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The influence of exogenous hormone on the fruit quality of strawberry (*Fragaria x ananassa* Duch)

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Abstract

The influence the exogenous hormone on strawberry fruit quality was investigated at the Malaysian Agricultural Research and Development Institute (MARDI) station in Cameron Highlands, Malaysia. Greenhouse factorial experiment (RCBD) was designed in three replicates, and two factors: cultivars (Camarosa and Camaroga) and foliage exogenous hormone (auxin (IBA), gibberellic acid (GA3) or cytokinin (6-BA) with 0 and 50 ppm used either singly or in multi-combinations. The result showed that fruits of 'Camarosa' was higher on percentage of Titratable acidity (TA %), vitamin C, dry and organic matters than 'Camaroga' cv. Singly application of GA3 increased the fruits contents of TA%, anthocyanin and vitamin C. Fruit levels of anthocyanin, TSS, dry and organic matters had increased by using the combination exogenous hormones. The results also indicated that the response of two cultivars to the effect of exogenous hormones was greatly appeared on fruit levels of TA %, anthocyanin and vitamin C. In contrast, the both cultivars showed similarity responding against affective of exogenous hormones in the TSS, dry matter, ash and organic matter contents. This finding might be useful in produce high quality of strawberry fruit.

Keywords: Fragaria × Ananassa; Exogenous Hormone; Fruit Quality

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1. Introduction

The cultivated strawberry, *Fragaria × ananassa* Duch., is a member of the Rosaceae family. It is cultivated worldwide in about 75 countries covering subtropical, temperate and high altitudes of tropical regions (Hancock, 1999; FAO, 2009). According to FAO (2009), the world production of strawberry increased by 385% between 1961 and 2009, where the estimated total world production in 2009 was about 4,178,152 tons with planting area of about 254,523 ha. Exogenous phytohormones are widely used in agriculture any of enhance quality and quantity or production management. In general, Plant Phytohormones have ability to affect the physiological process of the growth and development plants at certain concentration either as a supplement nutrients or vitamins (Rangan et al., 2004). On strawberry, application of exogenous hormone would give miscellaneous results, depending upon the concentration, time or stage and cultivars were applying. Previous studies showed that application of GA3 was slightly decreased levels of TSS, ascorbic acid and acidity (Mikhteleva and Petrovskaya-Baranova, 1974; Rajesh et al., 2008; Sharma and Singh, 2009). Application of GA3 at 75 ppm also has reported to increase the percentage of juice content (Sharma and Singh, 2009), as well as enhance the colour and level of anthocyanin content in strawberry fruit (Montero et al., 1998; Al-Najdawi et al., 2007). Meanwhile, alteration results reported on effect of 6-BA on quality of strawberry fruit. Shan et al. (2007) found that the application 6-BA was improves the fruit quality, whereas, study by Al-Najdawi et al., (2007) resulted on no significant different on V.C on both Camarosa and Sweet Charlie cvs. Comparison of exogenous hormone studied by Mir et al. (2004) where mixing of NAA with GA3 resulted to being the superior in improving yield and quality of strawberry fruit except vitamin C.

Up to date, very limited report on the effect of combination of exogenous phytohormones on the quality of strawberry fruit is available. Therefore, in the present study, the effect of GA3, 6-BA and IBA in singly or in combination on quality of strawberry fruits was scrutinized.

2. Material and methods

2.1. Planting materials and experimental plot

The field plot of this study was performed at the Malaysian Agricultural Research and Development Institute (MARDI) station in Cameron Highland, Malaysia (4°28'6.75"N and 101°23'6.83"E). Two strawberry cultivars, 'Camarosa' and 'Camaroga', were used. Planting materials consisting of runners were obtained from the mother plants and grown in the nursery until the 3.5 leaf stage. The plantlets were then transferred and grown in the 15 cm diameter pots without chilling exposure for subsequence growth and development. Plants kept under the plastic greenhouse and arranged according to the RCBD factorial design with two factors (cultivars and exogenous hormone). The plants were sprayed one time with three types of hormone; indolyl-3- butyric acid (IBA) (Merck, Germany), N-6-benzyladenine (BA) (R & M, UK), and GA3 (Merck-Schuchardt, Germany) with two levels of concentration, 0 and 50 ppm with single or combination of two or three. Three replicates were used in this study. The temperature and humidity, had been taken daily using

Thermo-Hygrograph. The fertilizer was supplied via the fertigation system. The plants were maintained following the normal strawberry cultivating practice in the Cameron Highlands.

