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Effect of abiotic stress on carotenoids accumulation in pumpkin plants under light and dark conditions

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Abstract

In nature, environmental factors highly influence the carotenoid composition in pumpkin plants and these factors were difficult to control; thus, carotenoid content is varied quantitatively and qualitatively. However, certain parameters can be controlled and this can be conducted in the laboratory through biogenesis manipulation. This approach uses environmental stress as a tool to alter the carotenoid pathway in the plants. The main objective of this study was to observe the inhibiting and enhancing effect of abiotic stress on individual carotenoid accumulation in pumpkin plants under light and dark conditions. The abiotic stresses used were plant elicitors which consisted of Ultra Violet light exposure, Polyethylene Glycol 4000, Salicylic Acid, and half strength nutrients using Murashige and Skoog Salt. After two weeks of treatments, the pumpkin leaves and stems were harvested, freeze dried and extracted to determine the carotenoids compound using High-Performance Liquid Chromatography (HPLC). Results showed that there was a significant difference ($p<0.005$) of lutein content (2.668 + 0.565 µg/g) in pumpkin leaves once exposed to Ultra Violet light with the absence of β-carotene compared to the control treatment; lutein (3.119 ± 0.210 µg/g) and β-carotene (0.838 + 0.05 µg/g). There were significant differences of carotenoids content under dark condition with the value of lutein at 0.472 + 0.008 µg/g to 1.247 + 0.047 µg/g and β-carotene from not detected to 1.360 + 0.003. The highest amount of lutein in pumpkin stem was detected in the Salicylic acid treatment under light condition (0.930 + 0.101 µg/g) and the highest amount of β-carotene in the pumpkin stem was detected in the Salicylic acid treatment under dark condition (0.234 + 0.018 µg/g). The carotenoid content varied in each treatment due to the adaptation of pumpkin plants with abiotic stress induced to them.

Introduction

Carotenoids are accumulated in most plant organs and they are lipophilic secondary metabolites, derived from the isoprenoid pathway. In plants, carotenoids are synthesised and localised in the plastids. Plastids may differentiate into several forms, which normally consist of chloroplasts, chromoplasts, gerontoplasts, and leucoplasts. Chloroplasts store carotenoids in thylakoid membranes while chromoplasts store high levels of carotenoids in membranes, oil bodies, or other crystalline structures within the stroma (Howitt and Pogson, 2006).

In pumpkin, carotenoids are synthesised in chloroplast and chromoplast (Nikola *et al*., 1991). Chloroplasts are organelles, specialised subunits, in plant and algal cells and their main role is to conduct the photosynthesis process, while chromoplast is heterogeneous organelles which are responsible for pigment synthesis and storage in specific photosynthetic eukaryotes (Whatley and Whatley, 1987). In chloroplasts, carotenoids constitute photosynthetic complexes in thylakoid membranes, where the formation of thylakoid membranes is believed to promote sequestration and storage of the synthesised carotenoids for a high level of accumulation in the chloroplasts. However, regulation of chromoplast biosynthesis plays a crucial role in controlling the carotenoid content in plants by enabling a great biosynthesis and high storage capacity (Lu and Li, 2008; Cazzonelli and Pogson, 2010; Ruiza-Sola and Rodriguez, 2012).

According to Shumskaya *et al.* (2012), phytoene synthase (PSY) isozymes exhibit differential plastid localisation, which could be linked to its activity in promoting carotenoid biosynthesis. Commonly, PSY

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is the major determinant for carotenoid content in plants, while other carotenoid biosynthetic enzymes would also become one of the factors that alter the carotenoids accumulation (Farre *et al.*, 2011). Apart from this, lycopene e-cyclase (LCY - e) plays a key role in controlling metabolic flux from one branch to another within the carotenoid biosynthetic pathway and was found to be a dominant regulator in the determination of the α-carotene and β-carotene ratio, especially in maize (Harjes *et al*., 2008; Bai *et al*., 2009).

