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FOLIAR APPLICATION OF SALICYLIC ACID IMPROVED THE GROWTH, YIELD AND LEAF'S BIOACTIVE COMPOUNDS IN RED AMARANTH (*AMARANTHUS TRICOLOR* L.)

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Summary

This study was conducted to determine the effect of foliar salicylic acid (SA) applications on growth, yield and bioactive compounds of red amaranth grown under greenhouse conditions in 2008 at Gifu University, Japan. SA was applied at three different concentrations (10^{-3}, 10^{-4} and 10^{-5} M), three times during the vegetation at 7-day intervals one week after sowing. Growth parameters (plant height, stem length, number and size of leaves, root length) and yield (fresh and dry matter weight) were recorded from treated and control plants on 28 days after sowing. Among bioactive compounds, betacyanins, chlorophyll, total polyphenol and antioxidant activity were the determined from the leaves of treated and control plants. All of three doses SA application enhanced the plant growth, yield and leaf's bioactive compounds compared to the control. The growth parameters and yield of red amaranth was significantly influenced by foliar SA applications. The highest yield, antioxidant activity, amount of betacyanins, chlorophyll and total polyphenol occurred in 10^{-5} M SA treatment. According to our results, applications SA at rate of 10^{-5} M should be recommended in order to improve yield and bioactive compounds in red amaranth.

key words: salicylic acid, biomass, total polyphenol, betacyanin, antioxidant activity

INTRODUCTION

Amaranth plants are sometimes exposed to environmental stress like drought, salinity, diseases and pests at different periods of growth. A possible approach to minimize environmental stress that induces crop losses is the foliar application with chemical desiccant on plants. Salicylic acid naturally occurs in plants in very low amounts and participates in the regulation of physiological processes in plant such as stomatal closure, nutrient uptake, chlorophyll synthesis, protein synthesis, inhibition of ethy-

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lene biosynthesis, transpiration and photosynthesis (Khan *et al*. 2003, Piatelli *et al*. 1969, Shakirova *et al*. 2003). It has been identified as an important signaling element involved in establishing the local and systemic disease resistance response of plants after pathogen attack (Abdel-Wahed *et al*. 2006). Salicylic acid, jasmonic acid (JA), and ethylene-dependent signaling pathways regulate plant responses to both abiotic and biotic stress factors (Rao *et al*. 2000). Moreover, SA treatments at 0.5 mM strongly or completely suppressed the Cd-induced up-regulation of the antioxidant enzyme activities of barley (Metwally *et al*. 2003). SA has a direct physiological effect through the alteration of antioxidant enzyme activities, induces flowering, increase flower life, retards senescence and increases cell metabolic rate. The sustained level of salicylic acid may be a prerequisite for the synthesis of auxin and/or cytokinin (Metwally *et al*. 2003). Salicylic acid promotes some physiological processes and inhibiting others depending on its concentration, plant species, development stages and environmental conditions (Senaratna *et al*. 2000). Salicylic acid at high doses inhibited plant growth and chlorophyll contents in tomato (Kord & Hathout 1992) and wheat plants (Iqpal *et al*. 2006, Shehata *et al*. 2001). Salicylic acid (SA), o-hydroxybenzoic acid, is one of the phenolic compounds produced in the plant. It has shown many important functions in the plant and can change physiological behavior of plant. Thus salicylic acid could be expected to influence the growth and yield of red amaranth plants.

Therefore, the present investigation was undertaken to study the impact of spraying salicylic acid on some morphological criteria, yield as well as some biochemical constituents of red amaranth (*Amaranthus tricolor* L.) cv. 'Rocto Alta' plants to improve growth, yield, and nutritional value.