2.2. Fruit quality measurements

2.2.1. The fruit dry matter, ash and organic matter content (%)

The sample of pulp (one gram) was weighed in a drying dish of known weight using an electronic weighing balance and its fresh weight was recorded as (Wt). The sample was placed in a constant-temperature oven set at a temperature of 60 °C until reach a stable weight, the reading was taken and sample was ignited to constant weight in a muffle furnace at 600° ± 25°C The percentage of ash with reference to the dried substance was calculated. And the Organic matter was calculated as difference from ash (100 – Ash).

2.2.2. The total soluble solids (TSS)

The total soluble solids (TSS) were determined by using 3 to 4 drops of fruit extract using a portable refractometer with the recommended scales (A.O.A.C., 1990).

2.2.3. Titratable acidity (TA %):

The percentage of titratable acidity (TA%) was measured by titrating 10 g of pulp after homogenising with distilled water. Samples were titrated with 0.1 N NaOH to reach a final pH of 8.2. The acidity was expressed as a percentage of citric acid equivalent to the quantity of NaOH used for the titration (Kamperidou and Vasilakakis, 2006). The TA% was calculated using the following formula:

$$\% \text{ TA} = \frac{\text{ml NaOH } x \text{ Normality NaOH } (0.01) x \text{ Total sample volume } x \text{ 56 x 100}}{\text{Volume of Sample taken (ml)} x \text{ Pulp wieght } x \text{ 1000}}$$

2.2.4. Anthocyanin content (mg/100g FW)

The total anthocyanin content of the fruit was determined using a modified pH differential method (Cheng and Breen, 1991). 1.5 gram of fruit pulp was homogenized and extracted with 4 ml methanol, then centrifuged (Universal 16R) at 3000 rpm for 5 min. The supernatant was poured carefully into a 10 ml volumetric flask, and make up to the volume with methanol. The amount of 0.5 extracted was pipetted into two volumetric flasks 10 ml and make up one with pH1.0 buffer and other with pH 4.5 buffer. The UV-1800 Shimadzu UV spectrophotometer was used to measure the absorbance at 510 and 700 nm in buffers at pH 1.0 and 4.5. The absorbance reading was converted into a total milligrams of cyaniding 3-glucoside (molecular weight 433 g mol^{-1} per) 100 g of fresh weight using the molar extinction coefficient of 22400 $Lmol^{-1}$ cm^{-1}(Cheng and Breen, 1991). All samples were prepared in three replications. Anthocyanin was calculated from the following formula:

$$\text{Anthocyanin} = [(A510 - A700)_{pH1.0} - (A510 - A700)_{pH4.5}].$$

2.2.5. Vitamin C (Ascorbic acid) content (mg/100FW)

The vitamin C (Ascorbic acid) content of the fruit was determined according to the method explained by Jagota and Dani (1982). Then 0.15 g of the fruit sample was homogenised with 1.0 ml of 10% trichloroacetic acid (TCA) under dim light and ice-cold conditions. The ground samples were centrifuged (Universal 16R) at 5000 rpm for 10 min at 4°C. Next, 300µl of the supernatant obtained was added into 1700 µl distilled water and 200 µl of 10% Folin reagent. The mixture was gently swirled and left on a bench under dim light for 10 min. Absorbance of the mixture was measured at 760 nm. A standard curve was prepared using standard ascorbic acid at various concentrations (0-300 µg/ml). Finally, 300 µl of ascorbic acid was added into the solution, as described above, and the amount of ascorbic acid in the samples was calculated based on the standard curve.

3. Data analysis and results

Data were analysed as factorial RCBD with exogenous hormone × cultivars as factors. The least significant difference (LSD) for individual factors and the mutable Duncan for interaction were calculated using GenStat 12.1 (PC/Windows program).

The variation between the strawberry cultivars in terms of fruit quality is presented in Table 1. It revealed that the amount of TA% of the 'Camarosa' fruit was greater than that of the fruit of 'Camaroga' by about 8.73%. The result also showed that the fruit of 'Camarosa' was greater than 'Camaroga' in vitamin C content, fruit dry matter (FDM%) and fruit organic matter (FOM %) with about 7.71%, 8.53 % and 0.85%, respectively. Meanwhile, 'Camaroga' was higher than 'Camarosa' in terms of the amount of the fruit TSS% and ash% by about 8.39% and 13.18%, respectively. No significant difference was observed between the two cultivars in terms of the anthocyanin content (Table.1).

However, the effect of the endogenous phytohormone was dependent on the type of cultivars. GA3 treatment decreased the content of FDM% in both cultivars by about 45.21% and 7.6% in 'Camarosa' and 'Camaroga' fruits, respectively. The TSS% also decreased with application of GA3 treatment on 'Camarosa', while, fruits of 'Camaroga' showed the same value as the control. The application of a combination of GA3 + 6-BA significantly increased the FDM%, FOM% and TSS%, to about 54.26%, 1.7% and 65.2% in 'Camaroga' fruits, respectively but reduced the fruit ash to about 31.54%. In 'Camarosa', the application of GA3+6-BA resulted in the same value as the control, and the lowest ash and higher FOM% were observed on application of GA3+IBA+6-BA.