Even though the individual biosynthetic enzymes are known, there is still a gap in the fundamental understanding of complexes and protein interactions involved in mediating the carotenoid biosynthesis. In previous studies, many researchers documented that the pathway of carotenoid biosynthesis was likely to function as multienzyme complexes to facilitate the metabolite channeling (Bonk *et al.*, 1996; Al- Babili *et al.*, 1996; Lopez *et al*., 2008). This indicates that the carotenoid biosynthesis process is a complex study and each process would require a multi-discipline research to investigate it in a holistic manner.

According to Biology online (2017), abiotic stress is defined as the negative impact of non-living factors on the living organisms in a specific environment; for example drought, high temperature, light, etc. In nature, environmental factors highly influence the carotenoid content and composition in pumpkin flesh and these factors are difficult to control, thus carotenoid content is varied quantitatively and qualitatively. However, certain parameters can be controlled and this can be conducted in the laboratory. Biogenesis manipulation is one of the new approaches to manipulate and produce the carotenoid compound. This approach uses abiotic stress as a tool to alter the carotenoid pathway in the plants or calluses. Thus, specific carotenoid can be produced either through enhancing or inhibiting the effects caused by the treatments.

Previous studies of abiotic stress effect on carotenoids accumulation were documented; for example, light exposure to Agria and Desiree minitubers led to the elevation of total and individual carotenoids up to 2-fold higher compared to the total and individual carotenoids produced by dark treatment, except for violaxanthin and treatment with Polyethylene glycol 4000 which were found to increase the total carotenoid content with higher amount of violaxanthin and total absence of zeaxanthin (Rashidi, 2009). He also found that nutrient strength influenced the development of carotenoid content in Agria minituber. From his observation, when Murashige Skoog salt of different strengths from 0.1 to 0.5 times lower were applied, the total carotenoid content, violaxanthin and β-carotene contents were decreased, while lutein concentration was increased. However, when MS0 salt of different strengths from 0.5 to 1.0 times higher were applied, the total carotenoid content of violaxanthin and β-carotene was increased, whereas lutein concentration was decreased. Gao *et al.* (2012) reported the treatment of 25 and 50 mg/L of Salicylic acid stimulated astaxanthin accumulation in *H. pluvialis*, while Vidhyavathi and Sarada (2011) reported that Salicylic acid treatment on *H. pluvialis* increased the proportion of astaxanthin which was 6.8-fold higher than the control treatment and high concentration of SA (500 lM) inhibited astaxanthin accumulation completely.

From the previous studies, carotenoids accumulation in plants and microorganism was documented. However, there were limited studies on carotenoids accumulation in pumpkin plants found. Thus, in this study, abiotic stress created using plant elicitors under light and dark conditions was applied on pumpkin plants and its effect on carotenoid accumulation was observed.

Materials and methods

Pumpkin tissue culture

Media of tissue culture was prepared as follows; 4.4 g of MS0 salt was weighed in a weighing boat and 1 litre of distilled water was mixed in a Schott bottle. The pH of the mixture was adjusted to 5.6 – 5.8. After the adjusted pH was obtained, 30 g of sucrose was added and stirred until the solution became homogenous. After that, 3.5 g of gelrite was added into the media. Then, the mixture was sterilised in the autoclave machine (15 minutes, 121^{o}C). The sterilised agar was poured in glass jam jars and was left in laminar air flow until the agar became hard.

By conducting the sterilisation technique to culture the pumpkin seeds, the procedure began with washing the pumpkin seeds with sterile distilled water until the seeds were clean. Then, the pumpkin seeds were washed with 70% alcohol solutions for three to five minutes and re-washed with distilled water for three times. By using a sterilised spatula, the seeds (five to 10 seeds/bottle) were placed on the prepared agar in the glass jam jars; the glass jam jars were covered, sealed with parafilm and placed on a rack in the culture room under a lamp to provide them with light. The culture room temperature was 20°C and monitored day and night. The pumpkin seeds culture was observed daily to ensure that there was no contamination during the culture period.

