Enhancement of Antioxidants Content in *Amaranths tricolor* L. by Adjustment of Cationic Ions in Fertilizer

Sopanat Sawatdee[1], Chattip Prommuak[2], Teeraya Jarunglumlert[3], Prasert Pavasant[4], and Adrian Evan Flood[1]
[1] School of Energy Science and Engineering, Vidyasirimedhi Institute of Science and Technology, Rayong, Thailand
[2] Energy Research Institute, Chulalongkorn University, Bangkok, Thailand
[3] King Mongkut's University of Technology North Bangkok (Rayong Campus), Thailand
[4] The Thailand Research Fund (TRF), Thailand
Email: 1530411@vistec.ac.th, chattip.p@chula.ac.th, teeraya.j@sciee.kmutnb.ac.th, supersert@gmail.com, adrian.flood@vistec.ac.th

***Abstract*—This study investigated the effect of cationic nutrient (K, Mg and Ca) concentrations on the biosynthesis of antioxidants while also maintaining the dietary fiber content and mass productivity in *Amaranthus tricolor* L. *Amaranthus tricolor* L. was chosen as a model vegetable as it was reported to contain high antioxidants in the leaves. Soilless culture was carried out using a modified Huett solution containing different cationic ion concentrations (K; 28.5, 200.5 and 372.5 ppm, Mg; 2.8, 20 and 37.2 ppm Ca; 9.8, 70 and 130.2 ppm). Mature leaves from each treatment was determined for total phenolic content, antioxidant activity and dietary fiber. It was found that an increase in potassium enhanced the antioxidant activity and total phenolic productivity whereas the mass yield and dietary fiber were not negatively affected. The lowest Mg proportion (2.8 ppm) resulted in no mass productivity. High concentration of Ca (130.2 ppm) could increase the mass yield by up to 34%, compared with the control (70 ppm Ca). However, neither significant effect on antioxidant activity nor total phenolic productivity was observed with varying calcium concentration. The results suggest that producing an antioxidant-rich vegetable, not lowering its original quality, could be achieved by optimization of these nutrient elements.**

***Index Terms*—*Amaranthus tricolor* L, total phenolic, antioxidant activity, DPPH, cationic elements**

I. Introduction

Amaranth tricolor L. (red amaranth) is a red to purple leaf plant which grows best in tropical areas (30-35°C) with tolerance for drought for a long period of time [1]. Raw or cooked leaves have a mild flavor and provide good nutrition including 3.5% protein, 0.25% fat, 6.6% carbohydrates and it is rich in vitamin A, B1 and C [2]. On top of that, its health benefits which come from the existence of polyphenols, anthocyanins and other antioxidants have been investigated by many research studies [3]-[6]. These antioxidants were suggested to be an excellent tool for preventing free radicals from oxidative reactions which are a main cause for aging and the development of cancer, as well as cardiovascular and inflammatory diseases [7], [8]. Moreover, the Centers for Disease Control and Prevention expected an increase in the number of cancer patients in the United State by 24% between 2010 and 2020 [9]. Consequently, consumers showed increasing concern about their diet and health.

Fruits and vegetables are a good source of natural antioxidants. In order to improve this content further, several studies have attempted to stimulate antioxidants production in plants. These studies have revealed that various environmental factors can influence plant growth and development [10]. These agricultural factors include light, temperature, humidity, carbon dioxide and especially the composition of the fertilizer used [3], [11]-[13]. For instance, blue polyethylene shade cloth could increase the betacyanin pigment content and antioxidant activity of red amaranth [3]. Elevated carbon dioxide (CO_2) conditions boosted the antioxidant activity and glutathione in red and green lettuce [14]. In soil cultivation, although nitrogen is an essential nutrition for plant growth, the highest nitrogen proportion treatment resulted in pumpkin with the lowest antioxidant capacity [15]. Besides, Beta-carotene and lycopene contents in tomato could be increased under treatment with high potassium concentrations [13], [16].

Somehow, there is still a lack of information on the development of antioxidant-rich red amaranth. Hence, the aim of this work was to indicate the effect of concentration of cationic ions (potassium (K), magnesium (Mg) and calcium (Ca)) in fertilizer solution on the antioxidants content in *Amaranths tricolor* L. The suitable fertilizer formula will enhance plant's nutrition along with the potential for the product to enter a more specific market with higher purchasing power from customers.

