

## FERTILIZER MANAGEMENT FOR AMARANTH (*AMARANTHUS* SPP.) CULTIVATION ON DARK-RED SOIL IN OKINAWA, JAPAN

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**Abstract.** *Amaranthus* is a promising food crop with unique nutritional compositions. Fertilizer treatments including 0 (Control), N, P, K, N + P (NP), N + K (NK), P + K (PK) and N + P + K (NPK) were evaluated based on growth, yield and quality of edible red leaf-amaranth (*Amaranthus* spp.) cultivated on dark-red soil (pH 6.6) to understand fertilizer management. N, P and K were applied at 50 g m<sup>-2</sup> according to the treatments in one of the experiments. Effects of the fertilizer NPK (N:P:K = 1:1:1) at 0, 10, 20, 30 and 40 g m<sup>-2</sup> were evaluated on red stem-amaranth and red leaf-amaranth in the same soil during another experiment. P and K fertilizers applied alone did not promote growth parameters and yield of amaranth, whereas N alone did. The fertilizer K promoted the function of N slightly but P did the same significantly regarding amaranth growth. Growth parameters and yield were the highest with the combined fertilizer NPK followed by NP. Yield and L-ascorbic acid content of amaranth were the highest with fertilizer NPK at 40 g m<sup>-2</sup>. This study indicates that fertilizer N and P are more effective, and combined fertilizer NPK at 40 g m<sup>-2</sup> is better for higher yield and quality of amaranth on dark-red soil in Okinawa.

**Keywords:** *growth characteristics, L-ascorbic acid, nutritive-value, vegetable crop, yield*

### Introduction

Growth, yield and quality of plant species differ with soil nutrient status and fertilizer management (Hossain and Ishimine, 2005; Akamine et al., 2007; Chowdhury et al., 2008; Hossain et al., 2011). Different plant species respond differently to fertilizer combination and rates, and plant species requires balanced fertilizer to maximize growth, yield and quality (Hossain et al., 2004; Akamine et al., 2007; Chowdhury et al., 2008; Shimray et al., 2019). The major nutrients N, P and K individually or in combination maintain growth, yield and quality of plants (Ivony et al., 1997; Nakano and Morita, 2009; Barbara et al., 2011). Nitrogen, the principal element of chlorophyll, 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 various minerals and promotes N uptake efficiency of plants. Insufficient K causes shoot yellowing, poor growth and low resistance to cold and drought in plants (Oya, 1972). Phosphorus enhances absorption of other nutrients and promotes plant growth when applied with other fertilizers together (Akamine et al., 2007).

*Amaranthus* is a promising food crop for its resistance to heat, drought, diseases and pests, as well as high nutritional value (Sreelathakumary and Peter, 1993; Rastogi and Shukla, 2013; Longato et al., 2017; Maurya and Arya, 2018; Soriano-García et al., 2018). Several *Amaranthus* species are popularly being cultivated as vegetable and grain in Africa, Bangladesh, Caribbean, China, Greece, India, Nepal and South Pacific Islands (Prakash and Pal, 1991; Dewan et al., 2017; Stallknecht and Schulz-Schaeffer, 1993; Svirskis, 2003). Vegetable amaranth, superior in taste to spinach (*Spinacia oleracea*), has higher carotenoids (90-200 mg kg<sup>-1</sup>), protein (14-30%), carbohydrate (5.0 g 100 g<sup>-1</sup>), fat

(0.1 g 100 g<sup>-1</sup>), calories (43 Kcal 100 g<sup>-1</sup>) and ascorbic acid (28 mg 100 g<sup>-1</sup>) (Abbott and Campbell, 1982; Dewan et al., 2017; Makus, 1984; Prakash and Pal, 1991; Shittu et al., 2006). *Amaranthus* has antioxidant, antimalarial and antiviral properties, which prevent cancer, cardiovascular diseases, diabetes, etc. (Dasgupta and De, 2007; Khandaker et al., 2008; Shukla et al., 2010; Adegbola et al., 2020).

*Amaranthus* grows very fast in tropical and subtropical areas under a variety of soils and agroclimatic conditions during summer when vegetables are not available (Dewan et al., 2017; Makus, 1984; Singh and Whitehead, 1996). In Okinawa, some amaranth species are found as weed in various crops and vegetables (personal survey) in the major soil types, dark-red soil, red soil and gray soil, and summer vegetables are very limited (Hossain and Ishimine, 2005; Okinawa Prefecture Agriculture, Forestry and Fisheries, 2008). Our previous study has selected some high-quality amaranth lines as summer vegetables in Okinawa (Ohshiro et al., 2015). Shittu et al. (2006) reported that balanced fertilizers in a specific soil provide higher yield and nutrient compositions of amaranth.

