Experiment 1: Effects of N, P and K fertilizers applied alone and in combination on amaranth cultivated from April to June, 2013

A glasshouse experiment was conducted using dark–red soil at the Subtropical Field Science Center of the University of the Ryukyus, from April 5 to June 15, 2013. The experiment was consisted of eight treatments with five replications (planters). The fertilizer treatments were control (Cont), N, P, K, N plus P (NP), N plus K (NK), P plus K (PK) and N plus P plus K (NPK). Nitrogen (N) at 500 kg ha^{-1} (5 g m^{-2}, 5.0 g per planter), P at 500 kg ha^{-1}(5 g m^{-2}, 5.0 g per planter) and K at 500 kg ha^{-1} (5 g m^{-2}, 5.0 g per planter) were mixed with 13 kg air dried soil per planter (size: 65E, 0.1 m^2) prior to the seed sowing according to the treatment design. Seeds of amaranth were sown on soil surface, and covered with 0.5 cm soil layer. The planters were placed randomly, and the plants were thinned to 8 healthiest stands per planter at 2- to 3-leaf stage. Water was applied as required for proper seedling emergence and plant growth.

4. Experiment 2: Effects of N, P and K fertilizers applied alone and in combination on amaranth cultivated from September to December, 2013.

A glasshouse experiment was conducted using dark-red soil at the Subtropical Field Science Center of the University of the Ryukyus, from September 18 to December 10, 2013. The experiment was consisted of eight treatments (similar to experiment 1) with five replications (planters). The same amaranth line, planters, treatments, fertilizer rates, seed sowing and management practices applied in the previous experiment 1 were taken in this experiment.

5. Experiment 3: Effects of NPK fertilizer rates on amaranth

A glasshouse experiment was conducted using dark-red soil at the Subtropical Field Science Center of the University of the Ryukyus, from July 15 to Sep 3, 2014. Two amaranth ines BB and BC were evaluated. Each experiment was consisted of five treatments with four replications (planters). The fertilizer treatments of Cont (control, 0 g m^{-2}, 0 g $planter^{-1}$), 10 g m^{-2} (1 g $planter^{-1}$), 20 g m^{-2} (2 g $planter^{-1}$), 30 g m^{-2} (3 g $planter^{-1}$) and 40 g m^{-2} (4 g $planter^{-1}$) were taken. The fertilizers of N ($CO(NH_2)_2$), P_2O_5 ($CaH_4(PO_4)_2H_2O$) and K_2O (KCl) were applied at the ratio of N:P:K=1:1:1. The fertilizers were mixed with 13 kg air dried soil per planter (size 65E) prior to the seed sowing according to the treatments. Seeds of amaranth were sown on soil surface, and covered with 0.5 cm soil layer. Water was applied as required everyday for proper seedling emergence and plant growth. The planters were placed randomly, and the plants were thinned to 10 healthiest stands per planter at 2- to 3-leaf stage.

6. Data collection

Amaranth is usually harvested at 20-35 cm in height; and both the leaf and stem are used as vegetable. Plant height and leaf number were recorded up to 43 days after seed sowing (DAS) at 7-day interval in the experiment 1. Five plants were harvested from each planter at

34 DAS, and plant height, stem diameter, internode length, leaf number, largest and total leaf area, and fresh and dry weights of leaf, stem and shoot were determined. In the experiment 2, five plants were harvested from each planter at 25 DAS, and similar growth parameters were measured. In the experiments 3, five plants were harvested from each planter at 26 DAS, and similar growth parameters were measured. Stem diametder was mesured at 5 cm from the soil surface, internode length was mesured from the third internode from the bottom.

7. Determination of SPAD value, leaf area and dry weight of edible amaranth

The SPAD value of the second and third fully expanded leaves from the top was measured with a chlorophyll meter SPAD-502 (Konica Minolta, Inc., Osaka, Japan). Leaf area was measured with an automatic area meter (AAM-8, Hayashi Denkoh Co.Ltd.). Various parts of amaranth plants were dried at 80 ℃ for 48 h using forced convection oven (DRLF23WA, Advantec) for dry weight measurement.

8. Determination of minerals, nitrogen, carbon and pH in soil, and nutrient status and L-ascorbic acid of amaranth

Various parts of amaranth plants were dried at 60 ℃ for 48 h using the same forced convection oven for chemical analysis. Soil samples were dried at room temperature of 25-28 ℃ for 5 days. The plant parts and soil were ground finely for chemical analysis. Mineral contents of soil and nutrients of amaranth were determined with Inductively Coupled Plasma Spectrometer (ICPS-8100, Shimadzu Co. Ltd.). Total C% and N% were determined with Gas Chromatograph (Soil GS-8A, Shimadzu Co. Ltd., NC-220F Juka analysis center) and Sumigraph(NC-90A, Shimadzu Co. Ltd.).Soil pH was determined with TOA pH meter (HM-20S, Toa Electronic Ltd.). L-ascorbic acid content in leaf and shoot were determined by using RQ Flex/agrocheck kid small-sized reflecting photometer (Kanto Chemical Co. Ltd.).

9. Statistical analysis

The statistical procedures used in the previous studies were used in this study.

Results

1. Effects of fertilizer N, P and K applied alone and in combination on edible amaranth

The plant grown from April to June, 2013 is shown in the Fig. VII-1. The plants with the NPK and NP showed best growth. The amaranth growth was very poor in the treatments where fertilizers of P, K or PK were applied, and the growth was also very poor in the control treatment (Cont) (Fig. VII-1).

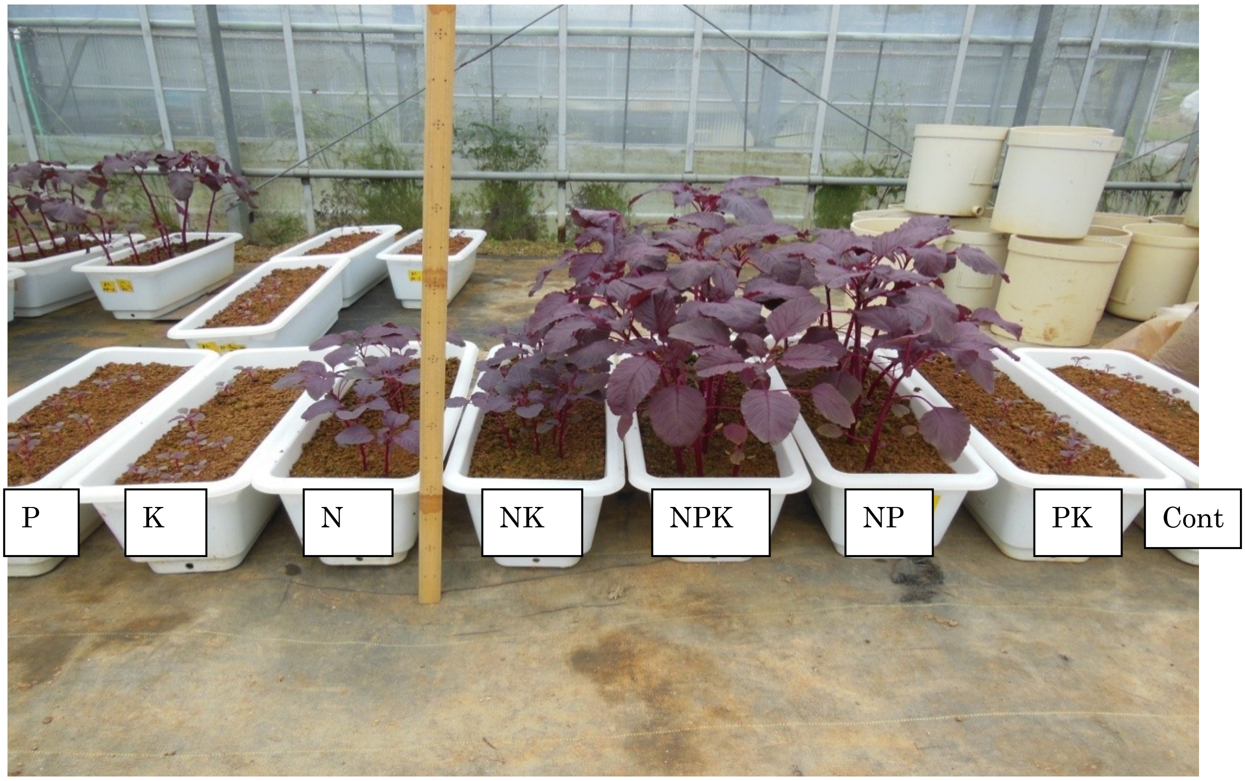


Fig VII-1. Effects of fertilizer N, P and K applied alone or in combination on growth of amaranth Line BC cultivated from April to June, 2013.

The plant grown from April to June, 2013 showed that plant height (Fig. VII-2 (A)) and leaf number (Fig VII-2 (B)) increased significantly with the fertilizers of N, NK, NP and NPK compared to that with the other treatments (Fig. VII-2). Plant height was about 3.0 times and leaf number was 1.3 times higher with the NP and NPK as compared to those with the N and NK (Fig. VII-1). Amaranth with the NPK and NP fertilizer grew faster and better than with the other fertilizer treatments (Fig. VII-2).

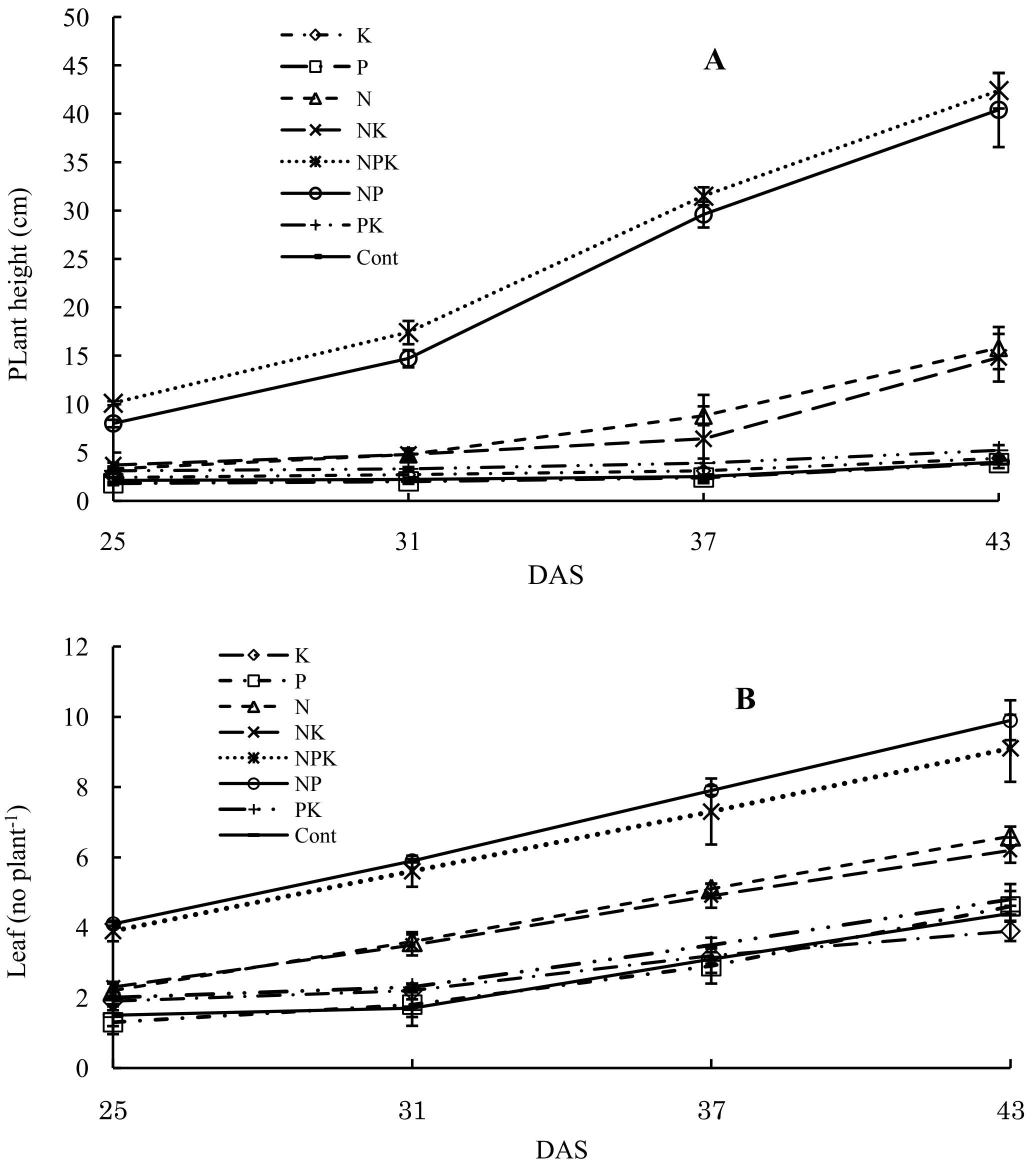


Fig. VII-2. Effects of fertilizer N, P and K applied alone or in combination on plant height (A) and leaf number (B) of amaranth line BC cultivated from April to June, 2013.

Separate application of P and K did not increase the growth parameters of amaranth, whereas N alone increased the growth parameters (Table VII-1). Largest leaf area, total leaf area, leaf weight and internode length were significantly higher with the NPK and NP compared to those with the other treatments. The values of the growth parameters were similar with the fertilizers of NPK and NP application (Table VII-1). Total leaf area and leaf weight of amaranth increased by 6-67 and 5-59 times, respectively when cultivated with the combined fertilizers of NP or NPK, as compared to those with the other fertilizer treatments. Stem diameter and stem weight were highest when amaranth was cultivated with the application of NPK followed NP. Stem weight increased by 12-165 and 8-115 times with the combined fertilizers of NPK and NP, respectively, as compared to that with the other fertilizer treatments. Yield (shoot dry weight) was highest with the NPK (1.61g per plant) followed by NP (1.36 g per plant). The fertilizer NPK and NP resulted in the 6.5 and 5.5 times higher yield, respectively as compared to the fertilizer NK. The fertilizer K did not influence the function of N in the growth parameters of amaranth, whereas fertilizer P did (Table VII-1).

Table VII-1. Effects of fertilizer N, P and K applied alone or in combination on growth parameters of edible amaranth cultivated during April to June, 2013

Treatment	Stem diameter (mm)	Internode length (cm)	Largest leaf area (cm^2 $plant^{-1}$)	Total leaf area (cm^2 $plant^{-1}$)	Fresh leaf weight (g $plant^{-1}$)	Dry leaf weight (g $plant^{-1}$)	Fresh stem weight (g $plant^{-1}$)	Dry stem weight (g $plant^{-1}$)	Fresh shoot weight (g $plant^{-1}$)	Dry shoot weight (g $plant^{-1}$)
K	1.26d	0.29d	1.79c	4.97c	0.11c	0.016c	0.06c	0.004d	0.17d	0.020d
P	1.25d	0.18d	1.76c	4.81c	0.10c	0.017c	0.05c	0.004d	0.15d	0.021d
N	3.81c	1.48b	17.38b	58.57b	1.55b	0.199b	1.00c	0.060c	2.55c	0.259c
NK	3.69c	1.22bc	14.58b	54.46b	1.39b	0.193b	0.97c	0.055c	2.36c	0.248c
NPK	8.05a	3.27a	56.26a	333.04a	9.23a	0.947a	13.9a	0.660a	25.49a	1.607a
NP	7.09b	3.02a	55.55a	326.47a	8.27a	0.896a	9.65b	0.459b	17.92b	1.355b
PK	1.41d	0.29d	1.69c	5.11c	0.14c	0.022c	0.07c	0.007d	0.21d	0.029d
Cont	1.23d	0.16d	1.93c	4.13c	0.09c	0.016c	0.04c	0.004d	0.21d	0.020d

Note: Data recorded at 35 day after planting. Data with the same letter within each column for each applied fertilizer are not significantly different at the 5% level, as determined by LSD test.

The amaranth grown from September to December, 2013 showed that the fertilizer NPK resulted in the best growth followed by NP (Fig. VII-3). The plant growth with the K, P and PK fertilizer or without fertilizer was very poor.