MATERIALS AND METHODS

Plants of red amaranth cultivar 'Rocto Alta' were raised from seeds planted in planters kept in a naturally illuminated greenhouse of Field science center at Gifu University, Japan during the month of June-July, 2008. Salicylic acid was dissolved in absolute ethanol then added drop-wise to water (ethanol/water: 1/1000, v/v). Salicylic acid was applied on the foliage of plants at a concentration of 10^{-3}, 10^{-4} and 10^{-5} M with a hand sprayer on 7, 15 and 21 days old plants. A control group of plants was grown sprayed with deionized water. A surfactant teepol (0.5%) was added with the control and SA treatment solutions. The volume of the spray was 30 ml per planter. Observations on plant height, length of stem, number of leaf, length and width of leaf, fresh yield and dry matter per plant, and root length were recorded on randomly selected nine plants from each planter. Nine apical leaves were harvested from 28 days old plant and dried overnight. Dried sample was grinded in fine powder and one gram of leaf sample dissolved in 40 mL of 90% methanol in a autoclaved bottle. The bottle was tightly capped and placed in a water bath with shaker (Thomastant T-N22S, Thomas Kagaku Co. Ltd., Japan), temperature was controlled at 80°C. After 1 hour the extract was cooled and filtered for further analytical assays of bioactive compounds i.e., total polyphenol, betacyanin, chlorophyll and antioxidant activity.

Determination of total polyphenols: Total phenolic content in red amaranth was determined with Folin-Ciocalteu reagent, (Slinkard & Singleton 1977) briefly, 50 µl of leaf extract solution was placed in a test tube, then 1 ml of Folin-Ciocalteu reagent (previously diluted by distilled water; reagent : water = 1:4) was added and the content was mixed thoroughly. After 3 min, 1 ml of Na_2CO_3 (10%) was added, the mixture was allowed to stand for 1 h in the dark. Absorbance was measured at 760 nm using a (U-1800, HITACHI, Tokyo, Japan) spectrophotometer. The concentration of total phenolic compounds in leaf extracts was determined as micrograms of gallic acid equivalent using an equation obtained from a standard gallic acid graph. Results are expressed as $mg \cdot 100\ g^{-1}$ gallic acid equivalent (GAE) of dry mass.

Determination of betacyanin: Approximately 0.2 g leaf disks of red amaranth were collected by cutting roller (12 base) and homogenized with a mortar and pestle and extracted with 20 ml 80% aqueous methanol containing 50 µM ascorbic acid, then shake for 30 min. After centrifugation at 14 g for 10 min at 4°C, the supernatant was removed and its betacyanin content was quantified by spectrophotometer (HITACHI U-1800, Tokyo, Japan) at a wavelength of 540 nm using the extinction co-efficient of betanin ($61.6 \times 10^6\ cm^2 \cdot mol^{-1}$) described by Wyler *et al*. (1959).

Determination of chlorophyll: Chlorophyll assay was performed by the method described by Lichtenthaler & Wellburn (1983). Leaf piece (1cm x 2cm) were cut and extracted by 20ml of 96% ethanol for 24 hours under dark condition. The extracts were measured at the wavelength of 665 nm and 649 nm for chlorophyll *a* and chlorophyll *b*, respectively, and total chlorophyll was estimated from them.

Anti-oxidant activity assay: Antioxidant activity was measured by the diphenylpicrylhydrazyl (DPPH) radical degradation method (Burits & Bucar 2000). Briefly, 10 µl of leaf extract solution (three replicates) was introduced into test tubes, and 4 mL distilled water and 1 mL of 250 µM DPPH solution was added. The tubes were mixed and allowed to stand for 30 min in the dark. Absorbance was read against a blank at 517 nm using a (U-1800, HITACHI) spectrophotometer. Antioxidant activity was calculated as the percent of inhibition relative to the control using the following equation: Antioxidant activity (%) = $(A_{blank} - A_{sample}/A_{blank}) \times 100$, where A_{blank} is the absorbance of the control reaction (control consisted of 10 µL, methanol instead of sample extract), and A_{sample} is the absorbance of the test compound.

