

Abscisic Acid Affects Strawberry Fruit Antioxidant Capacity

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ABSTRACT: Abscisic acid (ABA) is a plant growth regulator with roles in strawberry fruit ripening. The objective of this work was to investigate the effects of (*S*)-cis-abscisic acid (*S*-ABA) application timing and concentration on bioactive compound content and antioxidant capacities in the field. Results showed that significant increases in ascorbic acid, anthocyanin, total phenolics, total flavonoids, ferric-reducing antioxidant power (FRAP), and 2, 2-diphenyl-1-picrylhydrazylhydrate (DPPH) eliminating ability were observed in the treated fruit. These effects may, however, vary depending upon the timing and concentration of ABA application. In conclusion, ABA application can improve fruit qualities with a higher antioxidant activity and concentration of phenolic compounds.

Introduction

Strawberry is a popular fruit with high visual appeal and a desirable taste^[1] that is also rich in vitamins, anthocyanins, flavonoids, and polyphenolics^[2-3]. In China, the strawberry harvest season ranges from October to May. Normally, a cultivar that produced fruit before the Spring Festival is called “winter strawberry”, while one that produces fruit after the Spring Festival is called “spring strawberry”. As fewer fruit are produced in winter, the “winter strawberry” is gaining attention from farmers because of its higher economic value. Cultivars that are available earlier in the season result in higher earnings for the farmers. Farmers, therefore, use cultivation techniques to regulate strawberry flowering time and harvest date, including the use of earlier plantings or daughter plants stored at low temperatures.

Cultivation techniques aside, the development and ripening of strawberry fruit are also regulated by plant hormones^[4]. Abscisic acid (ABA) is an important phytohormone that is the main regulator of strawberry fruit ripening^[5]. ABA application is a potential method for regulating strawberry ripening. The length of fruit growth time is, however, closely related to fruit quality. This directly influences the commercial value of strawberry. It is not currently known whether the shortened growth period affects strawberry fruit quality. Previous studies have shown that exogenous ABA application significantly increased the in vitro anthocyanin content of strawberry^[6], but other quality parameters such as ascorbic acid (AsA), and antioxidant capability have not been studied. Therefore, the objective of this study was to investigate the effect of the timing and concentration of ABA application on the ripening-related antioxidant content and bioactive compound content of strawberry fruit, and may provide useful information about when and how to apply ABA during strawberry ripening in the field.

MATERIALS AND METHODS

Plant materials and ABA treatment

Strawberry (*Fragaria ananassa* ‘Benihoppe’) plants were grown in soil in a plastic greenhouse under natural culture conditions during the winter season in Ya'an, China. About 850 secondary flowers on 200 strawberry plants were tagged at flowering. Only targeted fruit were sprayed with *S*-ABA at five different concentrations (20, 25, 30, 35 or 40 mg/L), or with water as the control until run off, at the

de-greening (DG, 18 d after anthesis), white (W, 21 d after anthesis), and initial red (IR, 23 d after anthesis) stages.

A randomized complete block design was adopted with three replicates. Fifteen fruits were sampled per experimental treatment. To avoid the non-targeted fruit were sprayed, a very small hand-held sprayer was used. The number of full red strawberries was recorded at 4-d intervals from the beginning of treatment, and full red fruit were harvested for fruit analyses.

Determination of ascorbic acid content

The AsA measurements were based on the method of Sun (2007). About 5.0 g of mixed strawberry fruit was extracted using 30 mL of 5% (w/v) metaphosphoric acid and the extracts centrifuged at $22,000 \times g$ for 15 min and quantified at 525 nm. Results were expressed as mg of AsA per 100 g of fresh weight (FW). An AsA curve was utilized with an ascorbate standard prepared in metaphosphoric acid (0–40 nmol/mL).

Total phenolic, total flavonoid content, and antioxidant capacity assays

Approximately 5.0 g of mixed strawberry fruit was extracted with 25 mL of 80% acetone for 1 h at room temperature, followed by centrifugation (10 min, $4500 \times g$) at room temperature. The supernatant was collected for the measurement of total phenolic concentration (TPC), total flavonoid concentration (TFC), ferric reducing antioxidant power (FRAP), and 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity.