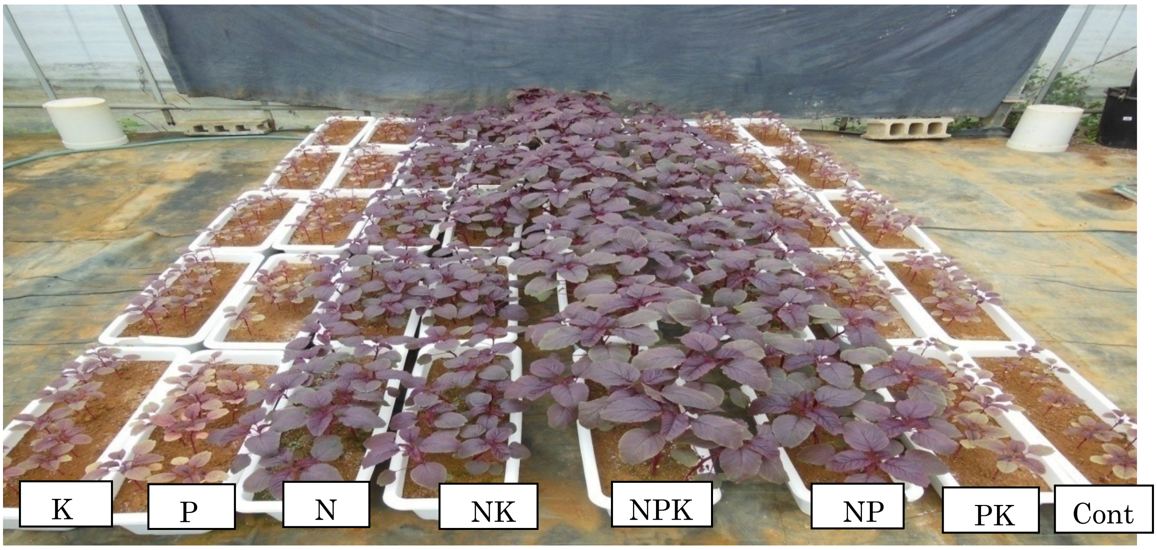


Fig. VII-3. Effects of fertilizer N, P and K applied alone or in combination on growth of amaranth line BC cultivated from September to December, 2013. Photo taken at 35 day after planting

The amaranth grown from September to December, 2013 showed that both the plant height (Fig. VII-4 (A)) and leaf number (Fig. VII-4 (B)) were highest with the fertilizer NPK followed by NP. The fertilizer N, NK and PK resulted in increased plant height and leaf number compared to control treatment. The fertilizer P and K applied alone did not show any positive effect on plant height and leaf number. The plant height was 1.7-2.0 times higher with the NPK and NP as compared to that with the N and NK (Fig. VII-4).

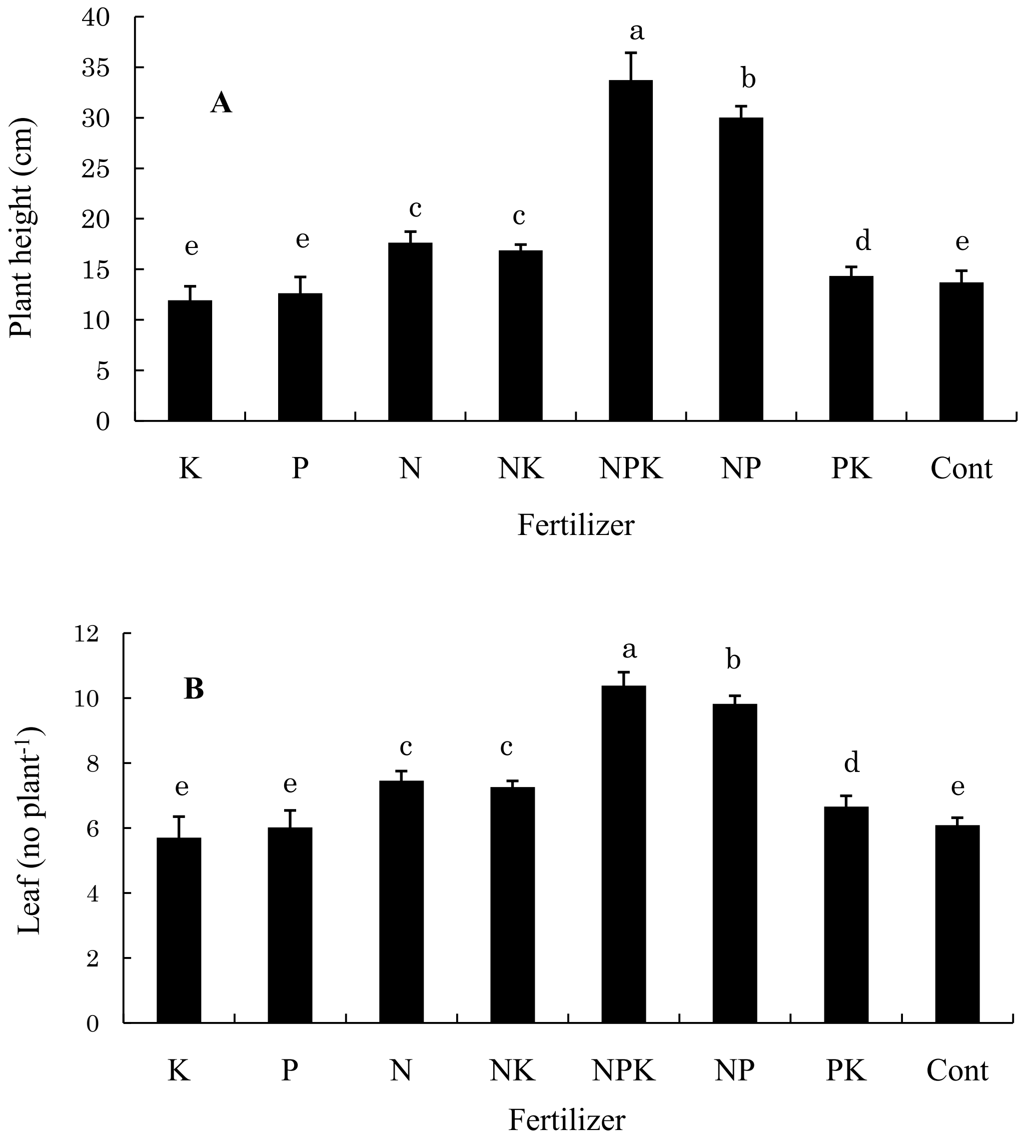


Fig. VII-4. Effects of fertilizer N, P and K applied alone or in combination on plant height (A) and leaf number (B) of amaranth line BC cultivated from September to December, 2013. Bars with the same letter are not significantly different at the 5% level (LSD test).

Separated application of P and K did not increase the growth parameters of amaranth, whereas, N alone increased the growth parameters (Table VII-2). All the growth parameters were highest with the NPK fertilization followed by NP. Total leaf area increased by 2-11 and 2-10 times with the combined fertilizer of NPK and NP, respectively, and dry leaf weight increased by 2-9 and 2-8 times accordingly, as compared to those with the other fertilizer treatments. Yield (shoot dry weight) was highest with the NPK fertilizer (1.76g per plant) followed by NP (1.46 g per plant) (Table VII-2). The fertilizer NPK and NP resulted in 2.2 and 1.8 times higher yield, respectively as compared to the fertilizer NK. The fertilizer K promoted the function of N fertilizer slightly or moderately but P did significantly in all the growth parameters of amaranth (Table VII-2).

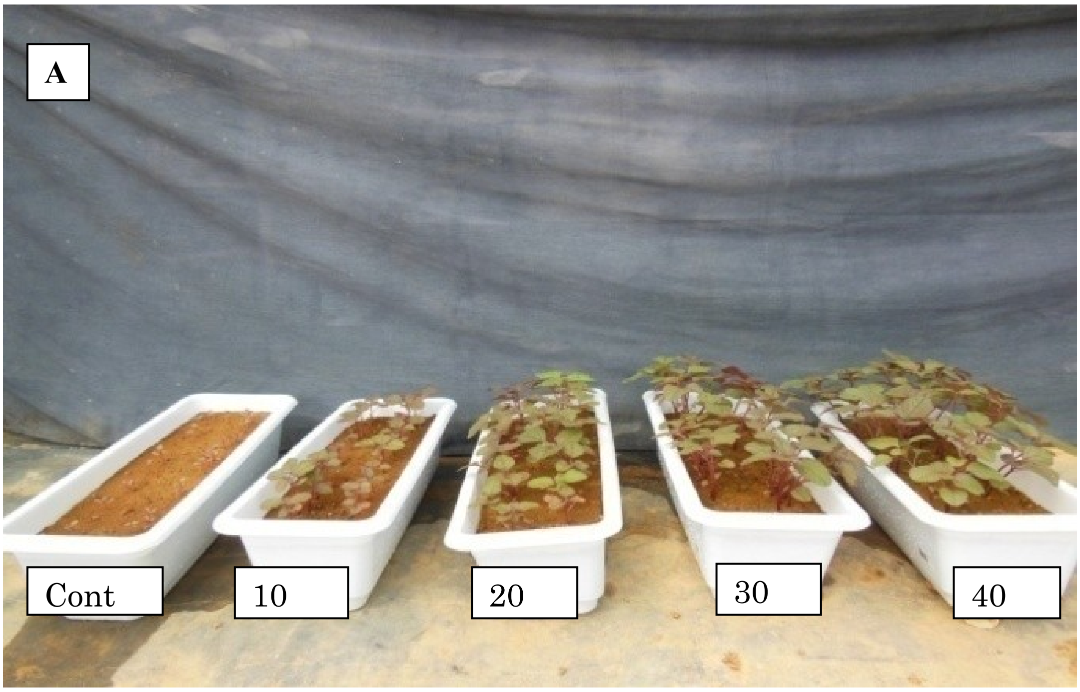
Table VII-2. Effects of fertilizer N, P and K applied alone or in combination on growth parameters of edible amaranth cultivated from September to October, 2013

Fertilizer treatment	Stem diameter (mm)	Internode length (cm)	Largest leaf area (cm^2 $leaf^{-1}$)	Total leaf area ($cm^2 plant^{-1}$)	Fresh leaf wt. (g $plant^{-1}$)	Dry leaf wt (g $plant^{-1}$)	Fresh stem weight (g $plant^{-1}$)	Dry stem weight (g $plant^{-1}$)	Fresh shoot weight (g $plant^{-1}$)	Dry shoot weight (g $plant^{-1}$)
K	2.68d	0.56d	9.15d	34.82d	0.97d	0.13d	0.48d	0.04e	1.45d	0.17d
P	2.92d	0.51d	9.33d	37.12d	1.09d	0.18d	0.58d	0.07de	1.67d	0.25d
N	5.04c	2.00c	33.02c	162.34c	4.06c	0.52c	3.05c	0.18c	7.11c	0.70c
NK	5.25c	2.16c	33.86c	168.61c	4.33c	0.48c	3.28c	0.18c	7.61c	0.66c
NPK	8.17a	4.16a	59.42a	393.64a	11.05a	1.15a	12.71a	0.61a	23.77a	1.76a
NP	6.57b	2.84b	52.94b	356.77b	8.72b	0.99b	9.62b	0.47b	18.34b	1.46b
PK	2.77d	0.70d	10.48d	52.55d	1.33d	0.21d	0.79d	0.09d	2.13d	0.30d
Cont	2.98d	0.63d	11.30d	50.20d	1.21d	0.19d	0.71d	0.08d	1.92d	0.27d

Note: Data recorded at 25 day after planting. Data with the same letter within each column are not significantly different at the 5% level, as determined by LSD test.

2. Effects of NPK fertilizer rates on growth and yield of amaranth BB and BC lines

Growth of amaranth lines BB (A) and BC (B) is shown in the Fig. VII-5. The growth of amaranth line BB was better with the increasing rates of fertilizer NPK, whereas the growth of amaranth BC line was almost similar with the 20-40 g m^{-2}.



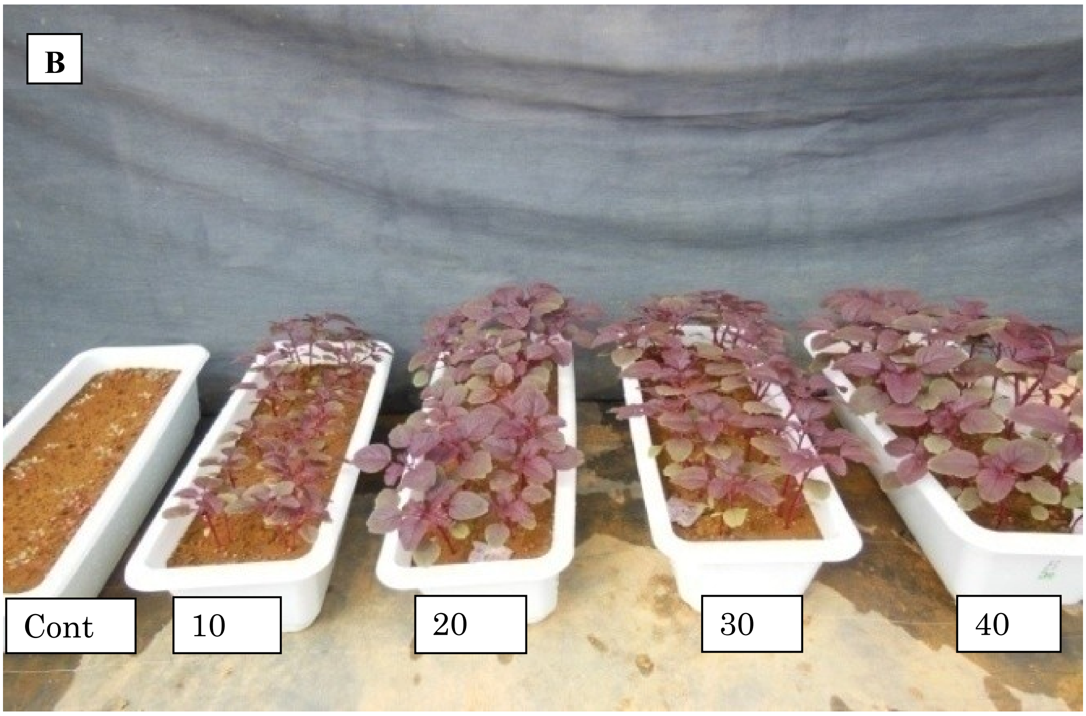
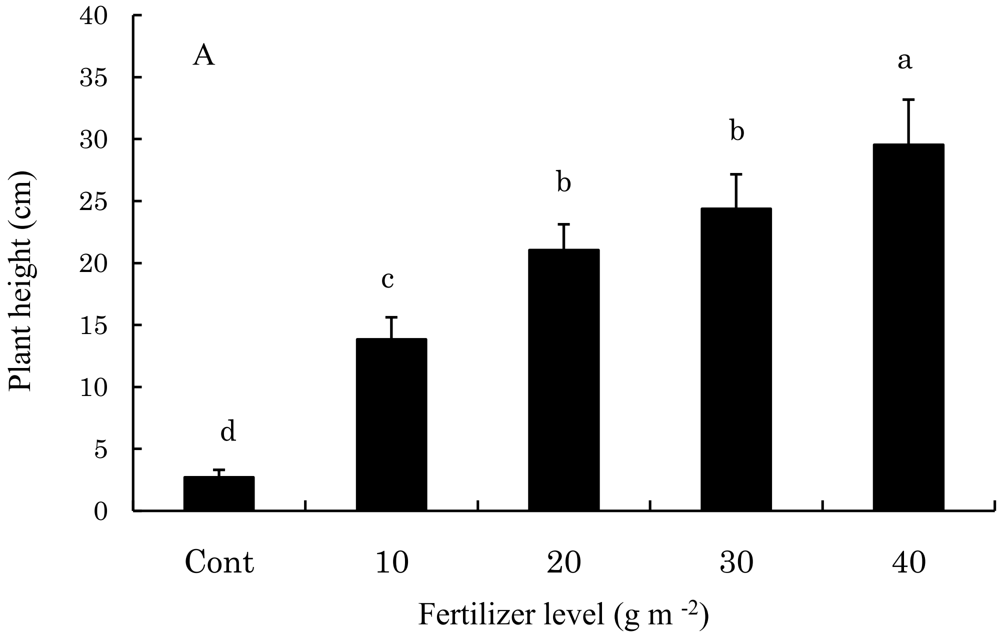


Fig. VII-5. Effects of combined fertilizer NPK rates on growth of amaranth (A: BB line; B: BC line) {(Cont(0g), 10 (10 g m^{-2}), 20 (20 g m^{-2}),30 (30 g m^{-2}), 40 (40 g m^{-2})}

Plants height of the amaranth lines BB (Fig. VII-6 (A)) and BC (Fig. 6 (B)) increased with the increasing rate of NPK fertilizer.



