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Original Research Article

Effect of Genotype and Organic Fertilizer on Red:Far Red Ratio, Stomatal Conductance, Leaf Temperature, and Dry Weight of Amaranth

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Abstract

Amaranth is a very important vegetable worldwide. Its leaves are consumed and the seeds are processed into various food products. Its production in the tropics is threatened by low yields which has a major impact on global production. A research aimed at improving the yield of this vegetable was carried out at Lady Bird organic farm Broga, 2.945^{0}N 101.874^{0}E, Semenyih, Selangor, Malaysia by studying the effect of genotype and organic fertilizer on the following growth indicators, red:far red ratio below canopy, stomatal conductance, leaf temperature, and dry weight. These growth indicators were monitored on a weekly basis over a period of six weeks. Three genotypes namely, *Amaranthus caudatus*, 3388 (green round leaf), *Amaranthus caudatus*, 3233 (green long leaf) and *Amaranthus cruentus*, 888 (red leaf) were grown on soils to which organic fertilizer was added at three different application rates (0 t/ha, 7.5 t/ha and 15 t/ha). There was no significant interaction effect of genotype and organic fertilizer on the growth indicators; however, each of these factors had significant effect on the growth of amaranth. A strong negative correlation occurred between dry weight and red:far red ratio below canopy likewise, between stomatal conductance and leaf temperature. Over a cultivation period of five weeks, the green long leaf genotype was superior showing that it is better adapted to growing in the rainforest agro ecological zone of Malaysia than the other two genotypes. Organic fertilizer application at a rate of 15 t/ha produced the lowest leaf temperature, after 4 weeks of cultivation implying better water status than 0 t/ha and 7.5 t/ha application rates. This study clearly shows that genotype and organic fertilizer have significant effect on the growth of amaranth. Therefore, these factors can be manipulated in order to enhance amaranth production.

KEYWORDS*:* Amaranth, genotype; organic fertilizer; stomatal conductance; growth indicators.

ABBREVIATIONS

RFR, red:far red ratio below canopy; SC, stomatal conductance; LT, leaf temperature; DW, dry weight; WAP, weeks after planting.

INTRODUCTION

Amaranth is one of the most important vegetables of the tropical regions of the world. It originates from South America and is an annual vegetable that requires four to six weeks to mature (Makinde et al., 2010). It is a C_4 plant that grows well in sunny, warm and humid environments. But it thrives also, in dry environments due to its low rate of water loss and high water use efficiency (Liu and Stutzel, 2002). This genus is made up of about 60 species out of which 15 are cultivated in many parts of the world especially Asia and Africa.

The leaves are widely consumed and in a few areas the stem and seeds are converted into various products. It is usually harvested before maturity if the leaves are the main interest of the farmer. However, if the seeds are of particular interest to the farmer it is allowed to grow to maturity. The seeds can be processed into flour and used for making bread. It serves as a good source of protein, calcium, iron and vitamins such as β carotene, ascorbic acid and niacin (Akparobi, 2009). The leaves are used by people in various parts of the world, especially in India and China for curing of bronchitis, fevers, dysentery, dropsy and cardiovascular diseases (Law-Ogbomo and Ajayi, 2009). Amaranth is also used in diets for broilers as a replacement for meat-and-bone meals (Pisarikova et al., 2006).

