Relationship of ethylene production on climacteric behavior in guava (*Psidium guajava* L.) leaf disks

Tanyakan Seephueng[1*], Ching-Chang Shiesh[2] and Rampai Nampila[1]

[1] Department of Plant Science and Agricultural Resources, Faculty of Agriculture Khon Kaen University 40002

[2] College of Agriculture and Natural Resources, National Chung Hsing University, Taiwan

[*] Corresponding author: Tanyakan.sp@gmail.com

Abstract

Guava (*Psidium guajava* L.) is a climacteric fruit although some cultivars behave in a non-climacteric manner. The objective of this work was to study about the ripening process in guava leaf disks by using kinetin induce ethylene for reduce time of selection and breeding. The results revealed that after 72 hours of incubation, the ethylene production greatly increased in climacteric cultivars and negligible in non-climacteric cultivars. The respiration rates in most cultivars were hardly different. It was predicted that respiratory pattern in hybrid seedlings from 'Pakistan' (Climacteric pattern) and 'Shyh-Jii Bar' (Non-Climacteric pattern) would eventually be climacteric. Moreover, it was also predicted that respiratory pattern in hybrid seedlings from 'Hawaii' (Climacteric pattern) and 'Jen-Ju Bar' (Non-Climacteric pattern) would be non-climacteric. The respiration rates were hardly different in all of cultivars. Therefore, ethylene production could be used as an index of climacteric manner to classify the cultivars of guava as either climacteric or non-climacteric type.

Keywords: Guava (*Psidium guajava* L.), ethylene production, climacteric behavior

Introduction

Guava (*Psidium guajava* L.) is a tropical fruit exhibiting rapid post-harvest ripening. However, the physiological basis involving in the ripening process of guava remains unclear. While guava is considered by several authors to be a climacteric fruit (Akamine and Goo, 1979; Brown and Wills, 1983), some cultivars behave in a non-climacteric manner (Biale and Barcus, 1970; Azzolini *et al*., 2005), making it difficult to develop technologies to efficiently enhance fruit storability during the postharvest periods. These limitations restrict commercial export and a trade of guava fruit. Therefore, guava improving and development of new varieties of guava are needed for the better storage lives than those local and traditional grown varieties. Non-climacteric varieties have longer postharvest storage lives and more suitable for commercial marketing. However, selection and breeding can even take up to 3 years (Pommer and Murakami, 2009).

In vegetative tissues, the rate of ethylene production is thought to be regulated by the endogenous level of free auxin (Bradford and Yang, 1980). Recently, Yu *et al.* (1979) demonstrate that IAA stimulate ethylene production by inducing the synthesis of the ACC synthase, which is the rate-limiting enzyme in the pathway of ethylene biosynthesis. Kinetin has a significant synergetic effect on IAA-induced ethylene production in hypocotyl of mungbean (*Phaseolus rmmgo* L.) seedling (Lau and Yung, 1974) and young leaf discs of guava (*Psidium guajava* L.) (Lin and Wang, 2006). Kinetin acts by suppressing the conjugation of IAA into IAAsp and thus sustaining a higher IAA-free level in the tissue which in turn contributes to the higher ethylene production rate (La and Yang, 1973).

The objective of this work was to study about the ripening process of guava leaf disks by using kinetin induce ethylene as an index of climacteric manner to classify the cultivars of guava as either climacteric or non-climacteric type.

Materials and Methods

Plant material. Guava leaves of Climacteric cultivars included 'Red guava', 'Li-Tzy Bar', 'Shyh-Jii Bar' and 'Red flesh guava', Non-Climacteric cultivars included 'Hung-Shin Bar' and 'Jen-Ju Bar', Two hybrid cultivars included one from Pakistan (Climacteric pattern) and Shyh-Jii Bar (Non-Climacteric pattern), and another from Hawaii (Climacteric pattern) and Jen-Ju Bar (Non-Climacteric pattern) were investigated. Guava leaves were collected from Agricultural Experiment Station in Beiguo, Wufeng District, Taichung County. The first pair of leaf was chosen from the apex, then

cut 5 disks of 1.1 cm. diameter and incubated in 2 mL test solution of 2% sucrose, 50 µg/mL chloramphenicol, 0.1 mM Kinetin and 50 mM MES buffer (pH 6.2) in a 25 mL erlenmeyer flask. The flasks were sealed with rubber serum caps and they put on constantly shaken with 90 rpm at 25°C for 24 hours. At the indicated time, ethylene production and respiration rate were determined.