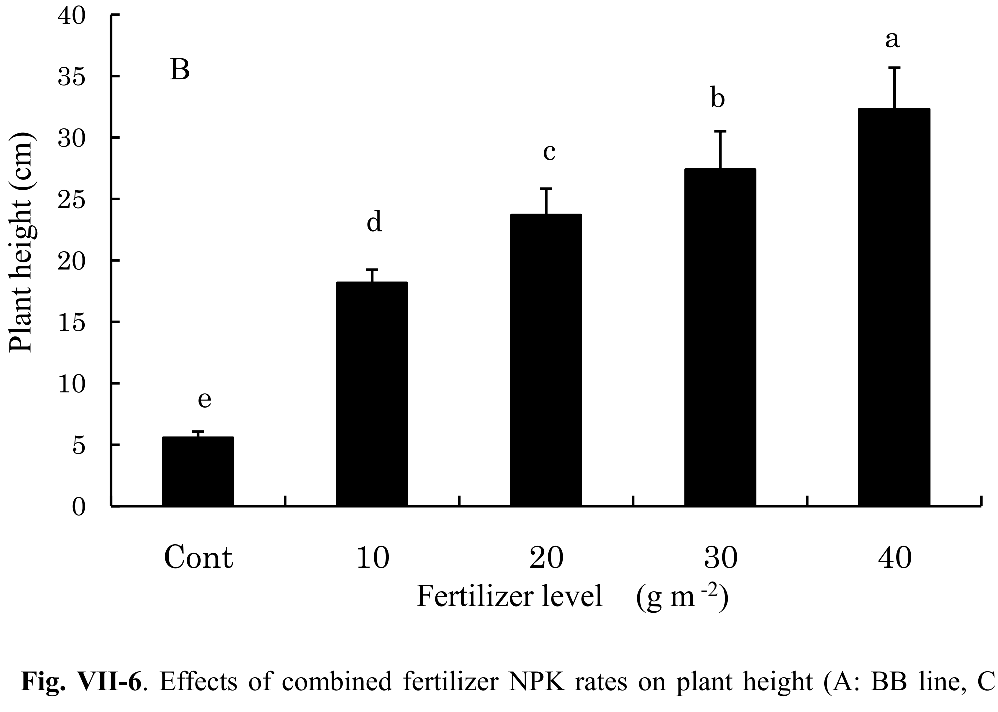
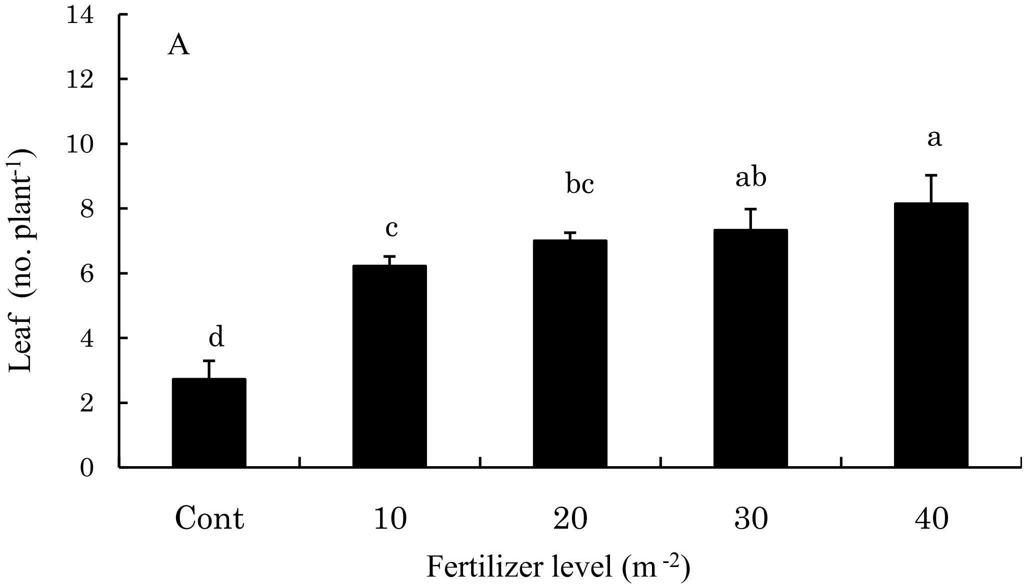


Fig. VII-6. Effects of combined fertilizer NPK rates on plant height (A: BB line, C: BC line) of amaranth cultivated from July to August, 2014 (Cont (0 g), 10 (10 g m^{-2}), 20 (20 g m^{-2}), 30 (30 g m^{-2}), 40 (40 g m^{-2}). Bars with the same letter are not significantly different at the 5% level, as determined by LSD test.

The leaf number of BB line (Fig. VII-7 (A)) was highest with the fertilizer rate of 40 g m^{-2} followed by 30 g m^{-2}. The BC line (Fig. VII-7 (B)) obtained highest leaf number when cultivated with the fertilizer rate of 40 g m^{-2} followed by 20-30 g m^{-2}.



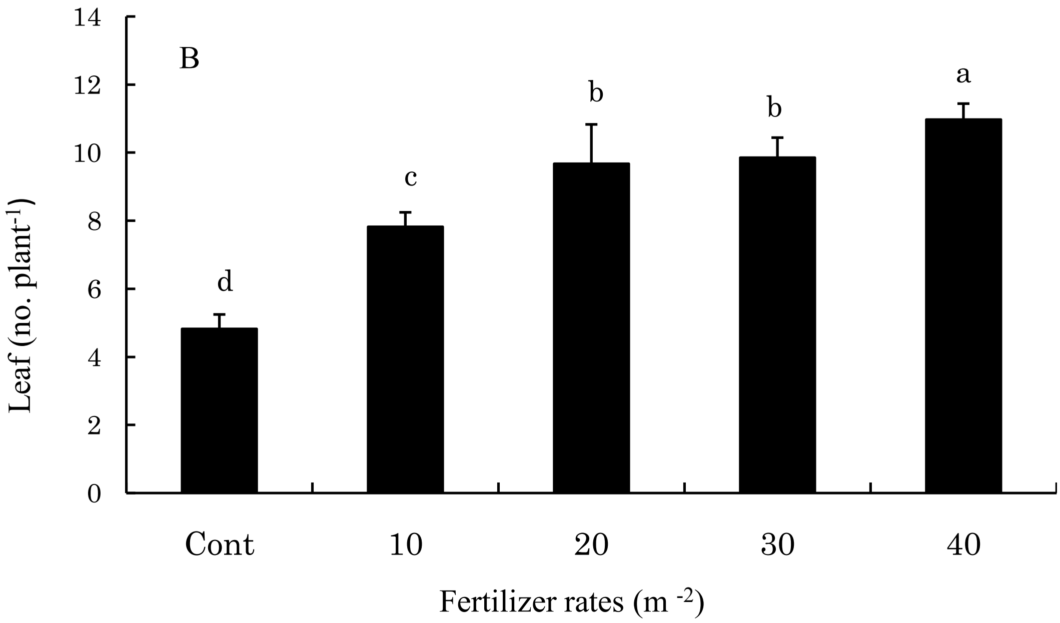


Fig. VII-7. Effects of combined fertilizer NPK rates on leaf number of amaranths (A: BB line, C: BC line) cultivated from July to August, 2014 (Cont (0 g), 10 (10 g m^{-2}), 20 (20 g m^{-2}), 30 (30 g m^{-2}), 40 (40 g m^{-2}). Bars with the same letter are not significantly different at the 5% level, as determined by LSD test.

SPAD value increased with all the fertilizer rates in both the amaranth lines (Fig. VII-8). The SPAD value was highest with the fertilizer of 40 g m^{-2} than that with the other fertilizer rates in BB line, but same with all the fertilizer rates in BC line.

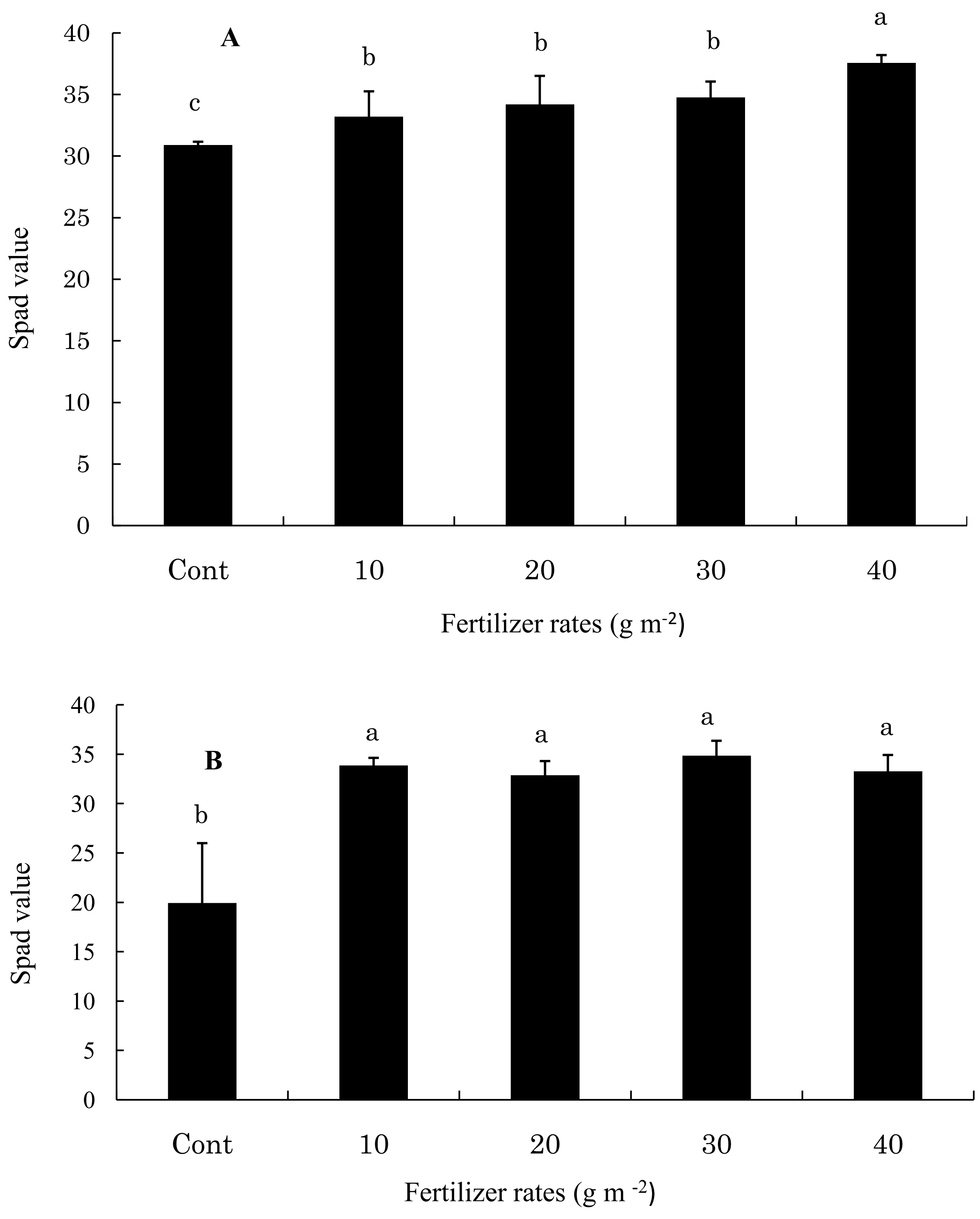


Fig. VII-8. Effects of combined fertilizer NPK rates on SPAD value of amaranths (A: BB line; B: BC line). (Cont(0g), 10 (10 g m^{-2}), 20 (20 g m^{-2}),30 (30 g m^{-2}), 40 (40 g m^{-2}). Bars with the same letter are not significantly different at the 5% level, as determined by LSD test.

Stem diameter, internode length, largest leaf area, total leaf area, leaf weight, stem weight and shoot weight (yield) of both the amaranth lines were increased with the increasing rate of fertilizer (Table 3). Yield per plant of BB and BC line was 2.30 and 3.89 g. respectively, when cultivated with the fertilizer rate of 40 g m^{-2}.

Table VII-3. Effects of combined fertilizer NPK rates on growth and yield parameters of amaranth lines BB and BC cultivated from July to August, 2014

Amaranth lines	Fertilizer rates (g m^{-2})	Stem diameter (mm)	Internode length (cm)	Largest leaf area ($cm^2 plant^{-1}$)	Total leaf area ($cm^2 plant^{-1}$)	Fresh leaf weight ($g plant^{-1}$)	Dry leaf weight ($g plant^{-1}$)	Fresh stem weight ($g plant^{-1}$)	Dry stem weight ($g plant^{-1}$)	Fresh shoot weight (g $plant^{-1}$)	Dry shoot weight ($g plant^{-1}$)
BB	Cont	0.89d	0.19d	0.75d	2.24d	0.05d	0.02c	0.03d	0.01d	0.08d	0.03d
	10	3.00c	1.70c	13.56c	48.75c	1.59c	0.64b	0.81c	0.21c	2.41c	0.85c
	20	3.95b	3.06b	23.00b	86.37b	2.95b	0.90b	1.86b	0.41b	4.80b	1.31bc
	30	4.38a	2.79b	23.25b	94.18b	3.16b	1.01b	2.63b	0.52b	5.78b	1.53b
	40	4.69a	4.05a	35.14a	151.97a	4.89a	1.60a	4.07a	0.70a	8.93a	2.30a
BC	Cont	1.28d	0.39d	1.21d	3.93d	0.12d	0.08d	0.09d	0.03d	0.18d	0.15d
	10	4.29c	1.52c	12.60c	53.90c	1.88c	1.00c	1.57c	0.52c	3.45c	1.52c
	20	4.93bc	1.96bc	20.31b	94.42b	3.46b	1.59b	3.33b	0.84b	6.79b	2.43b
	30	5.72b	2.33b	20.78b	102.49b	4.16b	1.84b	4.75b	1.15b	8.90b	2.99b
	40	6.99a	3.07a	31.42a	162.07a	5.83a	2.35a	7.85a	1.55a	13.68a	3.89a

Note: Data recorded at 26 day after planting. Data with the same letter within each column for each amaranth line are not significantly different at the 5% level, as determined by LSD test.

3. **Effects of N, P and K applied alone and in combination on mineral, nitrogen and carbon content of amaranth**

The experiment conducted from April to June, 2013 showed that Na content of amaranth was highest with the NP fertilizer, and K was highest with the NPK fertilizer (Table VII-4). The content of Ca in the amaranth plants decreased with all the fertilizers, compared to those with the control treatment. The Mg content in the amaranth increased with all the fertilizers, except K fertilizer. The Al content decreased with all the fertilizers, except P and PK fertilizers. The Fe content did not differ clearly with the fertilizers, except Fertilizer P. The P content in amaranth was the highest with the application of P and PK fertilizer, and was the second highest with the NP followed by NPK fertilizer. Total N and C content in the plant was not significantly influenced with the fertilizers, however total N in the plant was lower with the NPK fertilizer.

Table VII-4. Effects of fertilizer N, P and K applied alone or in combination on mineral, total N and total C in amaranth leaf cultivated from April to June, 2013

Fertilizer treatment	Na (mg g^{-1})	K (mg g^{-1})	Ca (mg g^{-1})	Mg (mg g^{-1})	Al (mg g^{-1})	Fe (mg g^{-1})	P (mg g^{-1})	TN %	TC %
K	2.16d	12.80d	10.94d	9.48c	0.67b	0.17cd	5.72c	-	-
P	3.80b	27.86b	15.16bc	15.68a	1.23a	0.97a	20.34a	-	-
N	3.38b	31.14a	13.38cd	15.28a	0.12c	0.02d	7.56c	4.46a	39.53a
NK	2.14d	30.20ab	13.42cd	13.18b	0.17c	0.01d	6.14c	4.71a	40.26a
NPK	3.10bc	35.06a	15.64bc	12.62b	0.15c	0.03d	11.58b	3.63a	39.57a
NP	7.32a	19.28c	15.16bc	16.50a	0.08c	0.22bc	12.62b	4.16a	40.21a
PK	2.98c	25.34b	16.96ab	16.98a	1.06a	0.26c	18.68a	-	-
Cont	3.76bc	26.40b	18.78a	8.12c	1.32a	0.42b	9.86c	-	-

Data with the same letter within each column are not significantly different at the 5% level, as determined by LSD test.

The experiment conducted from September to October, 2013 showed that Na content was highest with the NP fertilizer, K was highest with the NPK fertilizer, and Ca was lowest with the NP fertilizer (Table VII-5). The Mg content of the plant was lower with all the fertilizer treatments, except K fertilizer. The P content increased when amaranth plant was cultivated with the P, NP, PK and NPK fertilizers. The content of Al and Mn was not clearly influenced with the fertilizers applied alone or in combination. The Zn content in the plant increased with the fertilizer N and NK. Total N and C contents were highest with the NP followed by and NPK fertilizer (Table VII-5).