The yield of this vegetable per hectare is generally poor in the tropics (7.60t/ha) as compared to United States (77.27t/ha) and world average (14.27t/ha) (Law-Ogbomo and Ajayi, 2009). In order to meet local demands and for commercialization, factors that affect its yield need to be studied. One of such major challenges affecting its yield in Peninsular Malaysia and many other tropical regions of the world is the poor nature of soils in these regions (Law-Ogbomo and Ajayi, 2009). Another important factor is genotypic variations (Stagnari et al., 2007). Organic manure is cheap and can be prepared from readily available raw materials such as poultry dung, as opposed to chemical fertilizers which are more expensive and contribute significantly to environmental pollution. In addition, nutrients are released slowly by organic manure thereby enhancing the nutrient use efficiency and productivity of crops and reducing leaching and run off of nutrients into aquatic bodies which can result in eutrophication (Mbonu and Arifalo, 2006). Low red:far red ratio below canopy (a consequence of absorbing red wavelength of light by photosynthetic pigments and transmitting or reflecting the far red wavelength) has been reported to have positive effect on growth in many crops (Mascarini et al., 2006; Mazzella et al., 2008; Chia and Kubota, 2010). Botto and Smith (2002) reported that low red:far red ratio caused hypocotyl extension and accelerated flowering in *Arabidopsis*. Stomatal conductance determines the amount of CO_2 passing in and water vapor passing out of leaves of plants and so, has consequences on photosynthetic rate and transpiration, respectively. Leaf temperature is an indication of water status of crops. High leaf temperature is a consequence of reduced transpiration rate which indicates water stress. Plants with high water use efficiency are able to thrive in water limiting soils and consequently keep their leaf temperature moderate (Gonzalez-Dugo et al., 2005; Liu and Stutzel, 2002).

The main objective of this research was to study the effect of genotype and organic fertilizer on red: far red ratio below canopy, stomatal conductance, leaf temperature, and dry weight of amaranth.

MATERIALS AND METHODS

Experimental site

The study was conducted in a polythene house at Lady Bird organic farm, Broga, 2.945^{0}N 101.874^{0}E, Semenyih, Selangor, Malaysia. The soil used for the field studies was a combination of sandy soil and compost (chicken manure + rice bran + fish meal + sea weed + molasses + microbes) combined in the ratio of 2:1, respectively.

Plant material

Three different genotypes of amaranth were used for this research. These are: *Amaranthus caudatus*, 3388 (green round leaf), *Amaranthus caudatus*, 3233 (green long leaf) and *Amaranthus cruentus*, 888 (red leaf).

Treatments and experimental design

A 3 × 3 factorial experiment was conducted using randomized complete block design (RCBD) replicated three times. A total of 27 plots were used each with dimension 2 m × 1.95 m. Inter-row and intra-row spacing were 15 cm and 10 cm, respectively. Treatments consisted of 9 combinations ($G_1$0, G_1 7.5, $G_1$15, $G_2$0, G_2 7.5, $G_2$15, $G_3$0, G_3 7.5, and $G_3$15) and were assigned to plots using random numbers (Figure 1a). Planting was done according to the pattern shown in Figure 1b.

Procedure

Sowing beds were raised by mechanical cultivation using a mini cultivator. Each experimental plot was sized 1.3 m by 1.95 m. Organic fertilizer was added to the beds according to the worked out rates (0t/ha, 7.5t/ha and 15t/ha). Sowing operation was carried out on 18/02/2012 by employing the drilling method in row to row spacing of 15 cm. The beds were watered before transplanting was done. Hand weeding was performed in order to reduce competition for nutrients and create a disease free environment. Thinning operation was conducted two weeks after sowing. This was done by increasing intra-row spacing to 10 cm in order to reduce competition for nutrients among the amaranth plants (Green round leaf, green long leaf and red leaf) thereby enhancing optimal growth. Irrigation was applied twice daily using sprinkler system. Uniformity was maintained by regulating the tap for 5 minutes only. Irrigation was done in order to ensure that the plants received adequate amount of water required for optimal growth. Pest control was conducted once in a week using an organic pesticide formulation consisting of Neem, garlic and lemon grass (Oparaeke et al., 2005; Arannilewa et al., 2006; Asia-Pacific Association of Agricultural Research Institutions and Asia-Pacific Consortium on Agricultural Biotechnology, 2009). This was done in order to reduce pest population to below economic injury levels thereby, enhancing crop productivity. This operation was also necessary in order to prevent infection of the plants by pathogens carried by some pests. Harvesting was done after six weeks of planting.

Data collection

Red far red below canopy (RFR), stomatal conductance (SC), leaf temperature (LT), and dry weight (DW) were measured on a weekly basis for a period of six weeks. The instruments used for the measurement of RFR, SC and LT were RFR meter (Skye Instruments Ltd, SKR 110), leaf porometer (Decagon Devices, Inc., SC-1) and infrared thermometer (Omega, OS-DT8855W), respectively.