The two cultivars used in this study showed different responses to exogenous hormone treatment in producing vitamin C. 'Camaroga' treated with GA3 produced the highest amount of Vit.C, which was higher by about 21% when compared with the control fruit. Meanwhile, 'Camarosa' produced the highest content of Vit.C when IBA+GA3 was applied, which increased by about 39.86% higher than the control fruit. The lowest content of Vit. C in 'Camaroga' was found when IBA was applied to the plant. Meanwhile, in 'Camarosa' the vit.C content was lower when GA3+6BA was applied, which showed the same level with the control fruit (Table 1).

Table 1. means compression of the effect of cultivars, exogenous hormone, and their interaction on quality of strawberry fruits

Factor	Treatments	TSS (%)		Fruit DM %		Fruit Ash (%)		Fruit OM (%)		Titratable acidity %		anthocyanin (mg/100 g FW)		Vitamin C (mg/ 100 g FW)	
cv.	Camarosa	10.125	b	12.586	a	6.052	b	93.948	a	1.407	a	19.23	a	31.98	a
	Camaroga	10.975	a	11.596	b	6.85	a	93.15	b	1.294	b	19.44	a	29.69	b
EX- hormone (b)	GA3	9	f	10.4	f	6.743	b	93.26	c	1.599	a	19.93	bc	34.64	a
	6-BA	10.35	c	12.01	c	6.4	bc	93.6	bc	1.380	b	19.5	c	31.03	bc
	IBA	10.38	c	11.97	c	6.365	bc	93.64	bc	1.514	a	13.07	e	25.57	e
	GA3+ 6BA	13.8	a	16.1	a	5.185	d	94.81	a	1.416	b	15.83	de	29.99	cd
	GA3+IBA	9.97	d	11.55	d	6.781	b	93.22	c	1.218	c	19.12	cd	34.63	a
	IBA+6-BA	10.1	d	10.6	ef	8.788	a	91.21	d	1.208	c	24.01	a	34.01	ab
	GA3+6BA+IBA	9.63	e	10.93	e	5.297	d	94.7	a	1.139	c	23.28	ab	26.76	de
	Control	11.17	b	13.16	b	6.05	c	93.95	b	1.329	b	19.92	bc	30.07	cd
L.S.D		0.1584		0.3868		0.5944		0.5944		0.0969		3.398		3.334	
a x b															
Camaroga	GA3	9	i	10.34	g	7.14	b	92.86	d	1.301	def	19.37	bcdef	41.31	a
	6-BA	10.47	f	11.65	ef	6.181	bcd	93.82	bcd	1.337	def	18.89	bcdef	27.02	ef
	IBA	9	i	11.09	f	5.99	cd	94.01	bc	1.445	bcd	10.8	g	21.52	g
	GA3+6BA	14.87	a	17.17	a	4.965	e	95.03	a	1.501	bc	14.16	fg	35.69	b
	GA3+IBA	10.27	fg	12.06	de	6.799	bc	93.2	cd	1.195	fg	16.86	def	32.91	bcd
	IBA+6-BA	9.13	i	8.91	h	11.29	a	88.71	e	1.127	gh	28.89	a	33.7	bc
	GA3+6BA +IBA	9.13	i	10.42	g	5.903	cd	94.1	bc	1.237	fg	22.8	bc	29.58	cde
	Control	9.13	i	11.13	f	6.531	bc	93.47	cd	1.208	fg	23.7	b	34.14	bc
Camarosa	GA3	9	i	10.46	g	6.346	bcd	93.65	bcd	1.898	a	20.48	bcde	27.96	def
	6-BA	10.23	fg	12.38	cd	6.619	bc	93.38	cd	1.423	cde	20.11	bcde	35.04	b
	IBA	11.77	d	12.85	c	6.74	bc	93.26	cd	1.583	b	15.35	efg	29.63	cde
	GA3+6BA	12.73	c	15.03	b	5.405	de	94.59	ab	1.330	def	17.49	cdef	24.3	fg
	GA3+IBA	9.67	h	11.05	f	6.763	bc	93.24	cd	1.240	fg	21.37	bcd	36.36	b
	IBA+6-BA	11.07	e	12.29	cd	6.286	bcd	93.71	bcd	1.288	ef	19.12	bcdef	34.31	bc
	GA3+6BA +IBA	10.13	g	11.44	f	4.69	e	95.31	a	1.041	h	23.76	b	23.94	fg
	control	13.2	b	15.19	b	5.568	de	94.43	ab	1.450	bcd	16.13	def	25.99	efg
L.S.D		0.224		0.547		0.8407		0.8407		0.137		4.806		4.715	

Column of treatments in same factor with different letters indicates significant different.