Table VII-5. Effects of fertilizer N, P and K applied alone or in combination on mineral and total nitrogen and total carbon in amaranth leaf cultivated from September to October, 2013

Fertilizer treatment	Na mg g^{-1}	K mg g^{-1}	Ca mg g^{-1}	Mg mg g^{-1}	Al mg g^{-1}	Fe mg g^{-1}	P mg g^{-1}	Mn mg g^{-1}	Zn mg g^{-1}	TN %	TC %
K	7.17d	91.83a	23.98bc	46.23a	0.65ab	0.26d	8.70b	1.57a	0.11b	2.52d	38.54f
P	7.07d	77.17b	23.87bc	37.90bc	0.67a	0.24d	9.52ab	1.36b	0.16b	1.86f	39.51e
N	11.13b	90.83a	23.97bc	31.90cd	0.56abc	0.42ab	7.92b	1.44ab	0.36a	5.26bc	41.25c
NK	8.88c	76.5b	25.77ab	32.83c	0.51bc	0.36bc	7.96b	1.58a	0.34a	5.15c	40.44d
NPK	9.53c	91.25a	20.60cd	26.75de	0.50bc	0.43ab	9.70a	1.47ab	0.09b	5.58b	41.79b
NP	12.73a	87.83a	18.40d	22.60e	0.48c	0.44a	10.16a	1.34b	0.12b	6.13a	43.33a
PK	6.68d	78.33b	25.73ab	35.83bc	0.55abc	0.25d	10.35a	1.47ab	0.09b	2.14ef	39.76e
Cont	7.32d	93.33a	28.93a	39.30b	0.63ab	0.30cd	8.47b	1.51a	0.05b	2.41de	39.89e

Data with the same letter within each column are not significantly different at the 5% level, as determined by LSD test.

4. Effects of fertilizer rates on L-ascorbic acid, mineral, nitrogen and carbon content of edible amaranth

L-ascorbic acid content of amaranth leaf increased significantly with all the fertilizer rates in both BB and BC lines, and the L-ascorbic acid content was highest in BB line when grown with the 40 g m^{-2} fertilizer followed by 30 and 20 g m^{-2} (Fig. VII-9). In BC amaranth line, L-ascorbic acid content was highest with 40 g m^{-2} followed by 30, 20 and 10 g m^{-2} fertilizer.

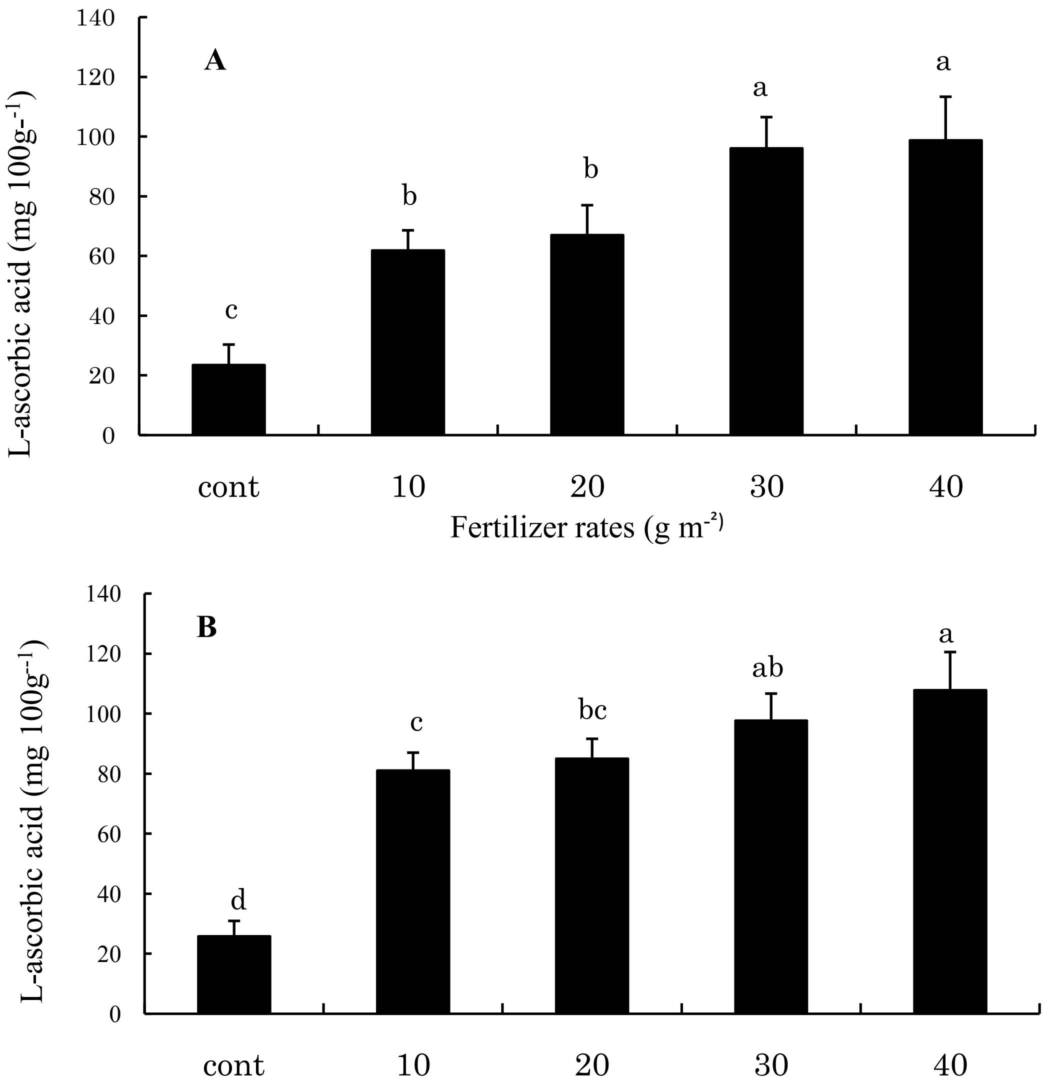


Fig. VII-9. Effects of combined fertilizer NPK rates on ascorbic acid content of amaranths (A: BB line; B: BC line). Cont(0g), 10 (10 g m^{-2}), 20 (20 g m^{-2}),30 (30 g m^{-2}), 40 (40 g m^{-2}). Bars with the same letter are not significantly different at the 5% level, as determined by LSD test.

The Na, K, Ca, Mg, P and Zn content of amaranth line BB increased with all the fertilizer rates (Table VII-6). The Al and Fe content was not clearly influenced with fertilizer rates. The Mn content decreased with the fertilizer rates of 20, 30 and 40 g m^{-2}. The Na content of BC line tented to increase with all the fertilizer rates, and K content increased with the 20, 30 and 40 g $m\text{-}^{2}$ (Table VII-6). The Ca content increased with the 10 and 20 g $m\text{-}^{2}$ but not with the 30 and 40 g $m\text{-}^{2}$, as compared to that with the control treatment, and Mg and Zn content was not influenced with the fertilizer rates. The Al, Fe and Mn content increased with all the fertilizer rates. The P content increased with the increasing fertilizer rates. Total N content was same with all the fertilizer rates in BB line, but increased with the increasing fertilizer rate in BC line. The C content of BB and BC lines was not influenced with the fertilizer rates.

Table VII-6. Effects of combined fertilizer NPK rates on mineral, total nitrogen and total carbon in amaranth lines BB and BC cultivated from July to August, 2014

Amaranth line	Fertilizer rates	Na	K	Ca	Mg	Al	Fe	P	Mn	Zn	TN	TC
	g m^{-2}	mg g^{-1}	mg g^{-1}	mg g^{-1}	mg g^{-1}	mg g^{-1}	mg g^{-1}	mg g^{-1}	mg g^{-1}	mg g^{-1}	%	%
BB	Cont	5.25c	127.67d	32.33c	24.73b	0.63a	1.02ab	14.03b	2.13a	0.32b	—	—
	10	6.63ab	141.50c	42.40a	27.10a	0.43b	0.89c	39.96a	1.98a	0.35a	3.30a	38.28a
	20	6.36ab	156.50b	40.90a	27.83a	0.64a	0.99b	41.43a	1.39b	0.36a	3.61a	39.40a
	30	6.15b	157.16b	35.70b	26.23a	0.66a	1.02ab	39.53a	1.28b	0.36a	3.47a	39.04a
	40	6.76a	183.33a	36.70b	26.13a	0.65a	1.02ab	41.70a	1.42b	0.35a	3.33a	38.77a
BC	Cont	6.50b	91.10c	24.23b	33.33a	0.42b	0.93c	21.35c	0.71a	0.34a	—	—
	10	6.53b	96.33c	29.13a	30.66a	0.66a	1.10a	24.26c	0.32b	0.37a	1.88c	39.43a
	20	6.95b	114.33b	28.33a	32.33a	0.60a	1.09a	25.63bc	0.36b	0.36a	2.25b	38.90a
	30	6.98b	124.66b	24.33b	29.30a	0.63a	1.08a	28.86b	0.36b	0.35a	2.32b	39.01a
	40	8.30a	152.50a	24.63b	30.86a	0.65a	1.04b	31.56a	0.37b	0.34a	2.90a	38.59a

Data with the same letter within each column for each amaranth line are not significantly different at the 5% level, as determined by LSD test. —, data not recorded due to insufficient sample.

Discussion

Both the experiments indicate that P and K applied alone were not effective for amaranth growth, but N was effective. Potassium (K) fertilizer influenced the function of N fertilizer slightly but P did significantly in all the growth parameters of amaranth. Combined application N and P resulted in the significantly higher growth parameters and yield than the combined application of NK. Combined application of NPK resulted in the highest growth parameters and yield followed by the combined application of NP. Similarly, other studies reported that higher N increases vegetative growth parameters of plants than other nutrients like P and K (Akamine et al., 2007; Chowdhury et al., 2008; Hossain et al., 2011, Sarker et al., 2002). The content of K and P in soil was 2.28 and 0.23 mg kg^{-1}, respectively. It is assumed that K content in soil was enough for amaranth growth but P was not enough. It is also considered that, amaranth needs higher amount of P than K for proper growth. The fertilizers N, P and K together provided balanced nutrients, which resulted in higher plant growth parameters and yield. Other studies found similar effects of balanced nutrients N, P and K for higher plant biomass production in various crop species (Oya, 1972; Akamine et al., 2007; Mazid, 1993; Hao and Papadopoulos, 2004; Hossain et al., 2012).

The growth of amaranth line BB was better with the increasing rates of fertilizer NPK, whereas the growth of amaranth line BC was similarly better with the 20-40 g m^{-2}. All the growth parameters and yield of both the amaranth lines were highest with the fertilizer rate 40 g m^{-2} followed by 30 g m^{-2}. These results indicate that the fertilizer NPK at 30-40 g m^{-2} is required for better yield of amaranth in dark-red soil in Okinawa.

The experiments conducted from April to June, and September to October, 2013 showed that Na content of amaranth was highest with the NP fertilizer, and K was highest with the NPK fertilizer (Tables VII-4, VII-5). The Ca content in the amaranth plants was lower with all the fertilizers when cultivated from April to June, but only with fertilizer NP when cultivated from September to October. The Mg content in the amaranth was higher with all the fertilizers when cultivated from April to June, but lower when cultivated from September to October. The P content increased when amaranth plant was cultivated with the P, NP, PK and NPK fertilizers for both the cultivation periods. The Al content was lower with all the fertilizers, except P and PK fertilizers when cultivated from April to June, but was not clearly influenced with the fertilizers when cultivated from September to August, 2013. Total N and C content in the plants was not significantly influenced with the fertilizers when cultivated from April to June, but significantly highest with the fertilizer NP followed by and NPK when cultivated from September to October. Similar results were recorded in other plants (Akamine et al., 2007; Hossain et al., 2011).

L-ascorbic acid content of amaranth increased significantly with all the fertilizer rates in both BB and BC lines, and the L-ascorbic acid content in the amaranth lines BB and BC was highest when grown with 40 g m^{-2} fertilizer followed by 30 and 20 g m^{-2}. The Na, K, Ca, Mg, P and Zn content of amaranth line BB increased with all the fertilizer rates. The content of Na, K, Al, Fe and P in the amaranth line BC increased with the fertilizer rates of 20- 40 g $m\text{-}^{2}$. The Ca content increased with the 10 and 20 g $m\text{-}^{2}$ but not with the 30 and 40 g $m\text{-}^{2}$, and Mg and Zn content was not influenced with the fertilizer rates. Total N content was same with all the fertilizer rates in line BB, but increased with the increasing fertilizer rate in line BC. The C content of BB and BC was not influenced with the fertilizer rates. Similar result was reported in other studies (Ivony et al., 1997; Barbara et al., 2011)

Conclusion

The fertilizer P or K applied alone was not effective for amaranth growth, because N in the soil was probably not enough and additional application of P or K resulted in unbalanced nutrient environment for amaranth growth. On the other hand fertilizer N applied alone was effective. It is thought that application of N created somewhat better nutrient environment for amaranth growth. Combined application of NP resulted in the significantly higher growth parameters and yield than the combined application of NK, which indicates that K fertilizer influenced the function of N fertilizer slightly but P did significantly in all the growth parameters of amaranth. In addition, it is assumed that K content in soil was enough for amaranth growth and P was not enough. Combined application of NPK resulted in the highest growth parameters, which indicates that fertilizer P and K applied alone was not effective but effective when applied together with the fertilizer N. All growth parameters and yield of both the amaranth lines were highest with the fertilizer NPK of 40 g m^{-2} followed by 30 g m^{-2}.

Amaranth contained highest Na and K with the fertilizer NP and NPK, respectively, and lower Ca with all the fertilizers. Amaranth contained higher Mg with all the fertilizers when cultivated from April to June, and lower Mg when cultivated from September to October. Total N content in the plants was not significantly influenced with the fertilizers when cultivated from April to June, but higher with the fertilizers NP and NPK when cultivated from September to October. Amaranth lines BB and BC contained higher L-ascorbic acid with all the fertilizer rates, which was highest with fertilizer 40 g m^{-2} followed by 30g m^{-2}. The Na, K, Ca, Mg and P content of amaranth line BB increased with all the fertilizer rates and Na, K, Al, Fe and P content in the line BC increased with the fertilizer of 20- 40 g m-^{2}. The Ca content increased with the 10 and 20 g m-^{2}, and Mg content was not influenced with the fertilizer rates. Total N content was same or higher with all the fertilizer rates in the lines BB and BC.

Combined application of NPK resulted in the highest growth parameters and yield with higher/similar minerals and higher L-ascorbic acid of BB line, compared to control plant. The lines BB and BC had higher growth parameters and yield with the NPK at 30-40 g m^{-2}, and lower /similar mineral and higher L-ascorbic acid with all NPK rates, compared to control plant. This study indicates that application of combined NPK fertilizer at 30-40 g m^{-2} is required for higher yield, mineral and L-ascorbic acid of amaranth in dark-red soil in Okinawa.

CHAPTER VIII

Effects of N, P and K Fertilizers Applied Alone and In Combination on Edible Amaranth *(Amaranthus* spp.) in Red Soil, Okinawa

Abstract: Fertilizer regimes were evaluated on growth, yield and quality of amaranth lines BB and BC in red soil (pH 5.1) in Okinawa. The fertilizer treatments were control (Cont), N, P, K, N plus P (NP), N plus K (NK), P plus K (PK), and N plus P plus K (NPK). Nitrogen (N) at 500 kg ha^{-1}, P at 500 kg ha^{-1} and K at 500 kg ha^{-1}were applied according to the experiment design. In the other experiment, the fertilizer NPK rates were control (Cont, 0 kg ha^{-1}), 100 kg ha^{-1}, 200 kg ha^{-1}, 300 kg $ha^{-1}$and 400 kg ha^{-1}. The plant cultivated with the fertilizer K, P, N, NK and PK was very poor, and many plants died. All the growth parameters and yield of amaranth were highest with the fertilizer NPK followed by NP. The fertilizers K, P and PK did not increase the growth parameters and yield. All the growth parameters and yield of amaranth line BB were significantly higher with the fertilizer 30-40 g m^{-2}, whereas the growth parameters and yield of amaranth line BC were significantly higher with the fertilizer 20-30 g m^{-2}. The Na and Ca contents in the amaranth plant were lower, whereas K, P and N contents were higher with the fertilizer NPK. The content of Mg was not influenced with the fertilizers. The Na and Ca contents were not influenced, but K, Fe and Mg were higher with the fertilizer NPK at 30-40 g m^{-2} in the amaranth BB. The content of Na, K, Ca, Mg and P in amaranth line BC was higher with the 30 g m^{-2}. The content of N in amaranth lines BB and BC was higher with the combined fertilizer 20-40 g m^{-2}. The results indicate that amaranth plant cannot grow without N and P fertilizers. All the growth parameters and major minerals were increased with the fertilizer rates of 30-40 g m^{-2}. The combined fertilizer NPK at 30-40 g m^{-2} is required for higher yield and quality of amaranth in red soil in Okinawa.

Introduction

Growth, yield and quality of a plant species differ with soil types and soil nutrient status; and a plant-species requires balanced fertilizer to maximize growth, yield and quality in a soil type (Hossain et al., 2004;Hossain and Ishimine, 2005; Akamine et al., 2007; Chowdhury et al., 2008; Hossain et al., 2011). Dark-red soil, red soil and gray soil are the major soil types in Okinawa, and red soil covers more than 60% of land (Hossain and Ishimine, 2005; Okinawa Prefecture Agriculture, Forestry and Fisheries, 2008). Previous studies have been conducted on fertilizer management in gray soil and dark-red soil, but no study has yet been conducted on red soil in Okinawa. Therefore, the objectives of these studies were to i) evaluate the effect of different fertilizer elements and to ii) evaluate rates of combined fertilizer on growth, yield and quality of edible amaranth lines BB and BC for developing management practices in red soil in Okinawa.