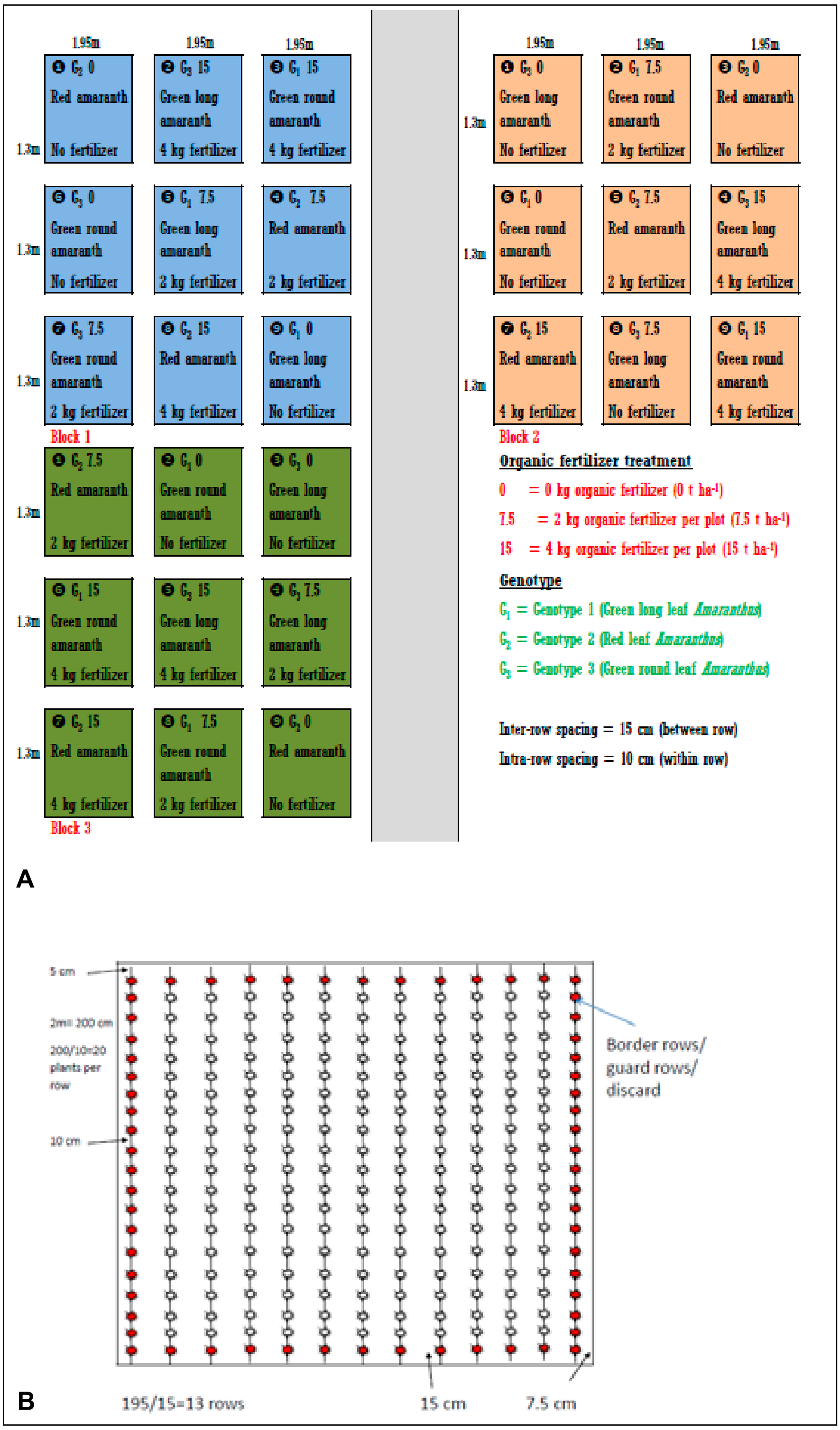

Figure 1. Experimental design A. Field layout for the experiment B. Planting pattern for the experiment.