Statistical Analysis: The treatments and leaf sampling for bioactive compound analysis were arranged in a completely randomized block design and replicated three times. Data were analyzed using Tukey's multiple comparison test (P<0.05) via ANOVA (Nagata & Yoshida 1997). The PC software 'Excel Statistics' (Version 5.0, Esumi Co. Ltd., Japan) was used for the calculations.

RESULTS AND DISCUSSION

Data presented in Table 1 show that foliar application of salicylic at 10^{-3}, 10^{-4} and 10^{-5} M promoted growth criteria of red amaranth's plant height, stem length, leaf number, leaf size, root length, as well as fresh and dry matter yield compared to corresponding untreated plants. In all cases, the increments in growth parameters were often highly significant in comparison with untreated ones. Salicylic acid at 10^{-5} M was the most effective treatment in increasing growth parameters, whereas, growth characters of red amaranth plants decreased by increasing salicylic concentration up to 10^{-3} M. Similarly Kord and Hathout (1992) found that foliar application of salicylaldehyde at 10^{-5} M stimulated different morphological and growth criteria of tomato plants but reduced effects were observed at 10^{-3} M. In a different experiment Yıldırım and Dursun (2009) found highest yield of tomato at 0.5 mM salicylic acid foliar treatment in greenhouse conditions. According to Gharib (2006), application SA of low concentration increased photosynthetic activity in basil and marjoram which enhanced their plant height, number of internodes, number of branches and leaves as well as leaf area, fresh and dry weights. In this respect, many investigators found that low concentrations of salicylic acid enhanced growth of soybean (Gutierrez-Coronado *et al*. 1998), maize (Shehata *et al.* 2001, El-Mergawi & Abdel-Wahed 2007) and wheat plants (Shakirova *et al*. 2003, Iqbal *et al*. 2006), whereas high concentrations caused an inhibitory effect on growth of tomato, lupine, wheat and maize plants (Kord & Hathout 1992, Haroun *et al*. 1998, Singh & Usha 2003, Abdel-Wahed *et al.* 2006). Low concentration salicylic acid foliar application also promote and influence the growth, development, differentiation of cells, and tissues of plants and enhanced the plant's growth parameters (Helgi *et al*. 2005). Thus, low dose (10^{-5} M) foliar application of salicylic acid is effective than higher doses in improving growth parameters and yield of red amaranth.

Table 1. The plant height, stem length, length of widest leaf, breath of widest leaf, no of leaf, root length, fresh weight and dry weight, of 'Rocto Alta' cultivars of Red amaranth in different doses of salicylic acid condition

Name of treatment	Growth parameter							
	Plant height (cm)	Stem length (cm)	Length of widest leaf (cm)	Breath of widest leaf (cm)	No. of leaf	Root length	Fresh weight/ plant (g)	Dry weight/ plant (g)
Control	29.4 a	21.53 a	5.26 a	3.78 a	13.66 a	3.63 a	6.54 a	0.72 a
10^{-3} M	29.5 ab	21.97 ab	5.28 a	4.52 b	14.11 ab	6.1 b	7.64 b	0.81 b
10^{-4} M	31.96 ab	22.87 ab	5.51 ab	4.32 ab	15.44 b	5.37 b	9.14 c	0.82 c
10^{-5} M	33.44 b	24.38 b	6.12 b	4.82 b	17.33 b	6.16 b	11.45 d	0.93 d

Means having the same letter within the column did not differ significantly according to Tukey's multiple comparison test ($P < 0.05$) via ANOVA

Likely red amaranth growth criteria foliar application of salicylic at 10^{-3}, 10^{-4} and 10^{-5} M enhanced accumulation of chlorophyll, betacyanin, total polyphenol and antioxidant activity of red amaranth compared to corresponding untreated plants.