Ethylene production. Measurement of ethylene production followed the method by Shiesh (1990). A 1 mL gas sample was withdrawn from the head space of the flask by a hypodermic syringe, and ethylene was injected into a gas chromatograph (Shimadzu, Model GC-8A, Japan) fitted with a stainless-steel column packed (6 mm × 2 m) and equipped with an alumina column (mesh size 80/100) and a flame ionization detector (FID). The temperatures of injection port, column and detector were 130, 90 and 130°C respectively. The ethylene production was calculated using the equation below.

$$\text{Ethylene } (\mu L C_2H_4 \cdot kg^{-1} \cdot hr^{-1}) = \frac{(\text{Sample peak (cm.)} \times \text{Fold}) - (\text{Air peak (cm.)} \times \text{Fold})}{(\text{Standard peak (cm.)} \times \text{Fold})} \times \frac{(\text{Volume (L.)})}{\text{Fresh weight (kg.)}} \times \text{Standard conc. (ppm)}$$

Respiration rates. Respiration rates were measured with CO_2 production, following the method by Shiesh (1990). One mL was obtained from each sample from the head space of the flask, and injected into IR-CO_2 analyzer (Maihak, Model UNOR610). The respiration rate was calculated using the equation below.

$$\text{Respiration rates } (mL CO_2 \cdot kg^{-1} \cdot hr^{-1}) = \frac{(\text{Sample peak (cm.)- Air peak (cm.)})}{\text{Standard peak (cm.)}} \times \frac{\text{Flow rate (L./hr.)}}{\text{Fresh weight (kg.)}} \times \text{Standard conc. (\%)} \times 10$$

Statistical analysis. Statistical analysis of the experiment data was performed by using SAS 9.3 (Statistical Analysis System) and subjected to one-way analysis of variance (ANOVA) in a complete randomized design (CRD) statistical model. There were 3 replicates in each treatment.

Results and Discussion

The six cultivars of guava, 'Red guava', 'Li-Tzy Bar', 'Red flesh guava' and 'Hung-Shin Bar' were found to be climacteric in their respiratory patterns. Other cultivars including 'Shyh-Jii Bar' and 'Jen-Ju Bar' exhibited a typical non-climacteric respiratory pattern (Table1). At 24 to 48 hours after the incubation, the differentiation of ethylene production in guava leaf discs was unclear. However, the ethylene production at 72 hours after the incubation greatly increased in 'Li-Tzy Bar' (63.77 $\mu LC_2H_4 \cdot kg^{-1} \cdot hr^{-1}$) followed by 'Red guava', 'Red flesh guava' and 'Hung-Shin Bar' (23.29, 21.63 and 19.10 $\mu LC_2H_4 \cdot kg^{-1} \cdot hr^{-1}$, respectively), and non-climacteric cultivars of 'Shyh-Jii Bar' and 'Jen-Ju Bar' were slightly changed at 1.82 and 2.08 $\mu LC_2H_4 \cdot kg^{-1} \cdot hr^{-1}$, respectively (Table 1, Figure 1).

Similar results of inducing ethylene biosynthesis were obtained by Burg and Burg (1969) who found that the duration of IAA-induced ethylene production was increased considerably with kinetin. In vegetative tissues, kinetin had an effect on IAA-induced ethylene production in hypocotyl of mungbean (*Phaseolus rmmgo* L.) seedlings (Lau and Yung, 1974) and young leaf discs of guava (*Psidium guajava* L.) (Lin and Wang, 2006).