Data analysis

Data collected were subjected to analysis of variance (ANOVA) procedure for randomized complete block design using SAS software. Mean separation was done using least significant difference (lsd).

RESULTS

Effect of genotype on Red Far Red ratio below canopy, Stomatal Conductance (mmol/m^2 s), Leaf temperature (^{0} C), and Dry Weight (g) of amaranth

RFR was significant (p < 0.05) only at 5 WAP resulting

Table 1. RFR, SC (mmol/m ^{2}s), LT (^{0} C), and DW (g) of three different genotypes of *Amaranthus* spp.

Genotype	Weeks after planting (WAP)											
	4				**5**				**6**			
	RFR	SC	LT	DW	RFR	SC	LT	DW	RFR	SC	LT	DW
Green round leaf	0.75	193.50^{b}	34.50	2.22	0.44^{ab}	207.38^{b}	32.62	11.85	0.33	752.30	28.84	24.24
Green long leaf	0.74	384.12^{a}	34.02	2.77	0.38^{b}	458.28^{a}	32.40	11.17	0.33	1910.50	28.97	26.43
Red leaf	0.80	267.26^{ab}	33.66	2.11	0.50^{a}	405.22^{a}	32.24	8.88	0.40	1222.40	28.96	21.82
SE	0.056	50.447	0.291	0.420	0.031	55.746	0.419	2.190	0.037	563.114	0.183	3.087

Values followed by different letters are significantly different at 5% level

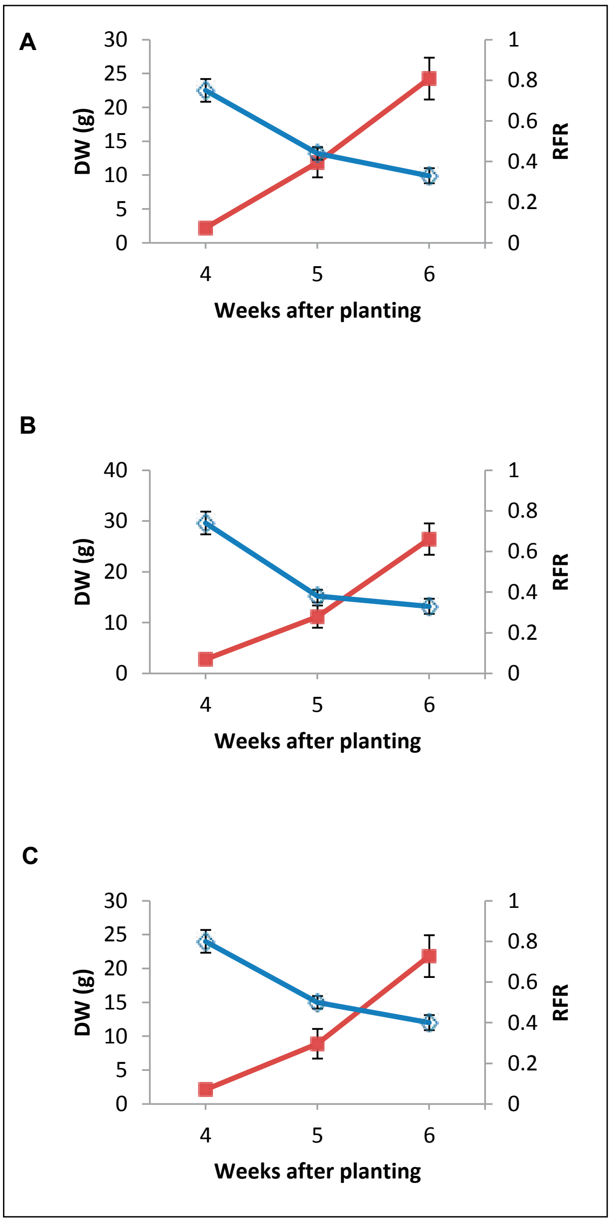

Figure 2. Relationship between dry weight and red:far red below canopy of different genotypes of amaranth. A. Green round leaf B. Green long leaf C. Red leaf. (DW, filled boxes; RFR, empty boxes).