Among the bioactive compoundss, the phenolics are widely distributed and have the ability to scavenge free radicals, superoxide and hydroxyl radical by a single-electron transfer (Deng *et al*. 1997). The total polyphenol content was the highest in leaves of red amaranth grown under 10^{-5} M doses of spraying salicylic acid condition and the lowest content determined in control condition (Fig.1). The enhancement occurred because SA it's self a plant-produced phenolic compound, also an endogenous growth regulator, which participates in the regulation of physiological processes in plants (Pila *et al*. 2010). In an earlier study, the SA treated (concentration 0.4 mM) tomato fruits accumulated increased phenolic contents. In this study, the profiling of phenolics showed fluctuations in quantity on three different doses indicating that their metabolism was affected by SA treatment. Yao & Tian (2005) demonstrated that SA stimulates phenylalanine ammonia lyase activity with consequent production of the main phenolic compounds and the synthesis of new polyphenolic substances in sweet cherry fruit. Salicylic acid (SA) (*o-hydroxybenzoic acid*), which belongs to a group of plant phenolics, is widely distributed in plants and is now considered as a hormone-like substance, which plays an important role in the regulation of plant growth and development (Raskin 1992, Klessig & Malamy 1994).

Betacyanin is a phytonutrient and an antioxidant and this pigment is responsible for the characteristic deep red color of red amaranth and have received considerable attention in recent years because of their possible role in the prevention of chronic diseases (Khandaker *et al*. 2009). The highest betacyanin content was determined from 10^{-5} M dose of SA treated leaves followed by 10^{-4} M, 10^{-3} M doses, where as untreated control leaves had the lowest content of betacyanin (Fig.2). Here again, the accumulations of chlorophyll were found highest in 10^{-5} M dose followed by control and 10^{-4} M doses of spraying salicylic acid where as lowest content was found in leaves of 10^{-3} M doses plants (Fig. 3). These results are in agreement with those obtained by Khurana and Makeshwari (1978), who found that in *Spirodela Polyrrhiza* SA at 10^{-5} M stimulated total chlorophyll synthesis whereas 10^{-3} M has a reverse effect. Gharib (2006) also obtained similar results, who found high total chlorophyll synthesis in sweet basil and marjoram plants salicylic acid at 10^{-5} mM, while 10^{-3} mM had the reverse effect. Chlorophyll is a photosynthesis dependant pigment and leaves stomata enable transfer of light and water and taking the CO_2 necessary for photosynthesis from the atmosphere. Stomatal index and stomatal density of pepper seeds were negatively affected by treatment with salicylic and sulfosalicylic acid, (10^{-3} M), on the other hand both at 10^{-4} and 10^{-5} M increased the stomatal index and its density on abaxial side, showing the opposite response in the adaxial side (Mendoza *et al*. 2002).

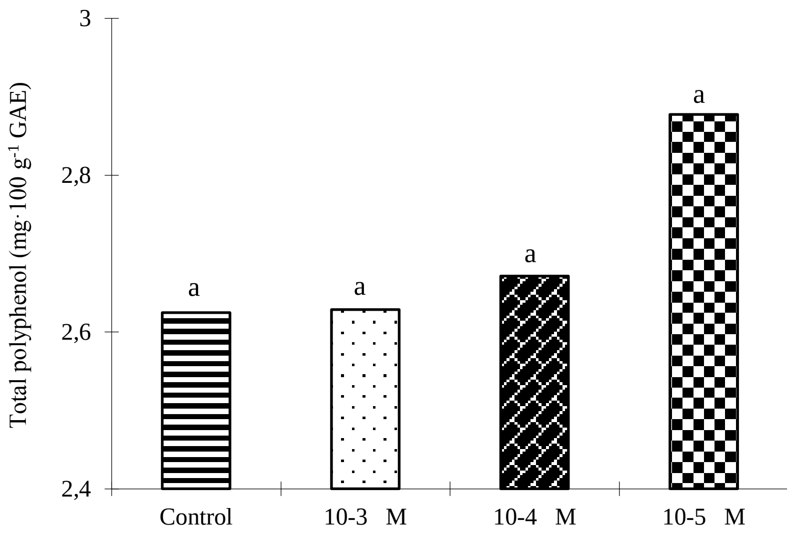

Fig. 1. Total polyphenol content of red amaranth cultivar 'Rocto Alta' in different doses salicylic acid condition.

