

Growth, Oxalate and Vitamin C Content of Red Amaranth (*Amaranthus tricolor* L.) Treated with Salicylic Acid

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ABSTRACT

Red amaranth (*Amaranthus tricolor* L.) contains phytochemicals that are important for human health, however it also contains oxalate that may cause uric acid problem in human health. This experiment was carried out to evaluate the effect of salicylic acid (SA) on growth, oxalate, chlorophyll, and vitamin C contents in red amaranth. Red amaranth seeds were germinated in a plastic pot containing a mixture of top soil and organic fertilizer. Three week-old seedlings were applied with SA of 0 M (control), 10^{-8} , 10^{-6} , 10^{-4} or 10^{-2} M. Five replicates were prepared for each treatment. Growth parameters observed were plant height, fresh weight, and dry weight of plant. The Ca-oxalate crystal density was determined by observing stem section under the microscope. Chlorophyll and oxalic acid content were determined by spectrophotometer method, whereas vitamin C content was determined by titration method. The results showed that plant height and root length were tend to decline by SA application, however SA of 10^{-6} M significantly increased chlorophyll, carotenoid and vitamin C content. All concentrations of SA applied were able to reduce oxalic acid content and Ca-oxalate crystal density in stem. It can be inferred that application of SA generally enhances nutrient quality of red amaranth.

1. Introduction

Red amaranth (*Amaranthus tricolor* L.) is one of vegetables that has been widely cultivated in Indonesia. Amaranth foliage contains high nutritive value and is an excellent source of bio-available iron and vitamin A (Funke 2011). It has been reported by Peter *et al.* (2014) that in fresh leaves of *A. cruentus*, β -carotene, iron and zinc content was 5.75 ± 0.04 , 8.47 ± 0.05 , and 3.18 ± 0.04 mg/100 g, respectively. Amaranth leaves and seeds are good source of protein. According to Andini *et al.* (2013) the average protein content in leaves of cultivated *A. cruentus* L. and *A. tricolor* L. were about 16%. Amaranth is also considered as a drought resistant plant and it can be appraised for future crop that can withstand climate change. However, beside its nutritive contents, amaranth leaves and grain contain oxalate. The presence of oxalate in food decline the availability of calcium and magnesium and for human being it can promote kidney stone development. From analysis of 30 genotypes amaranth's grain, the average concentration

of oxalate was 229 mg/100 g (Gelinas and Seguin 2017). Another report by Vityakon and Standal (1989) showed that total oxalate content was 91 g kg^{-1} on a dry weight basis in *A. gangeticus* L. grown in three different soil types and climatic factors. The average content of oxalate in plants is also affected by nitrogen source and the availability of inorganic ions (Libert and Francheschi 1987). Efforts in reducing oxalate content in vegetable are still important to be conducted.

It has been suggested that oxalic acid biosynthesis can be derived from glyoxylate/glycolate, ascorbate, and oxaloacetate (Cai *et al.* 2015). In spinach, the accumulation of oxalate can be regulated by ammonium and nitrate. Under normal condition, the transcription levels of genes that involved in oxalate biosynthesis, such as *SoGLO2*, *SoGLO3*, *SoOXACs*, *SoMLS*, *SoMDH1*, *SoMDH2*, and *SoMDH* were high and it leads to high oxalate concentration (Cai *et al.* 2018). According to Kostman *et al.* (2001) the potential precursor of oxalate is ascorbic acid. Another finding by Truffault *et al.* (2017) reported that through [^{14}C] ascorbate labelling in tomato, oxalate, threonate as well asoxalyl threonate, were found as the degradation products of ascorbate. The degradation of ascorbate in plants may occur enzymatically through

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dehydroascorbate or non-enzymatically (Green and Fry 2005). In plants subjected to adverse environmental condition, one of the plant's defense mechanism is through detoxifying reactive oxygen species (ROS) by ascorbate peroxidase, this enzyme converts H_2O_2 into H_2O , using ascorbate as a specific electron donor (Caverzan *et al.* 2012). However, the mechanisms underlying the biosynthesis and breakdown of oxalate in plant are still undetermined. It has been suggested that ascorbate, dehydro ascorbate and total ascorbate can be reduced by application of salicylic acid in pepper plants treated with either UV-A, UV-B or UV-C (Mahdavian 2018). Salicylic acid (SA) is one of plant hormone that has a major role in regulating plant's defense against biotic and abiotic stress (Pye *et al.* 2013), and affects oxalate oxidase for oxalate compound breakdowns (Maksimov *et al.* 2015). This study was aimed to evaluate effects of SA on growth, vitamin C and oxalate content in red amaranth.