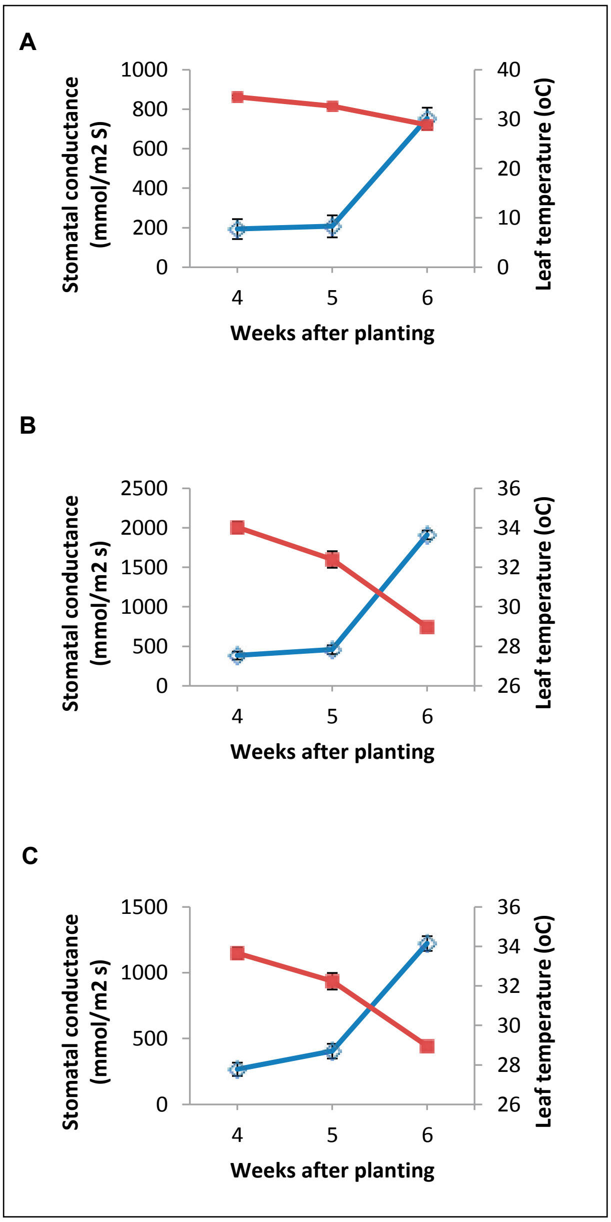

Figure 3. Relationship between stomatal conductance and leaf temperature of different genotypes of amaranth. A. Green round leaf B. Green long leaf C. Red leaf. (LT, filled boxes; SC, empty boxes).

into mean values of 0.44, 0.38, and 0.50 for green round leaf, green long leaf, and red genotype, respectively (Table 1). There was a progressive decline in RFR value from 4 WAP up till 6 WAP for all genotypes. The dry weight of the three genotypes was not significantly different at all weeks of monitoring. Nevertheless, there was a progressive increase in dry weight up till the harvest week for all genotypes. DW and RFR are inversely related, for all the genotypes (Figure 2), producing an overall correlation coefficient of -0.89 ($p < 0.01$). SC peaked at 6 WAP but a significant difference among the three genotypes was observed only at 4 and 5 WAP. SC was highest among the green long leaf and the red leaf genotypes at 4 WAP yielding mean values of 384.12 mmol/m^2 s and 267.26 mmol/m^2 s, respectively, while the green round leaf genotype produced the least mean value (193.50 mmol/m^2 s) although this value is statistically similar at 5 % probability level, to the mean value obtained for the red leaf genotype (Table 1). A similar pattern was observed at 5 WAP except that in this case, the mean SC values for the red leaf genotype and the green round leaf genotype were not statistically similar (405.22 mmol/m^2 s and 207.38 mmol/m^2 s, respectively). LT decreased progressively from 4 WAP up till 6 WAP. However, there was no significant variation in LT among the three genotypes at 4, 5, and 6 WAP. Results shows that a negative correlation exists between SC and LT (Figure 3) and a correlation coefficient value of -0.82 ($p < 0.01$) was obtained.