Materials and Methods

1. Soil collection

Red soil (Kunigami mahji) was collected from the top 50 cm layer of a field in Nago city, Okinawa. The soil pH was 5.1, and the soil contained 0.06% total nitrogen and 0.20% total carbon. Sodium (Na), K, Ca, Mg, Al, Fe, P and Mn contents in soil were 0.69, 0.93, 14.51, 1.64, 0.04, 0.25, 0.28 and 0.02 mg kg^{-1}, respectively. Physical properties of the soil are presented in the chapter III.

2. Amaranth lines

The *Amaranthus tricolor* lines BB and BC selected for higher yield in previous study (Ohshiro et al., 201%) was evaluated in this study.

3. Experiment 1: Effects of N, P and K fertilizers applied alone and in combination on amaranth cultivated from November 5 to December 15, 2014

A glasshouse experiment was conducted using red soil at the Subtropical Field Science Center of the University of the Ryukyus, from November 5 to December 15, 2014. The experiment was consisted of eight treatments with five replications (planters). The fertilizer treatments were control (Cont), N, P, K, N plus P (NP), N plus K (NK), P plus K (PK) and N plus P plus K (NPK). Nitrogen (N) at 500 kg ha^{-1} (5.0 g per planter), P at 500 kg ha^{-1} (5.0 g per planter) and K at 500 kg ha^{-1} (5.0 g per planter) were mixed with 13 kg air dried soil per planter (size: 65E, 0.1 m^2) prior to the seed sowing according to the treatment design. Seeds of *Amaranthus* spp. were sown on the soil surface, and covered with 0.5 cm soil layer. The planters were placed randomly, and the plants were thinned to 8 healthiest stands per planter at 2- to 3-leaf stage. Water was applied as required everyday for proper seedling emergence and plant growth.

4. Experiment 2: Effects of N, P and K fertilizers applied alone and in combination on amaranth cultivated from February 19 to April 4, 2015

A glasshouse experiment was conducted using the same red soil at the Subtropical Field Science Center of the University of the Ryukyus, from February 19 to April 19, 2015. The experiment was consisted of eight treatments with five replications (planters). The same amaranth lines, planters, treatments, fertilizer rates, seed sowing and management practices applied in the experiment 1 were taken in this experiment.

5. Experiment 3: Effects of NPK fertilizer rates on amaranth in red soil from June 20 to July 24, 2014

A glasshouse experiment was conducted using red soil at the Subtropical Field Science Center of the University of the Ryukyus, from June 20 to July 24, 2014. Theamaranth lines, BB and BC were evaluated. Each experiment was consisted of five treatments with four replications (planters). The fertilizer treatments of control (Cont, 0 kg ha^{-1}, 0 g $planter^{-1}$), 100 kg ha^{-1} (1 g $planter^{-1}$), 200 kg ha^{-1} (2 g $planter^{-1}$), 300 kg ha^{-1} (3 g $planter^{-1}$) and 400 kg ha^{-1} (4 g $planter^{-1}$) were taken. The fertilizers of N ($CO(NH_2)_2$), P_2O_5 ($CaH_4(PO_4)_2H_2O$) and K_2O (KCl) were applied at the ratio of N:P:K=1:1:1. The fertilizers were mixed with 13 kg air dried soil per planter (size 65E) prior to the seed sowing according to the treatments. Seeds of *Amaranthus* spp. were sown on the soil surface, and covered with 0.5 cm soil layer. Water was applied as required everyday for proper seedling emergence and plant growth. The planters were placed randomly, and the plants were thinned to 10 healthiest stands per planter at 2- to 3-leaf stage.

6. Data collection

Plant height and leaf number were recorded at 40 day after seed sowing (DAS) in the experiment 1. Five plants were harvested at 40 DAS from each planter, and stem diameter, internode length, leaf number, largest leaf area、total leaf area, and fresh and dry weights of leaf, stem and shoot were determined. In the experiment 2, five plants were harvested from each planter at 44 DAS, and similar growth parameters including plant height and leaf number were measured. In the experiments 3, five plants were harvested from each planter at 35 DAS, and similar growth parameters were measured. Stem diametder was mesured at5 cm from the soil surface, and internode length was mesured from the third internode from the bottom.

7. Determination of leaf area and dry weights of edible amaranth

Leaf area was measured with an automatic area meter (AAM-8, Hayashi Denkoh Co. Ltd.). Various parts of amaranth plants were dried at 80 ℃ for 48 hr using forced convection oven (DRLF23WA, Advantec) for dry weight measurement.

8. Determination of minerals, nitrogen, carbon and pH in soil, and nutrient status in amaranth

Various parts of amaranth plants were dried at 60 ℃f or 48 h for chemical analysis using the same forced convection oven. Soil samples were dried at room temperature of 25-28 ℃ for 5 days. The plant parts and soil were ground finely for chemical analysis. Mineral contents of soil and nutrients of amaranth were determined with Inductively Coupled Plasma Spectrometer (ICPS-8100, Shimadzu Co. Ltd.). Total C and N were determined with Gas Chromatograph (Soil GS-8A, Shimadzu Co. Ltd., NC-220F Juka analysis center) and Sumigraph(NC-90A, Shimadzu Co. Ltd.).Soil pH was determined with TOA pH meter (HM-20S, Toa Electronic Ltd.).

9. Statistical analysis

The same statistical procedures used in the previous studies were applied in this study.

Results

1. Effects of fertilizer N, P and K applied alone and in combination on growth and yield of amaranth line BC

Growth of amaranth line BC cultivated from November 5 to December 15, 2014 under different fertilizers in red soil is shown in the Fig. VIII-1. The plants cultivated with the fertilizer N and NK grew for some days but did not survive finally. The plant cultivated with the fertilizer K, P and PK was very poor, and many of plants died (Fig. VIII-1). The growth of amaranth was best with the NPK followed by NP.

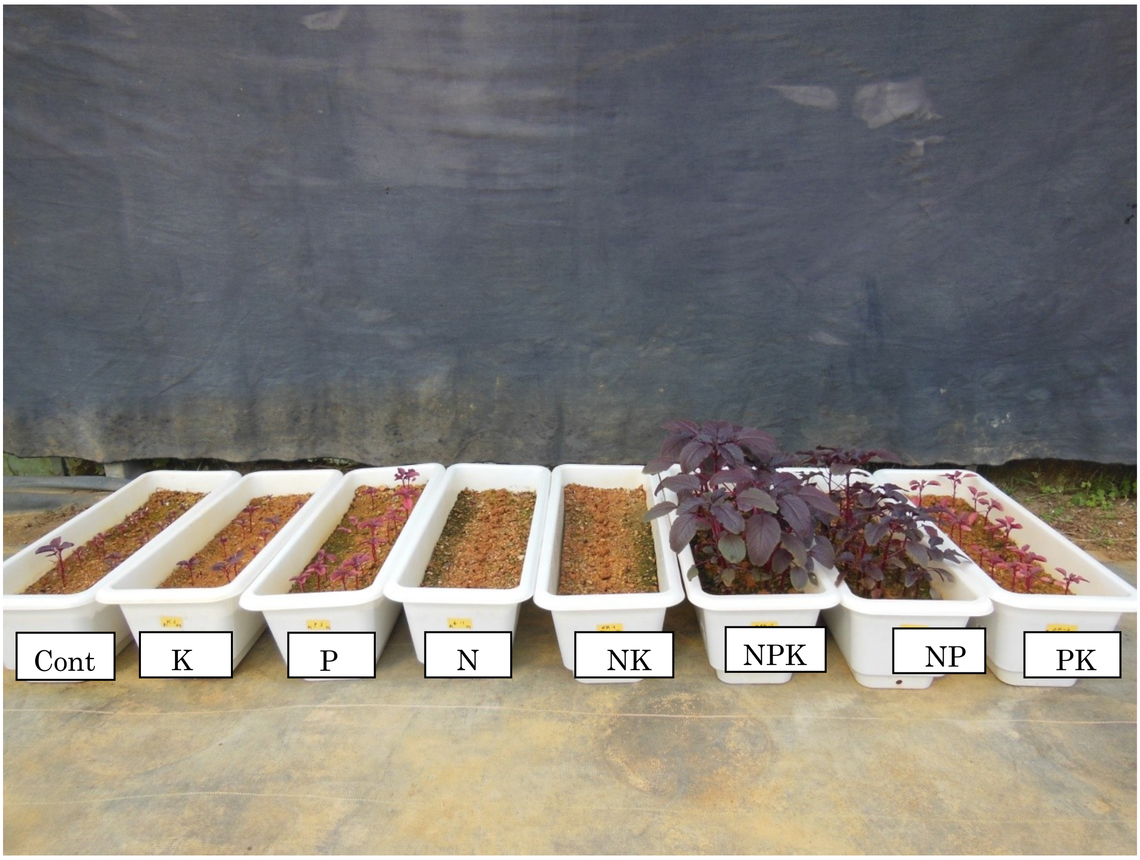


Fig. VIII-1. Effects of fertilizer N, P and K applied alone or in combination on growth of amaranth line BC cultivated from November 5 to December 15, 2014.

Plant height of amaranth BC cultivated from November 5 to December 15, 2014 under different fertilizers is shown in the Fig. VIII-2. The plant height was highest with the combined fertilizer NPK, which was 3.6 times higher than that with the control treatment. The fertilizer N or NK showed adverse effect on plant height. The other fertilizer treatments resulted in increased plant height. The fertilizer NP resulted in the second highest plant height. The plant height with the NPK was about two times higher than the plant with the NP (Fig. VII-2).

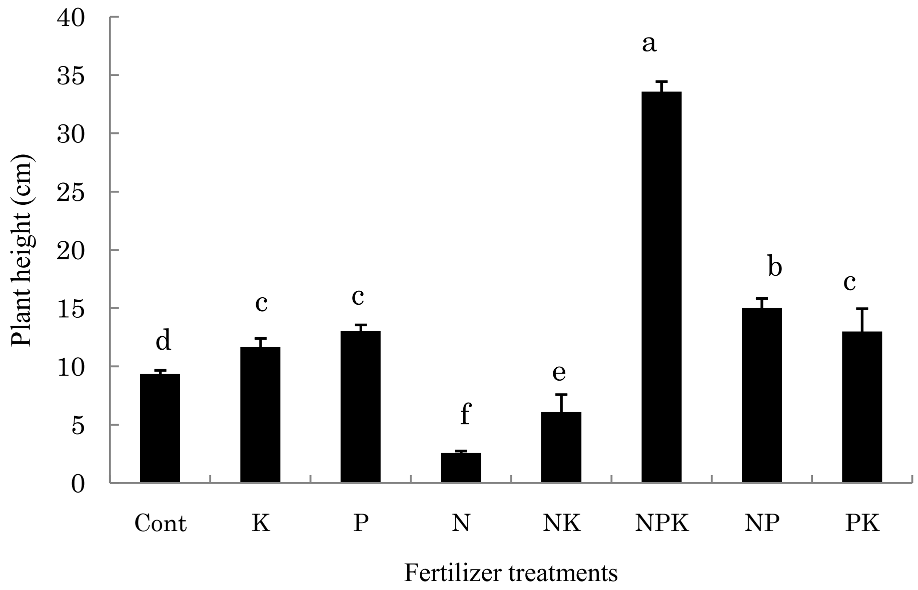


Fig. VIII-2. Effects of fertilizer N, P and K applied alone or in combination on plant height of amaranth line BC cultivated from November to December, 2014. Bars with the same letter are not significantly different at the 5% level, as determined by LSD test.

The plant with the fertilizer NPK had highest leaf number (11) followed by the fertilizer NP (8) (Fig. VIII-3). The fertilizer K or P applied alone did not result increased number of leaves per plant. The plant cultivated with the fertilizers N and NK resulted in the decreased number of leaves as compared with the control plant.

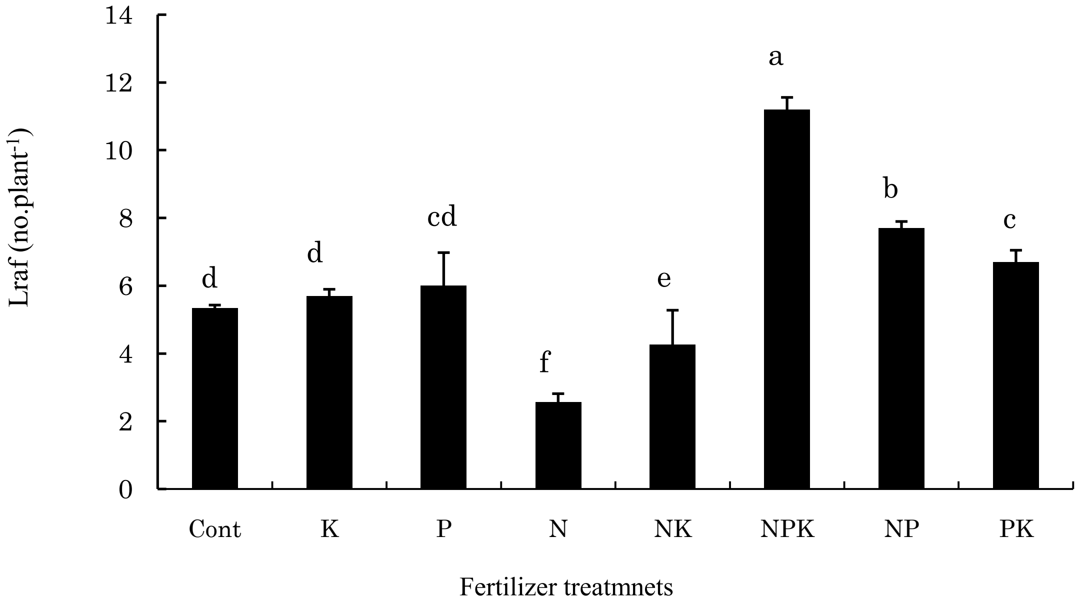


Fig. VIII-3. Effects of fertilizer N, P and K applied alone or in combination on leaf number of amaranth line BC cultivated from November to December, 2014. Bars with the same letter are not significantly different at the 5% level, as determined by LSD test.

Data were not recorded for the fertilizers of N and NK due to very poor plant growth. Stem diameter, internode length, largest leaf area, total leaf area, fresh leaf and dry leaf of amaranth increased with the fertilizers NPK and NP (Table VIII-1). Fresh and dry stem weight increased with the fertilizers NPK, NP and PK. Fresh and dry shoot (yield) weight increased significantly with the NPK and NP. The fertilizers K, P and PK did not increase the growth parameters and yield. All the growth parameters and yield were highest with the NPK followed by NP.

Table VIII-1. Effects of fertilizer N, P and K applied alone or in combination on growth parameters and yield of amaranth cultivated from November to December, 2014

Fertilizer treatment	Stem diameter (mm)	Inter-node length (cm)	Largest leaf area (cm^2)	Total leaf area (cm^2)	Freshleaf weight (g $plant^{-1}$)	Dry leaf weight (g $plant^{-1}$)	Fresh stem weight (g $plant^{-1}$)	Dry stem weight (g $plant^{-1}$)	Fresh shoot weight (g $plant^{-1}$)	Dry shoot weight (g $plant^{-1}$)
Cont	1.698c	0.210c	2.325c	6.535c	1.456c	0.120c	0.602c	0.066c	2.058c	0.186c
K	1.750c	0.243c	1.993c	6.675c	1.496c	0.210c	0.734c	0.048c	2.230c	0.258c
P	2.025c	0.247c	2.743c	7.873c	1.887c	0.287c	1.065b	0.085c	2.952c	0.372c
N	-	-	-	-	-	-	-	-	-	-
NK	-	-	-	-	-	-	-	-	-	-
NPK	6.593a	2.033a	26.760a	130.238a	15.561a	1.775a	17.566a	0.907a	33.127a	2.682a
NP	3.330b	0.690b	11.250b	45.983b	4.998b	0.685b	2.659b	0.240b	7.657b	0.925b
PK	2.393c	0.233c	5.486c	13.515c	1.660c	0.229c	1.689b	0.211b	3.349bc	0.440c

Data with the same letter within each column are not significantly different at the 5% level, as determined by LSD test. －, data not recorded due to poor growth

Growth of amaranth line BC cultivated in red soil from February 19 to April 4 under different fertilizers is shown in the Fig VIII-4. The plant growth was best with the fertilizer of NPK followed by NP. The plant growth was too poor with the fertilizer N and NK.