II. Materials and Methods

A. Red Amaranth Cultivation

Commercial red amaranth seeds were germinated in $1 \times 1 \times 1$ cm^3 sponge cubes and kept indoors with twice-a-

Manuscript received December 6, 2018; revised March 4, 2019.

doi: 10.18178/joaat.6.2.108-112

day watering. Ten-day-old flawless sprouts were selected to grow by soilless cultivation conducted in UV resistant roof greenhouses set up at Vidyasirimedhi Institute of Science and Technology, Rayong, Thailand. The experiments were carried out during June-July 2017 with atmospheric temperatures of 25-36°C. To determine the effect of nutrient variation on the stimulation of antioxidants, mass production and dietary fiber, four ingredients of nutrient solution with varying K, Mg and Ca were prepared following Table I. Other elements were fixed at: 116 ppm Nitrogen, 26 ppm Sulphur, 22 ppm Phosphorus, 2.5 ppm Iron, 0.22 ppm Manganese, 0.21 ppm Boron, 0.15 ppm Zine, 0.03 ppm Copper and 0.01 ppm Molybdenum [17]. During cultivation, the nutrient solutions were circulated perpetually, and the concentrations of each element were monitored and adjusted on a weekly basis. The Kjeldahl method [18] was conducted for the determination of N concentration and the content of other elements were investigated using an inductively coupled plasma - optical emission spectrometry; ICP-OES (710 ICP-OES, Agilent Technologies. USA). After 44 days of cultivation, mature leaves from each treatment were randomly harvested for analyses. Edible leaves from randomly selected plants in each treatment were weighted to calculate the mass productivity in the unit of g/m^2 day.

TABLE I. CONCENTRATION OF CATION ELEMENTS IN EACH TREATMENT

Treatment	Concentration (ppm)		
	K	Mg	Ca
Standard Huett's (control)	200.5	20.0	70.0
Low K	28.5	20.0	70.0
High K	372.5	20.0	70.0
Low Mg	200.5	2.8	70.0
High Mg	200.5	37.2	70.0
Low Ca	200.5	20.0	9.8
High Ca	200.5	20.0	130.2

B. Extraction of Plant Sample

Pre-freeze (-80°C) red amaranth leaves were lyophilized for 48 hours by the Alpha 1-4 LDPlus, Christ, UK then were pulverized into fine powder using a commercial kitchen blender. At room temperature, a gram of red amaranth powder was extracted with 20 mL of 80% aqueous methanol for 15 hours. The mixture was then centrifuged (Model 3700, KUBOTA Corporation, Japan) at 13,200 rpm for 20 min. Supernatant was decanted and stored at -80°C [19] until further determination of total phenolic compounds, antioxidant activity and dietary fiber.

C. Analyses

1) Determination of total phenolic compounds

A modified Folin–Ciocalteau colorime [3] was used to determine total phenolic content in red amaranth extracts. Briefly, 125 µL of plant extracts were reacted with 2.5 mL of Folin–Ciocalteu solution (previously diluted by distilled water; reagent: water = 1:4) in amber bottles and were kept stagnant for 3 minutes prior to be added with 2.5 mL of 10% aqueous sodium carbonate. The mixture was then incubated in the dark for an hour at room temperature. Using a spectrophotometer (Cary 60, Agilent Technologies, USA), the absorbance of the sample was measured at 760 nm against a blank containing 1 mL of deionized water and 2 mL of 10% aqueous sodium carbonate. Results were reported on the unit of milligram of Gallic Acid Equivalents (GAE) per gram of Dry Weight (DW).

2) Determination of antioxidant activity by DPPH assay

Antioxidant activity was determined using a modified DPPH free-radical scavenging assay [3]. The mixture of 200 µL of plant extract, 1 mL of deionized water and 4 mL of 0.1 mM DPPH in 80% aqueous MeOH were prepared in an amber bottle and was incubated in the dark for 30 min at room temperature. The concentration of plant extract was varied in the range of 0-25 mg/mL to estimate the IC50 which represents the concentration of the plant extract required to inhibit 50% of radicals. Absorbance was measured at 517 nm using a spectrophotometer (Cary 60, Agilent Technologies, USA) using absolute methanol as a blank. Antioxidant capacity was calculated as the presence of inhibition relative to the control (%DPPH scavenged), following (1):

$$\%\text{DPPH scavenged} = (A_{blank} - A_{sample}/A_{blank}) \times 100 \quad (1)$$

where A_{blank} is the absorbance of the control reaction (absolute methanol) and A_{sample} is the absorbance of the sample.