Effect of organic fertilizer on Red Far Red ratio below canopy, Stomatal Conductance (mmol/m^2 s), Leaf Temperature (^{0}C), and Dry Weight (g) of amaranth

Organic fertilizer had no significant effect on RFR. It is important to mention that RFR declined progressively during the course of crop monitoring for all organic fertilizer application rates (Table 2). The opposite trend was observed for DW. Again, there was no significant difference in DW of amaranth grown at the three organic fertilizer application rates. These two parameters (RFR and DW) are inversely related (Figure 4) at all organic fertilizer application rates. Pearson's correlation revealed a negative correlation between the two growth indicators at all organic fertilizer application rates. A correlation coefficient of -0.87 ($p < 0.01$) was obtained. SC increased progressively up the time of harvest for all organic fertilizer application rates. But there was no significant difference among them. Variation in LT among the three organic fertilizer application rates was significant only at 4 WAP (Table 2) yielding mean values of 34.38 ^{0}C, 34.31 ^{0}C, and 33.48 ^{0}C for 0 t/ha, 7.5 t/ha, and 15 t/ha application rates, respectively. LT decreased progressively from 4 WAP up till 6 WAP for all organic fertilizer application rates. Our results reveal that an inverse relationship exists between SC and LT (Figure 4). And a negative correlation coefficient value (-0.92) was obtained.

DISCUSSION

There was no significant interaction between the effect of genotype and organic fertilizer on the growth indicators measured; hence discussion was focused on main effects. Results as shown in Figure 2 show that a reciprocal relationship exists between RFR and DW. A reduction in RFR is perceived by light signal receptors called phytochrome, this triggers physiological responses leading to morphological changes, and consequently, dry matter accumulation. Mazzella et al. (2007) showed that lowering of red:far red ratio resulted in dry matter accumulation which occurred by a mechanism largely independent of metabolic changes. Stem elongation due to low red:far red ratio is often accompanied by stem dry weight gain (Casal and Sanchez, 1992). A correlation coefficient of -0.89 indicates that a strong negative relationship occurs between DW and RFR of the three genotypes of amaranth studied. Although there was a significant difference in RFR among the three genotypes at 5 WAP, there was no corresponding statistically significant difference in their dry weight. This could be due to the fact that the genetic variation in their light signal detection systems is not strong enough to produce significant difference in dry matter accumulation. Botto and Smith (2002) showed that by lowering red:far ratio flowering and elongation growth occurred in among different accessions

Table 2. RFR, SC (mmol/m ^{2} s), LT (^{0} C), and DW (g) of *Amaranthus*, as affected by different levels of application of organic fertilizer (t/ha).

Organic fertilizer	Weeks after planting (WAP)											
	4				5				6			
	RFR	SC	LT	DW	RFR	SC	LT	DW	RFR	SC	LT	DW
0	0.76	310.08	34.38^a	2.38	0.43	333.04	32.52	8.88	0.33	1623.00	29.04	21.64
7.5	0.80	250.24	34.31^{ab}	2.41	0.46	451.61	31.81	13.09	0.35	1097.10	28.72	26.96
15	0.73	284.56	33.48^b	2.32	0.43	286.22	32.93	9.93	0.37	1165.20	29.01	23.88
SE	0.056	50.447	0.291	0.420	0.031	55.746	0.419	2.190	0.037	563.114	0.183	3.087

