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Preharvest application of abscisic acid promotes anthocyanins accumulation in pericarp of litchi fruit without adversely affecting postharvest quality



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ABSTRACT

Pericarp colour of litchi fruit is an important quality attribute that determines its market value and consumer acceptance. Plant growth regulators (PGR) such as abscisic acid (ABA) and ethephon are known to play important roles in peel colour development during maturation and ripening of non-climacteric fruits (e.g. grape and litchi). Our aim was to investigate the effects of preharvest application of ABA, ethephon and their combination on pericarp colour and fruit quality of litchi (cv. Calcuttia) and also to assess the potential effects on postharvest performance of fruit. Exogenous application of ABA (150 or 300 mg L⁻¹) at the colour-break stage significantly increased the concentration of total anthocyanins and cyanidin-3-O-rutinoside, the major anthocyanin contributing ~71–96% of the total anthocyanins, in litchi pericarp compared to ethephon (500 μL L⁻¹). Among different anthocyanins quantified, the relative contribution of cyanidin-3,5-diglucoside to the total anthocyanins was significantly higher in all PGR-treated fruit compared to the control, but the concentration of cyanidin-3-O-glucoside was specifically enhanced by ABA. No significant effect on the concentrations of epicatechin, and quercetin-3-O-rutinoside was observed in response to PGR treatments. Ethephon (500 μL L⁻¹) treatment did not significantly increase the anthocyanin levels in pericarp, but it caused more degradation of chlorophyll pigments than control. Aril quality with regard to firmness, soluble solids and acidity was not significantly affected by PGR treatments, except that ethephon-treated fruit showed significant softening and lower acidity. Postharvest changes in fruit quality attributes including pericarp browning during cold storage at 5 °C for 14 d were mainly related to the storage duration effect, rather than PGR treatment. In conclusion, ABA treatment (150 or 300 mg L⁻¹) at the colour-break stage enhanced anthocyanins accumulation in litchi pericarp without adversely affecting postharvest quality and storage stability for 14 d.

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1. Introduction

Pericarp colour is an important quality attribute of litchi fruit that determines its consumer acceptance (Sivakumar et al., 2007). Anthocyanins are a class of flavonoids responsible for red/pink colouration of litchi pericarp (Lee and Wicker, 1991). The biosynthesis and accumulation of anthocyanins in litchi pericarp are known to be influenced by extrinsic and intrinsic factors (Tyas et al., 1998; Underhill and Critchley, 1992; Wei et al., 2011). The uniformity and intensity of pericarp colour is dependent on genotype.

Chinese litchi cultivars have been classified into three categories: non-red, unevenly red, and evenly red cultivars (Wei et al., 2011). In addition to the genetic factor, the orchard and environmental conditions play important roles in the pericarp colour development of litchi. For instance, fruit in the inner and lower parts of tree canopy are reported not to achieve pericarp colour due to poor accumulation of anthocyanin pigments (Tyas et al., 1998). We have observed poor pericarp colouration affecting litchi fruit quality in the warm sub-tropical climate of North Indian plains, leading to economic losses to the fruit growers. This suggested a need for pragmatic preharvest intervention to improve pericarp colour in litchi fruit without affecting its postharvest longevity.

The roles of plant growth regulators (PGR) such as abscisic acid (ABA) and ethylene have been established in skin colour development during maturation and ripening of non-climacteric fruits

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such as grape (Coombe and Hale, 1973; El-Kereamy et al., 2003; Peppi et al., 2006; Wheeler et al., 2009) and litchi (Wang et al., 2007; Wei et al., 2011). The exogenous application of ABA (300 or 400 mg L⁻¹) at veraison stage increased skin anthocyanin concentration in 'Flame Seedless' (Peppi et al., 2006), 'Crimson Seedless' (Cantín et al., 2007) and 'Cabernet Sauvignon' (Wheeler et al., 2009) grapes. Similar to the role of ABA in berry colour development in grape, ABA (25 and 200 mg L⁻¹) application 3–4 weeks before anticipated harvest has been reported to enhance biosynthesis and accumulation of anthocyanins in the pericarp of litchi fruit (Wang et al., 2007; Wei et al., 2011). Higher level of endogenous ABA in litchi pericarp at colour-break stage has been associated with increased activity of chlorophyllase enzyme leading to chlorophyll degradation and enhanced accumulation of anthocyanins (Wang et al., 2007). The expression of the UDP glucose: flavonoid 3-O-glucosyltransferase (UFGT) gene, a key gene in the anthocyanin biosynthesis pathway, was upregulated by ABA treatment in litchi (Wei et al., 2011) and grapes (Peppi et al., 2008; Koyama et al., 2010).

Ethephon (2-chloroethylphosphonic acid), an ethylene-releasing compound, has been known to increase anthocyanin accumulation in coloured grape through upregulation of some key enzymes in the anthocyanin biosynthesis pathway (Coombe and Hale, 1973; El-Kereamy et al., 2003). Similarly, ethephon (800 µL L⁻¹) and its combination with ABA have also been reported to enhance pericarp colour development in litchi fruit (Wang et al., 2007). These studies have demonstrated that the exogenous applications of ABA alone or in combination with ethephon have potential to improve on-tree pericarp colouration in litchi fruit. The efficacy of foliar application of various PGRs in fruit trees is influenced by environmental conditions (Stover and Greene, 2005). Therefore, the effectiveness of these PGRs for favourable outcomes in litchi fruit needs to be investigated.

Anthocyanins are a class of polyphenolic compounds which include monoglucosides and diglucosides of cyanidin, delphinidin, petunidin, peonidin and malvidin along with the glucoside esters of acetic, coumaric and caffeic acid (Kuhn et al., 2014). Cyanidin-3-O-rutinoside has been reported to be the principal anthocyanin pigment contributing 67% to >95% of total anthocyanins in different litchi cultivars from China ('Meiguli', 'Bailla', 'Baitangying', 'Guiwei', 'Nuomici' and 'Guinuo'), Mexico ('Brewster'), Thailand ('Hong Huey', 'Chacapat' and 'Kom') and Israel ('Mauritius') (Lee and Wicker, 1991; Rivera-López et al., 1999; Zhang et al., 2005; Somboonkaew and Terry, 2010a; Reichel et al., 2011; Wei et al., 2011). Cyanidin-3-O-glucoside, malvinidin-3-O-glucoside and peonidin-3-O-rutinoside are the minor anthocyanins identified in these litchi cultivars. In addition to the cultivar factor, the concentration of individual anthocyanins also varies according to growing location, climatic conditions, and orchard practices (Mori et al., 2005; Peppi et al., 2006; Wheeler et al., 2009). Previous studies have shown the effect of exogenous application of ABA and ethephon on total anthocyanins in litchi pericarp (Wang et al., 2007; Wei et al., 2011), but the effects on the composition of anthocyanins have not yet been reported. The anthocyanin composition is important for fruit quality because individual anthocyanins have different characteristics with regard to colour and stability (Mori et al., 2005). We, therefore, hypothesized that PGR applications aimed to promote total anthocyanins accumulation may influence the relative concentration of individual anthocyanins. In addition to the quality and biochemical attributes related to the skin colour, the potential effects of preharvest treatments with PGR must be considered on postharvest quality and storage behaviour of fruit (Cantín et al., 2007). Variable effects of ABA and ethephon treatments on quality attributes such as firmness, soluble solids and acidity of grape berries have been reported (Mori et al., 2005; Peppi et al.,

2006). To the best of our knowledge, no information is currently available on the effects of exogenous applications of ABA and ethephon on the postharvest storage behaviour of litchi fruit. Therefore, our aim was to evaluate the effects of preharvest application of ABA and ethephon on fruit quality including the concentration and composition of anthocyanins in litchi pericarp and also to assess the impact of these treatments on postharvest performance of fruit.

