MECHANISMS REGULATING APPLE CULTIVARS SUSCEPTIBILITY TO BITTER PIT

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INTRODUCTION

Bitter pit (BP) is a physiological disorder in apple fruit believed to be caused by lower levels of fruit tissue calcium (Ca^{2+}) content. This disorder develops during storage, but it can also develop before harvest in severe cases (Crisosto & Day, 1993). The symptoms are small spots that start as water-soaked tissue, becoming dark-brown and eventually depressed on the fruit surface (Ferguson & Watkins, 1989).

Previous studies have shown that total fruit tissue Ca^{2+} content is not always well correlated to fruit susceptibility to BP incidence. These studies suggested that mechanisms to regulate cellular Ca^{2+} partitioning and distribution are the most important factors controlling fruit susceptibility to BP (De Freitas et al., 2010). Cultivars have different susceptibility to BP, possibly due to different fruit Ca^{2+} uptake and regulation of cellular Ca^{2+} partitioning and distribution. The objective of this study was to analyze the effects of total fruit tissue and cell wall Ca^{2+} contents on fruit susceptibility to BP incidence.

MATERIALS AND METHODS

Apple fruit (*Malus domestica*) cultivars Granny Smith and Fuji were harvested in a commercial orchard in Stockton, California, USA, and stored for two months at 0ºC in the Postharvest Laboratory at the University of California, Davis, CA, USA. At two months of storage, fruit from cultivar Granny Smith were segregated into two lots, fruit with and fruit without visual BP symptoms. 'Fuji' apples had no BP incidence during storage. 'Granny Smith' fruit without and with BP and 'Fuji' fruit without BP were analyzed as different treatments. The experiment followed a completely randomized design, with four replications with 50 fruit for each treatment. Fruit were analyzed for flesh and skin Ca^{2+}, Mg^{2+}, N and K^{+} concentrations and N/Ca^{2+} and $[K^{+}+Mg^{2+}]/Ca^{2+}$ ratios at two months of storage. Cell wall was extracted as described by De Freitas et al. (2010) and total flesh cell wall Ca^{2+} concentration was determined at harvest, and after 1 and 2 months of storage. Nutrient concentrations were determined as described by De Freitas et al. (2010). The analysis of variance (ANOVA) was performed for each variable. The mean values of four replicate samples were compared using Tukey's test ($p < 0.05$).

RESULTS AND DISCUSSION

The results show that flesh and skin Ca^{2+} concentrations were higher in 'Granny Smith' fruit without visible BP symptoms than in 'Granny Smith' fruit with BP symptoms (Table 1). 'Fuji' fruit did not develop BP symptoms during 2 months of storage. Calcium concentrations in flesh and skin tissues were similar between 'Granny Smith' fruit with BP symptoms and 'Fuji' fruit (Table 1). Magnesium concentration in flesh tissue was similar in 'Granny Smith' and Fuji fruit, as well as in 'Granny Smith' fruit with and without BP (Table 1). In skin tissue, the highest Mg^{2+} concentration was observed in 'Granny Smith' fruit with BP symptoms and the lowest was observed in 'Fuji' fruit (Table 1). The lowest N and K^{+} concentrations in flesh and skin tissues were observed in 'Fuji' fruit (Table 1).

Table 1. Nutrient concentration and concentration ratios in fruit flesh and skin of 'Granny Smith' apples without and with visible BP symptoms, and in 'Fuji' without visible BP symptoms at 2 months of storage*.

Treatment	Ca^{2+} (g kg^{-1})	Mg^{2+} (g kg^{-1})	N (g kg^{-1})	K^{+} (g kg^{-1})	N/Ca^{2+}	$[K^{+}+Mg^{2+}]/Ca^{2+}$
			Flesh			
Granny Smith - BP	0.207 a	0.272 a	3.8 a	7.4 a	18.3 b	37,0 b
Granny Smith + BP	0.148 b	0.278 a	4.3 a	8.9 a	29.0 a	62.0 a
Fuji	0.126 b	0.240 a	2.8 b	4.5 b	22.2 b	37.6 b
CV (%)	12.8	8.0	13.1	13.7	22.1	13.8
			Skin			
Granny Smith - BP	0.570 a	0.954 b	5.4 b	8.9 b	9.5 c	17.2 b
Granny Smith + BP	0.352 b	1.097 a	6.1 a	11.6 a	17.3 a	36.0 a
Fuji	0.320 b	0.602 c	4.2 c	4.1 c	13.1 b	14.7 b
CV (%)	9.8	5.8	5.8	11.47	12.8	17.2

***** Mean values with the same letter within the columns are statistically equal according to Tukey's test ($p<0.05$).

The highest N/Ca^{2+} and $[K^{+}+Mg^{2+}]/Ca^{2+}$ ratios were observed in flesh and skin tissues of 'Granny Smith' fruit with BP symptoms (Table 1). Accordingly, previous studies also showed that these ratios have a much better correlation to BP incidence than Ca^{2+} concentrations alone (Ferguson & Watkins, 1989; Saure, 2005; Amarante et al., 2006; Amarante et al., 2009). High N levels could trigger faster cell expansion, which has been suggested to enhance fruit susceptibility to BP, especially with limited fruit Ca^{2+} uptake (Saure, 2005). High levels of Mg^{2+} and K^{+} may enhance these nutrient’s competition with Ca^{2+} for binding sites in the cell membranes, which has been suggested to make fruit tissues more susceptible to BP incidence (Schonherr & Bukovac, 1973; Yermiyahu et al., 1994; De Freitas et al., 2010).