3) Determination of dietary fiber content

A modified van Soest analysis [20], [21] was used to investigate cellulose and hemicelluloses content in the plant samples. The hot alcohol insoluble solids method was conducted to prepare the cell wall material [20]. Shortly, fresh leaves were cut into small section and boiled at 82-85°C in 96% ethanol (leaf: solvent = 1 g: 2.5 mL). After 20 min, the boiled leaves were pulverized by a kitchen blender for 15 min, then filtered off using a filter cloth. The solid parts were washed with 70% ethanol (20 min incubation before filtration each time), followed by 96% ethanol and acetone (washed three times in each solvent) and were dried in an oven at 40oC for at least 8 hours and stored in desiccator until further determination of cellulose and hemicellulose contents.

In the determination of cellulose content, the acid-Detergent Solution (AD) was first prepared. Briefly, 20 g of Cetyltrimethylammonium Bromide (CTAB) was diluted in the liter of 0.5 M sulfuric acid. Subsequently, 0.5 g of the dried sample was boiled at 210°C for 10 min in the mixture of 100 mL of AD solution and 2 mL of decahydronaphthalene using a reflux apparatus. Following that, the temperature was lowered and maintained at 185°C to avoid foaming for another hour. The sample was then filtered (Whatman No. 1) and rinsed twice with hot water (90-100°C), following by acetone. The solid residue was dried at 100℃ in an oven for at

least 8 hours and the weight was recorded as cellulose content.

In the determination of hemicellulose content, the Neutral-detergent Solution (ND) was prepared. Briefly, 18.61 g of disodium Ethylene Diamine Tetraacetate (EDTA), 6.81 g of sodium borate decahydrate, 30 g of sodium lauryl sulfate, 10 mL of 2-ethoxyethanol, and 4.56 g of disodium hydrogen phosphate were dissolved in 1 L of heated deionized water. 0.5 g of dry sample was boiled in a reflux apparatus at 210°C for an hour in 100 mL of ND solution, 2 mL of decahydronaphthalene and 0.5 g of sodium sulfite. The post-treatment steps were carried out following that described previously in the determination of cellulose content.

III. Results and Discussion

A. Mass Productivity and Dietary Fiber Content

The effect of cationic ions concentration on mass productivity of red amaranth are described by Fig. 1. Obviously, red amaranth grown by the highest proportion of Ca (130.2 ppm) resulted in the highest mass productivity (33.50 g/m2 day), which was 34% higher than the control treatment (25.00 g/m2 day). The result agrees with the research studies by [22], [23], where Ca^{2+} was found to possess the potential to promote plant growth. The increasing mass yield with Ca^{2+} enhancement could also be attributed to the enhancing capacity of cytokinin to postpone a growing older and leaf falling of plant [24], [25]. Mg plays a crucial role in photosynthetic process. A deficient level of magnesium in the nutrient solution could restrict the potential of chlorophyll in capturing light energy to be used in photosynthesis process [26] and thus resulting in immature plants. Accordingly, none of edible leaves was produced under the lowest Mg (2.8 ppm) treatment. Potassium is also a major nutrient for plant growth. Literally, K^+ plays essential role in water relations, photosynthesis and enzyme activity that definitely lead to increase crop yield and improvement of plant qualities [27], [28]. Although mass yield with increasing K (372.5 ppm) was found higher than that obtained from the low K (28.5 ppm) treatment, it was somehow lower than that from the control (200.5 ppm). This result could be attributed to the imbalance cationic ions, both monovalent and divalent in fertilizer solution which may negatively affect the yield productivity. High proportion of K often increases the possibility of Mg deficiency because of the absorption competition between K^+ and Mg^{2+} [29]-[31].

On the other hand, the result in Fig. 2 suggested that concentration of cationic ions did not affect significantly on dietary fiber content of red amaranth, except for K proportion. Hemicellulose content was found the highest (48.94 g/100 g DW) at 372.5 ppm of K treatment and the lowest (29.77 g/100 g DW) at 28.5 ppm of K treatment. The reduction of fiber content was related to carbohydrate metabolism which is the crucial role of K^+ [32], [33].

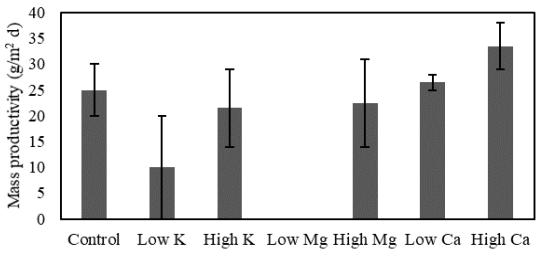

Figure 1. Effect of cationic ions concentration on mass productivity of red amaranth; Vertical bars indicate ± standard error from three replicates.

