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Some changes in postharvest physiology and activities of glutamine synthetase in broccoli head supplied with exogenous sucrose during storage

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Abstract

Sugars play indispensable roles in many metabolic processes in plants. In broccoli, the level of sugars, particularly sucrose, rapidly decline few days after harvest. This study investigated the influence of exogenous application of 10% (w/v) sucrose to broccoli heads during storage at 20°C. Hydration of the head was slowed down by sucrose treatment compared with the non-treated heads which gained weight by about 5% of the initial value at the end of the experimental period. Furthermore, sucrose application enhanced ethylene production as well as respiration rate. Glutamine synthetase (GS; EC 6.3.1.2) activity was higher in the florets of sucrose-treated heads but, like the non-treated heads, the activity continuously declined until the end of the storage period. The relatively higher GS activity during the early period of storage caused the delay of the onset of ammonia accumulation by about a day. In the branchlet portion, GS activity was higher in the sucrose-treated heads until day 2 but declined thereafter. The decline in GS activity in this portion, however, did not result to ammonia accumulation.

Key words: Ammonia, ethylene, postharvest life, respiration, broccoli

Introduction

Broccoli heads are harvested for commercial purposes when they are physiologically immature, thus making them susceptible to rapid quality deterioration when kept under ambient temperatures after harvest. During storage, major physio-biochemical changes occur in heads that lead to postharvest senescence. For instance, sucrose level rapidly declines (Pramanik *et al.*, 2004; Coupe *et al.*, 2003; Downs *et al.*, 1997) and ammonia accumulates (Baclayon *et al.*, 2004; Matsui *et al.*, 2004; King and Morris, 1994) in the florets few days after harvest. These changes have been singled out as the major contributing factors in the quality deterioration of the commodity.

Postharvest sugar application has been reported to increase the longevity of some important horticultural commodities such as roses (Ichimura *et al.*, 1999), carnation (Verlinden and Garcia, 2004) and broccoli (Nishikawa *et al.*, 2005; Irving and Joyce, 1995). It could be pointed out that the essential roles of sugars as sources of carbon skeleton for the complex biochemical metabolism in plants contribute to the postharvest life of perishable commodities. It was reported that exogenous sucrose application in broccoli can improve postharvest quality by altering ethylene metabolisms (Nishikawa *et al.*, 2005), keeping higher level of chlorophyll in the florets (Coupe *et al.*, 2003), and increasing ascorbic acid levels (Smirnoff and Pallanca, 1996). Furthermore, sucrose may affect the ammonia detoxification process by providing energy for the latter's assimilation by glutamine synthetase (GS; EC 6.3.1.2), a primary enzyme responsible for assimilating ammonia in plants. As high levels of ammonia are thought to be toxic to plant cells, it is incorporated by GS as the amide group into glutamate, thus enabling detoxification. Ratajczak *et al.* (1981) reported that GS activity in lupine embryo in media containing saccharose was induced. However, in this study, sucrose was considered since sugars are transported to the sink tissues as sucrose; other forms of sugars could be hardly transported into the cells (Nishikawa *et al.*, 2005).

As mentioned earlier, sugar application improves the shelf life of perishable produce. However, the influence of sucrose on glutamine synthetase activity and ammonia accumulation, which are believed to have important roles in the shelf life of broccoli, have not been investigated which formed the basis of this study. This report also presents the changes of some important physiological processes occurring in broccoli during storage.

Materials and methods

Plant material and treatment: Broccoli var. 'Pixcels' heads were harvested from Kagawa Agricultural Experiment Station, Miki, Kagawa, Japan. Right after harvest, the heads were trimmed and brought to the laboratory for treatment. The stem ends were immersed in a 10% (w/v) sucrose solution with 0.05% (v/v) NaClO. The same conditions were employed in the control samples except the addition of sucrose in solution. The solution was replaced with a newly prepared one every 24 h. The heads were enclosed with perforated plastic sheet and kept at 20°C. At the end of each storage period, the florets were separated from the branchlets and kept at -30°C until enzyme and ammonia extractions and analyses.

Daily weight determination: The weights of the heads were taken daily and expressed as percent weight loss or gain of the initial sample weight.

CO_2 and C_2H_4 production rate measurements: Each head was weighed and placed in a 6 L glass jar held at 20°C. Carbon dioxide and ethylene production were measured daily from an intact

head sealed in a glass jar for 1 h by taking a 10 ml (for CO_2) and 1 ml (for C_2H_4) gas sample from the glass jar and injecting the sample to the thermal conductivity detector (TCD) (GC-8 AIT, Shimadzu Co., Ltd.) and flame ionization detector (FID) (GC-14B, Shimadzu Co., Ltd.) gas chromatographs, respectively. The result was expressed in ml CO_2 $kg^{-1}h^{-1}$ for respiration rate and nl $g^{-1}h^{-1}$ for C_2H_4 production.