Values followed by different letters are significantly different at 5% level

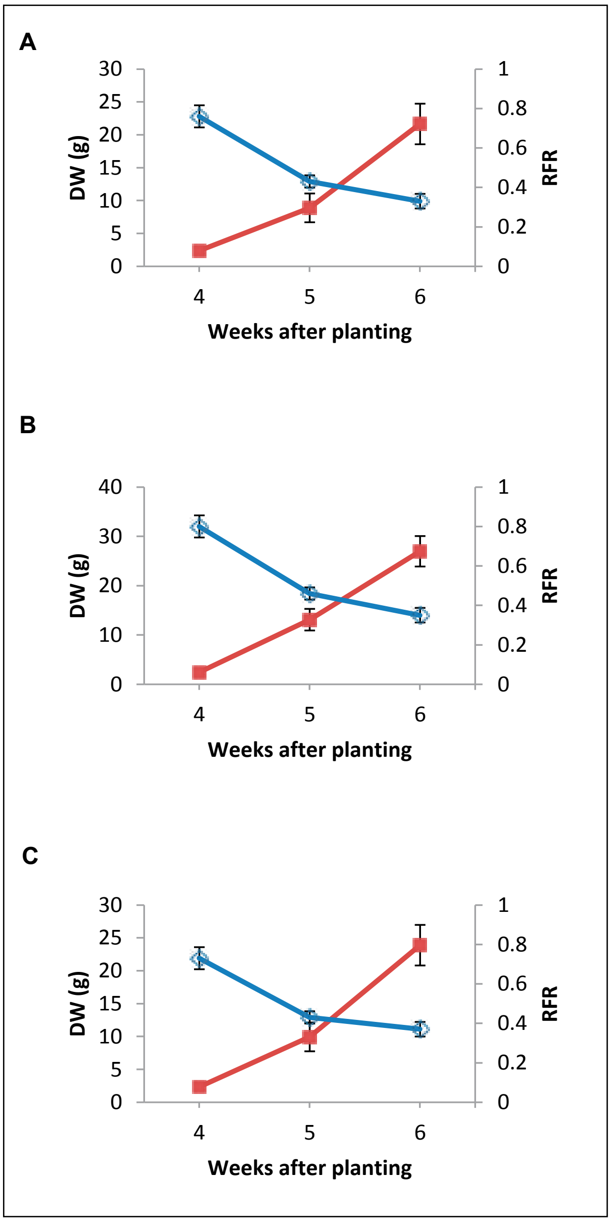

Figure 4. Relationship between dry weight and red: far red ratio below canopy of amaranth grown at different organic fertilizer application rates. A. 0 t/ha (control) B. 7.5 t/ha C. 15 t/ha. (DW, filled boxes; RFR, empty boxes).

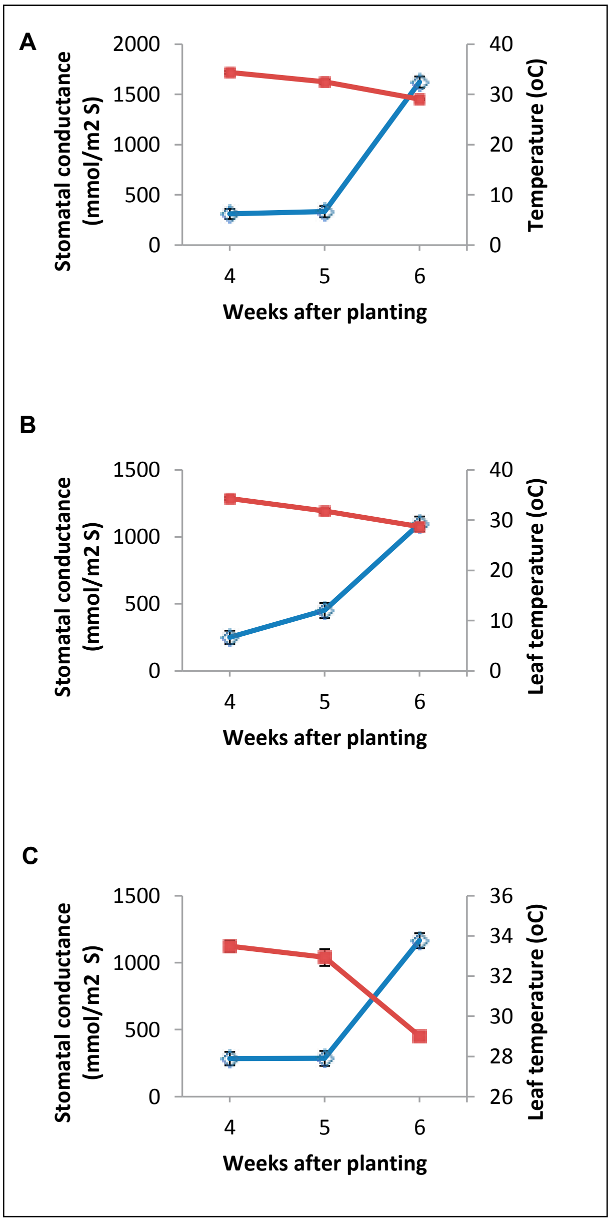

Figure 5. Relationship between stomatal conductance and leaf temperature of amaranth grown at different organic fertilizer application rates. A. 0 t/ha (control) B. 7.5 t/ha C. 15 t/ha. (LT, filled boxes; SC, empty boxes).