2. Materials and Methods

2.1. Sample Preparation, Design Experiment and Data Calculation

This study was conducted in Greenhouse of Faculty of Biology Universitas Gadjah Mada during April to July 2018 and repeated in 2019. Red amaranth seeds were germinated in a plastic pot containing a mixture of top soil and compost fertilizer (3:1 = v:v). Seedlings were selected and three seedlings were kept growth in each plastic pot. Three weeks old seedlings were then applied with salicylic acid of 0 M (control), 10^{-8} , 10^{-6} , 10^{-4} or 10^{-2} M. Two weeks later, root length, plant height, fresh weight and dry weight of shoot and root were measured. Chlorophyll and carotenoid content were determined spectrophotometrically, vitamin C was determined by titration method whereas distribution of crystal oxalate was determined through anatomical observation of stem section. Data were analyzed with One Way ANOVA in 95% confidence level, and continued by DMRT with 5% significant level if there is a significant difference between means.

2.2. Chlorophyll Content Determination

Two grams of leaves sampel was crushed using a mortar and pestle and extracted in 10 mL of 80% acetone. Leaf extract was filtered using a Whatman filter paper and the absorbance of leaf extract was measured with UV-Vis Spectrophotometer at 645 nm and 663 nm for chlorophyll content determination. The formula for calculating total chlorophyll content was as follow:

$$\text{Total chlorophyll content (mg/g)} = \frac{((20.2 \times A_{645}) - (8.02 \times A_{663})) \times V}{1000 \times W}$$

Where,

V : volume of filtrate,
W : leaf fresh weight, and
A : absorbance value of leaf extract (Arnon 1949)

2.3. Ascorbic Acid Content Determination

Vitamin C quantification was performed according to the method described by AOAC (1984) and results were expressed as mg ascorbic acid per 100 grams of leaf fresh weight. Two grams of leaf sample was mashed and diluted with 15 mL of aquadest. The solution was filtered and diluted into 250 mL with aquadest and then 50 mL of filtrate was added with 2 mL of amyllum 1% in 125 mL erlenmeyer and titrated with iodine of 0.01 N. The content of ascorbic acid was determined with the following formula:

$$\text{Ascorbic acid} = \frac{V \text{ Iodine} \times N \text{ Iodine} \times 0.88 \times df \times 100}{\text{sample weight (g)}}$$

With,

1 mg iod 0.01N = 0.88 mg of ascorbic acid

2.4. Oxalic Acid Content Determination

The oxalic acid content was determined according to Fitriani *et al.* (2016) with modification. Two grams of red amaranth leaves was crushed with a mortar and pestle and then added with 100 mL aquadest. The extract was heated for 20 minutes and then filtered. The filtrate was diluted with aquadest to 250 mL and then 50 mL of solution was taken and added with 1 mL of H_2SO_4 . The solution was titrated with $KMnO_4$ until the equivalent point was reached. The formula for calculating oxalic acid content is as follow:

$$\text{Oxalic acid normality} \quad V_K \times N_K = V_O \times N_O$$

$$V_O \times N_O = \frac{\text{Oxalic acid mass (mg)}}{\text{EW}}$$

$$\text{Oxalic acid content (mg/100g)} = \frac{\text{Oxalic acid mass (mg)}}{\text{sample weight (g)}} \times 10^2$$

Where,

V_K : Volume of potassium permanganate (mL)
 N_K : Normality of potassium permanganate (N)
 V_O : Volume of sample (mL)
 N_O : Normality of oxalic acid (N)
EW : Equivalent weight of oxalic acid = 63

2.5. Stem Section and Ca-oxalate Crystal Density Determination

The stem was free-hand sectioned and the sections was placed in 70% alcohol and each section was observed under microscope and documented using an optilab. Density of Ca-oxalate crystal was calculated by a formula mentioned by Harijati *et al.* (2011) as follow:

$$\text{Density} = \frac{\text{Total of observed crystal}}{\text{View area (mm}^2\text{)}}$$

3. Results

3.1. Growth of Red Amaranth

In general, application of SA up to 10^{-4} M through foliar spraying in red amaranth caused a slight reduction on the average of plant height, whereas the highest concentration of SA applied in this research (10^{-2} M) inhibited vegetative growth of red amaranth. Figure 1. illustrates the morphology of control and SA treated amaranth plants. In Figure 2, it can be seen that there was a trend for slight reduction in plant height of those plants treated with SA of 10^{-8} M, 10^{-6} M or 10^{-4} M and for plants treated with SA of 10^{-2} M the plant height was significantly decreased compared to control. Similar trend was also found for root length.

3.2. Biomass of Red Amaranth

From Table 1, it can be seen that SA concentration of 10^{-8} M, 10^{-6} M or 10^{-4} M did not significantly influence fresh weight of root and shoot. However, the highest concentration of SA (10^{-2} M) reduced



Figure 1. Phenotype of *Amaranthus tricolor* L. treated with salicylic acid. Note: SA1 = SA 10^{-8} M, SA2 = SA 10^{-6} M, SA3 = SA 10^{-4} M, SA4 = SA 10^{-2} (Courtesy of CB Asy Shyfa)

significantly red amaranth fresh weight compared to control. Total dry weight of red amaranth treated with SA decreased accordingly to the total fresh weight of shoot and roots in those plants treated with salicylic acid.

3.3. Chlorophyll Content of Red Amaranth

The result of chlorophyll determination is presented in Figure 3. It was showed that the highest chlorophyll content was found in plants treated with 10^{-6} M SA, however, the chlorophyll content decreased in plants treated with higher concentration of SA.