In previous study, soil types and fertilizer regimes were evaluated on growth, yield, and quality of some *Amaranthus tricolor* lines (Ohshiro et al., 2016). All the growth parameters and yield of the amaranth lines were the best in gray soil. We have evaluated fertilizer N levels and combined fertilizer NPK on the amaranth lines in three soil types in Okinawa. Nitrogen fertilizer alone increased growth and yield of amaranths in gray soil but not in dark red soil and red soil. The combined fertilizer NPK resulted in the highest growth parameters and yield of amaranths in all soils. Previous study reported that combined fertilizer NPK at 20–40 g m<sup>-2</sup> is effective for higher yield and minerals of amaranth in gray soil in Okinawa. Gray soil covers only 5% of land, whereas dark-red soil covers 35% of land and red soil covers 60% of land in Okinawa. Most of the dark-red soil field is used for crop cultivation, but only 40% of the red soil is used for crop cultivation in Okinawa. In addition, we did not evaluate separate and combined application of N, P and K on the amaranth lines in dark-red soil and red soil. Therefore, the objectives of this study were to (i) identify the effect of different fertilizer elements and (ii) to evaluate rates of combined fertilizer on growth, yield and quality of two edible amaranth lines to understand fertilizer management practices in dark-red soil in Okinawa.

## Materials and methods

### *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, Japan. The content of Na, K, Ca, Mg, Al, Fe, P and Mn in the soil was 1.24, 2.28, 18.88, 2.85, 0.05, 0.23 and 0.07 mg kg<sup>-1</sup>, respectively. Total N, total C and soil pH was 0.09%, 0.31% and 6.6, respectively. Coarse sand, fine sand, silt, clay and apparent density of the soil was 2.93%, 7.33%, 23.94%, 57.24% and 0.87 g cm<sup>-3</sup>, respectively (Hossain and Ishimine, 2005).

### *Amaranth lines*

Edible red stem-amaranth (BB line) and red leaf-amaranth (BC line) of *Amaranthus tricolor* selected for higher yield and quality in our previous studies (three experiments conducted from April, 2010 to May, 2011) were evaluated in this study (Ohshiro et al., 2015).

***Experiment 1: Effects of N, P and K fertilizers applied alone or in combination on edible amaranth cultivated during 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 the 5<sup>th</sup> April to the 15<sup>th</sup> June, 2013. The experiment consisted of eight treatments with five replications (planters). The fertilizer treatments were 0 (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), P and K at 50 g m<sup>-2</sup> (5.0 g per planter) were mixed with 13 kg of air-dried soil per planter (size 65E; 0.1 m<sup>2</sup>) prior to the seed sowing according to the treatment design. Seeds of *Amaranthus* BC line were sown on soil surface, and covered with 0.5 cm of soil layer. The planters were placed randomly, and the plants were thinned to the 8 healthiest stands per planter at 2- to 3-leaf stage. Water was applied as required (considering soil moisture checked by squeezing the soil sample firmly in hand to form an irregularly shaped “ball”, plant size and growth stage, daily weather condition, etc.) for proper seedling emergence and plant growth.

***Experiment 2: Effects of N, P and K fertilizers applied alone or in combination on edible amaranth cultivated during September to December, 2013***

A glasshouse experiment was conducted at the Subtropical Field Science Center of the University of the Ryukyus, from September 18 to December 10, 2013. The soil type, planter, soil amount per planter, fertilizer rate, seeds of *Amaranthus* BC line, treatment, replication, seed sowing procedure, and other management practices of this experiment were similar to those taken in the previous experiment (experiment 1). The plants were thinned to the 10 healthiest stands per planter at 2- to 3-leaf stage

***Experiment 3: Effects of NPK fertilizer rates on edible amaranth cultivated during July to September, 2014***

A glasshouse experiment was conducted using dark-red soil at the Subtropical Field Science Center of the University of Ryukyus, from the 15<sup>th</sup> July to the 3<sup>rd</sup> September, 2014. Two amaranth lines, BB and BC were evaluated. Each experiment consisted of five treatments with four replications (planters). The fertilizer treatments of the experiment were 0 (Cont), 10 g m<sup>-2</sup> (1 g planter<sup>-1</sup>), 20 g m<sup>-2</sup> (2 g planter<sup>-1</sup>), 30 g m<sup>-2</sup> (3 g planter<sup>-1</sup>) and 40 g m<sup>-2</sup> (4 g planter<sup>-1</sup>). The fertilizers of N (CO(NH<sub>2</sub>)<sub>2</sub>), P<sub>2</sub>O<sub>5</sub> (CaH<sub>4</sub>(PO<sub>4</sub>)<sub>2</sub>H<sub>2</sub>O) and K<sub>2</sub>O (KCl) were applied at a ratio of N:P:K = 1:1:1. The fertilizers were mixed with 13 kg of air dried soil per planter (size 65E) prior to the seed sowing according to the treatment. *Amaranthus* seeds were sown on soil surface and covered with 0.5 cm of soil layer. Water was applied according to the experiment 1 for proper seedling emergence and plant growth. The planters were placed randomly, and the plants were thinned to the 10 healthiest stands per planter at 2- to 3-leaf stage.