Table 1 Respiratory pattern and ethylene production of 6 guava cultivars

Cultivars	Respiratory pattern	Ethylene production ($\mu LC_2H_4 \cdot kg^{-1} \cdot hr^{-1}$)		
		0-24 hrs.	24-48 hrs.	48-72 hrs.
Li-Tzy Bar	Climacteric	0.30 b	42.01 a	63.77 a
Red guava	Climacteric	0.20 c	11.03 bc	23.29 b
Red flesh guava	Climacteric	0.42 a	12.04 b	21.63 b
Hung-Shin Bar	Climacteric	0.04 d	14.05 b	19.10 b
Shyh-Jii Bar	Non-climacteric	0.11 d	2.31 d	1.82 c
Jen-Ju Bar	Non-climacteric	0.09 d	3.41 cd	2.08 c

Means within a column followed by the same letter are not significantly different ($P > 0.05$).

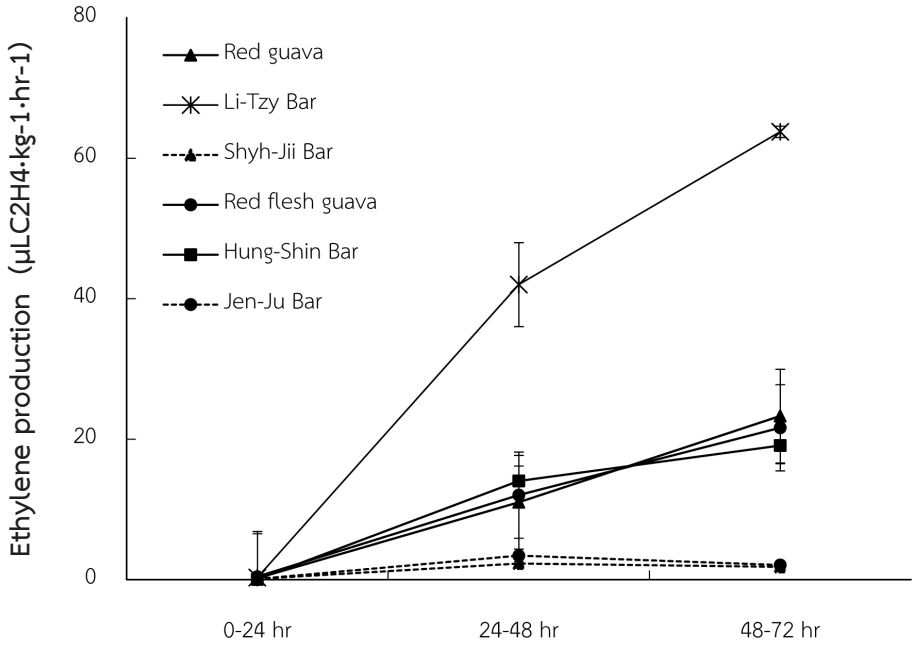

Figure 1 Ethylene production of 6 guava cultivars

The differentiation of respiration rates in 6 cultivars between climacteric and non-climacteric cultivars were uncleared. Respiration rate of 'Jen-Ju Bar' cultivar was found to be a non-climacteric pattern which had the highest rate in all periods of incubated time, but in 'Red guava' cultivar, a climacteric pattern, had the lowest rate in all periods of incubated time, and the other cultivars were rarely differences. (Table2, Figure 2)

Table 2 Respiratory pattern and respiration rate of 6 guava cultivars

Cultivars	Respiratory pattern	Respiration rates ($mLCO_2 \cdot kg^{-1} \cdot hr^{-1}$)		
		0-24 hrs.	24-48 hrs.	48-72 hrs.
Li-Tzy Bar	Climacteric	165.36 c	195.58 b	205.55 bc
Red guava	Climacteric	157.06 c	181.44 b	169.86 d
Red flesh guava	Climacteric	197.76 ab	197.92 b	201.00 cd
Hung-Shin Bar	Climacteric	172.08 bc	268.08 a	235.59 ab
Shyh-Jii Bar	Non-climacteric	169.13 bc	196.94 b	202.17 bcd
Jen-Ju Bar	Non-climacteric	222.74 a	302.13 a	246.20 a

Means within a column followed by the same letter are not significantly different ($P > 0.05$).