After two to three weeks, the pumpkin plants were ready for further treatments using Ultra visible (UV) light exposure, Polyethylene glycol, Salicylic acid and half strength of nutrients. Media for each treatment was prepared in a sterile tube. The pumpkin plants were transferred to the media and were observed after two weeks. The plant elicitors used for the pumpkin plants were MS30: 4.4 g/l (Control), Salicylic acid: 0.1 g/l (SA), exposure to UV light for 30 minutes daily for two weeks. (UV), Polyethylene glycol: 5 g/l (PEG) and Half strength: MS15, 2.2 g/l (HS). Each treatment was conducted under light versus darkness by incubation under cool-white, fluorescent lamps (80-85 µmol m^{-2} s^{-1}; 24 hours photoperiod) and with a dark condition imposed by carefully covering the pumpkin plant and callus in a sealed card box. After the treatment period for two weeks, the pumpkin plants were observed, harvested, and extracted for carotenoid analysis. The experimental design is shown in Table 1.

Table 1. Experimental Design of Effects of Abiotic Stress on Carotenoids Accumulation in Pumpkin Plants

Treatments	Amount
Ultra Visible Light Exposure (UV)	30 minutes daily
Polyethylene Glycol (PEG)	5 g/l
Salicylic Acid (SA)	0.1 g/l
Half Strenght Nutrient (HS)	2.2 g/l
Full Strenght (Control)	4.4 g/l

Each treatments were placed under light and dark conditions except for treatment of UV light exposure. UV light treatment was conducted under light condition with 30 minutes exposure to UV light daily for two weeks.

Carotenoid extraction

The extraction procedure described by Rashidi Othman (2009) was followed with slight modifications. 1 g of powdered freeze-dried pumpkin sample was weighed and mixed with 50 ml of acetone and methanol mixture (7:3, v/v) in a centrifuge tube. To allow efficient solvent penetration, the mixture was vortexed and allowed to stand overnight in darkness at room temperature. The next day, the samples were re-vortexed and centrifuged for 15 minutes at 13 500 g (Sorvall Biofuge Primo R, Thermo Scientific). Then, the supernatant was transferred to a new 50 ml centrifuge tube. This procedure was repeated for three times until the tissue turned colourless. Extraction was continued by adding an equal volume of 15 ml of hexane and 15 ml of distilled water to the combined supernatants. The solution was then centrifuged for 15 minutes at 13 500 g to allow better separation. The upper hexane layer containing the carotenoids was collected and dried under nitrogen gas. Vials/tubes were then capped and sealed with parafilm to exclude oxygen and immediately stored at -20°C for further analysis.

Saponification

Saponification was done as described by Rashidi Othman (2009) with slight modifications. This step was done to eliminate chlorophylls in the stems and leaves of the pumpkin plants as well as in the callus. The carotenoid extract which was dried after the extraction process was then subjected to certain steps. 20 µl of acetate was added to the samples, followed with 380 µl acetonitrile: distilled water (9:1 v/v). Then, 400 µl methanolic potassium hydroxide solution (10% w/v) was added. Base carotenoids were then extracted by adding 2 ml hexane with 0.1% butylated hydroxytoluene (BHT), followed with 10% of sodium chloride (NaCl). Finally, the extracts were washed with distilled water, the upper layer was collected and dried under a gentle stream of oxygen-free nitrogen and resuspended in ethyl acetate for HPLC analysis.

Identification of carotenoid using High-Performance Liquid Chromatography

The HPLC analysis of carotenoids was performed using Agilent model 2100 series which comprises of a binary pump with an auto sampler injector, micro vacuum degassers, thermostat column compartment and a diode array detector. The column used was an HPLC column: ZORBAX Eclipse XDB-C18, analytical 4.6 x 150 nm (5 microns) end capped 5 µm. The solvents used were (A) acetonitrile: water (9:1 v/v) and (B) ethyl acetate. The solvent gradient used was developed as follows: 0-40% solvent B (0-20 mins), 40-60% solvent B (20-25 mins), 60-100% solvent B (25-25.1 mins), 100% solvent B (25.1-35 mins) and 100-0% solvent B (35-35.1 mins) at a flow rate of 1.0 ml min^{-1}. The temperature of the column was maintained at 20ºC. The injection volume was 10 µl. Carotenoid standards of β-carotene, α-carotene and lutein were obtained commercially from Sigma-Aldrich. Detection of carotenoid peaks was in the range of 350 to 550 nm. Individual carotenoid concentrations were calculated by comparing their relative proportions, as reflected by the integrated HPLC peak areas.