3.4. Ascorbic Acid and Oxalate Content of Red Amaranth

In Table 2, it was found that application of

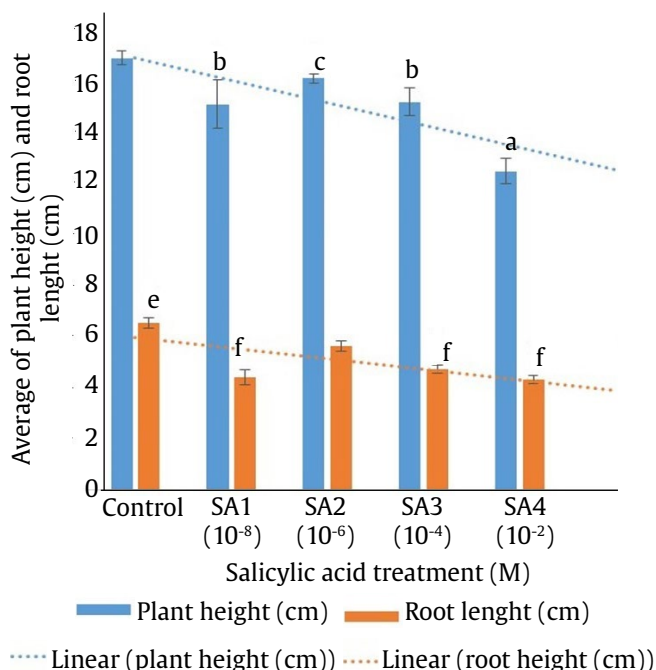


Figure 2. Effect of salicylic acid on average \pm SE of plant height and root length of *Amaranthus*

Table 1. Fresh and dry weight of *A. tricolor* L. treated with salicylic acid

Salicylic acid treatment (M)	Total of shoot and root fresh weight (g)	Total of shoot and root dry weight (g)
Control	2.4 \pm 0.33 ^b	0.213 \pm 0.026 ^c
SA1 (10^{-8})	2.3 \pm 0.33 ^b	0.150 \pm 0.008 ^b
SA2 (10^{-6})	2.1 \pm 0.46 ^b	0.168 \pm 0.021 ^b
SA3 (10^{-4})	2.1 \pm 0.22 ^b	0.160 \pm 0.022 ^b
SA4 (10^{-2})	1.5 \pm 0.33 ^a	0.110 \pm 0.031 ^a

The number followed by the same letter in the same column has no significant difference based on DMRT $\alpha=0.05$, n = 5

salicylic acid increased the content of ascorbic acid, on the other hand, the content of oxalic acid and the density of Ca-oxalate crystal in stem of red amaranth decreased by application of salicylic acid (Figure 4.).

4. Discussion

4.1. Growth of Red Amaranth

The results of this experiment showed that both plant height and root length tend to decline by application of SA. The highest concentration of SA applied caused a significant reduction in plant height whereas root length of those plants treated with SA was slightly decline compared to control plant. As a plant hormone, the physiological effect of salicylic acid may different amongst many species and it depends on concentration applied to the plant, frequency of application as well as the physiological age of plants. In many cases salicylic acid was reported to increase growth and yield of plant especially those grown under mild stress such as in *Dianthus superbus* L. It has been reported that

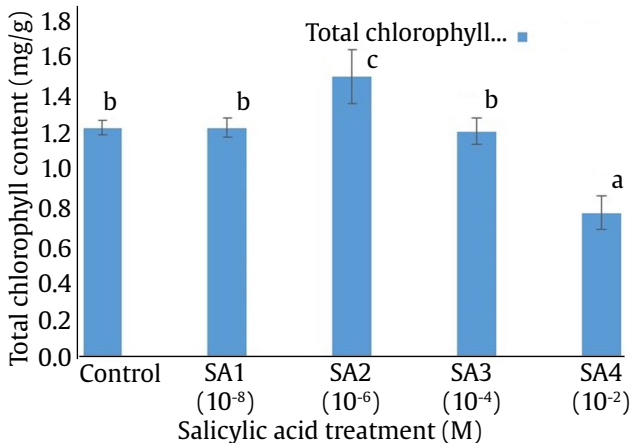


Figure 3. Effect of salicylic acid on chlorophyll content (mg/g). The same letter above the column showed no significant difference based on DMRT, $\alpha = 0.05$ n = 5