2. Materials and methods

2.1. Plant material and PGRs application

Litchi (*Litchi chinensis* Sonn. cv. Calcuttia) trees were selected in a commercial orchard (Sekhon Farms) located at Langroya, Shaheed Bhagat Singh Nagar, Punjab (31.13° N; 76.12° E). Twenty year-old experimental trees were under integrated orchard management practices. Six trees were randomly selected for each treatment which involved 2 trees per replicate. Ten fruit clusters (10–15 fruit/cluster) in each tree were randomly tagged from all directions and upper and lower parts in tree canopy from each direction. All the tagged clusters on a tree received the same treatment. Five different PGRs treatments were applied on tagged fruit clusters: (i) 500 µL L⁻¹ ethephon (Ethrel® 39% active ingredient; Bayer CropScience Ltd., Chandigarh, India); (ii) 150 mg L⁻¹ ABA (10% active ingredient; Panpan Industry Co., Limited, Zhengzhou, Henan, China); (iii) 300 mg L⁻¹ ABA; (iv) 150 mg L⁻¹ ABA + 500 µL L⁻¹ ethephon; and a control treatment with water. Powdered ABA was first dissolved in a small amount of 70% ethanol, which was then diluted to a final volume with water. The aqueous solutions of PGRs containing 0.1% (v/v) Tween-20 as a surfactant were sprayed 4 weeks before anticipated harvest (colour break stage) in the morning hours. Fruit clusters were sprayed until runoff with a hand-held sprayer. The weather data for temperature and relative humidity (maximum and minimum) were recorded after PGR application until harvest using an automatic weather station installed by the India Meteorological Department at Krishi Vigyan Kendra, Langroya, about 250 m away from the experimental site.

2.2. Harvesting and postharvest storage

Litchi fruit were harvested at commercial maturity in the morning hours and transported to the laboratory within 2 h in an airconditioned vehicle maintained at 20 °C. The fruit were sorted for various defects such as cracking, bruising and sunburn. To reduce the fruit sample size for postharvest experiment, the healthy fruit (out of 20 clusters/replicate) from respective treatments were pooled into 6 clusters (20 fruit/cluster) per replicate per treatment. Three fruit clusters packed in each PrimePro® bag (Chantler Packaging Inc., ON, Canada) constituted an experimental unit and was replicated three times for each treatment including control. The packed fruit in corrugated fibre board boxes were stored in controlled environment chambers (Percival Scientific, Inc., IA, USA) maintained at dark, 5 °C, 90–95% RH for 14 d.

2.3. Fruit quality evaluation

Fruit were evaluated for quality attributes such as pericarp colour (L^* , a^* , b^* , and hue angle), flesh firmness, soluble solids concentration (SSC), titratable acidity (TA), SSC:TA ratio, and browning index (for postharvest storage experiment). In addition to these attributes, profiling of polyphenolic (anthocyanins, epicatechin and quercetin-3-O-rutinoside) and non-polyphenolic (chlorophyll a, chlorophyll b and pheophytin) compounds in

pericarp tissue was conducted for fruit samples at harvest. Three clusters (20 fruit/cluster) of fruit per replicate per treatment were transferred after 7 and 14 d of cold storage and were allowed to warm to ambient conditions (22–25 °C) before quality evaluation.

Measurements of pericarp colour, firmness, SSC and TA were conducted on randomly selected 20 fruit per replicate per treatment. Pericarp colour was measured at four different points around the equatorial region of each fruit using a colour metre (ColorFlex EZ, Hunter Associates Laboratory, Inc., USA) which provided CIE L^* , a^* and b^* values. The lightness coefficient ' L^* ', represents brightness and darkness, ' a^* ' value represents greenish and redness as the value increases from negative to positive, and ' b^* ' represents bluish and yellowish. These values were used to calculate hue angle degree [$h^\circ = \arctan(b^*/a^{*-1})$], where 0° = red-purple; 90° = yellow; 180° = bluish-green; and 270° = blue.

Aril firmness was measured using a texture analyser (TA-HD Plus; Stable Microsystems Ltd, Surrey, UK) interfaced to a computer with Exponent[®] software. A 2-mm thick probe, with a 1 kg load cell on, punctured the peeled fruit at a crosshead speed of 2 mm s^{-1} to 5 mm depth. Each fruit was punctured on both the sides at the equatorial region. The firmness was expressed as Newtons (N). SSC of the juice was measured using an Atago automatic digital refractometer (Rx 5000 iplus, Atago Co. Ltd, Tokyo, Japan). TA was determined by titrating juice using 0.01 N NaOH and expressed as % (w/v) malic acid. Pericarp browning was visually assessed and expressed as browning index calculated according to Zhang and Quantick (1997). The browning scale includes: 1 = no browning; 2 = 1–2 brown spots, acceptable marketability; 3 = some spots with browning, limited marketability; 4 = 50%; 5 = 75% and entire fruit surface brown.

2.4. Extraction, identification and quantification of polyphenolic compounds

2.4.1. Extraction of polyphenolic compounds

Polyphenols from pericarp (2.0 g) were extracted using 10 mL of a solvent containing methanol:HCl (97:3; v/v). The extract in an amber coloured centrifuge tube was placed at -20°C for 2 h for protein precipitation and then centrifuged ($10,000 \times g$) at 4°C for 15 min. The supernatant was filtered through a $0.2 \mu\text{m}$ PTFE syringe filter (Sigma–Aldrich India Pvt. Ltd., Bangalore, India) and collected in an amber coloured autosampler vial.

2.4.2. Identification and quantification of polyphenolic compounds using HPLC

The chromatographic separation, identification and quantification of anthocyanins (cyanidin-3-*O*-rutinoside, cyanidin-3-*O*-glucoside, cyanidin-3,5-*O*-diglucoside, and cyanidin-3-*O*-galactoside), flavanol (epicatechin) and flavonol (quercetin-3-*O*-rutinoside) was performed on an Agilent HPLC (Infinity 1260; Agilent Technologies India Pvt. Ltd., Chandigarh, India) equipped with a Zorbax SB-C18 column ($4.6 \text{ mm} \times 150 \text{ mm} \times 3.5 \mu\text{m}$) preceded by a guard column of the same stationary phase maintained at 35°C . The injection volume was $10 \mu\text{L}$. Solvents used for elution were MQ water (A), methanol (B) and ethyl acetate (C). Elution was achieved in 35 min using the following gradient: 90% A and 10% B as the initial condition, then a linear gradient to 10% A, 40% B and 50% C in 15 min, which was held for 15 min. The system was returned to initial conditions during 30–31 min and equilibrated from 31 to 35 min before the next injection. A diode array detector (DAD) was used for detection of anthocyanins, epicatechin and quercetin-3-*O*-rutinoside at 530, 280 and 350 nm, respectively. The compounds were identified by comparing their retention times with those of authentic standards and co-chromatography approach. The quantification was

based on five-point calibration curves of respective standards using Agilent's EZ Chrom Software.