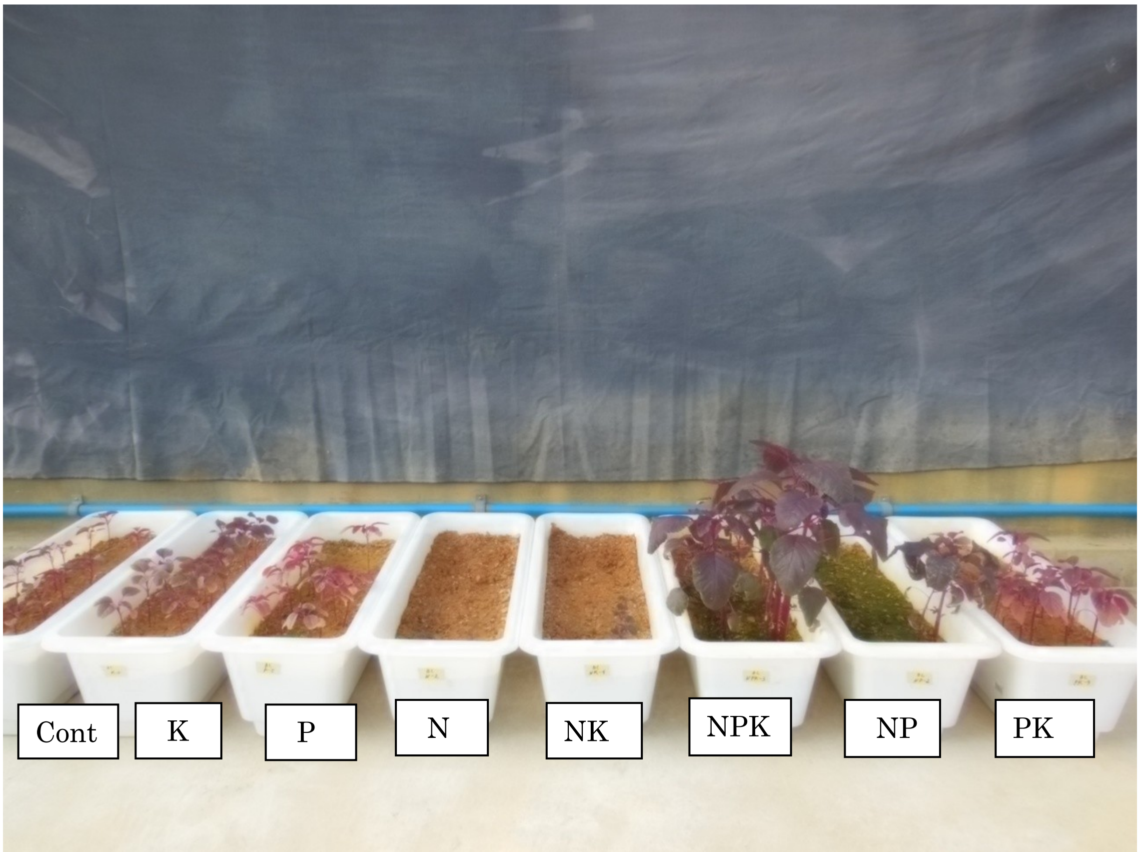
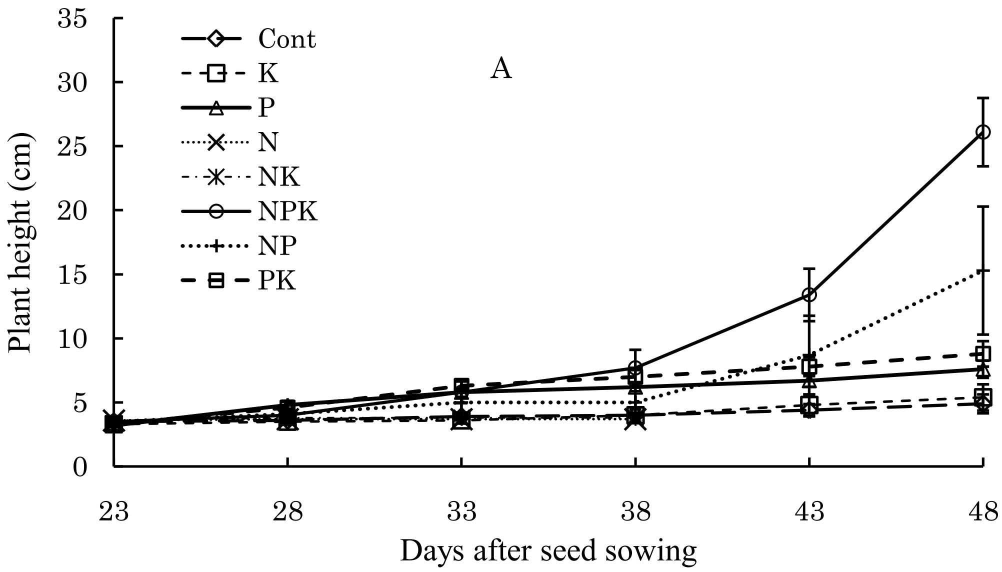


Fig. VIII-4. Effects of fertilizer N, P and K applied alone or in combination on growth of Amaranth line BC cultivated from February to April, 2015.

Plant height and leaf number of amaranth line BC cultivated from February 19 to April 4, 2015 under different fertilizers is shown in the Fig. 5. Plant height (Fig. VIII-5 (A)) and leaf number (Fig. VIII-5 (B)) were highest with the NPK followed by NP. The leaf number was 11, 9 and 6 with the fertilizer NPK, NP and NK, respectively. The other fertilizer treatments did not show positive effect on plant height. The fertilizers P and PK showed somewhat positive effect on leaf number.



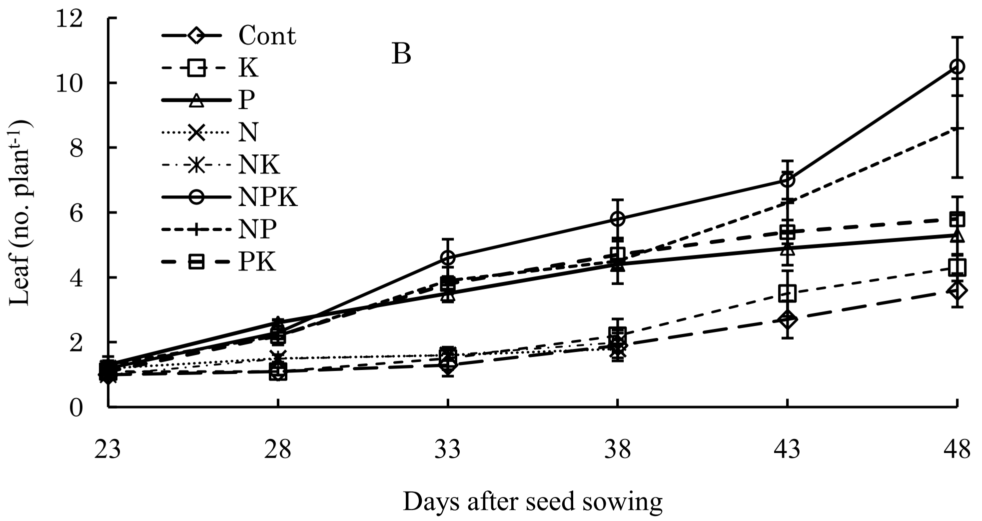


Fig. VIII-5. Effects of fertilizer N, P and K applied alone or in combination on plant height and leaf number of amaranth line BC cultivated in red soil from February to April, 2015

The growth of amaranth line BC was too poor, therefore growth parameters and yield were not measured (Table VIII-2). Stem diameter and internode length increased with the P, NPK and NP (Table VIII-2). Largest leaf area was lower with the K and PK, and higher with the NPK. Total leaf area, fresh leaf and dry leaf increased with only NPK. Fresh and dry stem increased with the P, NPK and NP. Fresh shoot increased with the NPK but decreased with the PK, Dry shoot increased with the P, NPK and NP (Table VIII-2). Most growth parameters and yield (shoot) were highest with the fertilizer NPK followed by NP (Table VIII-2).

Table VIII-2. Effects of fertilizer N, P and K applied alone or in combination on growth parameters and yield (shoot) of amaranth cultivated in red soil from February to April, 2015

Fertilizer treatment	Stem diameter (mm)	Inter-node length (cm)	Largest leaf area cm^2	Total leaf area cm^2	Fresh leaf weight (g $plant^{-1}$)	Dry leaf weight (g $plant^{-1}$)	Fresh stem weight (g $plant^{-1}$)	Dry stem weight (g $plant^{-1}$)	Fresh shoot weight (g $plant^{-1}$)	Dry shoot weight (g $plant^{-1}$)
Cont	1.880c	0.180c	6.807b	19.350b	1.456b	0.233b	0.602c	0.066c	2.058b	0.299c
K	1.946c	0.367bc	3.973c	13.680b	1.496b	0.210c	0.734c	0.058c	2.230b	0.268c
P	2.120b	0.480b	6.073b	17.893b	1.887b	0.287b	1.065b	0.148b	2.952b	0.436b
N	-	-	-	-	-	-	-	-	-	-
NK										
NPK	6.748a	2.025a	34.893a	147.510a	15.561a	1.773a	17.566a	0.907a	33.127a	2.682a
NP	2.705b	0.408b	7.518b	32.610b	3.066b	0.403b	1.689b	0.211b	4.755b	0.615b
PK	2.331b	0.192c	4.711c	8.953c	0.413c	0.201c	0.202d	0.120b	0.615c	0.321c

Data with the same letter within each column are not significantly different at the 5% level, as determined by LSD test. －, data not recorded due to poor growth

2. Effects of NPK fertilizer rates on growth and yield of amaranth BB and BC lines

Growth of amaranth lines BB (A) and BC (B) cultivated in red soil are shown in the Fig. VIII-5. The growth of amaranth line BB was better with the increasing fertilizer rates. However, the growth of amaranths is similarly better with the fertilizers 30 and 40 gm^{-2}. The growth of amaranth line BC increased with the fertilizer rates up to 30 gm^{-2}. The growth of amaranth is found to be lower with the fertilizer 40 gm^{-2} than with the 30 gm^{-2}. The plant grown without fertilizer was very poor in both the amaranth lines.

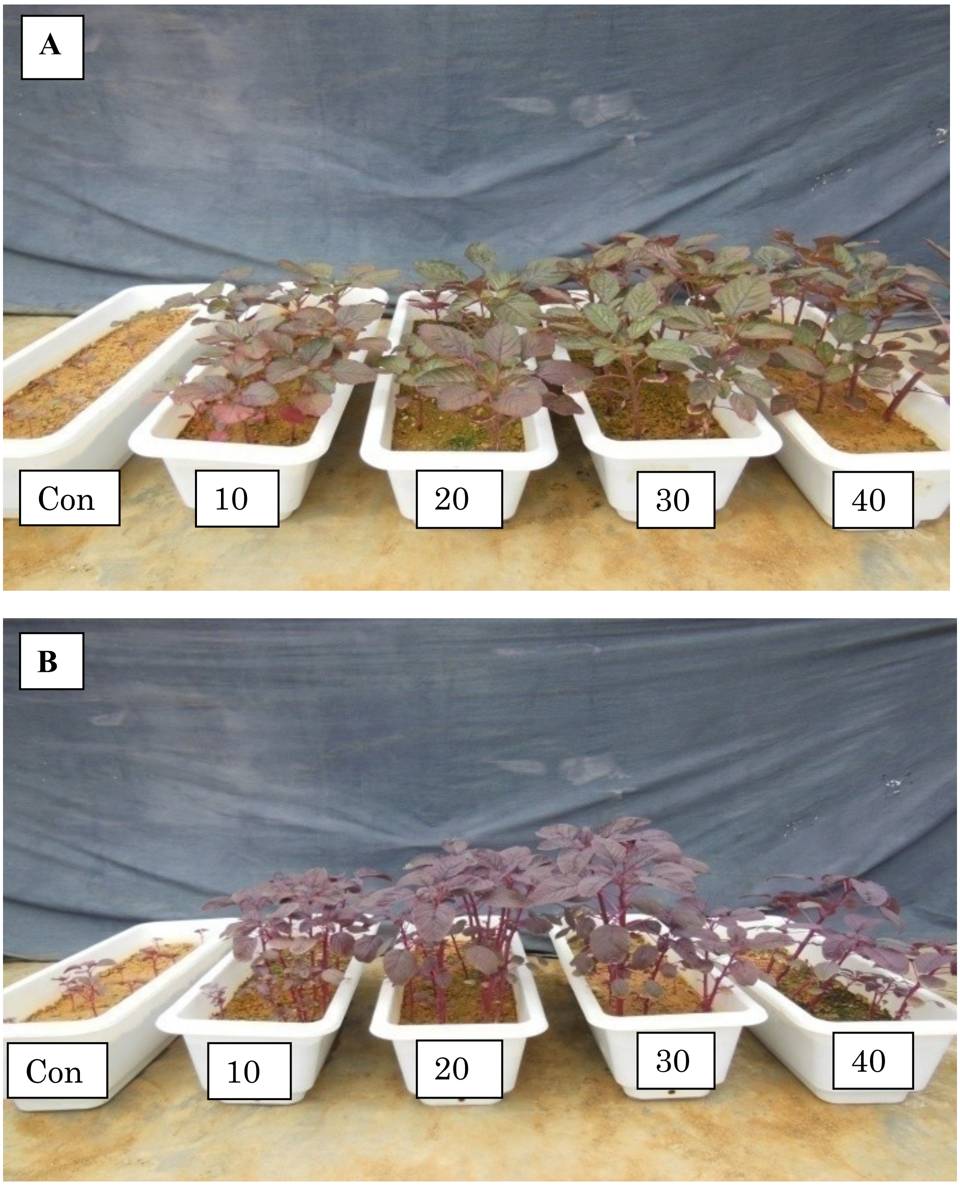
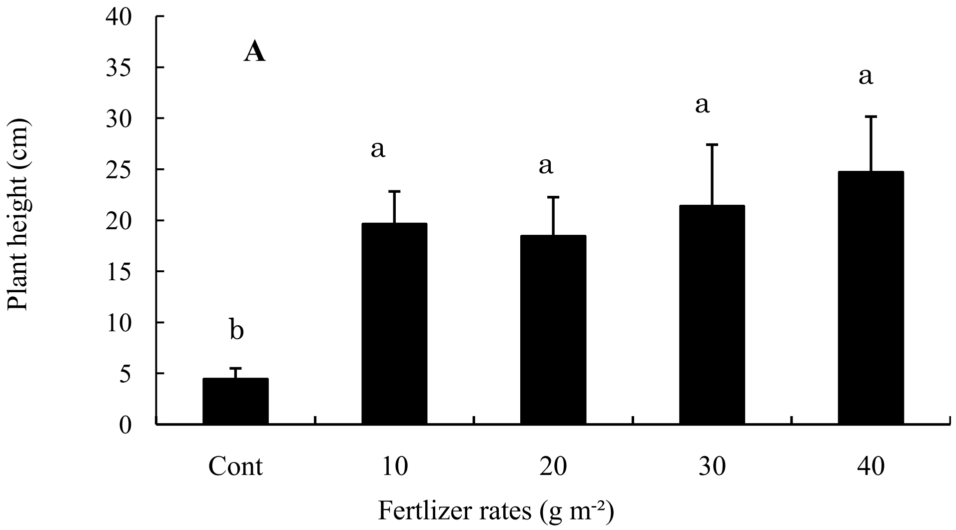


Fig. VIII-6. Effects of combined fertilizer NPK rates on growth of amaranth lines BB (A) and BC (B) cultivated from June to July, 2014 in red soil. Cont (0g), 10 (10 $g\ m^{-2}$), 20 (20 $g\ m^{-2}$),30 (30 $g\ m^{-2}$), 40 (40 $g\ m^{-2}$)

Plants height of the amaranth line BB increased similarly with all the fertilizer rates, however the plant height was highest with the 40 g m^{-2} followed by 30 g m^{-2} (Fig. VIII-7 (A)). On the other hand, plant height of the amaranth line BC increased with all the fertilizer rates, and the plant height was similarly highest with the 30 and 40 g m^{-2} (Fig. VIII-7(B)).



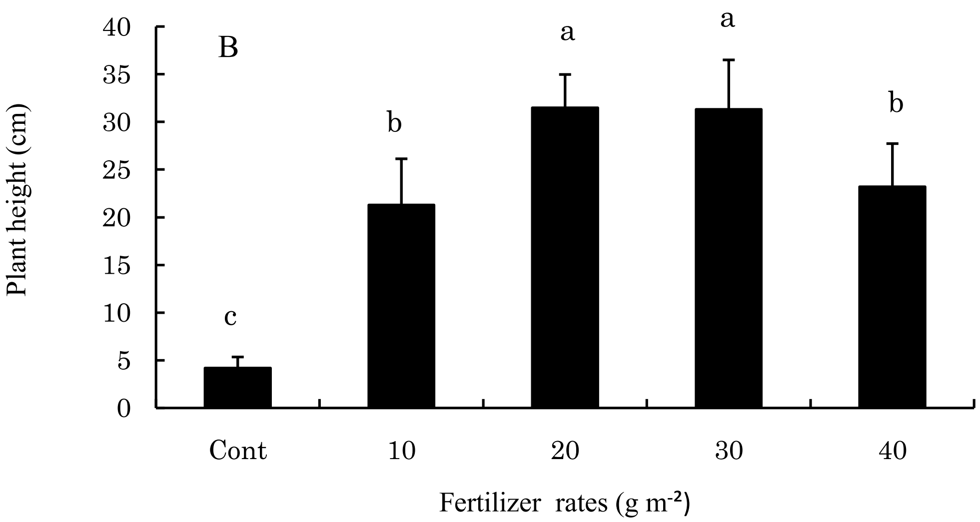


Fig. VIII-7. Effects of combined fertilizer NPK rates on plant height of amaranth lines BB (A) and BC (B) cultivated June to July, 2014 in red soil. Bars with the same letter are not significantly different at the 5% level, as determined by LSD test.