***Data collection***

Leaf-amaranth is usually harvested when 20-35 cm in height; and both the leaf and stem are used as a vegetable. Stem-amaranth is usually harvested at the young stage (20-35 cm) for the use of both leaf and stem, and at the pre-flowering stage (semi mature plant) for only the stem. Plant height and leaf number were recorded up to 43 days after seed sowing (DAS) at a 7-day interval in experiment 1. Five plants were harvested from each planter at 34 DAS, and plant height, stem diameter, internode

length, leaf number, largest leaf area, total leaf area, and fresh and dry weight of the leaf, stem and shoot were determined. Shoot (leaf plus stem) weight was considered as yield. In experiment 2, five plants were harvested from each planter at 25 DAS and the same growth parameters were measured. In experiment 3, five plants were harvested from each planter at 26 DAS and the same growth parameters were measured. Stem diameter was measured at five cm from the soil surface, and internode length from the third internode from the soil surface.

#### ***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 °C for 48 h using a forced convection oven (DRLF23WA, Advantec) for dry weight measurement.

#### ***Determination of mineral, nitrogen, carbon and pH in soil, and nutrient status and L-ascorbic acid in edible amaranth***

Various parts of amaranth plants were dried at 60 °C for 48 h using the same forced convection oven. Soil samples were dried at room temperatures of 25-28 °C 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 an Inductively Coupled Plasma Spectrometer (ICPS-8100, Shimadzu Co. Ltd.). Total C and N were determined with a 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 a TOA pH meter (HM-20S, Toa Electronic Ltd.). L-ascorbic acid content in leaves was determined by using a RQ Flex/agrocheck kid small-sized reflecting photometer (Kanto Chemical Co. Ltd.).

#### ***Statistical analysis***

Average data for each replication was calculated, and then mean and standard deviation (SD) of the replications were determined using analysis of variance. Fishers protected least significant difference (LSD) test at the 5% level was used to compare treatment means. The amaranth lines were analysed separately.

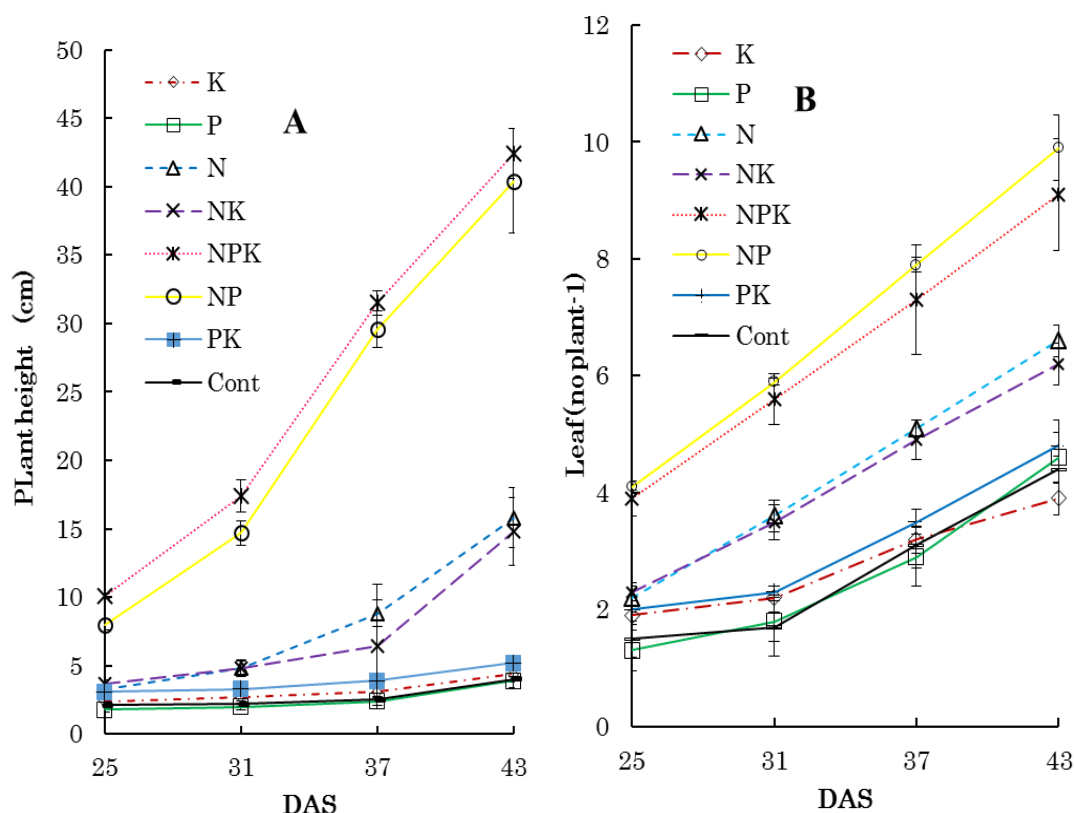
## **Results**

### ***Effects of N, P and K fertilizers applied alone or in combination on cultivated edible amaranth***

The plant grown from April to June, 2013 showed that plant height and leaf number increased significantly with the fertilizers of N, NK, NP and NPK compared to that with other treatments (*Fig. 1*). Plant height and leaf number were increased by 300% and 67%, respectively with the NP or NPK as compared to that with N and NK (*Fig. 1*).