2.5. Extraction, identification and quantification of non-polyphenolic pigments

For extraction of non-polyphenolic pigments (chlorophyll a and b, pheophytin), the pericarp (2.0 g) was homogenized with $2 \times 5 \text{ mL}$ cold acetone using a digital homogenizer (Ultra-Turrax T25; Ika India Pvt Ltd., Bangalore, India) and centrifuged at $10,000 \times g$ at 4°C for 30 min using a refrigerated centrifuge (5810 R, Eppendorf). The chlorophyll pigments were separated using a Zorbax SB-C18 column ($4.6 \text{ mm} \times 150 \text{ mm} \times 3.5 \mu\text{m}$) preceded by a guard column of the same stationary phase maintained at 35°C . The flow rate was 0.6 mL min^{-1} . The solvents used for elution were: 1% formic acid in MQ water (A) and methanol containing 1% formic acid (B). Gradient elution over 50 min run time was from 10% B to 50% B (20 min), from 51 to 90% B (20 min), 91 to 100% B (5 min), and from 100 to 10% B (5 min). Chlorophyll a, chlorophyll b and pheophytin were detected by DAD at 660 nm. The authentic standards of these pigments were used for comparing the retention times, co-chromatography and quantification in the litchi pericarp samples. The EZChrom software was employed for data processing and quantification of these pigments.

2.6. Statistical analyses

Data pertaining to the effects of PGRs on fruit quality at harvest were subjected to one-way analysis of variance (ANOVA) using GenStat Release 11.1 (VSN International Ltd., Hemel Hempstead, UK), while postharvest storage data was subjected to two-way ANOVA involving two factors, PGR treatment and storage duration. The effects of PGR treatment on different parameters at harvest and after postharvest storage were assessed within ANOVA and the least significant differences (LSD) were calculated at 5% level of significance after a significant *F*-test. The validity of statistical analysis was ensured by checking all the assumptions of ANOVA.

3. Results

3.1. Pericarp colour

Weather data recorded near the experimental site showed that minimum and maximum temperature during 4 weeks before harvest remained at more than 20 and 30°C , respectively (Fig. 1).

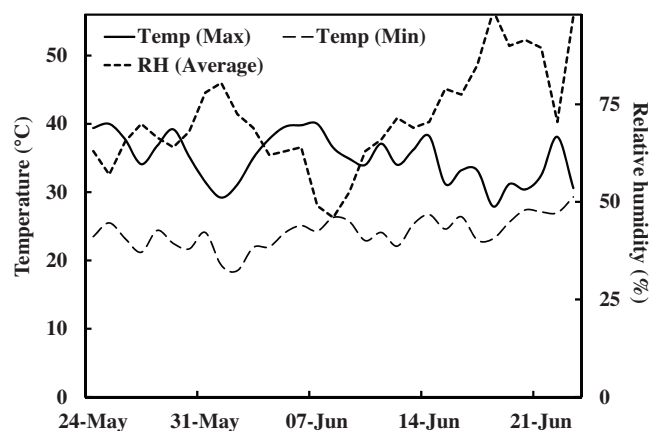


Fig. 1. Maximum and minimum temperature and average relative humidity recorded from the experimental site during 4 weeks before harvest using an automatic weather station.

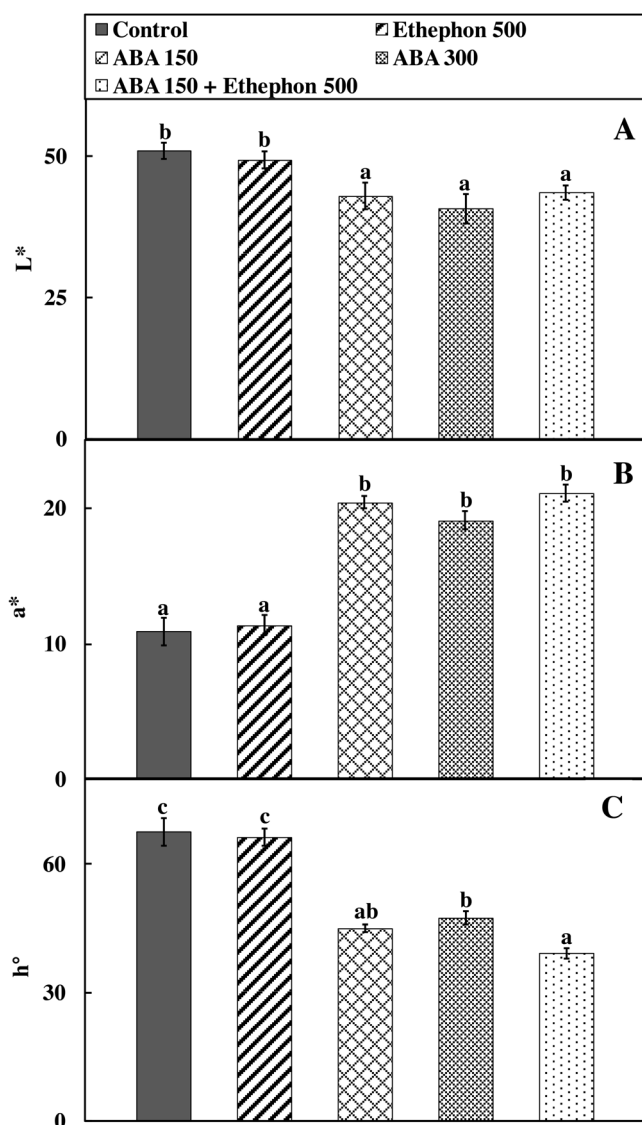


Fig. 2. Effect of preharvest application of plant growth regulators (PGRs) on litchi pericarp colour parameters L^* (A), b^* (B) and hue angle (C) at harvest. PGR treatments include ethephon $500 \mu\text{L L}^{-1}$, abscisic acid (ABA) 150 mg L^{-1} , ABA 300 mg L^{-1} and ABA 150 mg L^{-1} + ethephon 500 mg L^{-1} . The bars, showing mean values, bearing the same letter(s) are not significantly different ($P \leq 0.05$). Vertical bars represent standard error of means.