Extraction and assay of GS and ammonia: Approximately five grams (plus or minus % weight gain or loss of the initial fresh weight of the tissue) from each portion of the broccoli head was added with 1% polyvinyl polypyrrolidone (PVPP) proportional to the sample weight, 1 g sea sand and 5 ml buffer solution containing 50 mM tris-HCl (pH 7.6), 10 mM $MgSO_4$, 1 mM EDTA, 1 mM dithiothreitol (DDT), 12 mM 2-mercaptoethanol, 5 mM L-glutamate and 100 ml glycerol L^{-1}. The mixture was homogenized using a cooled mortar and pestle. The homogenate was squeezed through four layers of cotton cloth. The residual tissues were re-extracted with an additional 5 ml of the same buffer and the filtrate was centrifuged at 12000 x *g* at 2°C for 10 min. Enzyme activity was determined as described by Baclayon *et al.* (2006).

Ammonia content was assayed using the procedure of Kun and Kearney (1974) with few modifications. Briefly, 2 g of fresh-weight sample from each portion of the broccoli head was extracted with 20 ml 10% TCAA at 0°C (ice bath) and centrifuged at 12000 x *g* at 2°C for 10 min. The 1 ml assay mixture contained 200 µl 0.5 M tris-buffer (pH 8), 100 µl 0.1 M 2-oxoglutarate solution (pH 7.4), 30 µl 8 mM β-NADH solution, 150 µl distilled water and 500 µl of neutral extract sample. The decrease of NADH, as determined by the change of extinction at 365 nm, was used as a measure of the reaction.

Analysis of data: Data were analysed in randomized complete block design with three replications to see the significance of treatments using calculated *F*-value. Linear correlation was used to evaluate the relationships between GS activity and ammonia accumulation.

Results

Weight loss/gain: The weights of the broccoli heads treated with sucrose were constant over the first 3 days of storage, and decreased thereafter (Fig. 1). For the non-sucrose-treated heads,

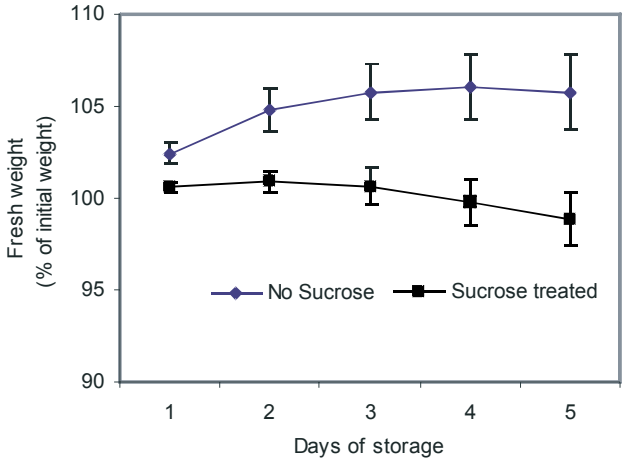

Fig. 1. Changes in fresh weight of intact broccoli heads supplied with exogenous sucrose during storage. Vertical bars indicate SE. SE bars are not shown when masked by the graph symbols.

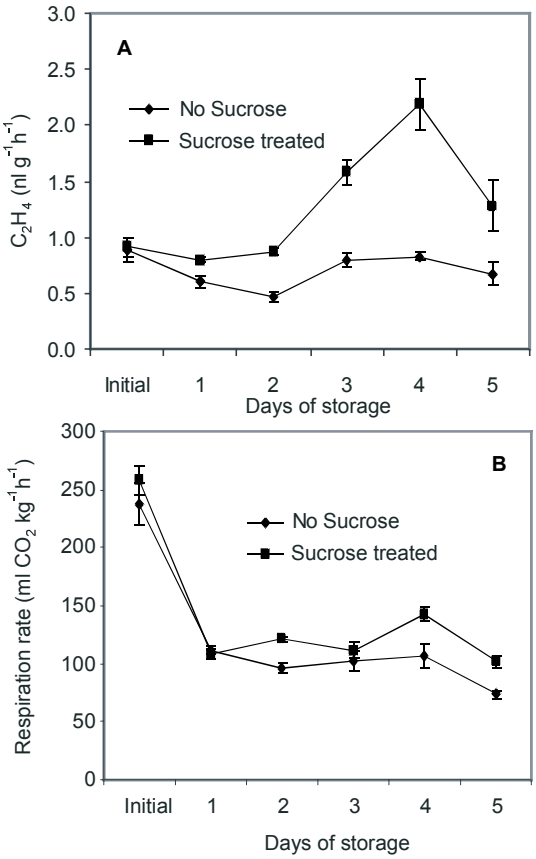

Fig. 2. Changes in ethylene production (A) and respiration rate (B) of intact broccoli heads supplied with exogenous sucrose during storage. Vertical bars indicate SE. SE bars are not shown when masked by the graph symbols.

the weights continuously increased until the last day of the storage. Weight gain was significantly higher in the heads without sucrose treatment than in the treated ones.