A combination treatment of IBA+6-BA produced the significantly highest amount of anthocyanin (mg/100 g FW) in the 'Camaroga' fruit. Meanwhile, no significant difference was observed on anthocyanin in the fruit of 'Camarosa' resulting from any of the hormone treatments. IBA treatment significantly decreased the level of anthocyanin (mg/100 g FW) of 'Camaroga' fruit, but only statistically decreased the level in the fruit of 'Camarosa' (Table 1).

The two cultivars of strawberry revealed different responses to hormone application in producing TA%. The application of GA3 in 'Camarosa' produced TA% by about 30.89% higher than the TA% of the control fruit. Meanwhile, the application of IBA or IBA+GA3 in 'Camaroga' produced a higher TA% with about 19.61% and 24.25%, respectively, when compared with those in the control fruit (Table 1).

4. Discussion

The result of this study is clearly indicated that the GA3 had increased the titratable acidity, vitamin C. Meanwhile, a combination of 6-BA with GA3 has increased the fruit dry matter.

IBA treatment decreased the anthocyanin, TSS% and vitamin. C. This may be due to the increase of the TA with the negative correlation of about -0.357 ($P>0.05$) with anthocyanin, and about -0.299 ($p>0.05$) with TSS %. However, the auxin led to acidify the cytoplasm of the cells (Rayle and Cleland, 1992). GA3 has an inhibitory effect on strawberry fruit ripening, evidenced by a decrease in the respiratory activity and a delay in anthocyanin synthesis and chlorophylls degradation (Martínez et al., 1994). It was found that the GA3 treatment was no effected on the level of anthocyanin in fruit, but GA3 led to decrease the TSS % on fruit, that probably due to the decrease the dry matter with the positive correlation of about 0.954 ($p>0.05$) between dry matter and TSS. While GA3 significantly increased the vitamin C on fruit, the data shows the same direction with the study done by Wahdan et al. (2011) in which GA3 significantly increased vitamin C in mango, as well as in sweet orange (Saleem et al., 2008). Cytokinin and auxin maybe inhibited or antagonized the role of gibberellins. This was found in this study via the negative significant effect on TSS %, TA %, Ash %. IBA+6-BA treatment increase the fruit anthocyanin and fruit Ash % that may be due to the decrease of the dry matter content % in the fruit with IBA+6-BA treatment with negative correlation of bout -0.400 ($p>0.01$) between dry matter and anthocyanin content and about -0.562 ($p>0.01$) between dry matter and ash. However, the treatment of GA3 and 6-BA increases the percentage of TSS %, fruit dry matters and organic matter content. In contrast, the combination of GA3 and 6-BA increases the fruit dry matter. These results are similar with those found in tomato, where the 6-BA effected on GA3 responses on hypocotyls length and GA3 effect on 6-BA response on anthocyanin (Fleishon et al., 2011). This result of percentage fruit set per plant also similar as suggested in Arabidopsis, where cytokinin has no effect on gibberellin responses (Greenboim-Wainberg et al., 2005). Furthermore, GA3 inhibited the IBA activity on fruit set when the two hormones are combined together (data not show). Cytokinin and auxin expressed antagonize effect to each other. They only enhanced the anthocyanin and ash content. This may be due to the increase the percentage of fruit set per plant on Camarosa *cv*. with a positive correlation of about 573 ($p>0.01$) .However, the role of cytokinin or auxin is determined by the concentration of other endogenous hormone in plant (Rangan et al., 2004).

Some of the effects are strawberry cultivar dependence, that is might be due to the genetic different between the two cultivars. Camarosa *cv*. This could be also explained by the reason that the response of cultivars to the exogenous hormone depended on the required hormone by the plant and gene expression and also related to the environment factor surrounding the plant and level or antagonistic interactions of the endogenous hormone (Gray, 2004).

5. Conclusion

Exogenous application of GA3 could be used to improve the quality of strawberry. The postharvest quality of strawberry fruit, which are Vitamin C, TA and anthocyanin could be improved by the application of foliar spray of exogenous GA3. The effect of exogenous PGR was varying among the varieties. Further study should be conducted on the effect of other PGR and at larger range of concentration. The interaction of exogenous on metabolites biosynthesis in strawberry fruits could be analyzed using the current technology including metabolomics and proteomic techniques.

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