During litchi fruit maturation and ripening, pericarp colouration involves an increase in a^* values and decrease in L^* , b^* and hue angle values. PGR treatments except ethephon increased the darkness of skin colour as evident from lower L^* values compared to the control (Fig. 2A). Preharvest application of ABA (150 or 300 mg L^{-1}) at the colour break stage significantly improved the pericarp colour as indicated by higher a^* (Fig. 2B) and lower hue angle (Fig. 2C) values compared to the control and ethephon. Ethephon ($500 \mu\text{L L}^{-1}$) treatment was not effective in improving pericarp colour and the L^* , a^* and hue angle values did not differ significantly from the control. The combination of ABA (150 mg L^{-1}) and ethephon ($500 \mu\text{L L}^{-1}$) did not influence the pericarp colour to a greater extent compared to ABA treatment alone at either concentration, but the hue angle was comparatively lower in the combination treatment compared to ABA (300 mg L^{-1}). Chromaticity (c^*) of litchi pericarp was not influenced by preharvest application of PGRs (data not shown).

3.2. Polyphenolic compounds (anthocyanins, epicatechin, and quercetin-3-O-rutinoside)

Total anthocyanins concentrations in litchi pericarp ranged between 15.0 and $37.0 \text{ mg } 100 \text{ g}^{-1}$ depending upon the treatment (Fig. 3A). Pericarp in fruit treated with ABA at both concentrations (150 and 300 mg L^{-1}) exhibited 2-fold higher concentrations of total anthocyanins compared to the control. The combination of ABA (150 mg L^{-1}) with ethephon ($500 \mu\text{L L}^{-1}$) did not show a significant increase in the concentration of total anthocyanins compared to ABA alone. Ethephon ($500 \mu\text{L L}^{-1}$) treatment also resulted in $\sim 25\%$ greater concentration of total anthocyanins than in the control, but the increase was statistically non-significant. The data showed that preharvest application of ABA was effective in promotion of anthocyanin accumulation in litchi pericarp.

PGR application had a great influence on the concentrations of individual anthocyanins (Fig. 3), leading to significant differences in the anthocyanin profiles of litchi pericarp subjected to different treatments. Cyn-3-O-rut was found to be the principal anthocyanin pigment contributing 71–96% of total anthocyanins depending upon the treatment. The variation in the contribution of cyn-3-O-rut to total anthocyanins emerged mainly due to the differential effects of different PGR treatments on the concentration of cyn-3,5-diglu (Figs. 3 and 4). ABA (150 or 300 mg L^{-1}) alone or in combination with ethephon (ABA 150 mg L^{-1} + ethephon $500 \mu\text{L L}^{-1}$) resulted in a significant increase in the concentrations of cyn-3-O-rut, cyn-3,5-diglu, and cyn-3-O-glu compared to the control. Interestingly, the magnitude of effects of ABA at 150 mg L^{-1} and ethephon at $500 \mu\text{L L}^{-1}$ were almost similar on the concentration of cyn-3,5-diglu. The increased concentration of ABA (300 mg L^{-1}) and its lower level (150 mg L^{-1}) in combination with ethephon ($500 \mu\text{L L}^{-1}$) resulted in further increase in the concentration of cyn-3,5-diglu. Though ethephon ($500 \mu\text{L L}^{-1}$) treatment was not effective in enhancing total anthocyanins and cyn-3-O-rut, it increased the concentration of cyn-3,5-diglu. No significant effect on the concentration of cyn-3-O-gal was observed in response to PGR application (data not shown). The data indicated that the relative contribution of individual anthocyanins towards total anthocyanins changed as a result of PGR application (Figs. 3 and 4). The concentration of quercetin-3-O-rutinoside, the major flavonol detected in litchi pericarp, was slightly increased by PGR treatments, but the increase was statistically nonsignificant (Fig. 4). Similarly, epicatechin concentration, the principal monomeric flavanol, was not influenced by PGR treatment (Fig. 4).

3.3. Non-polyphenolic compounds (chlorophyll and pheophytin)

Chlorophyll concentration in the litchi pericarp tissue was remarkably affected by the type and concentration of PGR application at the colour-break stage (Fig. 5). Total chlorophyll concentration, determined by the summation of chlorophyll a and b, was lower in all PGR-treated fruit compared to the control (Fig. 5A). Ethephon ($500 \mu\text{L L}^{-1}$) and ABA (150 mg L^{-1}) exerted similar effects on degradation of chlorophyll pigments in the pericarp. However, this effect was enhanced either by the higher concentration of ABA (300 mg L^{-1}), or by the combined treatment of ABA (150 mg L^{-1}), and ethephon ($500 \mu\text{L L}^{-1}$). Since chlorophyll a was the major contributor to the total chlorophyll concentration, the effect of various PGR treatments on chlorophyll a was almost similar to that on the total chlorophyll (Fig. 5B). The ratio of chlorophyll a and b was >2.5 in the pericarp tissue of commercially mature fruit, regardless of the PGR treatment and concentration of total chlorophyll. The effect of PGR treatments on chlorophyll b was almost similar to that observed on chlorophyll a. There was no significant difference among the PGR treatments for their effect