Statistical analysis

The statistical analysis was conducted using SPSS. All the data were reported as mean ± standard deviation using the One-Way ANOVA method. The significant difference among the samples

was compared using Duncan multiple tests at the significance level of $p<0.05$.

Results and discussion

Carotenoid profile in pumpkin plant

The carotenoid content in different pumpkin parts is shown in Table 2. From the results obtained, there were two types of individual carotenoid detected in different parts of the pumpkin plant: lutein and β-carotene. Analysis of variance ($p < 0.05$) showed that there was a significant difference in carotenoid content in different parts of the pumpkin plants. In pumpkin stems, lutein was the major carotenoid with the amount of 0.834 µg/g, followed with 0.344 µg/g of β-carotene compound. In contrast, for pumpkin leaves, the dominant carotenoid was lutein with the amount of 4.201 µg/g, which was five times higher than the amount detected in pumpkin stems. Meanwhile, β-carotene amount detected was 0.793 µg/g, which was two times higher than the pumpkin stems. However, a small amount of β-carotene (0.266 µg/g) was detected in pumpkin seeds and a very small trace amount of lutein (0.025 µg/g) was detected in pumpkin roots.

The results showed that carotenoid compounds were dominantly synthesised and accumulated in pumpkin leaves followed by pumpkin stems, as in line with a study conducted by Nikola *et al*. (1991), which showed that carotenoid compounds were synthesised in the chloroplast and chromoplast of pumpkin leaves. According to Li and Hui (2013), chromoplasts, which are commonly located in vegetable leaves, contain a lot of carotenoids and are associated with the red, orange, and yellow colours in flowers, fruits and roots. Besides, previous studies conducted by Marcela and Rodguirez-Amaya (2003); Liu *et al.* (2007); and Dias *et al.* (2009) found that the prominent carotenoid found in green leafy vegetables was lutein, similar to this study.

Effect of abiotic stress on pumpkin plants under light and dark conditions

Table 3 shows carotenoid content in pumpkin leaves and stems in five treatments of abiotic stress under light and dark conditions. Under light condition, the highest lutein content was detected in the control treatment (3.119 µg/g), followed by ultra violet exposure (2.668 µg/g), half strength treatment (1.1111 µg/g), polyethylene glycol treatment (0.532 µg/g) and salicylic acid treatment (0.283 µg/g). β-carotene content was detected only in the control treatment (0.838 µg/g).

Meanwhile, in dark treatments, the highest content of lutein was detected in Salicylic acid treatment (1.247 µg/g), followed by Polyethylene glycol (0.917 µg/g) and Half strength (0.771 µg/g) treatment. However, β-carotene was only detected in Salicylic acid treatment (1.360 µg/g) and Half strength treatment (0.701 µg/g).

Table 2. Carotenoid content in different part of pumpkin plant (µg/g)

Carotenoid	β- carotene	lutein
Stems	$0.344^{a} \pm 0.01$	$0.834^{a} \pm 0.05$
leaves	$0.793^{b} \pm 0.08$	$4.201^{b} \pm 0.50$
Seeds	$0.266^{a} \pm 0.02$	ND
Roots	ND	$0.025^{c} \pm 0.00$

Means (n=6) in the same column with different superscript are significantly ($p<0.05$) different
ND: Not Detected

For the carotenoid content in pumpkin stems, the highest lutein content was detected in half strength treatment under light condition (0.184 + 0.101 µg/g) and the highest lutein content in dark treatment was found in Salicylic Acid treatment (0.182 + 0.004 µg/g). However, β-carotene was only detected in half strength treatment (0.234 + 0.018 µg/g).