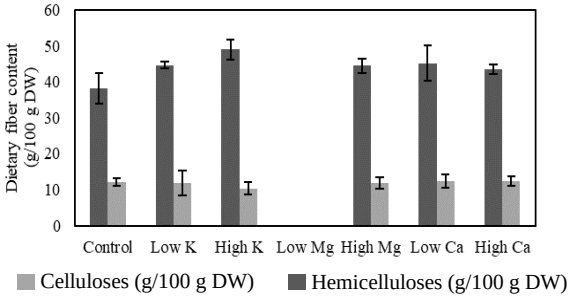

Figure 2. Effect of cationic ions concentration on dietary fiber content of red amaranth; Vertical bars indicate ± standard error from three replicates.

B. Antioxidant Activity (DPPH Assay)

The result (Fig. 3) suggests that different concentration of some cationic ions impacted antioxidants biosynthesis of red amaranth. The highest antioxidant activity (lowest IC50: 9.71 mg/mL) in plant extract was observed at high K treatment (372.5 ppm) whereas the low K treatment (28.5 ppm) resulted in the lowest activity (highest IC50: 15.42 mg/mL). This potentially relates to the fact where K^+ is the element involving the stimulation of enzymes in carbohydrate metabolism and thus helps encouraging antioxidant biosynthesis [10], [23], [29]. Hence, high performance of scavenge free radicals in red amaranth grown under high K concentration was a consequence. In contrary, the negative trend of inhibition of radicals were observed under Ca treatment as the antioxidant activity lowered with increasing Ca concentration. This is in accordance with the research study by Paiva *et al.* [34] where increasing Ca caused the decrease in lycopene content, a major antioxidant compound in tomato. However, in current study, high Mg, low and high Ca proportion did not affect significantly the antioxidant capacity when compared with the control treatment.

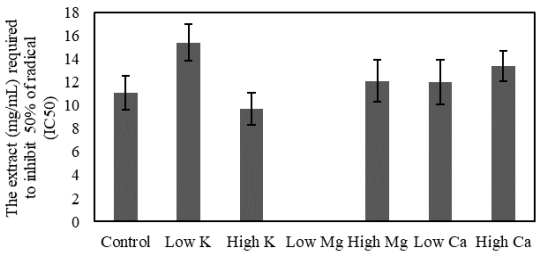

Figure 3. Effect of cationic ions concentration on antioxidant activity (IC50) of red amaranth; Vertical bars indicate ± standard error from three replicates.

C. Total Phenolic Content and Its Productivity

Phenolic compounds were determined as a representative of antioxidant compounds in this work since they are generally the main group of antioxidants in fruits and vegetables, including red amaranth [35]. The great antioxidant activity (the lowest IC50) obtained from the higher K treatment which could strongly be related to the positive effect of K on phenolic biosynthesis. Here, the total phenolic content per gram of dry matter was the highest (79.65 mg GAE/g DW) when treated with high K proportion (Fig. 4.). On the other hand, the effect of Ca concentration on total phenolic content was found to be negative. However, the unappropriated Mg vary (too low concentration) made us miss to study the effect of Mg concentration. Several reliable studies, however, have indicated that antioxidant accumulation in plants were increased under Mg^{2+} deficiency [36]-[38]. Although a high Ca treatment often led to low phenolic content in the final biomass (mg GAE/g DW), Ca helped enhance the mass productivity leading to the enhancement of total phenolic productivity. This work could preliminarily suggest that the high Ca concentration could be one good option for an antioxidant-rich red amaranth production as the highest total phenolic productivity (263.00 mg GAE/m^2 day) was observed (Fig. 5.).

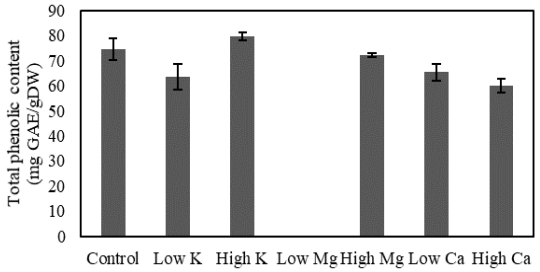

Figure 4. Effect of cationic ions concentration on total phenolic content of red amaranth; Vertical bars indicate ± standard error from three replicates.