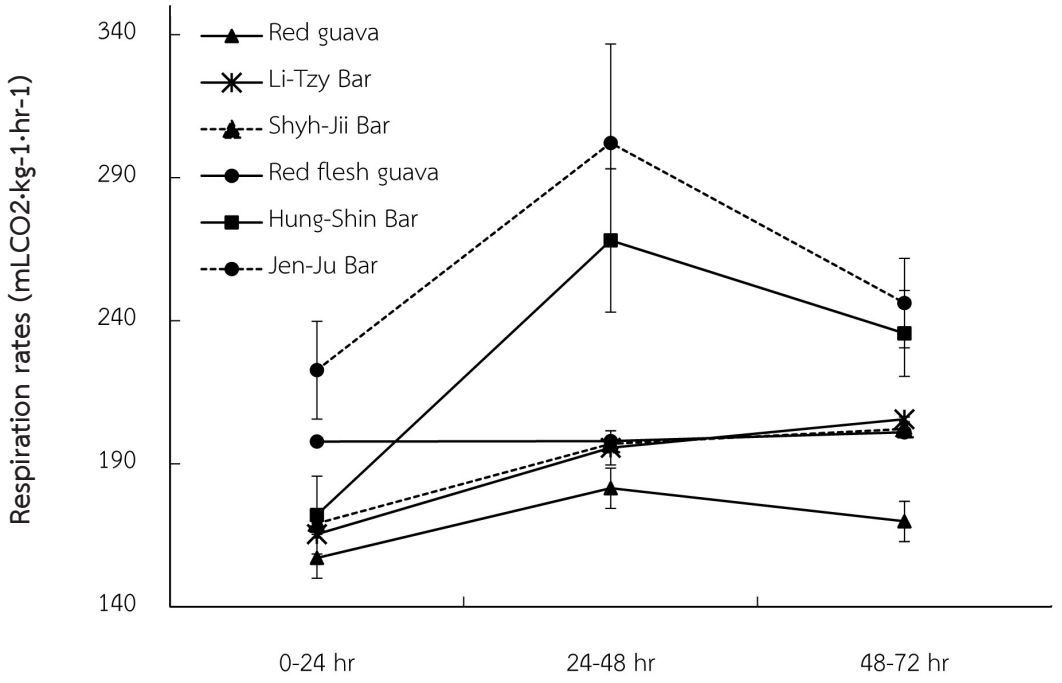

Figure 2 Respiration rates of 6 guava cultivars

The prediction of respiratory pattern from guava leaf disks obtained from hybrid seedling showed that 72 hours after the incubation, ethylene production increased in all of 4 hybrid seedlings from 'Pakistan' (Climacteric pattern) and 'Shyh-Jii Bar' (Non-Climacteric pattern) cultivars, predicting a climacteric respiratory pattern. However, both hybrid seedlings from 'Hawaii' (Climacteric pattern) and 'Jen-Ju Bar' (Non-Climacteric pattern) cultivar negligibly increased, predicting a non-climacteric respiratory pattern. For 2 unknown hybrid seedling cultivars, one could be predicted to be climacteric through their respiratory pattern, and another could be predicted to be non-climacteric in their respiratory pattern. The respiration rates were hardly different between these cultivars. (Table 3).

Table 3 Respiratory pattern prediction obtained from hybrid seedlings

Cultivars	Ethylene production ($\mu LC_2H_4 \cdot kg^{-1} \cdot hr^{-1}$)			Respiration rates ($mLCO_2 \cdot kg^{-1} \cdot hr^{-1}$)			Respiratory pattern prediction
	0-24hrs.	24-48hrs.	48-72hrs.	0-24 hrs.	24-48 hrs.	48-72 hrs.	
Pakistan (C) x Shyh-Jii Bar (NC)							
1	0.55	23.70	24.45	293.70	291.60	304.59	C
2	1.24	15.47	15.43	254.09	316.17	341.81	C
3	2.46	9.82	10.18	268.24	317.23	300.86	C
4	1.01	21.88	35.66	203.52	313.60	329.00	C
Hawaii (C) x Jen-Ju Bar (NC)							
1	0.63	4.46	3.83	214.59	246.28	241.69	NC
2	0.44	6.33	3.60	221.13	228.87	254.93	NC
Unknown Cultivars							
1	0.35	4.08	3.03	210.88	223.30	239.46	NC
2	0.86	18.29	22.40	245.09	279.75	291.98	C