Production of C_2H_4 and CO_2: Ethylene production was constant in the sucrose treated heads while declined in the non-treated heads until day 2 of storage (Fig 2A). A rapid increase was observed in the sucrose-treated heads while the level in the non-treated heads was at par with the initial value until day 4. At the end of the 5-day storage period, ethylene production declined in both treatments. Although ethylene was higher in the treated heads, the pattern of decline in greenness was nearly the same as that in heads without sucrose treatment (data not shown).

Carbon dioxide production drastically declined after 24 h from harvest in both the treated and non-treated heads (Fig. 2B). However, CO_2 production in the sucrose-treated heads was consistently higher than that in the non-treated heads from day 2 until day 5.

GS activity and ammonia accumulation: The glutamine synthetase activity varied between portions of broccoli head. In the floret portion, the GS activity in both treatments increased after 24 h from harvest and continuously declined thereafter (Fig. 3A). The sucrose-treated florets had relatively higher enzyme activity than the non-treated ones throughout the entire storage duration. In the branchlet portion, sucrose treatment increased the enzyme activity until day 2 while the activity in the non-treated portion was maintained until day 3. Enzyme activity in the sucrose-treated tissue was higher than that without sucrose treatment except on day 3.

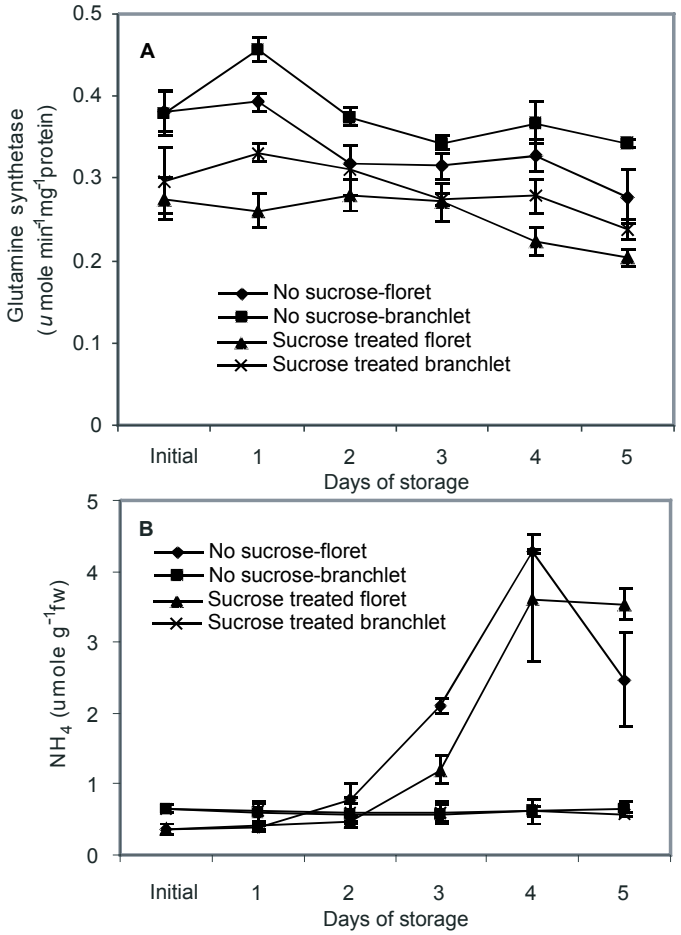

Fig. 3. Changes in glutamine synthetase activity (A) and ammonia content (B) in the branchlet and floret portions of broccoli heads supplied with exogenous sucrose during storage. Vertical bars indicate SE. SE bars are not shown when masked by the graph symbols.

Ammonia content in the florets treated with sucrose was slightly lower than that without sucrose until day 4 (Fig. 3B). Although ammonia content abruptly increased in both treatments, the onset of ammonia accumulation was delayed by about a day in the sucrose treated florets than that without sucrose. In the branchlet portion, the ammonia content was not significantly different between the non-treated and the sucrose-treated tissues, and there was no remarkable change in ammonia content throughout the storage duration.