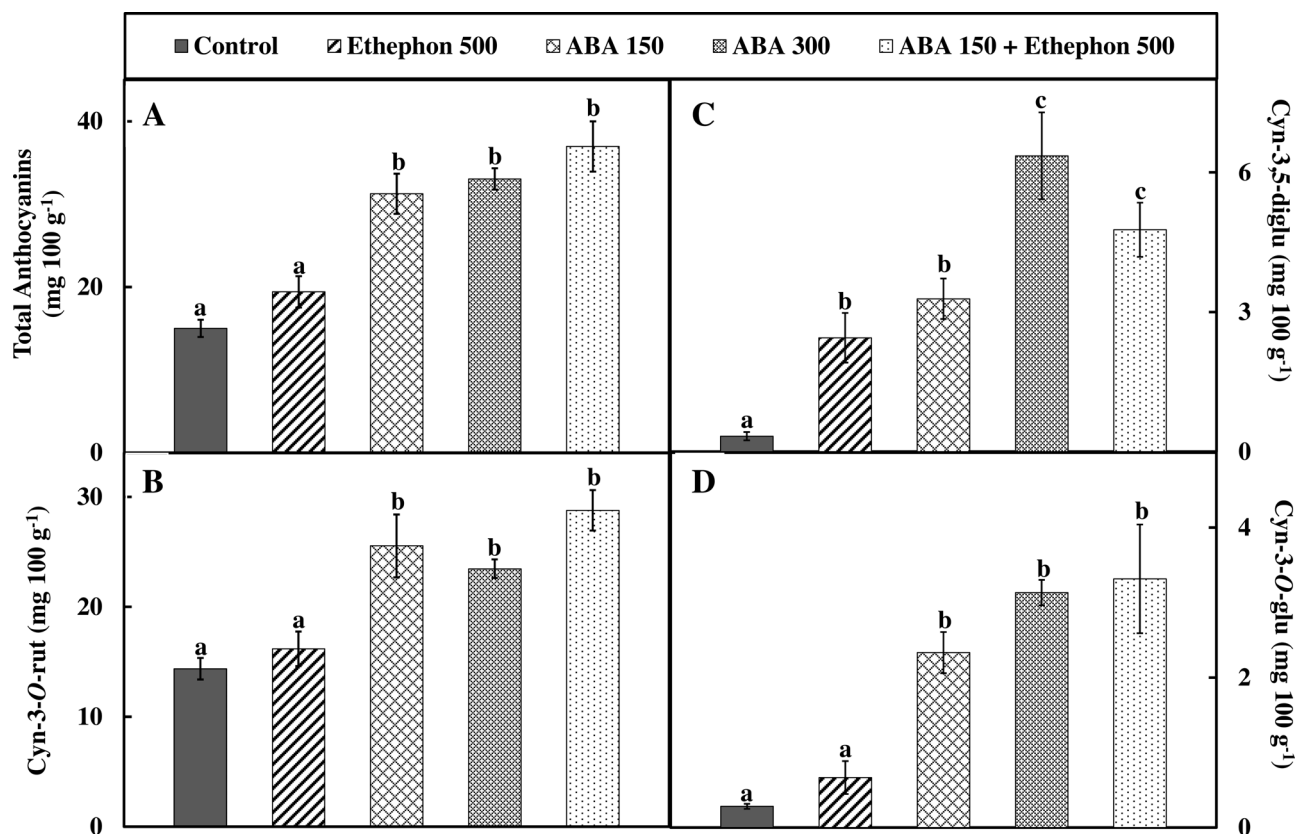


Fig. 3. Effect of preharvest application of plant growth regulators (PGRs) on the concentrations of total anthocyanins (A), cynidin-3-O-rutinoside (B), cynidin-3,5-diglucoside (C) and cynidin-3-O-glucoside (D) at harvest. PGR treatments include ethephon 500 $\mu\text{L L}^{-1}$, abscisic acid (ABA) 150 mg L^{-1} , ABA 300 mg L^{-1} and ABA 150 mg L^{-1} + ethephon 500 mg L^{-1} . The bars, showing mean values, bearing the same letter(s) are not significantly different ($P \leq 0.05$). Vertical bars represent standard error of means.

on pheophytin concentration, which is a degradation product of chlorophyll (data not shown).

3.4. Fruit quality at harvest

Preharvest application of ethephon (500 $\mu\text{L L}^{-1}$) caused a significant reduction in aril firmness compared to other PGRs (Fig. 6A). ABA treatment at both concentrations (150 and 300 mg L^{-1}) did not significantly influence TA compared to the control, but ethephon (500 $\mu\text{L L}^{-1}$) alone or in combination with ABA (150 mg L^{-1}) caused a significant reduction in TA of the aril (Fig. 6B). ABA alone or in combination with ethephon did not influence aril firmness at

harvest. SSC was not affected, regardless of PGR treatment (Fig. 6C). The reduction in TA in ethephon alone or in combination with ABA led to a significant increase in SSC:TA ratio in the litchi aril (Fig. 6D).

3.5. Postharvest quality of PGR-treated fruit

The changes in pericarp colour measured by CIE colour coordinates L^* , a^* , and hue angle were primarily influenced by duration of cold storage (Table 1). A general trend of decrease in L^* value indicating increase of pericarp darkness was observed during 14 d of cold storage in all fruit irrespective of the PGR treatment, but the decrease was comparatively more in control fruit. As a function

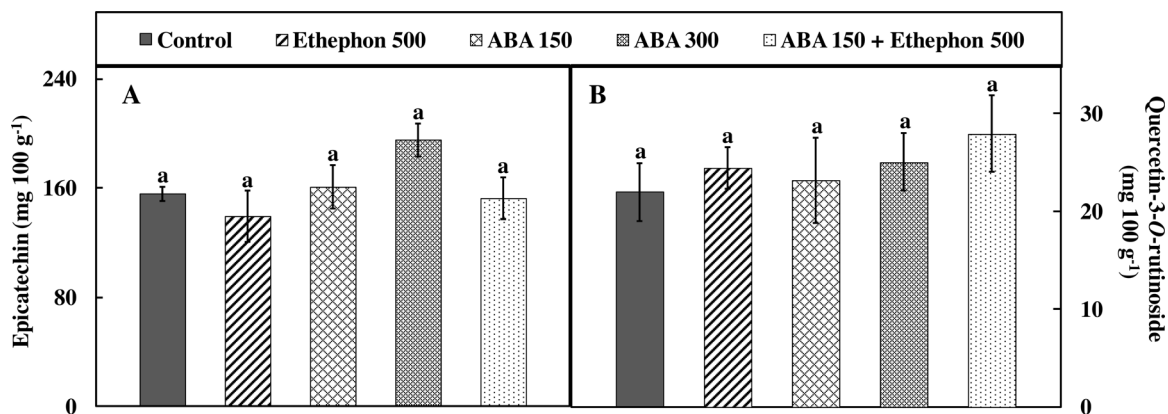


Fig. 4. Effect of preharvest application of plant growth regulators (PGRs) on the concentrations of epicatechin (A), and quercetin-3-O-rutinoside (B) at harvest. PGR treatments include ethephon 500 $\mu\text{L L}^{-1}$, abscisic acid (ABA) 150 mg L^{-1} , ABA 300 mg L^{-1} and ABA 150 mg L^{-1} + ethephon 500 mg L^{-1} . The bars, showing mean values, bearing the same letter(s) are not significantly different ($P \leq 0.05$). Vertical bars represent standard error of means.

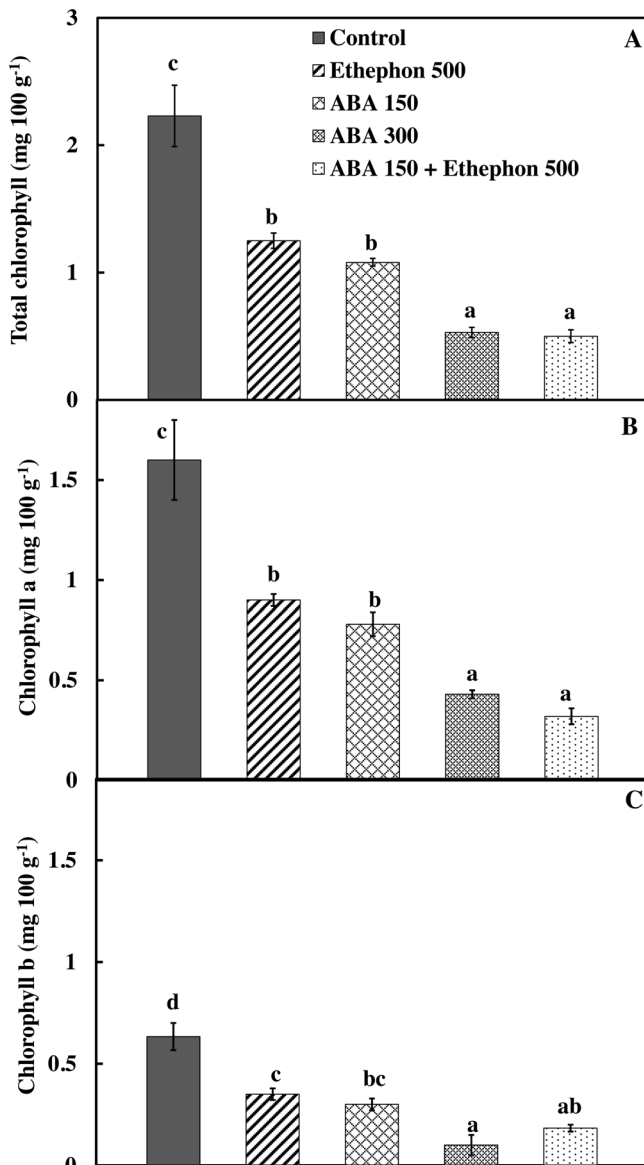


Fig. 5. Effect of preharvest application of plant growth regulators (PGRs) on the concentrations of total chlorophyll (A), chlorophyll a (B) and chlorophyll b (C) at harvest. PGR treatments include ethephon 500 $\mu\text{L L}^{-1}$, abscisic acid (ABA) 150 mg L^{-1} , ABA 300 mg L^{-1} and ABA 150 mg L^{-1} + ethephon 500 mg L^{-1} . The bars, showing mean values, bearing the same letter(s) are not significantly different ($P \leq 0.05$). Vertical bars represent standard error of means.