under moderate salinity stress, foliar application of SA effectively increased leaf biomass, soluble protein and sugar content, and up regulated the expression of MYB and P5CS in the *D. superbus*. However, there was no significant difference in plant's physiological responses as well as relevant gene expression between plants treated with or without SA when the plants were subjected to an extreme salt stress (0.9% NaCl) (Zheng *et al.* 2018). Foliar application of SA (3 mM) increased vegetative growth, number of flower and earliness in strawberry (Mohamed *et al.* 2017). According to (Canakci 2011), SA application of 1.5 mM to *Capsicum annuum* L. caused a stimulating effect while inhibition of seedling growth was found when SA was applied at higher concentration (5 mM or 10 mM). The inhibition of shoot brought by highest SA concentration probably due to the interaction of SA with other hormones such as auxin, or abscisic acid. In Arabidopsis mutants *cpr5*, *cpr6*, and *snc1* mutants that contain increased endogenous SA levels, the plants possess reduced apical dominance and stunted growth phenotypes and it reminiscent of AUX-deficient or AUX-insensitive mutants. In addition, these SA-accumulating mutants contain lower endogenous levels of free IAA and reduced sensitivity to AUXs compared with wild-type plants (Wang *et al.* 2007). In cucumber (*Cucumis sativus* L.) it was reported that SA treatment increased the endogenous content of abscisic acid and this abscisic acid leads to stomatal closure, whereas the endogenous content of auxin and gibberellins were not affected (Hao *et al.* 2011). It has been reported by Pasternak *et al.* (2019), that low concentration of SA (below 50 μ M) induced adventitious roots and altered architecture of the root apical meristem in *Arabidopsis thaliana*, whereas high-concentration of SA (greater than 50 μ M) inhibited all growth processes in the root. Auxin synthesis and transport are altered in plants subjected to exogenous SA. It was suggested that auxin synthesis might be promoted

Table 2. Oxalate content and Ca-oxalate density in *A. tricolor* treated with salicylic acid

Concentration of SA (M)	Ascorbic acid (mg/100 g)	Oxalate content	
		Oxalic acid (μ g/100 g)	Ca-oxalate crystal density (crystal/mm ²)
Control	0.2751±0.0739 ^a	112±15 ^c	43.00±6.63 ^d
SA1 (10 ⁻⁸)	0.4499±0.023 ^b	103±11 ^{bc}	17.75±2.20 ^b
SA2 (10 ⁻⁶)	0.5720±0.120 ^b	93±6 ^b	14.12±1.42 ^{ab}
SA3 (10 ⁻⁴)	0.5720±0.196 ^b	90±7 ^b	11.03±1.49 ^a
SA4 (10 ⁻²)	0.5257±0.108 ^b	66±4 ^a	30.59±1.79 ^c

The number followed by the same letter in the same column has no significant difference based on DMRT $\alpha = 0.05$ n = 5

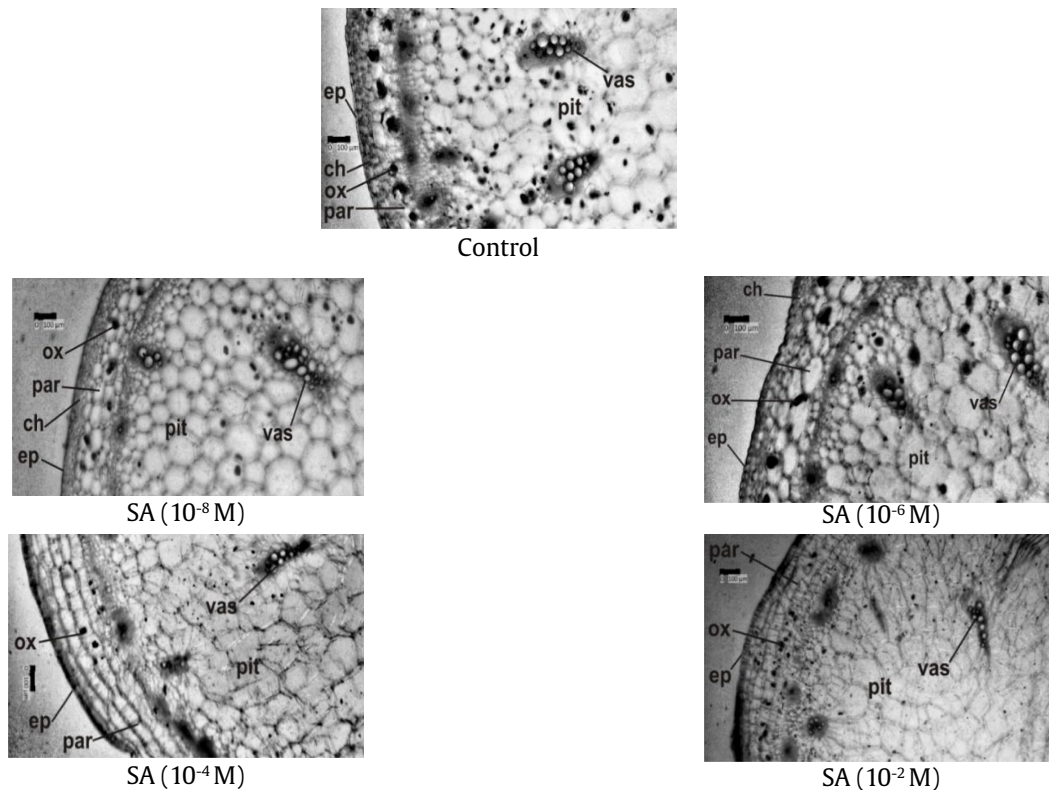


Figure 4. Stem anatomical structure of *A. tricolor* treated with salicylic acid. Magnified 4 x 10, Note: ep = Epidermis, ox = Ca-oxalate crystal, ch = collenchyma, par = parenchyma, pit = pith, vas = vascular bundle, Bar = 100 μ M (Courtesy of C.B. Asy Shyfa)

by a wide range of SA concentrations, but the auxin transport was dose dependent on SA concentration. The decline in plant height and root length of red amaranth treated with higher SA possibly associated with the reduction in endogenous auxin and/or inhibition of auxin transport as well as an increased in the endogenous abscisic acid. The mechanism by which high concentration of SA inhibit shoot and root growth in red amaranth still warrant further examination.