The leaf number of BB line increased with all the fertilizer rates, which was similarly highest with the fertilizer rate of 30 and 40 g m^{-2} (Fig VIII-8. (A)). The BC line obtained similarly higher leaf number with all the fertilizer rates, however the leaf number was found to be highest with the fertilizer 20 and 40 g m^{-2} (Fig. VIII-8 (B)).

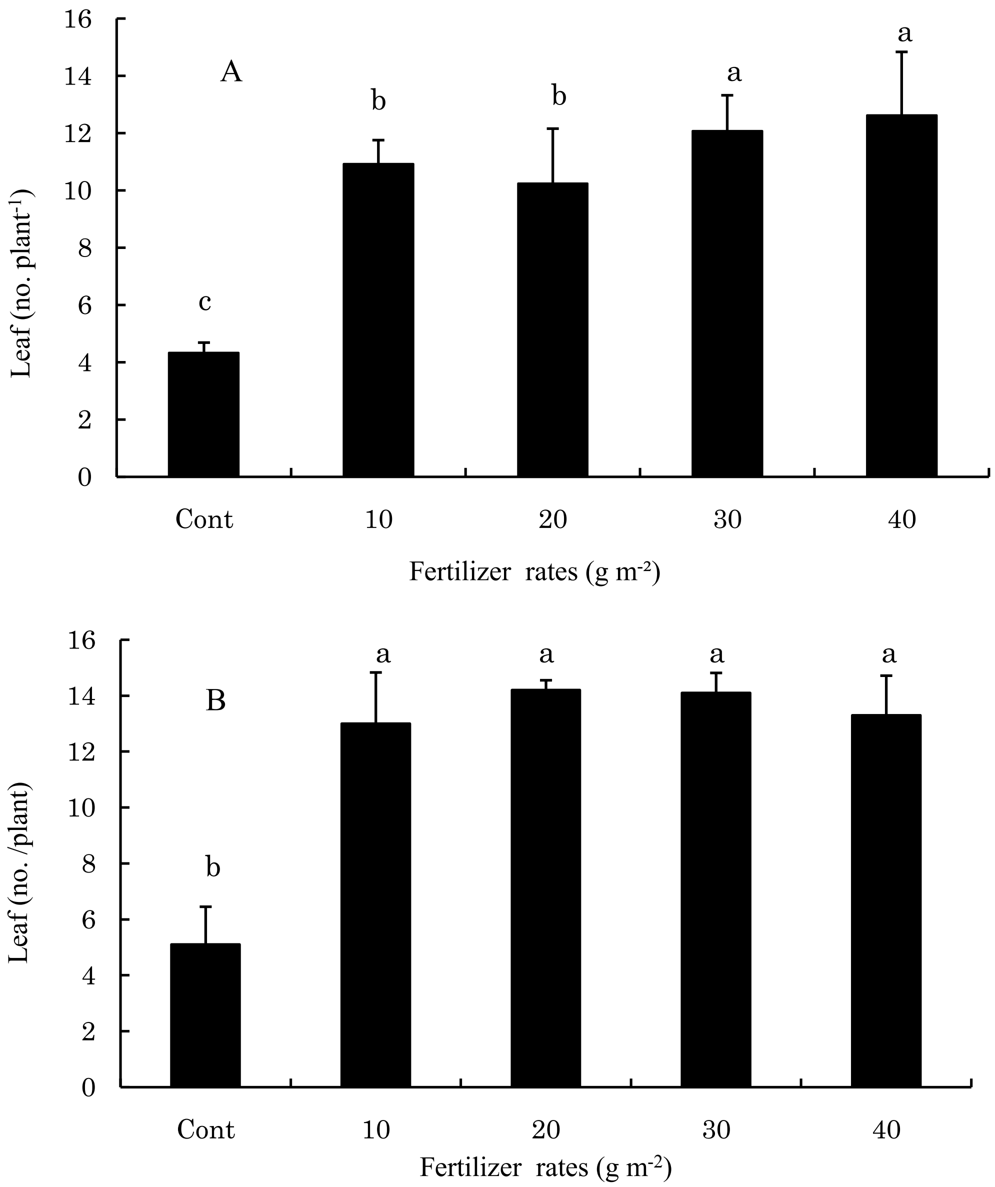


Fig. VIII-8. Effects of combined fertilizer NPK rates on leaf number of amaranth lines BB (A) and BC (B) cultivated from June to July, 2014 in red soil.
Bars with the same letter are not significantly different at the 5% level, as determined by LSD test.

Stem diameter of amaranth line BB increased with all the fertilizer rates, and was similarly higher with the 20-40 g m^{-2} (Table VIII-3). The internode length was similarly higher with all the fertilizer rates. Largest leaf area and total leaf area were similarly highest with the 30-40 g m^{-2}. Fresh and dry leaf and stem were similarly higher with the fertilizer rate of 20-40 g m^{-2}. Fresh and dry shoot weight was highest with the 40 g m^{-2} followed by 30 g m^{-2} (Table VIII-3).

Amaranth line BC obtained increased growth parameters and yield (shoot) with all the fertilizer rates (Table VIII-3), and the growth parameters and yield were similarly higher with the fertilizer rates of 20-30 g m^{-2}. The growth parameters and yield were found to be decreased with the fertilizer 40 g m^{-2}(Table VIII-3).

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Table VIII-3. Effects of combined fertilizer NPK rates on plant growth parameter and yield (shoot) of amaranth lines BB and BC cultivated in red soil from June to July, 2014

Lines	Fertilizer rates (g m^{-2})	Stem diameter (mm)	Internode length (cm)	Largest leaf area ($cm^2 leaf^{-1}$)	Total leaf area ($cm^2 plant^{-1}$)	Fresh leaf weight ($cm^2 plant^{-1}$)	Dry leaf weight ($cm^2 plant^{-1}$)	Fresh stem weight ($cm^2 plant^{-1}$)	Dry stem weight ($cm^2 plant^{-1}$)	Fresh shoot weight ($cm^2 plant^{-1}$)	Dry shoot weight ($cm^2 plant^{-1}$)
	Cont	1.28c	0.38c	2.08c	5.61d	0.15c	0.03c	0.16c	0.01c	0.31e	0.04d
	10	4.16b	1.32a	22.69b	101.60c	4.62b	0.61b	2.30b	0.21b	6.92d	0.82c
BB	20	5.40ab	1.29ab	22.56b	131.39b	5.51ab	0.78ab	3.39ab	0.35a	8.90c	1.13b
	30	5.61ab	1.25b	24.37ab	138.75ab	6.73a	0.84a	3.55ab	0.38a	10.28b	1.22ab
	40	6.01a	1.26ab	26.70a	153.19a	7.58a	0.97a	4.54a	0.41a	12.12a	1.38a
	Cont	1.57c	0.15c	1.71d	7.54d	0.25d	0.14c	0.13c	0.05c	0.38c	0.19c
	10	4.88b	2.07ab	16.76c	75.74c	2.96c	0.41b	3.48b	0.30b	6.44bc	0.72b
BC	20	7.06a	2.02ab	27.75a	121.82ab	6.55a	0.58a	9.22a	0.50a	15.77a	1.07a
	30	6.69ab	2.26a	27.56a	134.95a	6.25a	0.57a	9.51a	0.47a	15.76a	1.04a
	40	5.85b	1.46b	24.44b	105.91b	4.72b	0.39b	5.36b	0.23b	10.08b	0.62b

Data with the same letter within each column for each applied fertilizer are not significantly different at the 5% level, as determined by LSD test.

3. Effects of fertilizer N, P and K applied alone and in combination on minerals, N and C content of amaranths

The Na content increased with the fertilizer P and NP, but decreased with the NPK and PK (Table VIII-2). The K content increased with all the fertilizer treatments except NP, which was highest with the fertilizer NPK followed by fertilizer P. The Ca content increased with the fertilizer K, but decreased with the fertilizer NPK and NP. The content of Mg was not influenced with the fertilizers. The P content increased with only the fertilizer NPK. The N content was highest with the fertilizer NPK followed by NP. The C content was lower with the fertilizer application (Table VIII-4). Data was not recorded for the treatments of N and NK due to very poor growth.

Table VIII-4. Effects of fertilizer N, P and K applied alone or in combination on mineral, total nitrogen and total carbon content of amaranth cultivated from November to December, 2014.

Fertilizer Treatment	Na ($mg\ g^{-1}$)	K ($mg\ g^{-1}$)	Ca ($mg\ g^{-1}$)	Mg ($mg\ g^{-1}$)	Fe ($mg\ g^{-1}$)	P ($mg\ g^{-1}$)	TN (%)	TC (%)
Cont	6.1bc	61.6d	29.0b	20.5a	0.70ab	14.3b	2.43b	39.27a
K	6.9b	74.3c	46.6a	23.9a	1.01a	15.2b	2.49b	37.88c
P	10.4a	95.6b	27.7b	25.5a	0.53b	10.9c	1.64c	37.85c
N	—	—	—	—	—	—	—	—
NK	—	—	—	—	—	—	—	—
NPK	4.5c	118.2a	13.7d	11.5b	0.69ab	29.5a	6.49a	38.69b
NP	10.6a	43.8e	18.1cd	19.1a	0.48b	3.9e	3.87ab	37.87c
PK	4.3c	76.3c	25.6bc	19.1a	0.22c	6.7d	1.73c	37.22d

Data with the same letter within each column are not significantly different at the 5% level, as determined by LSD test. —, data not recorded due to poor growth

4. Effects of fertilizer NPK rates on mineral, N and C content of amaranths

In the amaranth line BB, the Na and Ca contents increased with the fertilizer rate of 10-20 g m^{-2} and 10 g m^{-2}, respectively (Table VIII-5). The content of K and Mg was similarly higher with the 20-40 g m^{-2} and 10-30 g m^{-2}, respectively. The content of Al, Fe, P and Mg increased with all the fertilizer rates, and Zn was not influenced with the fertilizer rates. The content of N and C was higher with the 20 g m^{-2}. In the amaranth line BC, the content of Na, K, Fe and P increased with all the fertilizer rates (Table VIII-5). The content of Ca, Mg and Al increased with the 10-30 g m^{-2}, 20-40 gm^{-2} and 40 g m^{-2}, respectively, but Mn and Zn did not differ with the fertilizer application. The K content was highest with the 30 g m^{-2} followed by 40 g m^{-2}, and Ca was highest with the 20 g m^{-2} followed by 30 g m^{-2}. The Mg content was highest with the 30 g m^{-2} followed by 40 g m^{-2}, Al was highest with the 40 g m^{-2} followed by 30 g m^{-2}, Fe was highest with the 10 g m^{-2} followed by 20 g m^{-2}, and P was highest with the 30 g m^{-2}, followed by 40 g m^{-2}. The N content was highest with the 40 g m^{-2} followed by 30 g m^{-2} and C content was highest with the 10 g m^{-2} followed by 20 g m^{-2}.

Table VIII-5. Effects of combined fertilizer NPK rates on mineral, nitrogen and carbon content of amaranth lines BB and BC cultivated in red soil from June to July, 2014

Line	Fertilizer rates (gm^{-2})	Na (mg g^{-2})	K (mg g^{-2})	Ca (mg g^{-2})	Mg (mg g^{-2})	Al (mg g^{-2})	Fe (mg g^{-2})	P (mg g^{-2})	Mn ((mg g^{-2})	Zn (mg g^{-2})	TN (%)	TC (%)
BB	Cont	6.73b	80.16c	52.48b	35.93b	0.45c	0.79c	22.63d	0.56c	0.36a	—	—
	10	7.63ab	112.00b	70.53a	44.36a	0.53b	0.83b	31.23c	0.71a	0.37a	2.75d	36.99b
	20	8.30a	135.66a	56.02b	44.25a	0.56a	0.81bc	30.53c	0.72a	0.36a	3.78a	37.21a
	30	6.83b	142.83a	55.73b	41.65a	0.57a	0.91a	44.00b	0.67b	0.37a	3.40b	36.93b
	40	6.86b	151.66a	55.60b	36.06b	0.58a	0.92a	49.26a	0.66b	0.37a	3.06c	37.00b
BC	Cont	6.23c	73.03d	36.40c	36.36bc	0.51b	0.68b	13.63d	0.73a	0.34a	—	—
	10	8.86b	117.66c	43.70b	32.53c	0.53b	0.90a	22.13c	0.74a	0.36a	2.23d	38.03a
	20	10.90a	144.33b	53.43a	38.16b	0.54ab	0.82a	23.03c	0.65a	0.36a	2.98c	37.40a
	30	10.93a	188.66a	48.53ab	44.93a	0.56ab	0.74b	31.60a	0.70a	0.36a	3.99b	37.07a
	40	9.56ab	175.66a	37.40c	44.10a	0.60a	0.72b	28.06b	0.74a	0.36a	5.07a	35.73b

Data with the same letter within each column for each amaranth lines are not significantly different at the % level, as determined by LSD test. —, data not recorded due to poor growth (sample not enough).

Discussion

The growth of amaranth was best with the NPK followed by NP. The plants cultivated with the fertilizer N and NK grew for some days but did not survive finally. The plant cultivated with the fertilizer K, P and PK was very poor, and many of plants died (Fig. VIII-1).The plant height was highest with the combined fertilizer of NPK followed by NP, and the plant height with the NPK was about two times higher than the plant with the NP (Fig. VIII-2). The fertilizer N or NK showed adverse effect on plant height. The plant with the fertilizer NPK had highest leaf number (11) followed by the fertilizer NP (8) (Fig. VIII-3). The fertilizer K or P applied alone did not result increased leaf, and the plant cultivated with the fertilizers N and NK resulted in the decreased number of leaves as compared with the control plant. Stem diameter, internode length, largest leaf area, total leaf area, leaf weight, stem weight and shoot (yield) weight of amaranth were highest with the fertilizer NPK followed by NP (Table VIII-1). Similarly, Oya (1972) reported that K fertilizer regulates activities of various minerals and promotes N uptake efficiency of plants, which result in higher plant growth. The fertilizers K, P and PK did not increase the growth parameters and yield. The results indicate that amaranth plant cannot grow without N and P fertilizer. Similarly other studies reported that N fertilizer contributes to 26-41% of crop yield, and P promotes absorption of other nutrients and plant growth (Maier et al., 1994; Ivony et al., 1997; Akamine et al., 2007).

The growth of amaranths line BB was found to similarly better with the fertilizers 30 and 40 gm^{-2}. The growth of amaranth line BC increased with the fertilizer rates up to 30 gm^{-2}, and was found to be lower with the fertilizer 40 gm^{-2} than with the 30 gm^{-2}. Plant height of the amaranth line BB was highest with the 40 $g\ m^{-2}$ followed by 30 $g\ m^{-2}$ (Fig. VIII-7(A)). On the other hand, plants height of the amaranth line BC was similarly highest with the 30-40 $g\ m^{-2}$ (Fig. VIII-7(B)). The amaranth line BB obtained similarly highest leaf number with the fertilizer rate of 30-40 $g\ m^{-2}$, and the BC line obtained similarly higher leaf number with the fertilizer 20- 40 g m^{-2} (Fig. VIII-8). The stem diameter of amaranth line BB was similarly higher with the 20-40 g m^{-2} (Table VIII-4). Largest leaf area and total leaf area were similarly highest with the 30-40 g m^{-2}. Fresh and dry leaf and stem were similarly higher with fertilizer rates of 20-40 g m^{-2}. Fresh and dry shoot weight was highest with the 40 g m^{-2} followed by 30 g m^{-2}. All the growth parameters and yield of amaranth line BC were similarly higher with the fertilizer rates of 20-30 g m^{-2} and the growth parameters and yield were found to be decreased with the fertilizer of 40 g m^{-2}. Similarly, Hossain et al. (2004) reported that over rate of fertilizer reduced shoot biomass of *Panicum repens*. The amaranth line BB provided higher shoot (yield) biomass which required higher rate of combined fertilizer NPK, compared to line BC. Similarly, *Panicum repens* required increasing rate of fertilizer with the increasing shoot biomass (Hossain et al. 2004).