of storage period, a significant decrease in a^* value was observed in litchi fruit treated with PGR containing ABA. Hue angle changes in litchi pericarp were significantly influenced by the treatment effect rather than storage duration effect. The hue angle values of fruit subjected to ABA (150 mg L^{-1}) and combination treatment increased during 14 d cold storage indicating loss of red colour. Browning index, determined subjectively by visual observations, increased as a function of storage duration, and was not influenced by PGR treatments. In general, preharvest exogenous application of PGR did not have any negative effect on the changes in skin colour during postharvest cold storage for 14 d (Table 1).

Similar to the effect on pericarp colour changes during cold storage, the aril firmness, SSC, TA and SSC:TA were mainly influenced by the storage duration (Table 2). A significant decrease in aril firmness and TA was observed during storage of litchi fruit for 14 d at 5 °C. Fruit treated with ethephon alone had the lowest firmness compared to other treatments and control during 14 d storage. As a

consequence of storage period, a marginal increase in SSC was also observed in PGR-treated and control fruit. Interestingly, the loss of TA during 14 d storage was significantly higher in ABA-treated fruit compared to those treated with either ethephon or a combination of ABA and ethephon (Table 2).

4. Discussion

Pericarp colour in litchi determines the appearance quality and thus consumer acceptance of fruit (Holcroft and Mitcham, 1996; Sivakumar et al., 2007). Several factors are known to influence the concentration and composition of anthocyanins in various coloured cultivars of grape and litchi (Underhill and Critchley, 1992; Tyas et al., 1998; Mori et al., 2005; Peppi et al., 2006; Wei et al., 2011). Among environmental factors, temperature has been reported to play an important role in biosynthesis and stability of anthocyanin pigments (Mori et al., 2005). The weather data recorded at the experimental site showed prevalence of high maximum and minimum temperatures during final stages of fruit maturation and ripening of litchi, which might have interfered with the development of uniform pericarp colour (Figs. 1 and 2). High temperatures have also been reported to inhibit the anthocyanin accumulation in coloured cultivars of grape (Mori et al., 2005; Peppi et al., 2006), but no information is available on the effects of temperature on anthocyanins in litchi.

Regulation of skin colour in coloured fruit is critical to meet quality standards and consumer expectations. In the present study, an increase in concentrations of total anthocyanins was observed in response to preharvest application of PGRs, but the increase was significant only in case of ABA and its combination with ethephon. Our data are consistent with the previous findings suggesting exogenous ABA-induced promotion of anthocyanin accumulation in litchi (Wang et al., 2007; Wei et al., 2011) and grapes (Peppi et al., 2006; Cantín et al., 2007; Wheeler et al., 2009). Litchi colour break stage, about 4 weeks before harvest, could be correlated with the veraison stage of grape berry maturation which involves the initiation of anthocyanin biosynthesis and also shares the nonclimacteric physiology of fruit maturation and ripening with litchi. Exogenous application of ABA might have increased the endogenous levels of ABA, enhancing anthocyanin biosynthesis through the upregulation of UFGT gene in pericarp tissue (Wang et al., 2007; Peppi et al., 2008; Wei et al., 2011). The UFGT gene is a candidate marker gene widely studied for its expression levels due to its closer association with the anthocyanin levels in fruit and with the resultant phenotype (Peppi et al., 2008; Wei et al., 2011). Preharvest treatment with ABA (25 mg L^{-1}) has been reported to enhance the expression of UFGT in litchi pericarp, while on the other hand, lower expression levels of UFGT were associated with poor pericarp colour phenotype caused by fruit cluster bagging and CPPU (4 mg L^{-1}) application (Wei et al., 2011). Similarly, in 'Crimson Seedless' grapes, the upregulation of UFGT gene within a week in response to ABA treatment was concentration-dependent, resulting in 3- and 6-fold increases in its expression levels by 150 and 300 mg L^{-1} concentration of ABA, respectively (Peppi et al., 2008). Our data show that increase in ABA concentration from 150 to 300 mg L^{-1} did not increase the concentrations of total anthocyanins significantly, indicating a plateau effect. In addition to the effect of ABA on the expression of anthocyanin biosynthesis genes, there is another possibility that externally applied ABA may feed forward to elevate the level of ABA biosynthesis pathway gene transcripts such as 9-cis-epoxycartenoid dioxygenases (NCEDs) and hence its own synthesis as reported in 'Cabernet Sauvignon' grapes, which showed higher endogenous levels of ABA in response to both 4 and 400 mg L^{-1} concentrations of ABA (Wheeler et al., 2009). This could possibly explain the response of litchi

Table 1
Postharvest changes in pericarp colour of litchi fruit during 14 days cold storage at 5 °C as influenced by preharvest application of plant growth regulators (ethephon 500 $\mu\text{L L}^{-1}$, ABA 150, 300 mg L^{-1} , and ABA 150 mg L^{-1} + ethephon 500 $\mu\text{L L}^{-1}$) and storage period.

Treatment	Storage period (d)	<i>L</i> *	<i>a</i> *	<i>h</i> ^o	Browning index
Control	0	50.91	10.87	67.50	1.0
	7	37.93	10.70	63.91	1.7
	14	35.92	12.60	58.75	2.0
Ethephon 500	0	49.30	11.36	66.23	1.0
	7	41.44	10.85	61.26	1.7
	14	40.56	11.77	55.24	2.1
ABA 150	0	42.97	20.41	44.97	1.0
	7	38.16	17.86	43.13	1.7
	14	35.82	12.09	51.89	2.1
ABA 300	0	40.68	19.09	47.42	1.0
	7	39.93	17.66	44.07	1.8
	14	37.59	15.25	47.33	2.2
ABA 150 + ethephon 500	0	43.56	21.09	39.14	1.0
	7	41.08	19.85	40.55	1.7
	14	31.25	16.73	43.74	2.3
Least significant differences of means at 5% level and levels of significance for a two-way ANOVA					
Treatment (T)		3.57*	1.69***	4.40***	NS
Storage period (SP)	2.76***	1.31***	NS	0.10**	
T × SP	NS	2.93**	7.63*	NS	

NS, non-significant.

* $P \leq 0.05$.