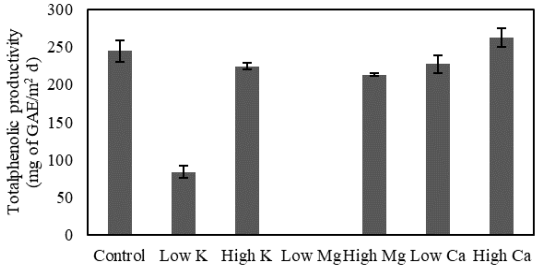

Figure 5. Effect of cationic ions concentration on total phenolic productivity of red amaranth; Vertical bars indicate ± standard error from three replicates.

IV. Conclusions

In summary, it could be suggested that the concentration of cationic ions (K, Mg and Ca) in fertilizer significantly affected the performance of antioxidant production and original qualities (mass yield and dietary fiber content) of *Amaranth tricolor* L. The potassium concentration was directly related to the level of antioxidant activity as well as antioxidants biosynthesis, especially phenolic compounds. Furthermore, significant yield increased with increasing proportion of Ca in fertilizer. The results from this work can be further employed to adjust the liquid fertilizing recipe to reach the target quality of *Amaranth tricolor* L. as needed.

Acknowledgment

The fund for this research was provided by Vidyasirimedhi Institute of Science and Technology, Thailand.

References

[1] G. K. O'Brien and D. M. L. Price, *AMARANTH Grain & Vegetable Types*, ECHO, 1983.
[2] B. E. Reid, "A translation of an ancient Chinese book on edible wild foods," Southern Materials Centre, 1977.
[3] L. Khandaker, A. S. M. G. M. Ali, M. B. Ali, and S. Oba, "Biomass yield and accumulations of bioactive compounds in red amaranth (Amaranthus tricolor L.) grown under different colored shade polyethylene in spring season," *Scientia Horticulturae*, 2010.
[4] L. Khandaker, M. B. Ali, and S. Oba, "Total polyphenol and antioxidant activity of red amaranth (Amaranthus tricolor L.) as affected by different sunlight level," *Japan Soc. Hort. Sci.*, vol. 77, no. 4, 2008.
[5] L. Khandaker, M. B. Ali, and S. Oba, "Influence of cultivar and growth stage on pigments and processing factors on Betacyanins in red amaranth (Amaranthus tricolor L.)," *Food Sci. Tech. Int.*, vol. 15, no. 3, 2009.
[6] P. Pasko, H. Barton, P. Zagrodzki, S. Gorinstein, M. Fołta, and Z. Zachwiej, "Anthocyanins, total polyphenols and antioxidant activity in amaranth and quinoa seeds and sprouts during their growth," *Food Chemistry*, vol. 115, 2009.
[7] J. Kanner, S. Harel, and R. Granit, "Betalainss- a new class of dietary cationized antioxidants," *Agric. Food Chem.*, vol. 49, 2001.
[8] Z. Yosefi, R. Tabaraki, H. A. A. Gharneh, and A. A. Mehrabi, "Variation in antioxidant activity, total phenolics, and nitrate in spinach," *Vegetable Science*, 2010.
[9] H. K. Weir, *et al.*, "The past, present, and future of cancer incidence in the United States: 1975 through 2020," The United States Cancer Statistics (USCS), 2015.
[10] M. G. Lefsrud, D. A. Kopsell, D. E. Kopsell, and J. Curran-Celentano, "Air temperature affects biomass and carotenoid pigment accumulation in kale and spinach grown in a controlled environment," HortScience, 2005.
[11] M. B. Ali, L. Khandaker, and S. Oba, "Changes in pigments, total polyphenol, antioxidant activity and color parameters of red and green edible amaranth leaves under different shade levels," *Food, Agriculture & Environment*, vol. 8, 2010.
[12] P. M. Nguyen and E. D. Niemeyer, "Effects of nitrogen fertilization on the phenolic composition and antioxidant properties of basil (Ocimum basilicum L.)," *Agric. Food Chem.*, vol. 56, 2008.
[13] S. Fanasca, *et al.*, "Changes in antioxidant content of tomato fruits in response to cultivar and nutrient solution composition," *Agric. Food Chem.*, vol. 54, 2006.
[14] U. Pérez-López, J. Miranda-Apodaca, A. Mu˜noz-Rueda, and A. Mena-Petite, "Lettuce production and antioxidant capacity are differentially modified by salt stress and light intensity under ambient and elevated CO_2," *Plant Physiology*, 2013.
[15] F. M. Oloyede, G. O. Agbage, E. M. Obuotor, and I. O. Obisesan, "Nutritional and antioxidant profiles of pumpkin (Cucurbita pepo Linn.) immature and mature fruits as influenced by NPK fertilizer," *Food Chemistry*, vol. 135, 2012.
[16] M. J. Ozbun and J. L. Orbun, "Influence of potassium on carotenoid content of tomato fruit," *American Society for Hort. Sciences*, vol. 96, no. 6, 1971.
[17] S. Parks and C. Murray, "Leafy Asian vegetables and their nutrition in hydroponics," *Industry and Investment*, 2011.
[18] S. Tonprasert. Total Nitrogen determination. [Online]. Available: http://9somkid.com/labtest/TN.html