The highest Ca^{2+} concentrations in fruit cell walls at harvest and after 1 and 2 months of storage were observed in 'Fuji' apples, and the lowest in 'Granny Smith' apples with BP symptoms after 2 months of storage (Table 2). The ratios between 'Granny Smith' apples without/with BP for flesh Ca^{2+} (207.3/148.7=1.3) and cell wall Ca^{2+} (768.0/521.1=1.4) concentrations were similar. This indicates that the higher cell wall Ca^{2+} concentrations observed in 'Granny Smith' apples without BP symptoms was due to the higher total flesh tissue Ca^{2+} concentration observed in that fruit (Table 1). Considering that the ratio of Ca^{2+} concentrations in 'Granny Smith' apples without/with BP was the same for flesh and cell wall Ca^{2+}, the ratio of non-cell wall bound Ca^{2+} for 'Granny Smith' apples without/with BP must also be the same, suggesting that non-cell wall bound Ca^{2+} concentration was higher in 'Granny Smith' fruit without BP than in fruit with BP. According to previous studies, lower concentrations of non-cell wall bound Ca^{2+} could enhance fruit susceptibility to BP due to lower availability of Ca^{2+} to other cellular functions (De Freitas et al., 2010). In addition, 'Fuji' apples did not develop BP and had higher total cell wall Ca^{2+} concentrations during storage than 'Granny Smith' apples. In this case, the specific cell wall localization may also play an important role in defining fruit susceptibility to BP. Calcium strongly bound to the water insoluble pectin fraction may be unavailable to other cellular functions, whereas Ca^{2+} loosely bound to short pectin chains in the water soluble pectin fraction may be exchangable and supply Ca^{2+} to other cellular functions as needed. In this context, more specific analyses are required to explore where Ca^{2+} is located in the cell wall, attempting to further understand the role of cell wall bound Ca^{2+} as a mechanism involved in BP development in apple fruit.

Table 2. Calcium concentration in fruit flesh cell walls during storage.

Treatment	mg Ca^{2+} kg^{-1} cell wall		
	At harvest	1 month	2 months
Granny Smith - BP	279.0 b*	365.3 b	768.0 b
Granny Smith + BP	-	-	521.0 c
Fuji	678.6 a	628.6 a	939.7 a
CV (%)	20.9	12.0	18.3

***** Mean values with the same letter within the columns are statistically equal according to Tukey’s test ($p<0.05$).

CONCLUSIONS

High N/Ca^{2+} and [K^{+}+Mg^{2+}]/Ca^{2+} ratios in flesh and skin tissues are associated to BP incidence in apple fruit. Low levels of non-cell wall bound Ca^{2+} concentration may enhance fruit tissue susceptibility to BP development.

REFERENCES

AMARANTE, C.V.T.; CHAVES, D.V.; ERNANI, P.R. Composição mineral e severidade de "bitter pit" em maçãs 'Catarina'. **Revista Brasileira de Fruticultura**, v.28, p.51-54, 2006.

AMARANTE, C.V.T.; ERNANI, P.R.; STEFFENS, C.A. Predição de "bitter pit" em maçãs 'Gala' por meio da infiltração de frutos com magnésio. **Revista Brasileira de Fruticultura,** v.31, p.962-968, 2009.

CRISOSTO, C.H.; DAY, K. Management and control of bitter pit in apples. **Central Valley Postharvest Newsletter,** v.2, p.4, 1993.

DE FREITAS, S.T.; AMARANTE, C.V.T.; LABAVITCH, J.M.; MITCHAM, E.J. 2010. Cellular approach to understand bitter pit development in apple fruit. **Postharvest Biology and Technology**, v.57, p.6-13, 2010.

FERGUSON, I.B.; WATKINS, C.B. Bitter-pit in apple fruit. **Horticultural Reviews**, v.11, p.289-355, 1989.

SAURE, M.C. Calcium translocation to fleshy fruit: its mechanism and endogenous control. **Scientia Horticulturae**, v.105, p.65–89, 2005.

SCHONHERR, J.; BUKOVAC, M.J. Ion exchange properties of isolated tomato fruit cuticular membrane: exchange capacity, nature of fixed charges and cation selectivity. **Planta**, v.109, p.73–93, 1973.

YERMIYAHU, U.; NIR, S.; BEN-HAYYIM, G.; KAFKAFI, U. Quantitative competition of calcium with sodium or magnesium for sorption sites on plasma membrane vesicles of melon (*Cucumis melo* L.) root cells. **The Journal of Membrane Biology**, v.138, p.55–63, 1994.