The Na content was lower with the NPK and PK (Table VIII-2). The K content increased with all the fertilizer treatments, which was highest with the fertilizer NPK followed by fertilizer P. The Ca content increased with the fertilizer K, but decreased with the fertilizer NPK and NP. The content of Mg was not influenced with the fertilizers. The P content in amaranth increased with the fertilizer NPK. The N content was highest with the fertilizer NPK followed by NP. The results indicate that a fertilizer does not show the same trend in the accumulation of all the minerals and N; some minerals increased but other minerals decreased with a fertilizer treatment. Similar results were reported in other plants (Hossain et al. 2011). It is still difficult to clarify the fertilizer effects on the mineral accumulation in amaranth plants, many unknown factors are involved. This result agreed the results reported by Oya et al. (1972).

The Na content of amaranth BB increased with the fertilizer rate of 10-20 g m^{-2}, and Ca content increased with the 10 g m^{-2} (Table VIII-5). The content of K and Mg was similarly higher with the 20-40 g m^{-2} and 10-30 g m^{-2}, respectively. The content of Al and Fe was similarly higher with the 20-40 g m^{-2} and 30-40 gm^{-2}, respectively. The content of N was higher with the 20 g m^{-2}. The Na content of amaranth line BC was similarly higher with the 20-40 g m^{-2}. The K content was highest with the 30 g m^{-2} followed by 40 g m^{-2}, and Ca was highest with the 20 g m^{-2} followed by 30 g m^{-2}. The Mg content was highest with the 30 g m^{-2} followed by 40 g m^{-2}, Al was highest with the 40 g m^{-2} followed by 30 g m^{-2}, Fe was highest with the 10 g m^{-2} followed by 20 g m^{-2}, and P was highest with the 30 g m^{-2} followed by 40 g m^{-2}. The N content was highest with the 40 g m^{-2} followed by 30 g m^{-2}. These results indicate that mineral accumulation influenced by fertilizer rates differ with the amaranth lines, and a amaranth line requires a certain level of fertilizer NPK to accumulate a particular mineral. However, the major minerals were increased with the fertilizer rates of 20-40 g m^{-2} in both the amaranth lines. In addition, it is thought that positive or negative interactions occur among the minerals and N existed in the soil and fertilizers supplied, which influence differently in accumulation of mineral and N in the amaranth plants.

Conclusion

The growth of amaranth was best with the NPK followed by NP. The plant cultivated with the fertilizer K, P, N, NK and PK was very poor, and many of plants died. All the growth parameters and yield of amaranth were highest with the fertilizer NPK followed by NP. The fertilizers K, P and PK did not increase growth parameters and yield. The results indicate that amaranth plant cannot grow without N and P fertilizer, and combined fertilizer NPK is required for amaranth cultivation in red soil in Okinawa.

The amaranth line BB grew better and similarly with the combined fertilizer NPK at 30-40gm^{-2} and the amaranth line BC grew best with the fertilizer 30 gm^{-2}. All the growth parameters and yield of the amaranth line BB were significantly higher with the fertilizer 30-40 g m^{-2}. Whereas, the growth parameters and yield of amaranth line BC were significantly higher with the fertilizer20-30 g m^{-2}, but lower with the fertilizer 40 g m^{-2}.

The content of Na and Ca in the amaranth plants was lower, whereas K, P and N were higher with the fertilizer NPK. The content of Na, Ca and Mg in the amaranth BB was not influenced, whereas K, Fe and Mg were higher with the fertilizer 30-40 g m^{-2}. The Na, K, Ca, Mg and P content of amaranth line BC was higher with the 30 g m^{-2}. The Fe content was not influenced with the fertilizer 30-40 g m^{-2}. The N content was higher with the 30-40 g m^{-2}. The results indicate that mineral accumulation influenced by fertilizer rates differ with amaranth lines, however, the major minerals were increased with the fertilizer rates of 20-40 g m^{-2}.

The above results indicate that amaranth plant cannot grow without N and P fertilizer, and combined fertilizer NPK provided the highest yield in red soil. The content of K, P and N in the amaranth plants was higher with the fertilizer NPK, and Mg content was not influenced with the fertilizers. Yield, major minerals and N were higher with the combined fertilizer NPK at 30-40 g m^{-2} in both the amaranth lines, which indicates that combined fertilizer NPK at 30-40 g m^{-2} is required for higher yield and quality of amaranth in red soil in Okinawa.

CHAPTER IX

GENERAL DISCUSSION

Amaranth (*Amaranthus* spp.), is a promising food crop for its resistance to heat, drought, diseases and pest, and high nutritional value. Many amaranth species are popularly consumed as vegetable in African and Asian countries. Vegetable amaranth, equal or superior in taste to spinach (*Spinacia oleracea*), possesses carotenoids, protein, minerals, fat, carbohydrate, ascorbic acid, polyphenols and antioxidant properties. Amaranth, a functional vegetable, prevents cancer, cardiovascular diseases and diabetes. Amaranth grows very fast under a variety of soils and agroclimatic conditions in tropical and subtropical areas. Okinawa is situated in the subtropical area of south-eastern part of Japan. The sun radiation intensity and air temperature are very high, and typhoon strikes several times in summer season, especially from June to September in Okinawa, which limits vegetable production in Okinawa, and causes about 80% of vegetables imported. Amaranth could be cultivated in Okinawa for supplying vegetable in summer season because it is tolerant to heat and grows very fast, and contains high nutrition. Therefore a series of experiments has been conducted to evaluate growth characteristics, yield and quality of some amaranth lines under seasonal variations, soil types and fertilizers to determine suitable amaranth lines, cultivation time, soil, fertilizers and fertilizer rates for the plant cultivation as vegetable in Okinawa.

Seven leafy-vegetable amaranth lines were taken among 12 lines to evaluate growth characteristics, yield and quality for selecting suitable line(s) for cultivation in Okinawa. The amaranth lines Bangladesh B (BB), Bangladesh C (BC), Bangladesh Red (BR), India Bengal (IB), Vietnam (V), Taiwan (TW) and Biam Tricolor (BT, domestic) were evaluated

Leaves were broad type and stems were soft in all amaranth lines evaluated, indicating that all the lines could be cultivated as the vegetables in Okinawa. Leaves and stems were mostly in redish and green color, which are colorfull as vegetbles. Most of the amaranth lines initiated inflorescences 45 days after seed sowing. Amaranths are not considered as quality vegetables when inflirencences are developed. All the plants grew well and provided enough shoots within 30-44 days after seed sowing, which indicating that these amaranth lines are fast growing vegetables. Among the amaranth lines evaluated in this study, the lines BB, BC, BR and IB grew faster and had higher growth parameters and shoot (yield) than other lines. Shoot weight was the highest in the line BC followed by BB in all the experiments, and the lines BB and BC had higher Na, K, Ca, Mg, Fe, crude protein and ascorbic acid. Considering growth, yield and quality parameters, the lines BB and BC were selected as the suitable lines for amaranth cultivation in Okinawa.

The average temperature in Okinawa ranges from 16.7 to 28.7 C, and higher temperature prevails from June to Okinawa. Temperature varies a little with the year. Average sun radiation is 7.0-23.4 MJ m^{-2} d^{-1}, which is higher from June to September. Average sun radiation varies significantly with the years, even with the months. Monthly average rainfall is 119 to 674 mm, which is high from May to August. Rainfall differs greatly with the years. The vast differences in sun radiation and rainfall are found from May to September in Okinawa. The average humidity is 61-85%, which is higher from May to August. In addition, typhoon strikes several times in summer seasons in Okinawa. The amaranth lines BB and BC selected in previous study were evaluated in spring (April-June) and summer (August-September) seasons to determine proper cultivation time in Okinawa. The amaranth lines grew well throughout the years in Okinawa, indicating that the amaranth plants could be cultivated with the temperature range of 16-29 ℃. The amaranth lines BB and BC required about 44 days in spring and 26 days in summer after seed sowing to harvest, indicating that amaranth plant is a short duration vegetable in summer season. All the growth parameters and yield of the lines were higher in summer than in spring. Minerals and total N in the amaranths were not clearly influenced with the cultivation season/time. The L-ascorbic acid in the amaranths was higher in summer. The results suggest that summer season is better than spring season for higher yield and quality of amaranth in Okinawa.

Gray soil (pH 8.4), dark-red soil (pH 6.6) and red soil (pH 5.4) are the major soils in Okinawa, which are significantly different in chemical and physical properties. Study on growth characteristics of a plant-species in local soils is important to understand suitable soils for higher yield with good quality. *Amaranthus tricolor* lines, IB (India Bengal), TW (Taiwan), BB (Bangladesh B) and BC (Bangladesh C) were cultivated in gray soil, dark-red soil and red soil to evaluate growth, yield and quality for determining suitable soil. All the *Amaranthus* lines grew faster and better in gray soil than in other soils. Plant height, number of leaves, stem diameter, leaf area, and fresh and dry shoot of all lines were highest in gray soil. The lines contained highest Na in gray soil followed by dark-red soil. All the lines had higher K in gray soil than in other soils. Calcium and Mg of the lines were higher in gray soil. Phosphorous did not differ with the soil types in IB, but TW had higher P in gray soil and dark-red soil, and BB had highest P in dark-red soil and BC had highest P in gray soil. The lines IB, BB and BC had highest N in dark-red soil, and TW had highest N in red soil. The results of this study indicate that gray soil is better for *Amaranthus* cultivation in Okinawa for higher yield, K, Ca and Mg.

Growth, yield and quality of a plant species differ with soil nutrient status and fertilizer management. Different plant species response differently to fertilizer rates and combination and a plant-species requires balanced fertilizers to maximize growth, yield and quality in a specific soil. The major nutrients N, P and K individually or in combination maintain growth, yield and quality of plants. Effects of N levels and combined NPK fertilizers on amaranth lines BB and BC were evaluated in gray soil, dark–red soil and red soil. The fertilizer treatments were control Cont (0 g m^{-2}), LN (nitrogen 50 g m^{-2}), HN (nitrogen, 100 g m^{-2}) and NPK (150 g m^{-2}; 50 g N, 50 g P, 50 g K) for each soil type. Leaf number was similarly increased with the LN, HN and NPK fertilizer, whereas it was significantly highest for NPK in dark-red soil and red soil. Leaf area and fresh and dry shoot weight of amaranth were highest for the NPK treatment in all soils. The fertilizer LN and HN increased fresh and dry weight of amaranth in gray soil, but not in other soils. Calcium was highest with HN in gray soil, with LN and NPK in dark-red soil, and with LN and HN in red soil. Magnesium was higher with the NPK in gray soil and with LN and HN in red soil. Nitrogen did not differ with the fertilizer treatments in gray soil, but was higher with HN in dark-red soil and with LN in red soil. This study indicates that amaranth plant responses differently with the different fertilizers in different soils may due to different levels of nutrients and pH in the soils. The amaranth plant requires combined fertilizers of N, P and K for proper growth, and could be cultivated in all soils in Okinawa by applying appropriate rate of N, P and K fertilizers.

Fertilizer requirement differs with the soil types. Previous study demonstrated that combined fertilizer of N, P and K is better for amaranth cultivation in gray soil. Present study has been conducted to evaluate rates of combined fertilizer NPK (0, 20, 30, 40, 50 60 and 80 g m^{-2}) on growth, yield and quality of amaranth lines BB and BC in gray soil in Okinawa. The fertilizer of 30, 40 and 50 g m^{-2} resulted in a similarly higher growth parameters and dry shoot (yield) in BB line, and agronomic efficiencies of the fertilizer were 0.11 g g^{-1} fertilizer, 0.11 g g^{-1} fertilizer and 0.10 g g^{-1} fertilizer, respectively. Dry shoot weight was similarly higher 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. In the experiment 2012, the growth parameters and yield of amaranth line BC increased almost similarly with all fertilizer levels, 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. In the experiment 2015, the amaranth line BC resulted in the highest growth parameters and yield with the 60 g m^{-2}. The SPAD value of amaranth leaf was highest with the 60 g m^{-2}, which contributed to higher photosynthesis and resulted in higher plant growth and yield. The shoot (yield) of amaranth line BC cultivated in 2015 was higher than that cultivated in 2012, which required higher rate of fertilizer NPK in 2015. In the experiments 2012 and 2015, mineral accumulation in both amaranth BB and BC lines were not clearly influenced with the fertilizer NPK rates. Total N increased with the fertilizers in both the BB and BC lines. The overall results of this study indicate that the requiring rate of fertilizer NPK differ with the plant size and cultivars/lines. Considering the growth parameters, yield and quality of amaranth lines BB and BC under different rates of NPK fertilizer application, the fertilizer rates of 50-60 gm^{-2} is required for the amaranth cultivation in gray soil in Okinawa.

Dark-red soil and red soil are the main soils, which cover more than 90% of land in Okinawa. Fertilizer regimes (Cont, N, P, K, NP, NK, PK and NPK; combined fertilizer NPK at 0 g m^{-1}, 10 g m^{-1}, 20 g m^{-2}, 30 g m^{-2} and 40 g m^{-2}) were evaluated on growth, yield and quality of amaranth lines BB and BC in dark-red soil (pH 6.6) and in red soil (pH 5.1) in Okinawa. In dark-red soil, the plants cultivated without N and P fertilizers did not grow well, and all the growth parameters and yield were highest with the combined fertilizer NPK followed by NP in both experiments. Growth parameters and yield of the amaranth lines were highest with the combined fertilizer NPK at 40 g m^{-2} followed by 30 g m^{-2}. Minerals and L-ascorbic acid contents of the amaranth lines were higher or same with some rates of the fertilizer NPK. In red soil, some of the amaranth plants cultivated without N and P fertilizers died, and all growth parameters and yield of amaranth lines were highest with the fertilizer NPK followed by NP. The growth parameters and yield of amaranth line BB and BC were significantly higher with the fertilizer 20-40 g m^{-2}. Some minerals were higher with the fertilizer 30-40 g m^{-2}. The combined fertilizer NPK at 30-40 g m^{-2} is required for higher yield and quality of amaranth in dark-red soil and red soil in Okinawa.

Overall results indicate that all the amaranth lines grow well in Okinawa and provide satisfactory yield. The appearances and quality parameters of the amaranth lines evaluated are suitable as vegetables. However amaranth lines BB and BC are best for higher yield, minerals, crude protein and L-ascorbic acid. The amaranth plants could be cultivated throughout the years. The amaranth plants require about 26 days in summer and 44 days in spring season from seed sowing to harvest. Amaranth plants cannot grow without N and P fertilizers in all soils in Okinawa. On the other hand, the plants provide higher yield with the N fertilizer in gray soil but not in other soils. The amaranth lines contain different values of minerals, crude protein and L-ascorbic acid. Minerals and L-ascorbic acid accumulations were not clearly influenced with the soil types, cultivation time, fertilizer elements and fertilizer rates. However, the amaranth lines provide higher yield and nutritional values with the combined fertilizer of NPK at 50-60 gm^{-2} in gray soil, and 30-40 g m^{-2} in dark-red oil and red soil in Okinawa.

Future Research Directions: Growth characteristics and yield parameters of amaranths were clarified in relation to amaranth lines, seasonal variations, soil types and fertilizers, but quality parameters were not clarified in this study. Therefore further experiments are needed to evaluate temperature, light, and separate and combined fertilizers of N, P and K on growth, yield and quality of amaranth under control environments.

CHAPTER X

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ACKNOWLEDGEMENT

I would like to express my warm gratitude to my major advisor Dr. Md. Amzad Hossain, Associate Professor of the University of the Ryukyus for his excellent guidance and encouragement during this study. I am deeply grateful to vice advisor Dr. Hilkaru Akamine, Associate Professor of the University of the Ryukyus for his constructive suggestions on this research work. My deep appreciation goes to vice advisor Dr. Shao-Hui Zheng, Professor of Saga University for his critical advices and suggestions in this study. I am grateful to Dr. Akihiro Nose, Emeritus Professor of Saga University for his advices and encouragement in my study. Acknowledgements are extended to Professor Masanobu Tamaki for his valuable suggestions and corrections in my dissertation. My sincere appreciations are due to Dr. Jun-Ichiro Sakagami, Professor of Kagoshima University for his critical comments, corrections and suggestions in this dissertation. I would like to express my deep gratitude to Dr. Ichiro Nakamura, Assistant Professor of the University of the Ryukyus for his excellent guidance in this work.

Acknowledgements are extended to Professor Prasanta Chitta Bhowmik of the Massachusetts University for his encouragement in my study. I am grateful to all the previous and present students in the laboratory of Dr. Hossain for their cordial and continuous assistance in my research work.

Many thanks are due to the staffs of the Subtropical Field Science Center and Instrumental Research Center of the University of the Ryukyus for their support in many aspects.

My sincerest gratitude goes to my wife Michiko Ohshiro, sons Fuminori Ohshiro and Naotake Ohshiro, grandson Yushin Ohshiro and other relatives for their inspiration in my study.