** $P \leq 0.01$.

*** $P \leq 0.001$.

pericarp to lower and higher concentrations of ABA in terms of increased anthocyanin accumulation observed in this study and previous reports (Wang et al., 2007; Wei et al., 2011).

The ethephon treatment was not effective in improving pericarp colouration. The combination of ethephon (500 $\mu\text{L L}^{-1}$) with

ABA (150 mg L^{-1}) also did not contribute to the anthocyanin-enrichment of litchi pericarp compared to ABA alone, which is contrary to the findings of Wang et al. (2007) showing significant synergistic effect of combined treatment with ABA and ethephon. The ethephon-induced increase in anthocyanins was reported to

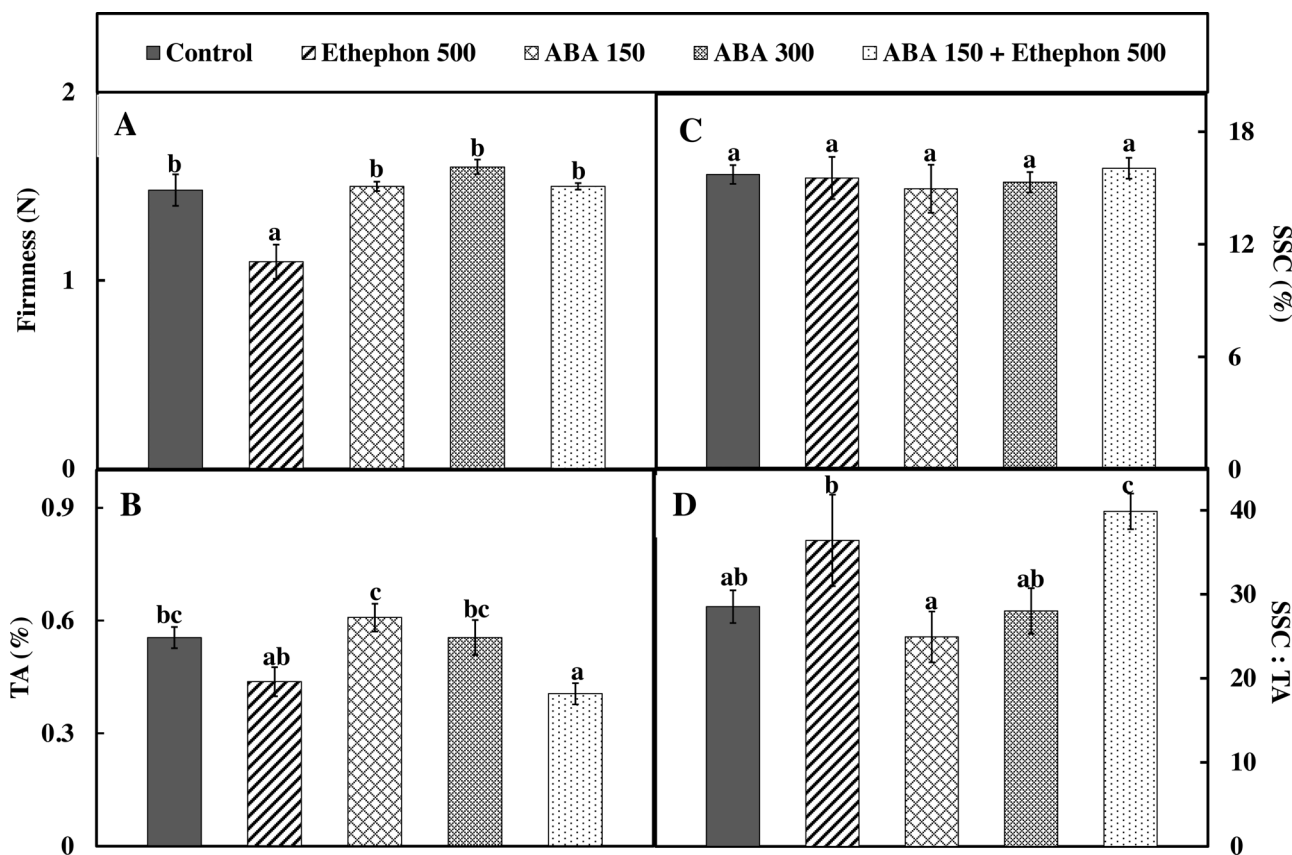


Fig. 6. Effect of preharvest application of plant growth regulators (PGR) on the aril firmness (A), titratable acidity (B) soluble solids concentration (C) and SSC to TA ratio (D) at harvest. PGR treatments include ethephon 500 $\mu\text{L L}^{-1}$, abscisic acid (ABA) 150 mg L^{-1} , ABA 300 mg L^{-1} and ABA 150 mg L^{-1} + ethephon 500 mg L^{-1} . The bars, showing mean values, bearing the same letter(s) are not significantly different ($P \leq 0.05$). Vertical bars represent standard error of means.

Table 2

Postharvest changes in firmness, SSC, TA, and SSC:TA of litchi fruit during 14 days cold storage at 5 °C as influenced by preharvest application of plant growth regulators (ethephon 500 $\mu\text{L L}^{-1}$, ABA 150, 300 mg L^{-1} , and ABA 150 mg L^{-1} + ethephon 500 $\mu\text{L L}^{-1}$) and storage period.

	Firmness (N)	SSC (%)	TA (%)	SSC:TA
Treatment				
Control	1.27	16.54	0.45	39.57
Ethephon 500	0.92	17.31	0.40	44.57
ABA 150	1.28	15.97	0.48	36.07
ABA 300	1.34	16.08	0.46	37.99
ABA 150+ethephon 500	1.28	16.68	0.39	44.40
Storage period (d)				
0	1.44	15.51	0.51	31.54
7	1.42	16.93	0.46	39.45
14	0.79	17.10	0.35	50.57
Least significant differences of means at 5% level and levels of significance for a two-way ANOVA				
Treatment (T)	0.12***	NS	NS	NS
Storage period (SP)	0.09***	1.16**	0.07***	7.93***
T \times SP	NS	NS	NS	NS

NS, non-significant.

** $P \leq 0.01$.

*** $P \leq 0.001$.

be achieved only at higher concentrations (800 $\mu\text{L L}^{-1}$), while the pigment levels in 400 $\mu\text{L L}^{-1}$ ethephon-treated fruit were almost similar to the control (Wang et al., 2007). Application of ethephon has been reported to hasten the accumulation of anthocyanins in grape skin through stimulated long-term expression of chalcone synthase (CS), flavanone 3 β -hydroxylase (F3H) and UFGT genes (El-Kereamy et al., 2003). In addition to the other factors, the warm weather conditions are known to negatively influence the efficacy of ethephon as an ethylene-releasing compound (Stover and Greene, 2005). Furthermore, the role of ethylene in pericarp colouration in litchi has been reportedly linked with the degradation of chlorophyll pigments, while the higher anthocyanins concentration in litchi pericarp has been associated with the increased levels of endogenous ABA (Wang et al., 2007).