of *Arabidopsis thaliana*. They added that these growth responses were significantly different among the different accessions. Progressive increase in SC from 4 WAP up till 6 WAP (Table 1) indicates an increase in photosynthetic rate, a prerequisite for growth which is evident by the progressive dry matter accumulation seen for all the genotypes. The three genotypes, however, do not have significant genetic variation in the system controlling SC; hence the absence of significant difference in SC among them. All genotypes were not water stressed throughout the period of crop monitoring as evident by the LT values at 4, 5 and 6 WAP (Table 1). The progressive decline in LT for each of the three genotypes indicates increase in transpiration rate (transpiration causes cooling), a result of increased crop growth with time. Results show that a negative correlation exists between SC and LT (Figure 3). A correlation coefficient of -0.82 was obtained. This makes a lot of sense because transpiration is affected by stomatal conductance. Therefore increase in SC can cause increase in transpiration and consequently, decrease in LT. The absence of statistically significant difference in LT among the three genotypes is an indication of similarity in their water status. Furthermore, it can be implied that they have similar pattern of transpiration. Nutrient supply is necessary for the biosynthesis of macromolecules such as photosynthetic pigments among many others. Our thought was that application of organic fertilizer would result in decrease in RFR (due to increased synthesis of photosynthetic pigments), increase in DW and increase in SC. Our results, however, show that organic fertilizer application did not produce any significant effect on RFR. Application of organic fertilizer above agronomic rate can result in reduction of crop performance which may be due to accumulation of heavy metals in soil and plant tissues. Phytotoxic release of salts may impair the ability of soil microorganisms for mineralization required for the slow and efficient release of nutrients to plants (Oikeh and Asiegbu, 1993). Weil and Kroonje (1979) reported the toxic effects of poultry manure on soil microorganisms that enhance accessibility of plants to soil nutrients. Neher (1999) showed that synchronization of nutrient release and water availability with demand by plants is required for optimal growth. It is, however, pertinent to note that DW and RFR have a negative correlation (Figure 4) and a correlation coefficient of -0.87 was obtained. Progressive decline in RFR with time is an indication of growth (especially increase leaf number and area). This implies that more red wavelength was intercepted (lower RFR) as time passes. Growth on the contrary, results in dry matter accumulation. Thus, DW increased with time. Liu et al. (2011) reported that dry weight of *Stevia rebaudiana* bertani increased with duration of cultivation. Increase in SC with time at the three organic fertilizer application rates is a consequence of increase in photosynthetic rate as earlier mentioned. Significant difference in LT at 4 WAP at the three organic fertilizer application rates is an indication that they had different water status. But this was not the case at 5 and 6 WAP where results show that amaranth grown at three organic fertilizer application rates may have similar water use efficiency. A strong negative correlation exists between SC and LT at the different organic fertilizer application rates (Figure 5). A correlation coefficient value of -0.92 was obtained.

CONCLUSION

DW and RFR have a strong negative correlation. The same relationship was observed between SC and LT. DW and SC increased with duration of cultivation while the opposite trend occurred in the case of RFR and LT. For a cultivation period of 5 weeks, the green long leaf genotype was better adapted to growing in the rainforest agro ecological zone of Malaysia than the other two genotypes. So, an amaranth farmer who intends to harvest after 5 weeks of planting should be encouraged to sow the green long leaf genotype. Organic fertilizer had a significant effect only on LT and that occurred at 4 WAP. Amaranth grown at Organic fertilizer application at a rate of 15 t/ha had the lowest LT value and thus, have better water status than those grown at other rates.

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