Separate application of P and K did not increase the growth parameters of amaranth, whereas N alone did (*Fig. 1; Table 1*). Amaranth grew faster and better with fertilizer NPK and NP than with other fertilizer treatments (*Fig. 1*). Internode length, largest leaf area, total leaf area and leaf weight were significantly and similarly higher with the

NPK and NP compared to those with the other treatments (*Table 1*). Total leaf area and leaf weight increased by 6-67 and 5-59 times, respectively when cultivated with the combined fertilizer NP or NPK, as compared to those with other fertilizer treatments. Stem diameter was the highest with the NPK followed by NP. Stem weight increased by 12-165 and 8-115 times with the combined fertilizer NPK and NP, respectively, as compared to that with other fertilizer treatments. The fertilizer NPK and NP resulted in 6.5 and 5.5 times higher yield, respectively as compared to the fertilizer NK.



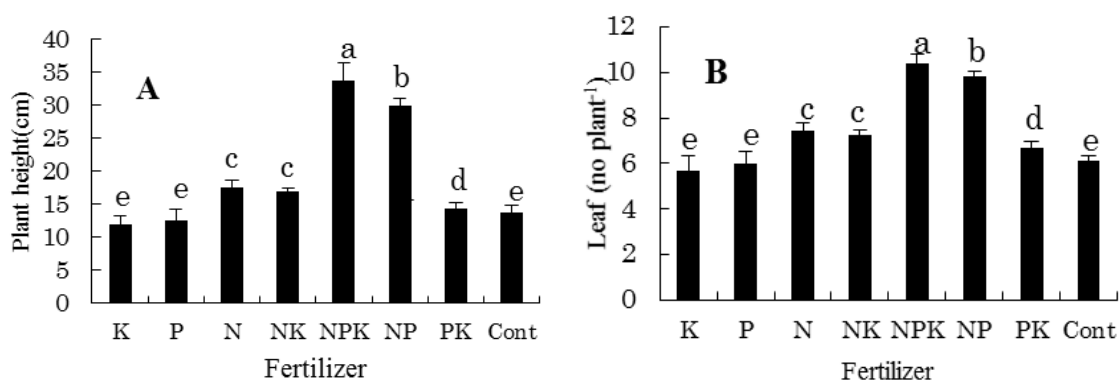
**Figure 1.** Effects of N, P and K applied alone or in combination on plant height (A) and leaf number (B) of edible amaranth BC line cultivated during April to June, 2013

**Table 1.** Effects of N, P and K fertilizers applied alone or in combination on growth parameters of edible amaranth BC line cultivated during April to June, 2013

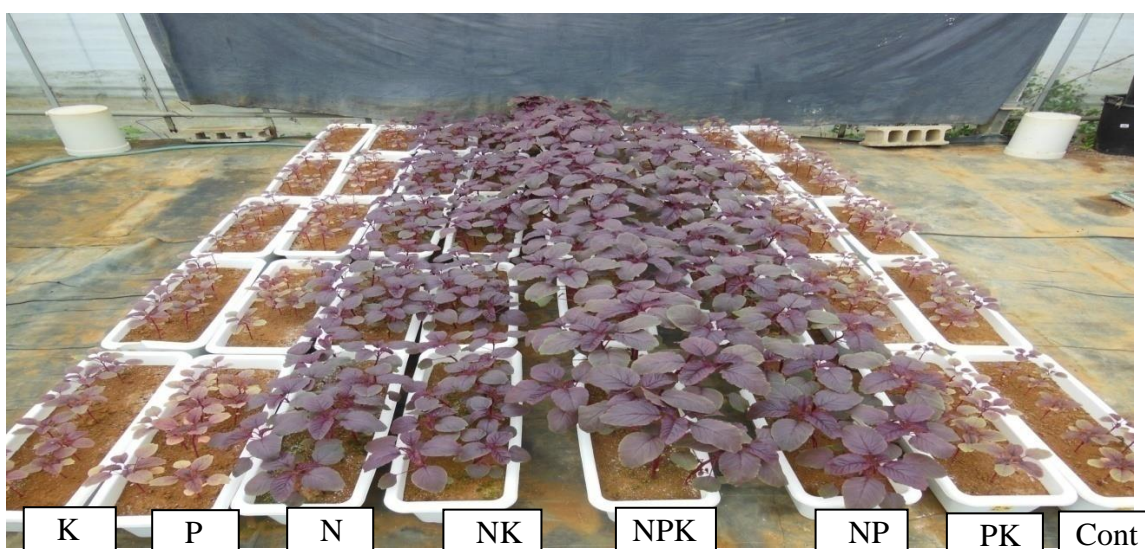
Treatment	Stem diameter mm	Internode length cm	Largest leaf area cm <sup>2</sup> leaf <sup>-1</sup>	Total leaf area cm <sup>2</sup> leaf <sup>-1</sup>	Fresh leaf weight g plant <sup>-1</sup>	Dry leaf weight g plant <sup>-1</sup>	Fresh stem weight g plant <sup>-1</sup>	Dry stem weight g plant <sup>-1</sup>	Fresh shoot weight g plant <sup>-1</sup>	Dry shoot weight g plant <sup>-1</sup>
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

Data were recorded at 34 day after seed sowing. Data with the same letter within each column are not significantly different at the 5% level, as determined by LSD test