The identification of anthocyanins in 'Calcuttia' litchi pericarp revealed the presence of cyn-3-O-rut, cyn-3,5-diglu, cyn-3-O-glu and cyn-3-O-gal as the major anthocyanin pigments. Cyn-3-O-rut was observed to be the major anthocyanin pigment contributing 71–96% to total anthocyanins pool which is in agreement with the previous studies on other litchi cultivars (Lee and Wicker, 1991; Rivera-López et al., 1999; Zhang et al., 2005; Somboonkaew and Terry, 2010a; Reichel et al., 2011; Wei et al., 2011). The anthocyanin profiling of litchi pericarp subjected to different PGRs treatments showed that relative concentrations of individual anthocyanin were significantly impacted by the concentration and type of PGR. The relative contribution of cyn-3,5-diglu to the total anthocyanins pool in ethephon (500 $\mu\text{L L}^{-1}$), ABA (300 mg L^{-1}), and ABA (150 mg L^{-1}) + ethephon (500 $\mu\text{L L}^{-1}$) treated fruit was comparatively larger than the control and ABA (150 mg L^{-1}) treated fruit. These results indicate that effects of PGRs are not only limited to the biosynthesis of anthocyanidins, rather glycosylation at downstream level is also likely to be affected by PGR. The higher proportion of cyn-3,5-diglu in some PGR treatments mentioned above could have positive effect on postharvest stability of anthocyanin pigments in litchi pericarp, leading to longer retention of red colour. Rommel et al. (1990) reported that cyan-3-O-glu was more susceptible to degradation than di- or tri-glucoside anthocyanins in raspberry juice or raspberry wine in the dark during storage at 2 °C and 20 °C. It has been proposed that diglucoside anthocyanins are comparatively more stable than monoglucoside anthocyanins in the aqueous solution, partly due to reduced electron density of the flavylum ring in the diglucoside (Kim et al., 2010). The decrease in pericarp a^* value during 14

d of cold storage was comparatively lower in the fruit having relatively higher concentration of cyn-3,5-diglu (Table 1), which supports the hypothesis of higher stability of diglucosides than monoglucosides (Rommel et al., 1990; Kim et al., 2010). However, this hypothesis needs more scientific evidence and testing for valid conclusions.

The concentrations of total chlorophyll, chlorophyll a and b were significantly reduced by all PGR treatments and the magnitude of effect varied with type and concentration of PGR. Changes in total chlorophyll in response to PGRs shared strong negative correlation with changes in total anthocyanins ($r = -0.83$) and a^* values ($r = -0.79$). Therefore, lower level of total chlorophyll was associated with higher total anthocyanins concentration and a^* values, which implied more reddening of litchi skin. The data on concentration of total anthocyanins and chlorophyll in relation to PGR treatments was consistent with the pericarp b^* and hue angle values (Figs. 2, 3 and 5). The ethephon (500 $\mu\text{L L}^{-1}$) treatment, which was otherwise not effective to promote anthocyanins concentration, enhanced the degradation of chlorophyll pigment in the litchi pericarp (Fig. 5). Ethephon-induced chlorophyll degradation was evident from the data on pericarp colour and concentration of chlorophyll pigment in the pericarp. The increase in level of anthocyanins has been reported to be concurrent with reduction in total chlorophyll concentration in fruit pericarp during maturation and ripening of litchi (Wang et al., 2007).

The potential effects of preharvest applications of ABA and ethephon on litchi fruit quality attributes such as firmness, soluble solids and TA have not been investigated previously (Wang et al., 2007; Wei et al., 2011). But, these quality attributes are known to be significantly influenced by the PGR treatments aimed to improve skin colour in grapes (Mori et al., 2005; Peppi et al., 2006). Preharvest application of ethephon treatment reduced aril firmness at harvest compared to control and other PGR treatments containing ABA which partially agrees with published reports of variable effects on grape berry texture in response to similar treatments at veraison stage (Peppi et al., 2006; Cantín et al., 2007). Ethephon treatment might have increased the utilization of malate as a substrate during enhanced rate of respiration in response to ethylene and thus leading to minor reduction in TA. Exogenous application of PGR did not influence the concentration of soluble solids as reported in grapes that ABA did not interfere in the sugar metabolism of fruit (Mori et al., 2005; Peppi et al., 2006; Wheeler et al., 2009). The effects of PGRs on firmness and TA have been

found to be variable in grapes due to interaction among various factors such as cultivar, timing of application, concentration of PGR and climatic conditions. Though, the application of ABA alone and in combination with ethephon resulted in minor changes in firmness and TA, but these were least likely to have negative perceptible effect on fruit quality from consumer perspective.

Preharvest application of PGRs did not negatively affect the changes in fruit quality during postharvest storage for 14 d at 5 °C (Tables 1 and 2). Aril softening, decrease in acidity and pericarp browning during cold storage could be ascribed to the effect of storage duration rather than the PGR-treatment effect as this is the general trend of biochemical changes in litchi fruit during cold storage for 2–3 weeks (Sivakumar and Korsten, 2006; De Reuck et al., 2009; Somboonkaew and Terry, 2010b), and the magnitude of these changes is temperature- and relative humidity-dependent (Somboonkaew and Terry, 2010a). Modified atmosphere packaging of PGR-treated and control fruit and cold storage at 5 °C in this study could have alleviated the vapour pressure deficit around the fruit, leading to better retention of pericarp colour as reported in previous studies on South African (Sivakumar and Korsten, 2006; De Reuck et al., 2009) and Thai litchi cultivars (Somboonkaew and Terry, 2010a,b). The severity of pericarp browning increased as a result of cold storage regardless of preharvest PGR treatment. As per the Zhang and Quantick (1997) browning index, most fruit had acceptable market quality after 7 d of cold storage with browning index (1–2), while the acceptability decreased marginally as the browning index was slightly more than 2 after 14 d (Table 1). Our data agree well with the previous finding of Cantín et al. (2007) who did not report any significant negative effect of preharvest application of ABA on postharvest performance of 'Crimson Seedless' grape stored at 0 °C for 60 d. In general, regulation of skin colour in grape berries through preharvest application of ABA has been shown to be more promising and superior to ethephon (Peppi et al., 2006; Cantín et al., 2007).

In conclusion, exogenous application of ABA (150 mg L⁻¹) at colour-break stage significantly increased the concentration of total anthocyanins in pericarp of 'Calcuttia' litchi. The higher concentration of ABA (300 mg L⁻¹) and combined treatment of ABA (150 mg L⁻¹) and ethephon (500 µL L⁻¹) enhanced the concentration of cyn-3,5-diglu in litchi pericarp resulting in a significant effect on anthocyanin profiles of litchi pericarp. The ABA treatments had a remarkable effect on anthocyanins accumulation in litchi pericarp compared to the minor effects on other quality attributes such as aril firmness, soluble solids and TA which could have negligible effect on the consumer acceptance of fruit. The changes in fruit quality attributes including pericarp browning during cold storage were mainly related to the storage duration effect, rather than PGR treatment. Therefore, the preharvest application of ABA (150 mg L⁻¹) at colour-break stage has potential to increase the economic value of litchi grown in warm climates unfavourable for proper pericarp colouration.

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