[19] E. M. Kwee and E. D. Niemeyer, "Variations in phenolic composition and antioxidant properties among 15 basil (Ocimum basilicum L.) cultivars," *Food Chemistry*, vol. 28, 2011.
[20] C. M. G. C. Renard, "Variability in cell wall preparations: Quantification and comparison of common methods," *Carbohydrate Polymers*, vol. 60, 2005.
[21] H. K. Goering and P. J. V. Soest, "Forage fiber analyses," *Agriculture Handbook*, vol. 379, 1970.
[22] A. O. Company, Calcium (Ca): Essential for Plant Growth and Development, 2015.
[23] P. K. Hepler, "Calcium: A central regulator of plant growth and development," *Plant Cell*, vol. 17, no. 8, 2005.
[24] B. W. Poovaiah and A. C. Leopold, "Inhibition of abscission by calcium," *Plant Physiol.*, vol. 51, 1973.
[25] B. W. Poovaiah and A. C. Leopold, "Deferral of leaf senescence with calcium," *Plant Physiol.*, vol. 52, no. 3, 1973.
[26] S. Patterson and M. Gardener, "Fixing magnesium deficiency in plants: How magnesium affects plant growth," *Soil, Fixes & Fertilizer*, 2018.
[27] *Functions of Potassium in Plants*, Better Crops, vol. 82, 1998.
[28] W. T. Pettigrew, "Potassium influences on yield and quality production for maize, wheat, soybean and cotton," *Physiologia Plantarum*, vol. 133, 2007.
[29] E. G. Mulder, "Nitrogen-Magnesium relationships in crop plants," *Plant and Soil*, vol. 7, 1956.
[30] M. Senbayram, H. Thiel, and A. Gransee, "Role of magnesium fertilisers in agriculture: Plant-soil continuum," *Crop and Pasture Science*, 2015.
[31] K. L. Kabu and E. W. Toop, "Influence of potassium. Magnesium: Antagonism on tomato plant growth," *Can. J. Plant Sci*., vol. 50, 1970.
[32] P. Adams and L. C. Ho, "Effects of environment on the uptake and distribution of calcium in tomato and on the incidence of blossom-end rot," *Plant and Soil*, vol. 154, 1993.
[33] K. Prajapati, "The importance of potassium in plant growth – A review," *Plant Sciences*, vol. 1, 2012.
[34] E. A. S. Paiva, R. A. Sampaio, and H. E. P. Martinez, "Composition and quality of tomato fruit cultivated in nutrient solutions containing different calcium concentrations," *Plant Nutrition*, vol. 21, 1998.
[35] N. Balasundram, K. Sundram, and S. Samman, "Phenolic compounds in plants and agri-industrial by-products: Antioxidant activity, occurrence, and potential uses," *Food Chemistry*, vol. 99, 2006.
[36] B. Sinilal, *et al.*, "Increased accumulation and decreased catabolism of anthocyanins in red grape cell suspension culture following magnesium treatment," *Planta*, vol. 234, 2011.
[37] J. Gershenzon, "Changes in the levels of plant secondary metabolites under water and nutrient stress," *Phytochemical Adaptations to Stress*, 1984.
[38] W. Guo, H. Nazim, Z. Liang, and D. Yang, "Magnesium deficiency in plants: An urgent problem," *The Crop*, vol. 4, 2016.

Sopanat Sawatdee was born on 19th February 1993 in Rayong, Eastern of Thailand. She got her bachelor's degree in Chemical Engineering from King Mongkut's University of Technology Thonburi, Thailand. She is currently studying in the 5-year program of Doctor of Philosophy in School of Energy Science and Engineering at Vidyasirimedhi Institute of Science and Technology, Rayong, Thailand. All support in the institute are provided by PTT Public Company Limited with its intention to increase country's competitiveness and foster the best people in science and technology.