From this study, the amount of carotenoids was lower in most cases compared to the control experiments. This was due to the enhancing and inhibiting effects of abiotic stress on individual carotenoids that existed in the plants. In control treatments, all nutrients including light were provided to ensure the optimum growth of the plants. However, in abiotic stress treatments, pumpkin plants were put under stress conditions which led to the enhancing and inhibiting of individual carotenoids in the plants, and in most cases, abiotic stress decreased the carotenoids content in all treatments with the enhancing and inhibiting effects of individual carotenoids that existed in the plants (lutein and β-carotene).

For examples, as recorded from previous studies, photosynthetic pigments which consisted of chlorophyll a, b and carotenoids and mineral content decreased with the increasing of Polyethylene Glycol 6000 concentration in Apple geranium (*Pelargonium odoratissimum* L.) (Khalid *et al.*, 2010); methyl jasmonate totally inhibited lycopene accumulation and stimulated β-carotene accumulation in ripened tomatoes *(Solanum lycopersicum)* (Saniewski and Czapski, 1983); Salicylic Acid decreased the carotenoid content in barley plants (*Hordeum vulgare* L.)(Khalaf and Fayez, 2013); and 25 and 50 mg/L of Salicylic Acid stimulated astaxanthin accumulation

Table 3. Effect of abiotic stress on carotenoid content in pumpkin leaves under light condition

	Leaves				Stems			
	Light		Dark		Light		Dark	
Plant elicitors	Lutein (ug/g)	Beta Carotene (ug/g)	Lutein (ug/g)	Beta Carotene (ug/g)	Lutein (ug/g)	Beta Carotene (ug/g)	Lutein (ug/g)	Beta Carotene (ug/g)
Murashige Skoog Salt (Control)	$3.119^{a} \pm 0.210$	0.838 ± 0.05	$0.472^{a} \pm 0.008$	ND	$0.096^{a} \pm 0.002$	ND	$0.135^{a} \pm 0.008$	ND
Ultra Violet Exposure (UV)	$2.668^{b} \pm 0.565$	ND	NA	NA	$0.153^{b} \pm 0.003$	ND	NA	NA
Polyethylene Glycol (PEG)	$0.532^{c} \pm 0.017$	ND	$0.917^{b} \pm 0.072$	ND	$0.099^{a} \pm 0.005$	ND	$0.182^{a} + 0.004$	ND
Salicylic Acid (SA)	$0.283^{d} \pm 0.038$	ND	$1.247^{c} \pm 0.047$	$1.360^{a} \pm 0.003$	$0.930^{c} \pm 0.101$	ND	$0.221^{b} + 0.010$	ND
Half Strength (HS)	$1.111^{a} \pm 0.033$	ND	$0.771^{a} \pm 0.129$	$0.701^{b} \pm 0.039$	$0.184^{b} \pm 0.101$	ND	$0.077^{c} + 0.004$	$0.234 + 0.018$

Means (n=6) in the same column with different superscript are significantly ($p<0.05$) different
ND: Not Detected
NA: Not Applicable

in Green alga *(Haematococcus pluvialis)* (Gao *et al.*, 2012).

Effect of ultra violet exposure treatment in pumpkin plants

In the Ultra Violet exposure treatment, lutein was the only carotenoid compound detected in pumpkin leaves and stems, and the amount of this compound was significantly higher compared to the control treatment. Scientifically, lutein compound serves as a barrier to protect the photosynthetic apparatus from photodestruction and perhaps functions as additional antennae to trap light energy of a shorter wavelength (UV) than those absorbed by the chlorophylls (visible light). Exposure to UV light will trigger the carotenoid compound in plants to act as photoprotection by protecting the chlorophyll pigments from any harmful effects caused by the photodynamic reactions (Juan *et al.*, 2007). The accessory of light-harvesting pigments which consist of carotenoid will transfer the light energy to the chlorophylls in the light-harvesting complexes which will then be transferred to the photosynthetic reaction centre for photosynthesis process (Richard *et al.*, 1994; Thorsten *et al.*, 2000).