4.2. Biomass of Red Amaranth

The results presented in Table 1 showed that there was no significant difference in the fresh weight of shoots and roots of red amaranth plants treated with SA up to 10^{-4} M compared to control whereas the highest concentration of SA applied (10^{-2} M) reduced the fresh weight of shoots and roots. However, the dry weight of shoots and roots in those plants treated with all concentration of SA were less compared to control. The reduction in the dry weight probably due to the increased content of abscisic acid that caused stomatal closure. When the stomata were closed the transpiration will also decline and consequently the absorption of

water by roots will be limited. This condition will reduce cell growth and eventually the biomass. Endogenous hormonal content in *Arabidopsis thaliana* mutant *sid2* having deficiency in SA content has been evaluated by Abreu and Munne-Bosch (2009), and it has been found that the cytokinin content as well as abscisic acid in leaves of that mutant were also low. They suggested that there is a cross-talk between salicylic acid and other phytohormones during plant development. In *Arabidopsis*, Seo and Park (2010) reported that MYB96 transcription factor acts as a signaling link that integrates ABA and SA signals and regulates a synergistic interaction between the two stress hormones. The synergistic interaction between salicylic acid and abscisic acid was also reported in wheat in which SA increased the endogenous content of abscisic acid as well as H_2O_2 (Wang *et al.* 2018). In *Brassica juncea*, it has been reported that the dry matter accumulation was significantly enhanced when lower concentrations of SA were sprayed, however, higher concentrations of SA had an inhibitory effect (Fariduddin *et al.* 2003). It is possible that application of high concentration of SA could increase the endogenous abscisic acid and as a consequence it inhibits growth as well as biomass

accumulation on red amaranth. The growth stimulation or inhibition by SA is related to change in hormonal status (Shakirova *et al.* 2003), therefore the complete hormone profile determination in red amaranth treated with different concentration of SA is needed to elucidate the mechanism of SA in increasing or decreasing plant's biomass.

4.3. Chlorophyll Content of Red Amaranth

In barley, application of high concentration of SA (1-5 mM) reduced photosynthesis rate and RuBisCO activity (Pancheva *et al.* 1996) and also reduced chlorophyll content in cowpea (rao *et al.* 1997). In this experiment, application of 10^{-6} M of salicylic acid slightly increased total chlorophyll content of red amaranth. Similar finding has been reported by Khandaker *et al.* (2011) in which application of 10^{-6} M SA to red amaranth increased chlorophyll content and other biochemical compound such as betacyanin and total polyphenol. Li *et al.* (1992) suggested that application of SA inhibited the activity of ACC synthase in tomato fruit and it lead to the limitation of ethylene synthesis as well as chlorophyll degradation. It is possible that the slight increase in chlorophyll content of red amaranth treated with 10^{-6} M salicylic acid also due to the reduction of ethylene biosynthesis or through other mechanisms. Highest concentration of SA applied to red amaranth in this experiment (10^{-2} M) also reduced chlorophyll content. In barley, high concentration of SA (1-5 mM) reduced photosynthesis rate and RuBisCO activity (Pancheva *et al.* 1996) and similar finding was also reported in cowpea (Rao *et al.* 1997). According to Singh and Chaturvedi (2012), the decline in chlorophyll content following higher concentration of SA application probably due to chlorophyll degradation or inhibition of chlorophyll biosynthesis and it depends on species and both internal or external factors. The mechanism by which salicylic acid application increased or decreased chlorophyll content in red amaranth still warrants further examination.

4.4. Ascorbic Acid and Oxalate Content of Red Amaranth

The results presented in Table 2. Showed that the content of ascorbic acid increased in red amaranth plants treated with SA. It was suggested that ascorbic acid in plants functions as a protective molecule against reactive oxygen species that are formed from photosynthetic and respiratory processes. In addition, it also plays role as a co-factor for many enzymes. Involved in the cell cycle as well as other mechanisms of plant's cell growth and