Correlation between GS activity and ammonia accumulation: Correlation analysis revealed that there is a significant negative correlation between GS activity and ammonia accumulation in the floret portion of the sucrose–treated heads (Table 1). On the other hand, no significant correlation between GS and ammonia content was found in both portions of the non-treated heads and the branchlet portion of the treated ones.

Table 1. Correlation coefficient (*r*) values computed from linear regression analyses between glutamine synthetase activity and ammonia accumulation in the branchlet and floret portions of broccoli heads treated with exogenous sucrose during storage

Treatment	Portion	Correlation coefficient (*r*) value
Without Sucrose	Florets	-0.356
	Branchlets	-0.277
Sucrose-treated	Florets	-0.503*
	Branchlets	0.359

*significant correlation at $P<0.05$ (0.468); n=18

Discussion

Major physio-biochemical changes have been observed in broccoli head during storage (Deschene *et al.*, 1991; Pogson and Morris, 1997; Matsui *et al.*, 2004; Pramanik *et al.*, 2004). In this study, immersing the cut base of the broccoli head in a solution either with or without sucrose during storage affected the hydration rate of the head as manifested by the changes in weight during storage (Fig.1). The weights of the sucrose-treated heads remained constant until day 3, but declined thereafter. In the non-sucrose-treated heads, the weights increased continuously to about 5% of the initial content at the end of the storage period. The decline in weight after day 3 may imply that transpiration rate exceeded the hydration rate in broccoli head. The presence of solute, sucrose in this case, could have impaired the hydration process. In cut rose, treatment in a solution containing sucrose significantly retarded hydration (Durkin, 1979). Furthermore, the application of sucrose enhanced ethylene production (Fig. 2A). After day 3 of storage, ethylene rapidly increased until day 4 in the sucrose treated heads but no remarkable increase was observed in the non-treated heads. Although ethylene production was significantly higher in the sucrose-treated heads, the rate of yellowing (data not shown) was almost the same as in the non-treated ones. Nishikawa *et al.* (2005) attributed this result to the decreased sensitivity of the florets to ethylene. They suggested that sucrose may be easily transported into the sink cells, the florets, and glucose produced by sucrose hydrolysis in the cells may enhance the ethylene biosynthesis. However, it was unclear as to what regulates ethylene sensitivity, sucrose or its hydrolyzed product, glucose, in the cell. On the other hand, the respiration rate was initially high in all heads but abruptly declined after 24 h from harvest and stabilized thereafter (Fig. 2B). The high initial CO_2 production can be attributed to stress imposed during harvest and subsequent trimmings. In the sucrose-treated heads, respiration rate was consistently higher than the non-treated heads from day 2 until the end of the storage period. The ready availability or abundance of respiratory substrate could have caused the higher CO_2 production.

GS activity in the florets of both treatments reached the maximum, a day after harvest, and continuously declined thereafter (Fig. 3A). The enzyme activity was higher in the florets of the sucrose-treated heads than in the non-treated ones. The changes in enzyme activity in the floret portion of sucrose-treated heads were negatively correlated with ammonia accumulation (Table 1). In senescing wheat leaves, the accumulation of ammonia has been found to coincide with almost complete disappearance of GS (Peters and Van Laere, 1992). The result implies that due to the relatively higher GS activity in sucrose-treated head, the onset of ammonia accumulation was delayed compared to the non-treated heads. Ammonia started to rise on days 2 and 3 in the non-treated and the sucrose-treated florets, respectively (Fig. 3B). In the branchlet portion, GS activity was higher in the sucrose-treated tissue during the earlier period of storage but dropped after day 3. Although there was a decline in GS activity in this portion, there was no significant increase in ammonia content until the end of the 5-day storage period. It is likely that the level of ammonia has been efficiently assimilated by GS. The ammonia produced from protein catabolism, amino acid deamination and some biosynthetic reactions (Lam *et al.*, 1996) may not have

exceeded the required amount to repress GS activity. In addition, the higher sugar content of broccoli branchlet (Pramanik *et al.*, 2004) favoured the activity of the enzyme.

The results of this study suggest that sucrose treatment delays the onset of ammonia accumulation due to relatively higher GS activity in the earlier period of storage of broccoli heads. However, as in the non-treated heads, the GS activity continued to decline after 24 h from harvest. The relatively higher respiration rate and ethylene production in the sucrose treated heads have enhanced senescence. Thus, the benefit of delayed onset of ammonia accumulation may be diminished by the effects of other deteriorative processes. Further study to a wider range of cultivars is needed to validate these suggestions.

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