C: Climacteric pattern, NC: Non-climacteric pattern

Conclusion

Inducing ethylene production of guava leaf disks could be used as an index of ripening manner to classify the cultivar of guava (*Psidium guajava* L.) as either climacteric or non-climacteric type at 72 hours after the incubation. Ethylene production in climacteric cultivars including 'Red guava', 'Li-Tzy Bar', 'Red flesh guava', and 'Hung-Shin Bar' increased greatly, but negligibly in non-climacteric cultivars including 'Shyh-Jii Bar' and 'Jen-Ju Bar'. Therefore, this result can be used to predict the unknown or hybrid cultivars. It was predicted that respiratory pattern in hybrid seedlings from 'Pakistan' (Climacteric pattern) and 'Shyh-Jii Bar' (Non-Climacteric pattern) would eventually be climacteric type. Moreover, it could be also predicted that respiratory pattern in hybrid seedling from 'Hawaii' (Climacteric pattern) and 'Jen-Ju Bar' (Non-Climacteric pattern) would be non-climacteric. The respiration rates were hardly different between these studied cultivars.

References

Akamine, E.K. and T. Goo. 1979. Respiration and ethylene production in fruits of species and cultivars of *Psidium* and species of *Eugenia*. J. Am. Soc. Hortic. 10: 632-635.

Azzolini, M., A.P. Jacomino, I.U. Bron, R.A. Kluge and A. Schiavinato. 2005. Ripening of 'Pedro Sato' guava: study on its climacteric or non-climacteric nature. Braz. J. Plant Physiol. 17: 299-306.

Biale, J.B. and D.E. Barcus. 1970. Respiratory patterns in tropical fruits of the Amazon basin. Trop. Sci. 12: 93-104.

Bradford, K.J. and S.F. Yang. 1980. Stress-induced ethylene production in the ethylene-requiring tomato mutant diagotropica. Plant Physiol. 65: 327-330.

Brown, B.I. and R.B.H. Wills. 1983. Postharvest changes in guava fruits of different maturity. Sci. Hortic. 19: 237-243.

Burg, S. P. and E. A. Burg. 1969. Interaction of ethylene, oxygen and carbon dioxide in the control of fruit ripening. Plant. Masttr. V,g. XIX. 1-3: 185-200.

Lau, O. L. and S. F. Yang. 1973. Mechanism of a synergistic effect of kinetin on auxin-induced ethylene production: suppression of auxin conjugation. ibid. 51: 1011-1014.

Lau, O.L. and K.H. Yung. 1974. Synergistic effect of kinetin on IAA-induced ethylene production. Plant & Cell Physiol. 15: 29-35.

Lin, H.L. and T.T. Wang. 2006. Relationship between leaves kinetin-induced ethylene biosynthesis and fruit climacteric behavior of *Psidium guajava* L. Abstracts 27th International Horticultural Congress & Exhibition 8: 270-270.

Pommer, C.V. and K.R.N. Murakami. 2009. Breeding Guava (*Psidium guajava* L.). pp. 83-120. S.M. Jain and P.M. Priyadarshan (eds.). Breeding Plantation Tree Crops: Tropical Species.

Shiesh, C.C. 1990. Studies on the Ripening Physiology and Postharvest Handling of 'Irwin' Mangoes. Ph.D. Thesis, Institute of Horticulture, National Taiwan University.

Singh, S.P. and R.K. Pal. 2008. Response of climacteric-type guava (*Psidium guajava* L.) to postharvest treatment with 1-MCP. Postharvest Biol. Technol. 47: 307-314.

Yu, Y.B., D.O. Adams and S.F. Yang. 1979. Regulation of auxin-induced ethylene production in mungbean hypocotyl: Role of L-aminocyclopropane-1-carboxylic acid. Plant Physiol. 63: 589-590.