The plant grown from September to December, 2013 showed that plant height and leaf number were the highest with the application of fertilizer NPK followed by NP (Figs. 2 and 3). The fertilizer N, NK and PK also resulted in increased plant height and leaf number compared to the control treatment. The plant height was 1.7-2.0 times higher with the NPK or NP compared to that with the N and NK. Individual application of P and K did not increase the growth parameters of amaranth, whereas N alone did (Table 2). All the growth parameters were the highest with the NPK fertilizer followed by NP. Total leaf area and dry leaf weight increased by 2-11 and 2-9 times, respectively with the combined fertilizer NPK or NP, compared to those with other fertilizer treatments. Yield was the highest with the NPK followed by NP (Table 2). The fertilizer NPK and NP resulted in a 2.2 and 1.8 times higher yield than the fertilizer NK.



**Figure 2.** Effects of N, P and K fertilizers applied alone or in combination on plant height (A) and leaf number (B) of edible amaranth BC line cultivated during September to December, 2013. Bars with the same letter are not significantly different at the 5% level (LSD test)



**Figure 3.** Effects of N, P and K fertilizers applied alone or in combination on growth of edible amaranth BC line cultivated during September to December, 2013. Photo taken at 34 day after planting. Alone K (K), alone P (P), alone N (N), N plus K (NK), N plus P plus K (NPK), N plus P (NP), P plus K (PK), Control (Cont)

**Table 2.** Effects of N, P and K fertilizers applied alone or in combination on growth parameters and yield of edible amaranth BC line cultivated during September to December, 2013

Fertilizer treatment	Stem diameter mm	Internode length cm	Largest leaf area cm <sup>2</sup> leaf <sup>-1</sup>	Total leaf area cm <sup>2</sup> plant <sup>-1</sup>	Fresh leaf weight g plant <sup>-1</sup>	Dry leaf weight g plant <sup>-1</sup>	Fresh stem weight g plant <sup>-1</sup>	Dry stem weight g plant <sup>-1</sup>	Fresh shoot weight g plant <sup>-1</sup>	Dry shoot weight g plant <sup>-1</sup>
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

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

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

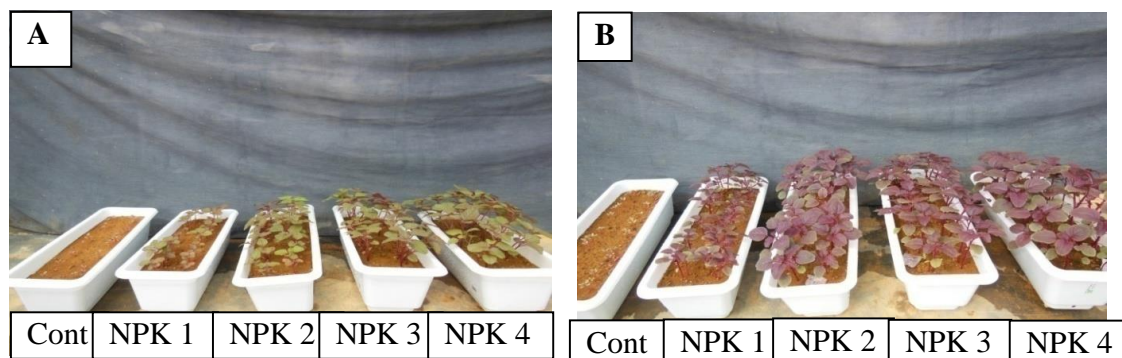
SPAD value increased with all the fertilizer rates in both amaranth lines (Table 3). The SPAD value was the highest with the fertilizer at 40 g m<sup>-2</sup> than with other fertilizer rates in the BB line, but same with all the fertilizer rates in the BC line. Plant heights of both BB and BC lines increased with the increasing rate of fertilizer (Figs. 4 and 5). The leaf number of the BB line was the highest with the fertilizer NPK at 40 g m<sup>-2</sup> followed by 30 g m<sup>-2</sup>. The BC line obtained similarly higher leaf number when cultivated with the fertilizer rates of 20, 30 and 40 g m<sup>-2</sup> (Fig. 5). Stem diameter, internode length, largest leaf area, total leaf area, leaf weight, stem weight and shoot weight of both amaranth lines were increased with the increasing rate of fertilizer (Table 3). The yield of the BB and BC lines was the highest when cultivated with the fertilizer rate at 40 g m<sup>-2</sup>.