In the light harvesting complex (LHC-II) of green plants, 30% of the total protein present in the chloroplast membranes is a trimeric protein and each monomer contains 14 chlorophyll and four carotenoid molecules of two lutein, one violaxanthin and one neoxanthin. As lutein compound was the dominant carotenoid in the chloroplast membrane, this is compound prominently present as individual carotenoid, even though it is exposed to the environmental stress created by the UV light exposure (Peter and Thornber, 1991; Liu *et al.*, 2004; Standfuss *et al*., 2005)

From this treatment, carotenoids present in pumpkin leaves and stems were manipulated. Dominant carotenoid which was lutein compound was left remained, while the other types of carotenoids were inhibited and suppressed, either by inhibition of certain enzymes responsible for the carotenoid biosynthesis or retardation of the carotenoid compound itself. However, the lutein compound in pumpkin leaves under dark condition treatment was decreased and this was in line with the decrease of photosynthesis process in plants. This theory was not applicable to the pumpkin stems as photosynthesis reactions mostly take place in the leaves. Thus, the amount of lutein compound in pumpkin stems under light and UV exposure treatment was left remained and increased.

Effect of polyethylene glycol treatment in pumpkin plants

Under drought stress condition using PEG plant elicitors, lutein compound was decreased to be six times lower than the control (MS30) treatment under light condition. While under dark condition, lutein compound in PEG treatment was slightly higher compared to the control (MS30) treatment under the same condition. The same result was also reported by El-Houssein *et al.* (1997) and Khalid *et al*. (2010). They reported that carotenoid content was decreased in their samples when PEG 4000 was applied.

Abiotic stress such as drought stresses, insufficient light and insufficient nutrient provided to the plants would lead to abscisic acid (ABA) synthesis, a phytohormone that modulates developmental and stress processes (Koorneef, 1986; Pfander and Packer, 1992). Under drought stress, plant roots will synthesise abscisic acid (ABA) and transport it into the shoots. According to Ingram and Bartels (1996),

ABA will become an essential mediator in triggering plant responses, especially carotenoid biosynthesis to overcome the adverse environmental effect; thus reflecting the accumulation of carotenoid content in pumpkin plants.

According to Seo and Koshiba (2002), ABA which is synthesised in the plastidial 2-C-methyl-D-erythritol-4-phosphate (MEP) pathway is an isoprenoid plant hormone. The C15 backbone of ABA is formed after the cleavage of C40 carotenoids in MEP. Zeaxanthin is the first to be committed to the ABA precursor. It is involved in a series of enzyme-catalysed epoxidations and isomerisations through violaxanthin and final cleavage of the C40 carotenoid by a deoxygenation reaction, which is then further oxidised to ABA.

Lutein was synthesised from an α-carotene compound which was derived from a different pathway of β-carotene, and zeaxanthin compound which was involved in the ABA synthesis was derived from the hydrolysis process of β-carotene (Figure 1). From this series of carotenoid biosynthesis pathway, it would best explain that there is a probability that this compound might undergo certain reactions in response to drought stress, thus reflecting the absence of β-carotene compound in pumpkin plants under drought stress condition.

In this study, pumpkin stems treated with PEG under dark condition had an increase in the production of lutein compound. Even though they showed an increasing amount of lutein compound, a better yield of this compound was still provided by pumpkin leaves, both under light and dark conditions.

Effect of salicylic acid treatment in pumpkin plants

In pumpkin leaves treated with SA under the light condition, the individual carotenoid detected was lutein compound. The amount of this compound was 11 times lower than the (MS30) control treatment, while β-carotene was not detected. The same finding was also reported by Moharekar (2003) and Khalaf and Fayez (2013) for their samples. However, the result obtained by SA treatment under dark condition was slightly different. The amount of lutein compound detected was three times higher compared to the (MS30) control treatment and β-carotene compounds were slightly consistent with the control (MS30) treatment.