TPC was measured according to the method described by Molan et al. (2009). A 12.5 μL aliquot of extract was mixed with 250 μL of 2% sodium carbonate solution in a 96-well microplate and allowed to react for 5 min at room temperature. Next, 12.5 μL of Folin–Ciocalteu phenol reagent (50%) was added and allowed to stand for 30 min at room temperature. Absorbance at 650 nm was measured using a model 680 microplate reader (Bio-Rad Instruments Inc., Japan). The TPC of each sample was expressed as g gallic acid equivalent per 100 g of sample on an FW basis, using a gallic acid calibration curve.

TFC was assayed according to the method described by Chang *et al.* (2002). Briefly, 30 μL of extract was added to a mixture of 90 μL of 95% ethanol, 6 μL of 10% aluminum chloride hexahydrate, 6 μL of 1 M potassium acetate, and 168 μL of deionized water. After incubation at room temperature for 40 min, the reaction mixture absorbance was measured at 415 nm using a model 680 microplate reader (Bio-Rad Instruments Inc., Japan). The data were expressed as g quercetin equivalents per 100 g of sample on a FW basis, using a quercetin calibration curve.

The FRAP assay was carried out according to the method described by Benze and Strain (1996) with some minor modifications. An 8.5 μL aliquot of sample extract was mixed with 275 μL of FRAP reagent using a 96-well microplate and the plates were incubated at 37°C for 30 min in the dark. Absorbance at 595 nm was measured using a model 680 microplate reader (Bio-Rad Instruments Inc., Japan). The antioxidant capacity of the sample was calculated using a standard curve and expressed as g $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ equivalent per 100 g FW of sample.

The DPPH radical scavenging activity was estimated as described by Brand-Williams et al. (1995). A 2.8 mL aliquot of DPPH-methanol solution was mixed with 200 μL of sample extract, vigorously shaken, and maintained for 30 min at room temperature. Absorbance at 517 nm was measured using a model 680 microplate reader (Bio-Rad Instruments Inc., Japan).

Statistical analysis

Experiments were performed according to a completely randomized design. ANOVAs were carried out to examine statistical differences between treatments, using the Tukey test. Data were expressed as mean. Statistical differences were evaluated at the 5% level of significance. All analyses were conducted using SPSS software (Release Version 20; IBM, USA)

RESULTS AND DISCUSSION

Effect of exogenous ABA on AsA of strawberry fruit

Strawberry is rich in AsA and a previous study has indicated that injection of ABA enhanced AsA synthesis (LI et al. 2015). Consistently, we also observed that ABA application had positive effect on AsA accumulation depending on the application time and concentrations (Table 1). The AsA content of fruit treated with 20 mg/L, 25 mg/L, 30 mg/L or 35 mg/L ABA at the DG stage increased 1.39 to 2.01 fold, and the fruit treated with 25 mg/L, 30 mg/L, 35 mg/L or 40 mg/L ABA at the W stage increased 1.33 to 1.68 fold, and the fruit treated with 25 mg/L or 30 mg/L ABA at the IR stage increased 1.84 to 2.22 fold respectively, compared with the control.

Table 1 AsA content of strawberry fruit

Treated stage	ABA concentrations (mg/L)	Ascorbic acid content (mg/100g)
DG	0	28.40 d
	20	27.57 d
	25	45.19 b
	30	56.99 a
	35	39.59 c
	40	30.77 d
W	0	41.19 c
	20	33.18 c
	25	67.61 a
	30	66.83 a
	35	69.02 a
	40	54.81 b
IR	0	27.57 c
	20	39.17 bc
	25	61.22 a
	30	50.80 ab
	35	36.38 bc
	40	45.19 abc

Effect of exogenous ABA on bioactive compounds and antioxidant activity of strawberry

Strawberry is a good source of phenolic compounds such as anthocyanin, phenolic acid, and flavonoids that might be helpful for human health. The ABA application affected phenolic compounds contents and antioxidant activity of ripe strawberry. The fruit treated with ABA at the W and IR stage accumulated more anthocyanin than the control fruit and only fruit treated with 40 mg/L ABA at the DG stage had increased anthocyanin levels compared with the control (Figure 1 A-C). Meanwhile, anthocyanin content increased more at the IR stage than the W stage after ABA treatment. These observations were similar with these studies in which ABA significantly increased the anthocyanin content in grape, strawberry and red-leaf lettuce. Interestingly, although ABA treatment increased the anthocyanin content of ripe strawberries, the color of treated fruit did not differ significantly from the control. These results indicate that the increased anthocyanin levels did not significantly affect the appearance of these fruit.