**Table 3.** Effects of NPK fertilizer rates on SPAD value, growth parameters and yield of edible amaranth BB and BC lines cultivated during July to September, 2014

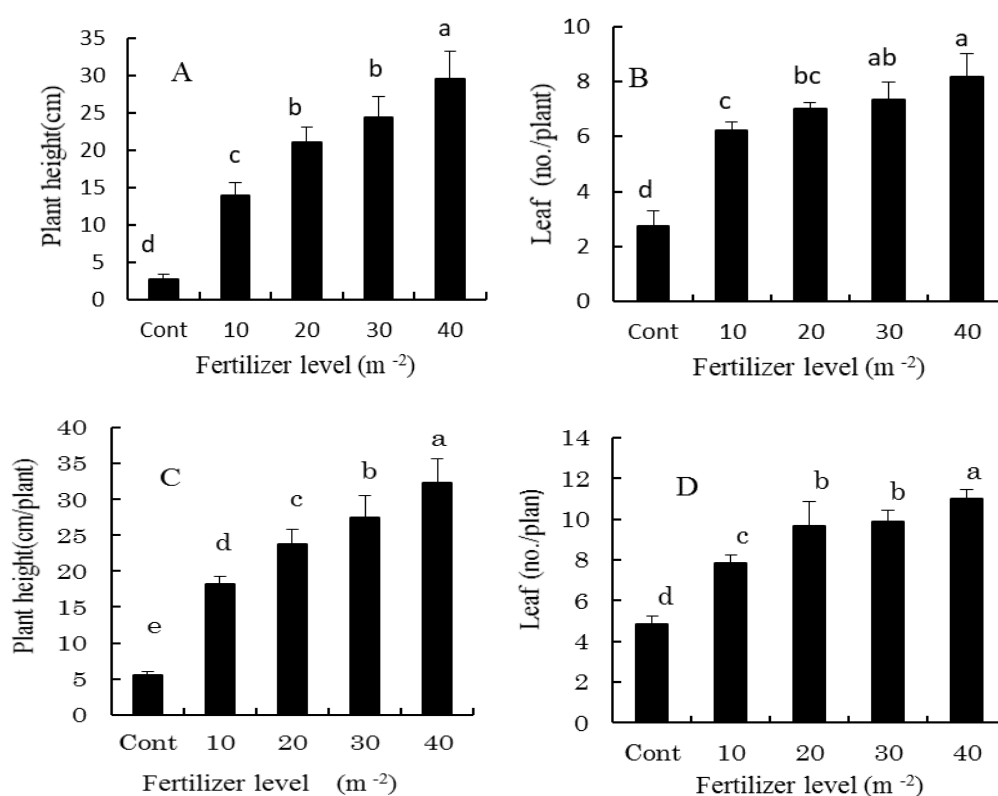
Amaranth lines	Fertilizer rates g m <sup>-2</sup>	SPAD value	Stem diameter cm	Largest leaf area cm <sup>2</sup> plant <sup>-1</sup>	Total leaf area cm <sup>2</sup> plant <sup>-1</sup>	Fresh leaf weight g plant <sup>-1</sup>	Dry leaf weight g plant <sup>-1</sup>	Fresh stem weight g plant <sup>-1</sup>	Dry stem weight g plant <sup>-1</sup>	Fresh shoot weight g plant <sup>-1</sup>	Dry shoot weight g plant <sup>-1</sup>
BB	Cont	19.92b	0.89d	0.75d	2.24d	0.05d	0.02c	0.03d	0.01d	0.08d	0.03d
	10	33.85a	3.00c	13.56c	48.75c	1.59c	0.64b	0.81c	0.21c	2.41c	0.85c
	20	32.87a	3.95b	23.00b	86.37b	2.95b	0.90b	1.86b	0.41b	4.80b	1.31bc
	30	34.85a	4.38a	23.25b	94.18b	3.16b	1.01b	2.63b	0.52b	5.78b	1.53b
	40	33.50a	4.69a	35.14a	151.97a	4.89a	1.60a	4.07a	0.70a	8.93a	2.30a
BC	Cont	30.90c	1.28d	1.21d	3.93d	0.12d	0.08d	0.09d	0.03d	0.18d	0.15d
	10	33.22b	4.29c	12.60c	53.90c	1.88c	1.00c	1.57c	0.52c	3.45c	1.52c
	20	34.20b	4.93bc	20.31b	94.42b	3.46b	1.59b	3.33b	0.84b	6.79b	2.43b
	30	34.77b	5.72b	20.78b	102.49b	4.16b	1.84b	4.75b	1.15b	8.90b	2.99b
	40	37.57a	6.99a	31.42a	162.07a	5.83a	2.35a	7.85a	1.55a	13.68a	3.89a

Data were recorded at 26 day after seed sowing. 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





**Figure 4.** Effect of NPK fertilizer rates [(Cont (0 g), NPK 1 (10 g m<sup>-2</sup>), NPK 2 (20 g m<sup>-2</sup>), NPK 3 (30 g m<sup>-2</sup>), NPK 4 (40 g m<sup>-2</sup>)] on growth of amaranth BB (A) and BC (B) lines at 26 DAS



**Figure 5.** Effects of NPK fertilizer rates on plant height and leaf number of amaranth lines (A and C: BB line, C and D: BC line). Bars with the same letter are not significantly different at the 5% level (LSD test)

### Effects of N, P and K fertilizers applied alone or in combination on mineral, nitrogen and carbon content of amaranth

The experiment conducted from April to June, 2013 showed that the Na content of amaranth was the highest with the fertilizer NP, and K was the highest with the fertilizer NPK (Table 4). The Ca content in the plants decreased, but Mg content increased with all the fertilizers, except fertilizer K, compared to those with the control treatment. Al content decreased with all the fertilizers, except fertilizers P and PK, whereas Fe content



did not differ clearly with the fertilizers, except fertilizer P. The P content in amaranth was the highest with the fertilizer P followed by fertilizer PK, NP and NPK. Total N and C content in the plant was not significantly influenced by the fertilizers, however N in the plant was lower with the fertilizer NPK.