division (Smirnoff 1996). By feeding the *Pistia stratoites* with 1-[14 C]-ascorbic acid, Kostman *et al.* (2001) showed that ascorbic acid is the precursor of oxalic acid. In tomato, it has been reported that application of SA 10^{-2} M increased ascorbic acid content compared to control (Javaheri *et al.* 2012). Similar finding has been shown in Mango stored at 5°C, in which application of SA increased both enzymatic and non-enzymatic antioxidants including ascorbic acid (Junmatonga *et al.* 2015). They suggested that SA may prevent the loss of ascorbic acid through inhibition of ascorbic acid oxidase (AAO) activity that breakdown ascorbic acid. The mechanism by which SA treatment increased ascorbic acid content in red amaranth was possibly through the decrease activity of ascorbic acid oxidase but this assumption needs further examination. It has been reported by Debolt *et al.* (2007) that ascorbic acid is a precursor of oxalic acid biosynthesis in plants. However, the finding in red amaranth argued that ascorbic acid is the main precursor of oxalic acid since the content oxalic acid decreased in those plants having higher ascorbic acid content. According to Cai *et al.* (2018), the high concentration of oxalate in spinach grown under normal condition was due to the high expression of several genes that involved in oxalate biosynthesis like *SoGLO2*, *SoGLO3*, three *SoOXACs*, *SoMLS*, *SoMDH1*, *SoMDH2*, and *SoMDH4*. They also suggested that the level of oxalate in plants is controlled by a complex regulatory mechanism and it also depends on plant varieties. Beside ascorbic acid, it was suggested that glyoxylate/glycolate and oxaloacetate could become a precursor of oxalic acid (Cai *et al.* 2015). Another possibility of oxalate precursor was the oxidative degradation of oxaloacetate which presumably catalyzed by oxaloacetate acetylhydrolase (OXAC) (Chang and Beevers 2007). Further metabolism and molecular examination still need to be carried out to elucidate the main precursor of oxalate found in red amaranth treated with salicylic acid.

It has been reported by Maksimov *et al.* (2015) that in wheat callus, the activity of oxalate oxidase increased when it was applied with exogenous SA. It was known that oxalate oxidase will breaks down oxalic acid into CO_2 and H_2O_2 (Walker and Farmiani 2018). In *Amaranthus hybridus* treated with abscisic acid (ABA), oxalate was not detected in the leaf and it was suggested that there was a rapid breakdown of oxalate into CO_2 and H_2O_2 by oxalate oxidase (Tooulakou *et al.* 2016). The red amaranth treated with SA probably also possessed high endogenous ABA, thus the level of oxalate decreased in those plants treated with SA. Most

of the plants produce oxalate endogenously and it has been considered that oxalate plays an important role in calcium regulation (Rahman and Kawamura 2011). The calcium oxalate crystal formed inside the idioblast and it also depends on the membrane, chamber, or secretory products found within the vacuole (Horner and Wagner 1995). Efforts for lowering oxalate accumulation has been carried out such as in transgenic tomato (*Solanum lycopersicum*) plants expressing an oxalate decarboxylase (OXDC) from the fungus *Flammulina velutipes* (FvOXDC) specifically in the fruit. This OXDC is an enzyme that catalyze decarboxylative degradation of oxalic acid to form formic acid and carbon dioxide. This transgenic tomato fruit showed up to a 90% reduction in oxalate content, which correlated with concomitant increases in calcium, iron, and citrate (Chakraborty *et al.* 2013). However, the explanation about factors which can affect the size or type of calcium oxalate crystal is still limited. A study about calcium oxalate ultrastructure change seems important to be conducted.

The cross section of *A. tricolor* stem treated with SA is shown in Figure 4. It was noted that structure of chlorenchyma and parenchyma tissue in red amaranth stem treated with 10^{-4} M or 10^{-2} M SA were slightly expanded compared to control. The collenchyma and parenchyma cell expansion found in those plants treated with SA probably caused by H_2O_2 that could be emitted from oxalate breakdowns. According to (Xiong *et al.* 2015), H_2O_2 could cause cell expansion because it affected cell wall structure and hence the collenchyma cells did not form a normal thickening in the cell wall.

5. Conclusion

From the results and discussions, it can be inferred that application of 10^{-2} M salicylic acid to red amaranth plants reduced plant height, root length, chlorophyll content, biomass accumulation, oxalic acid, and crystal Ca-oxalate distribution in stem, however, the content of ascorbic acid increased by application of SA. This SA treatment which lowers oxalate content in forms of oxalic acid and calcium oxalate crystal yet increased vitamin C level in red amaranth seems beneficial in respect of healthy food despite the biomass was reduced by SA application. For obtaining a relatively good performance in biomass and low oxalate level in red amaranth, treatment with SA of 10^{-6} M is recommended since this treatment also increased chlorophyll as well as vitamin C content. Further examination on hormonal

and other phytochemical contents, enzymatic activity and expression of various genes involved in oxalate regulation still need to be conducted.