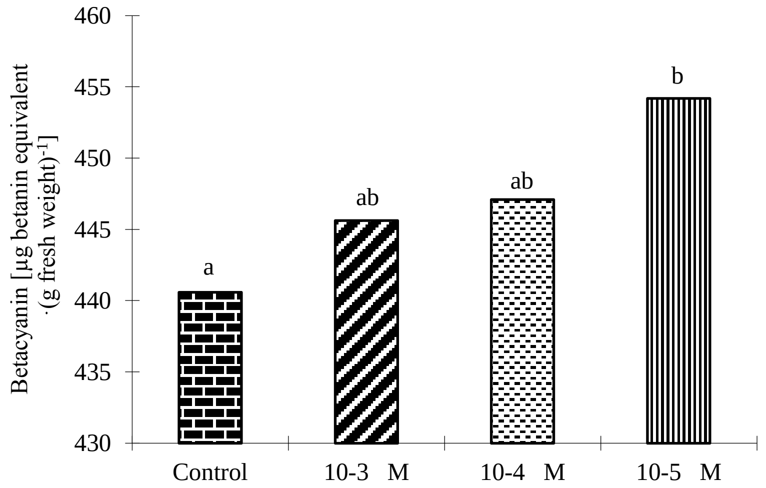

Fig. 2. Betacyanin content of red amaranth cultivar 'Rocto Alta' in different doses salicylic acid condition.

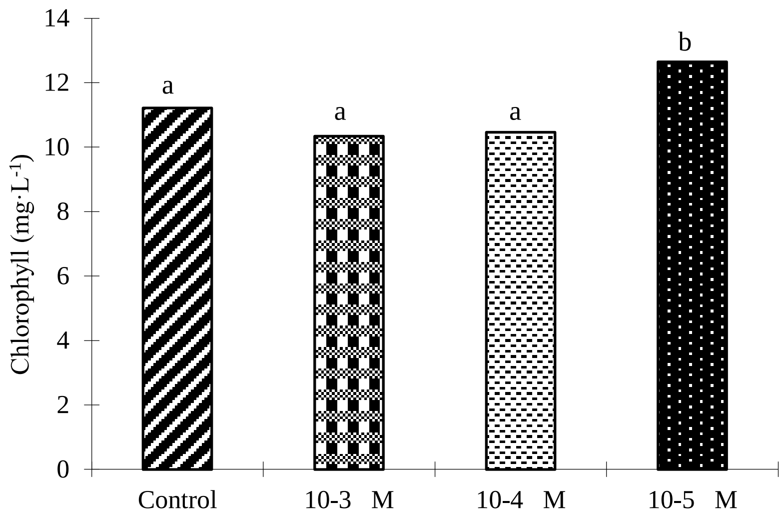

Fig. 3. Chlorophyll content of red amaranth cultivar ‘Rocto Alta’ in different doses salicylic acid condition

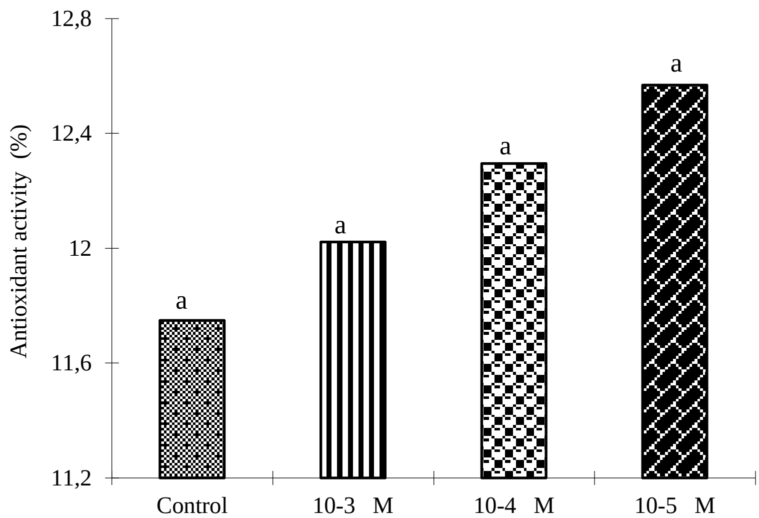

Fig. 4. Antioxidant activity of red amaranth cultivar ‘Rocto Alta’ in different doses salicylic acid condition