**Table 4.** Effects of N, P and K fertilizers applied alone or in combination on minerals, total N and total C in amaranth BC line cultivated during April to June, 2013

Fertilizer treatment	Na mg g <sup>-1</sup>	K mg g <sup>-1</sup>	Ca mg g <sup>-1</sup>	Mg mg g <sup>-1</sup>	Al mg g <sup>-1</sup>	Fe mg g <sup>-1</sup>	P mg g <sup>-1</sup>	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 were recorded at 34 day after seed sowing. 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

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

**Table 5.** Effects of N, P and K fertilizers applied alone or in combination on mineral and total nitrogen and carbon in amaranth BC line cultivated during September to December, 2013

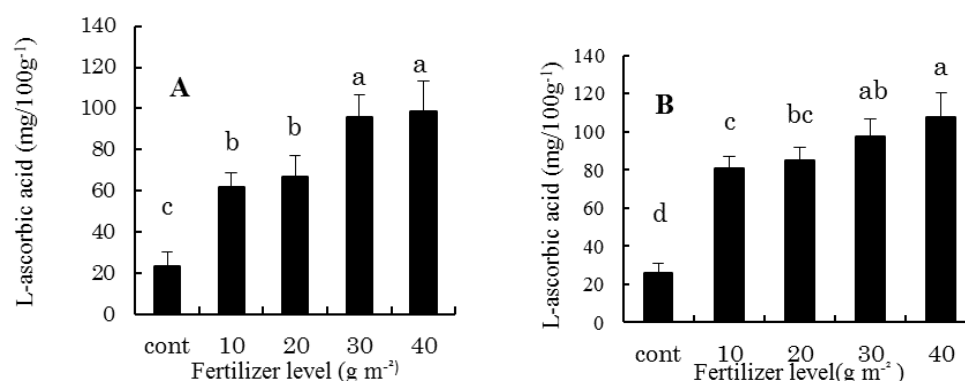
Fertilizer treatment	Na mg g <sup>-1</sup>	K mg g <sup>-1</sup>	Ca mg g <sup>-1</sup>	Mg mg g <sup>-1</sup>	Al mg g <sup>-1</sup>	Fe mg g <sup>-1</sup>	P mg g <sup>-1</sup>	Mn mg g <sup>-1</sup>	Zn mg g <sup>-1</sup>	TN %	TC %
K	7.17d	91.83a	23.98bc	46.23a	0.65ab	0.26d	8.70b	1.57a	0.11b	2.52d	38.54f
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 were recorded at 25 day after seed sowing. Data with the same letter within each column are not significantly different at the 5% level, as determined by LSD test

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

L-ascorbic acid content of the amaranth increased significantly with all the fertilizer rates in both BB and BC lines, and L-ascorbic acid content was the highest when the amaranth was grown with the fertilizer NPK at 40 g m<sup>-2</sup> followed by 30 and 20 g m<sup>-2</sup> (Fig. 6).

The Na, K, Ca, Mg, P and Zn content of the amaranth BB line increased with all the fertilizer rates, whereas the Al and Fe content was not clearly influenced with the fertilizer rates (Table 6). The Na content of the BC line tended to increase with all the fertilizer rates, and K content increased with the 20-40 g m<sup>-2</sup> (Table 6). The Ca content increased with the 10 and 20 g m<sup>-2</sup> but not with the 30 and 40 g m<sup>-2</sup>, compared to that with the control treatment, and the Mg and Zn content was not influenced by the fertilizer rates. The Al, Fe and P content increased with all the fertilizer rates. The Mn content decreased with all the fertilizer rates in both amaranth lines. The total N content was the same with all the fertilizer rates in the BB line, but increased with the increasing fertilizer rate in the BC line. The C content of the amaranth lines was not influenced with the fertilizer rates.



**Figure 6.** Effects of NPK fertilizer rates on L-ascorbic acid of amaranth BB (A) and BC (B) lines at 26 DAS. Bars with the same letter are not significantly different at the 5% level (LSD test)

**Table 6.** Effects of NPK fertilizer rates on mineral, total nitrogen and total carbon in amaranth BB and BC lines cultivated during July to September, 2014