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References

- Abreu ME, Munne-Bosch S. 2009. Salicylic acid deficiency in *NahG* transgenic lines and *sid2* mutants increases seed yield in the annual plant *Arabidopsis thaliana*. *Journal of Experimental Botany* 60:1261–1271. DOI:10.1093/jxb/ern363
- Andini RS *et al.* 2013. *Amaranthus* genetic resources in Indonesia: morphological and protein content assessment in comparison with worldwide amaranths. *Genetic Resource of Crop Evolution* 60:2115–2128. DOI:10.1007/s10722-013-9979-y
- [AOAC] Association of Official Analytical Chemists. 1984. *Official Method of Analysis*. Washington DC: Association of Official Analytical Chemists.
- Arnon DI. 1949. Copper enzymes in isolated chloroplasts, polyphenol oxidase in *Beta vulgaris*. *Plant Physiology* 2:1-15. DOI:10.1104/pp.24.1.1
- Cai XF *et al.* 2015. The oxalic acid in plants: biosynthesis, degradation and its accumulation regulation (Review in Chinese). *Journal of Plant Physiology* 51:267–272. DOI:10.3390/molecules23061286
- Cai XF *et al.* 2018. Expression analysis of oxalate metabolic pathway genes reveals oxalate regulation patterns in spinach. *Molecules* 23:1286–1301. DOI:10.3390/molecules23061286
- Canakci S. 2011. Effects of salicylic acid on growth, biochemical constituents in pepper (*Capsicum annum* L.) seedlings. *Pakistan Journal of Biological Sciences* 14:300-304. DOI:10.3923/pjbs.2011.300.304
- Caverzan AP *et al.* 2012. Plant responses to stresses: role of ascorbate peroxidase in the antioxidant protection. *Genetics and Molecular Biology* 35:1011-1019. DOI:10.1590/S1415-47572012000600016.
- Chakraborty NR *et al.* 2013. Reduction of oxalate levels in tomato fruit and consequent metabolic remodeling following overexpression of a fungal oxalate decarboxylase. *Plant Physiology*. 162:364–378. DOI:10.1104/pp.112.209197
- Chang CC, Beevers H. 2007. Biogenesis of oxalate in plant tissues. *Plant Physiology* 43:1821-1828. DOI:10.1104/pp.43.11.1821
- Debolt S *et al.* 2007. Ascorbate as a biosynthetic precursor in plants. *Annals of Botany* 99:3–8. DOI:10.1093/aob/mcl236
- Fariduddin Q *et al.* 2003. Salicylic acid influences net photosynthetic rate, carboxylation efficiency, nitrate reductase activity, and seed yield in *Brassica juncea*. *Photosynthetica*. 41:281–284. DOI:10.1023/B:PHOT.0000011962.05991.6c
- Fitriani H *et al.* 2016. Kandungan asam oksalat sayur bayam. *Medical Laboratory Technology Journal* 2:51-55.