ABA treatment does increase the TPC and TFC in strawberry and grape, and there are many reports showing that ABA can also increase antioxidant capacity in lettuce, pepper, and grape. In the present study, ABA application increased or had no effect on TPC, TFC, and antioxidant capacity depending on the application time and concentration (Figure 1 D–O). We also observed that a higher antioxidant capacity was accompanied by a higher TPC and TFC. These non-enzymatic antioxidants may, therefore, account for the higher antioxidant capacity observed.

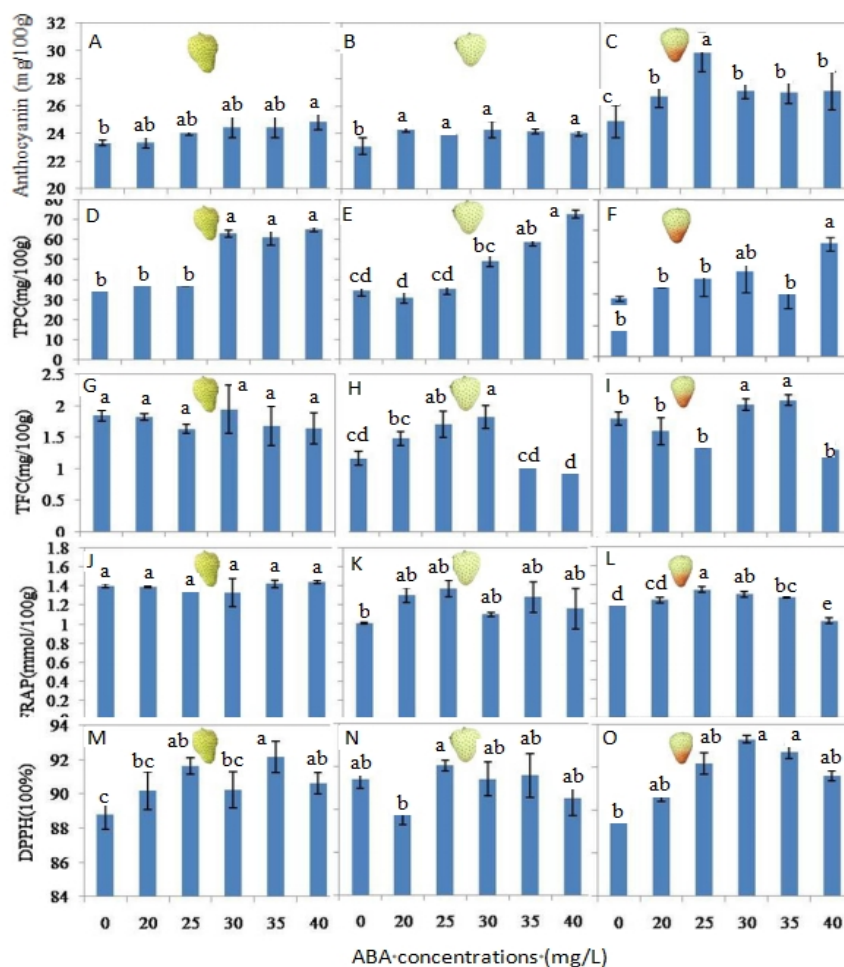


Figure 1 bioactive compounds and antioxidant activity of strawberry fruit

Conclusions

This study demonstrated that ABA application increased anthocyanin content, and antioxidant capacity. Moreover, this suggests that ABA has no effect on yield but may improve fruit quality while shortening the growth period. Furthermore, the effect of ABA on fruit quality, and antioxidant capacity is dependent upon the application time and concentration. This field trial offers real insights into the regulation of strawberry fruit ripening and quality by ABA.

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