Amaranth line	Fertilizer rates g m <sup>-2</sup>	Na	K	Ca	Mg	Al	Fe	P	Mn	Zn	TN	TC
		mg g <sup>-1</sup>	mg g <sup>-1</sup>	mg g <sup>-1</sup>	mg g <sup>-1</sup>	mg g <sup>-1</sup>	mg g <sup>-1</sup>	mg g <sup>-1</sup>	mg g <sup>-1</sup>	mg g <sup>-1</sup>	%	%
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 were recorded at 26 day after seed sowing. 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 experiments indicate that P and K applied alone were not effective for amaranth growth, but N was effective. Combined application of NP resulted in significantly higher growth parameters and yield than the combined application of NK. This result indicates that fertilizer P enhanced the function of fertilizer N significantly but K did slightly in all the growth parameters of amaranth. Combined application of NPK resulted in the highest growth parameters and yield followed by the combined application of NP, indicating that fertilizer K enhanced the function of fertilizers N and P for the growth of amaranth plant. Similarly, other studies reported that N is more effective than P and K for vegetative growth of plants (Akamine et al., 2007; Chowdhury et al., 2008; Hossain et al., 2011; Sarker et al., 2002). The content of K and P in the soil was 2.28 and 0.23 mg kg<sup>-1</sup>, respectively, indicating that K content in the soil was about 10 times higher than P, which was sufficient for amaranth growth. This result also indicates that amaranth needs a higher amount of P than K for proper growth on dark-red soil in Okinawa. The fertilizers N, P and K together provided balanced nutrients, which resulted in higher plant growth parameters and yield. Similarly, other studies reported higher plant biomass in various crop species with the combined fertilizer of N, P and K (Oya, 1972; Akamine et al., 2007; Hao and Papadopoulos, 2004; Hossain et al., 2012).

The growth of the amaranth BB line increased with the increasing rate of fertilizer NPK, whereas, the growth of the BC line was similarly higher with the 20-40 g m<sup>-2</sup>. Similarly, Ohshiro et al. (2016) reported that fertilizer requirement varies with the plant species or cultivars. All the growth parameters and yield of the amaranth lines were the highest with the fertilizer NPK at 40 g m<sup>-2</sup> followed by 30 g m<sup>-2</sup> due to the higher SPAD value which probably contributed to higher photosynthesis (Sarker et al., 2002)

The experiments conducted from April to June, and September to December showed that Na content of amaranth was the highest with the NP fertilizer, while K was the highest with the NPK fertilizer followed by NK (*Tables 4 and 5*). The results indicate that P fertilizer influenced the absorption of K fertilizer by the plant. The Ca content in the amaranth was lower with all the fertilizer treatments, except K and PK. The Mg content in the amaranth decreased with all the fertilizers when cultivated from April to June, but increased when cultivated from September to December, which may be due to the differences in temperature and solar radiation during the cultivation time. The P content increased in the amaranth with the P, NP, PK and NPK fertilizers, which indicates that fertilizer P was the cause of increased P mineral in the amaranth plant. Total N content in the plant was not significantly influenced with the fertilizers when cultivated from April to June, but significantly the highest with the N followed by NK when cultivated from September to December. The total N in the plant with the fertilizer NPK was lower due to the highest biomass production, which is supported by other studies (Akamine et al., 2007; Hossain et al., 2011)

L-ascorbic acid content in the amaranth BB and BC lines was the highest with fertilizer NPK at 40 g m<sup>-2</sup> followed by 30 g m<sup>-2</sup>. The Na, K, Ca, Mg and P content of the amaranth BB line increased with all the fertilizer rates, whereas the content of Na, K, Al, Fe and P in the amaranth BC line increased with the fertilizer NPK at 20-40 g m<sup>-2</sup>. The Ca content was lower in the BC line, and Mg and Zn content was not influenced by the fertilizer rates. The total N content was the same with all the fertilizer rates in the BB line, but increased with the increasing fertilizer rate in the BC line. The results showed that all the minerals were not increased or decreased with the fertilizer rates.

Similar result was reported in other studies (Ivony et al., 1997; Barbara et al., 2011). Overall results indicate that the fertilizer NPK at 40 g m<sup>-2</sup> is better for a higher yield and quality of the amaranth plant on dark-red soil.

## Conclusions

The fertilizer P and K applied alone were not effective for amaranth growth, because the P and K content in the soil was probably sufficient or there was a lack of N content. Combined application of NP resulted in significantly higher growth parameters and yield than the combined fertilizer of NK, which indicates that P fertilizer influenced the function of N fertilizer significantly but K did slightly in the amaranth. It is also considered that K content in the soil was sufficient for amaranth growth but P was not sufficient. Combined application of NPK resulted in the highest growth parameters, which indicates that fertilizer P and K applied alone were not effective because of insufficient N, but effective when applied together with N. All the growth parameters and yield of the amaranths were the highest with the combined fertilizer NPK at 40 g m<sup>-2</sup> followed by 30 g m<sup>-2</sup>. The P, K and N content in the amaranth increased but Ca and Mg content decreased with the fertilizer NPK. However, total amount of the minerals significantly increased. The Na, K, Ca, P, N and L-ascorbic acid content in the amaranth lines increased, and Mg, Al and Fe content was the same with the fertilizer NPK at 30-40 g m<sup>-2</sup>. Overall results indicate that combined fertilizer NPK at 40 g m<sup>-2</sup> is better for higher yield, mineral and L-ascorbic acid of amaranth on dark-red soil in Okinawa. However, it is very difficult to clarify the actual effects of individual or combined fertilizers of N, P and K on amaranth cultivation in field soil. In addition, a specific plant species needs a specific ratio and amount of fertilizers N, P and K for proper growth, yield and quality. Therefore, further experiments should be conducted to evaluate ratio and amount of fertilizers N, P and K on amaranth cultivation by using nutrientless soil.

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