- Funke OM. 2011. Evaluation of nutrient contents of amaranth leaves prepared using different cooking methods. *Food and Nutrition Sciences* 2:249-252. DOI:10.4236/fns.2011.24035
- Gelinas B, Seguin P. 2017. Oxalate in grain amaranth. *Journal of Agricultural and Food Chemistry* 55:4789-4794. DOI:10.1021/jf070384d
- Green MA, Fry SC. 2005. Vitamin C degradation in plant cells via enzymatic hydrolysis of 4-O-oxalyl-L-threonate. *Nature* 433:83-7. DOI:10.1038/nature03172
- Hao JH *et al.* 2011. Salicylic acid induces stomatal closure by modulating endogenous hormone levels in cucumber cotyledons. *Russian Journal of Plant Physiology* 5:906-913. DOI:10.1134/S1021443711050098
- Harijati N *et al.* 2011. Pengaruh pemberian kalsium terhadap ukuran dan kerapatan kristal kalsium oksalat pada porang (*Amorophallus muelleri* Blume). *Jurnal Pembangunan dan Alam Lestari* 1:72-139.
- Horner HT, Wagner BL. 1995. Calcium oxalate formation in higher plants. In: Khan SR (Eds.). *Calcium Oxalate in Biological System*. Boca Raton: CRC PRESS, pp. 53-70.
- Li N *et al.* 1992. Accumulation of wound-inducible ACC synthase transcript in tomato fruit is inhibited by salicylic acid and polyamines. *Plant Molecular Biology* 18:477-487. DOI:10.1007/BF00040664.
- Javaheri M *et al.* 2012. Effects of salicylic acid on yield and quality characters of tomato fruit (*Lycopersicon esculentum* Mill.). *International Journal of Agriculture and Crop Sciences* 4:1184-1187.
- Junmatonga CB *et al.* 2015. Cold storage in salicylic acid increases enzymatic and non-enzymatic antioxidants of Nam Dok Mai No. 4 mango fruit. *Science Asia* 41:12-21. DOI:10.2306/scienceasia1513-1874.2015.41.012
- Khandaker L *et al.* 2011. Foliar application of salicylic acid improved the growth, yield and leaf's bioactive compounds in red amaranth (*Amaranthus tricolor* L.). *Vegetable Crops Research Bulletin* 74:77-86. DOI:10.2478/v10032-011-0006-6
- Kostman TA *et al.* 2001. Biosynthesis of l-ascorbic acid and conversion of carbons 1 and 2 of l-ascorbic acid to oxalic acid occurs within individual calcium oxalate crystal idioblasts. *Plant Physiology* 125:634-640. DOI:10.1104/pp.125.2.634
- Libert B, Francheschi VR. 1987. Oxalate in crop plants. *Journal of Agriculture and Food Chemistry* 35:926-938. DOI:10.1021/jf00078a019
- Mahdavian K. 2018. Effect of salicylic acid spray on leaf ascorbate, proline and quercetin content in ultraviolet stressed Pepper seedlings. *Journal of Plant Process and Function* 6:55-61.
- Maksimov IV *et al.* 2015. Changes in the phytohormone levels in wheat calli as affected by salicylic acid and infection with *Tilletia caries*, a bunt pathogenic agent. *Russian Journal of Plant Physiol.* 51:228-233. DOI:10.1023/B:RUPP.0000019219.83823.49
- Mohamed RA *et al.* 2017. Salicylic acid effects on growth, yield, and fruit quality of strawberry cultivars. *Journal of Medicinally Active Plants* 6:1-11.
- Pancheva TV *et al.* 1996. Effects of salicylic acid on growth and photosynthesis in barley plants. *Journal of Plant Physiology*. 149:57-63. DOI:10.1016/S0176-1617(96)80173-8
- Pasternak A *et al.* 2019. Salicylic acid affects root meristem patterning via auxin distribution in a concentration-dependent manner. *Plant Physiology* 180:1725-1739. DOI:10.1104/pp.19.00130
- Peter C *et al.* 2014. Retention of B-Carotene, Iron and Zinc in solar dried amaranth leaves in Kajiado County, Kenya. *IJSBAR* 13:329-338.
- Pye MF *et al.* 2013. Induced resistance in tomato by sar activators during predisposing salinity stress. *Frontiers Research* 4:82-91. DOI:10.3389/fpls.2013.00116
- Rahman MM, Kawamura O. 2011. Oxalate accumulation in forage plants: some agronomic, climatic and genetic aspects. *Asian-Australian Journal of Animal Science* 24:439-448. DOI:10.5713/ajas.2011.10208
- Rao MV *et al.* 1997. Influence of salicylic acid on H₂O₂ production, oxidative stress, and H₂O₂-metabolizing enzymes. *Plant Physiology* 115:137-149. DOI:10.1104/pp.115.1.137
- Seo PJ, Park CM. 2010. MYB96-mediated abscisic acid signals induce pathogen resistance response by promoting salicylic acid biosynthesis in *Arabidopsis*. *New Phytologist* 186:471-483. DOI:10.1111/j.1469-8137.2010.03183.x
- Shakirova FM *et al.* 2003. Changes in the hormonal status of wheat seedlings induced by salicylic acid and salinity. *Plant Science* 164:317-322. DOI:10.1016/S0168-9452(02)00415-6
- Singh PK, Chaturvedi VK. 2012. Effects of salicylic acid on seedling growth and nitrogen use efficiency in cucumber (*Cucumis sativus* L.). *Plant Biosystems-An International Journal Dealing with all Aspects of Plant Biology* 146:302-308. DOI:10.1080/11263504.2011.602991
- Smirnoff N. 1996. The function and metabolism of ascorbic acid in plants. *Annals of Botany* 78:661-669. DOI:10.1006/anbo.1996.0175
- Tooulakou GA *et al.* 2016. Alarm photosynthesis: calcium oxalate crystals as an internal CO₂ source in plants. *Plant Physiology* 171:2577-2585. DOI:10.1104/pp.16.00111
- Truffault V *et al.* 2017. Ascorbate degradation in tomato leads to accumulation of oxalate, threonate and oxalyl threonate. *The Plant Journal* 89:996-1008. DOI:10.1111/tbj.13439
- Vityakon P, Standal BR. 1989. Oxalate in vegetable amaranth (*Amaranthus gangeticus*): forms, contents, and their possible implications for human health. *Journal of the Science of Food and Agriculture* 48:469-474. DOI:10.1002/jsfa.2740480409
- Walker RP, Famiani F. 2018. Organic acid in fruits: metabolism, function and content. *Horticultural Reviews* 45:393-430. DOI:10.1002/9781119431077.ch8
- Wang DK *et al.* 2007. Salicylic acid inhibits pathogen growth in plants through repression of the auxin signaling pathway. *Current Biology* 17:1784-1790. DOI:10.1016/j.cub.2007.09.025
- Wang W *et al.* 2018. Hydrogen peroxide and abscisic acid mediate salicylic acid-induced freezing tolerance in wheat. *Frontiers in Plant Science* 9:1137-1150. DOI:10.3389/fpls
- Xiong J *et al.* 2015. Novel roles of hydrogen peroxide (H₂O₂) in regulating pectin synthesis and demethylesterification in the cell wall of rice (*Oryza sativa*) root tips. *New Phytologist* 206:118-126. DOI:10.1111/nph.13285
- Zheng J *et al.* 2018. Salicylic acid promotes plant growth and salt-related gene expression in *Dianthus superbus* L. (Caryophyllaceae) grown under different salt stress conditions. *Physiological Molecular Biology of Plants*. 24:231-238. DOI:10.1007/s12298-017-0496-x