It has long been recognized that naturally occurring substances in the fruits and vegetables have antioxidant activity. Antioxidant activity of red amaranth varied in a wide range, and the 10^{-5} M doses of spraying salicylic acid treatment had the highest and the control condition had the lowest level (Fig. 4). It was reported that the antioxidant capacity and phenolic content of tomato plants was increased at 10^{-4} M SA pretreatment condition (Khan *et al*. 2003). SA has a direct physiological effect through the alteration of antioxidant enzyme activities and salicylic acid can enhance photosynthesis and nutrient uptake, phenolic, antioxidant activity and many metabolic substrates (Alvarez 2000). According to the Renhua *et al*. (2008), exogenous SA pretreatment could change the antioxidant system and maintain the nutritional value of fruits and vegetables, which have a higher ability to withstand oxidation injuries.

Collectively foliar application of SA can significantly regulate the plant growth parameters, yield as well as bioactive compounds in red amaranth. From the preceding results and discussion, it can be concluded that foliar application on red amaranth with salicylic acid at 10^{-5} M dose enhance biosynthesis of photosynthetic pigment chlorophyll and bioactive compounds betacyanins, total polyphenol and their antioxidant activity. Same dose of salicylic acid application also increased number of leaf per plant, leaf size, and fresh and dry matter yield of red amaranth. Thus foliar application of salicylic acid improved red amaranth yield and nutritional valued bioactive compounds.

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DOLISTNE STOSOWANIE KWASU SALICYLOWEGO POPRAWIA WZROST, PLONOWANIE ORAZ ZWIĄZKI BIOAKTYWNE W LIŚCIACH AMARANTU CZERWONEGO (*AMARANTHUS TRICOLOR* L.)

Streszczenie

Niniejsze badania przeprowadzono w celu określenia wpływu dolistnego stosowania kwasu salicylowego na wzrost, plonowanie i związki bioaktywne roślin amarantu czerwonego uprawianego w warunkach szklarniowych w roku 2008 na Uniwersytecie Gifu w Japonii. Kwas salicylowy stosowano w trzech stężeniach (10^{-3}, 10^{-4} i 10^{-5} M), trzy razy w okresie wegetacji w odstępach 7-dniowych po tygodniu od wysiewu nasion. Notowano parametry wzrostu (wysokość rośliny, długość łodygi, liczbę i wielkość liści, długość korzeni) oraz plonowania (świeżą i suchą masę) roślin traktowanych i kontrolnych 28 dni po siewie. Spośród związków bioaktywnych w liściach roślin traktowanych i kontrolnych określano betacyjaniny, chlorofil, polifenole ogółem i aktywność przeciwutleniającą. Wszystkie trzy zastosowane stężenia kwasu salicylowego zwiększały wzrost roślin, plonowanie oraz związki bioaktywne w liściach w porównaniu z kontrolą. Stosowanie kwasu salicylowego miało istotny wpływ na parametry wzrostu i plonowanie amarantu czerwonego. Najwyższy plon, aktywność przeciwutleniającą, ilość betacyjanów, chlorofilu i polifenoli ogółem uzyskano po traktowaniu roślin kwasem salicylowym w stężeniu 10^{-5} M. Na podstawie naszych wyników zalecane jest stosowanie kwasu salicylowego w stężeniu 10^{-5} M, w celu poprawy plonu i zawartości związków bioaktywnych w amarancie czerwonym.