A different pattern was observed in the pumpkin stem. Treatment with SA was likely to enhance lutein compound by 10 times higher than the control (MS30) treatment under light condition. While under dark condition, the lutein compound was two times higher than the control (MS30) treatment. According to

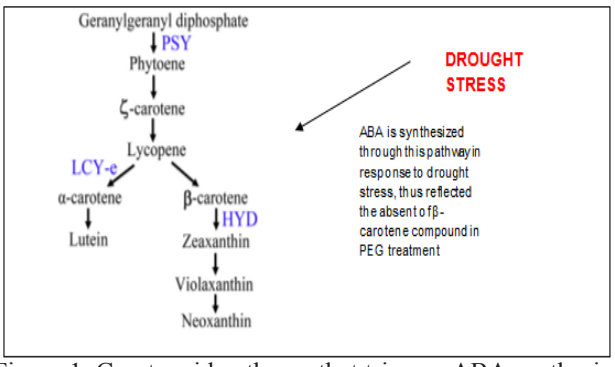

Figure 1. Carotenoid pathway that triggers ABA synthesis

Munné-Bosch and Lalueza (2007), SA is a hormone-like substance that helps the plants to be more resistant to pathogens and also participates in the plant response to adverse environmental conditions. From the result obtained in this study, abiotic stress caused by salicylic acid in dark condition had a positive impact on carotenoid biosynthesis, especially in lutein accumulation. However, in the presence of light, negative effect on carotenoid accumulation in pumpkin leaves was observed in this treatment. Based on the overall observations, we could assume that there were many possibilities that contributed to the carotenoid accumulation in pumpkin leaves under SA treatments.

Abiotic stress caused by SA in dark condition positively affected the lutein accumulation. Abiotic stress will alter the gene expression and trigger cellular metabolism in plants (Buchanan *et al*., 2000). The stress recognition then may activate the signal transduction pathways that transmit information within the individual cell and throughout the plant. This may induce changes in the gene expression that modifies the growth and development of the plants and also influences the carotenoid biosynthesis and accumulation (Rashidi Othman, 2009). In short, we could say that a stress recognised by SA in light and dark conditions will trigger and alter the cellular metabolism in plants, thus reflecting the secondary metabolite produced by the plants. However, the resistance or sensitivity of plants to stress depends on the species, genotype and development age and further study should be conducted to confirm this hypothesis.

Effect of half strength treatment in pumpkin plants

The amount of lutein compound in pumpkin leaves with HS treatment under light condition was two times lower than the control (MS30) treatment. Different to the dark condition, consistent amounts of lutein and β-carotene were detected. Meanwhile, lutein compound was detected in the control (MS30) treatment under the same condition with the absence

of β-carotene compound. This is in line with the study conducted by Rashidi Othman (2009); when half strength of MS30 salt was applied, the total carotenoid content was decreased due to insufficient nutrient provided.

For pumpkin leaves under dark condition, lutein compound was slightly higher than the control (MS30) treatment, whereas the β-carotene compound was only detected in HS treatment and this compound was absent in the control (MS30) treatment. Scientifically, each nutrient and light intensity provided to the pumpkin plants would affect the carotenoid biosynthesis and accumulation of this compound would be varied quantitatively and qualitatively. However, from the result observed, it seemed to indicate that the absence of light would increase the lutein compound in pumpkin leaves, but the synthesis of β-carotene could not occur without the presence of light.

Conclusion

The objective of this study was to observe the inhibiting and enhancing effects of abiotic stress on individual carotenoids accumulation in pumpkin plants under light and dark conditions. Under abiotic stress, pumpkin plants were forced to adapt to the stress by altering the gene expression and by triggering the cellular metabolism in them. Through this adaptation, the secondary metabolite including carotenoid was also affected. The effect would be either positive or negative. Among the treatments, lutein compound in pumpkin leaves could be enhanced using the control (MS30) treatment under light condition and this compound could be manipulated and produced as a single carotenoid in pumpkin leaves by exposing the plants for 30 minutes daily under UV light for two weeks. The β-carotene compound could be enhanced in the pumpkin leaves by using SA under dark condition. Inhibiting effects of lutein and β-carotene in pumpkin leaves with SA and PEG treatments under light condition was observed, as well as in the control (MS30) treatment under dark condition.

From this study, we can conclude that all treatments involved were not suitable to increase the carotenoids content. However, there were enhancing and inhibiting effects in each treatment. To identify the most suitable condition to enhance the individual carotenoids (lutein or beta-carotene), further study should be focused on the suitable ratio of plant elicitors used in each treatment.

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