貯蔵温度による収穫後のタケノコのGS、ACC合成、ACC酸化酵素とPAL活性の変化

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Postharvest Changes in Activities of GS, ACC Synthase, ACC Oxidase, and PAL in Bamboo Shoots at Different Storage Temperatures

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In this study, we investigated the postharvest changes in glutamine synthetase (GS; EC 6.3.1.2), l-aminocyclopropane-1-carboxylate (ACC) synthase (EC 4.4.1.14), ACC oxidase (EC 1.4.3) and phenylalanine ammonia lyase (PAL; EC 4.3.1.5) activities in relation to ammonia content, ethylene production, respiration rate, and texture and color changes after harvest of moso bamboo (Phyllostachys edulis) shoots stored at 1 and 20°C for up to 9 days. GS activity decreased in shoots stored at 20°C with increasing ammonia content up to approximately 6-fold the initial content after a 9-day storage period. Although the highest ACC oxidase activity corresponded to the highest ethylene production, the highest ACC synthase activity was delayed 8 h or more compared with that in the previous study. The development of PAL activity coincided with an increase in breaking strain at 20°C up to 3 days. Low-temperature storage of shoots at 1°C retained good shoot color and quality since it was effective in decreasing respiration rate, ammonia accumulation, ACC synthase, ACC oxidase, and PAL activities after the harvest of moso bamboo shoots.

(Mosoe bamboo shoots accumulate ammonia during postharvest storage. GS is the primary enzyme responsible for assimilating ammonia in plants. As high levels of ammonia are thought to be toxic to plant cells, ammonia is incorporated by GS as the amide group into glutamate, thus enabling detoxification.

The plant hormone ethylene is produced in response to various types of environmental stress, such as wounding, physical load, disease, exposure to low temperatures and chemicals, and water stress. ACC synthase and ACC oxidase play essential roles in the ethylene biosynthetic pathway. ACC synthase catalyzes the conversion of s-adenosylmethionine (SAM) to ACC, whereas ACC oxidase catalyzes the oxidation of ACC to ethylene.

Lignin, the substance that lends fibers their toughness, is polymerized from cinnamyl alcohol derived from the shikimic acid pathway and the enzymatic browning substrate chlorogenic acid in bamboo shoots, is metabolized from p-coumaric acid or caffeic acid via the same pathway. PAL catalyzing the conversion of l-phenylalanine to trans-cinnamic acid has been considered to play an important role as the key enzyme for lignification and for producing a polyphenol substrate. CHEN et al. observed a rapid increase in PAL activity in harvested bamboo shoots stored at relatively high temperatures.

In this study, we investigated respiration rate, color changes and the activities of the aforementioned enzymes and their substrates that occur in shoots stored for up to 9 days at 1 and 20°C.

Materials and Methods

1. Plant materials

Moso bamboo shoots were harvested from a farmer's field in Kagawa Prefecture, Japan in April, 2004. The shoots were immediately brought to the
laboratory (Faculty of Agriculture, Kagawa University). The bract was peeled off and enclosed with perforated plastic bags with 8 holes (6 mm φ) or left unbagged at 1 and 20°C for 9 days in the dark. At the end of the storage period, the shoots were weighed (both bagged and unbagged) and the changes in color, respiration rate and ethylene production for shoots only enclosed with plastic bags were measured. Samples were immediately frozen at −80°C until analysis.

2. Weight loss of peeled shoots

Weight loss was determined by weighing the peeled shoots 0, 3, 6 and 9 days after harvest. It was expressed as a decrease in sample weight (g) as determined every 3 days.

3. Respiration rate and ethylene production measurement of peeled shoots

A peeled bamboo shoot was placed in a 5 l glass jar held at 20°C. Carbon dioxide and ethylene productions were measured at 0, 3, 6 and 9-day periods by taking 10 ml of CO₂ and 1 ml of C₂H₄ gas from the glass jar sealed for 1 h and injecting the gasses into thermal conductive detector (TCD) (GC-8 AIT, Shimadzu Co., Ltd.) and flame ionization detector (FID) (GC-14 B, Shimadzu Co., Ltd.) gas chromatographs, respectively. The results were expressed as ml CO₂ kg⁻¹ h⁻¹ and µl C₂H₄ kg⁻¹ h⁻¹, respectively.

4. Color change/hue angle determination of peeled shoots

Surface color was measured using a chromometer (Nippon Denshoku, Kogyo Co., Ltd.) equipped with an 8 mm measuring head and a C illuminant. The meter was calibrated using the manufacturer's standard white plate. Color changes were quantified using the L, a and b colorimetric system. L refers to the lightness of the head, and ranges from black = 0 to white = 100. Positive and negative a values indicate red-purple and green, while positive and negative b values indicate yellow and blue, respectively. Hue angle was calculated in a manner similar to that reported in the previous paper.

5. Texture measurement

Texture was measured rheologically based on the measurement of strain to pressure or shearing. Breaking strain, which indicates the fiber content in shoots, was determined with a creep meter (YAMADEN RHEONER RE-3305) equipped with software Ver. 2.0 for automatic analysis. With a running load cell of 2 kgf, a cross-sectional cut of 1 cm thickness was made at a rate of 1 mm per second using the reverse side of a blade with a thickness of 0.04 mm. The circumference of the cut tissue was further divided into 5 cm portions and breaking strain (fragility strain rate, 100% strain rate means sample thickness and a large number indicates strong fragility) reading was made at 3 points on each shoot portion.

6. Extraction and assay of GS and ammonia

The extraction and assay procedures for the determination of GS activity and ammonia content were performed as described by MATSUI et al. The result of GS activity was expressed as µmol glutamine produced per min per mg of protein and that of ammonia as µmol N₄H₄ produced per g fresh weight.

7. Extraction and assay of ACC synthase and ACC oxidase

The extraction and assay procedures for the determination of ACC synthase and ACC oxidase activities were carried out as described by BHOWMIK et al. The results of ACC synthase and ACC oxidase activities were expressed as nmol ethylene produced per h per mg of protein.

8. Extraction and assay of PAL activity

The extraction and assay procedures for the determination of PAL activity were performed as described by BHOWMIK et al. The results of PAL activity were expressed as nmol of trans-cinnamate formed per h per mg of protein.

9. Statistical analysis

The experiment was performed using a randomized complete block design with three replications. The difference between the means of two populations was calculated by T-test.

Results

1. Changes in weight, color, respiration rate and texture

At both storage temperatures, the weight of the shoots kept in a perforated bag was almost maintained throughout the 9-day storage period. On the other hand, the unbagged shoots stored at 1 and 20°C had lost approximately 16.7 and 52.8% of their water content by the last day of storage, respectively (Fig. 1, upper part). The hue angle at 1°C was higher than that at 20°C until day 3. However, the severe browning caused by polyphenol oxidase resulted in zero values of L, a and b. Due to this condition, it was impossible to
calculate the hue angle changes thereafter (Fig. 1, middle part). The decrease in hue angle during storage was evident from the corresponding decrease in yellowish green on the head parts of bamboo shoots. All heads except those stored at 20°C for 6- and 9-day periods were substantially yellowish green at the end of the experimental period. The respiration rate abruptly decreased during the first 24 h of storage at 1°C and was almost constant until the end of the experimental period, while at 20°C, the CO₂ production of the shoots gradually decreased until day 9 (Fig. 1, lower part). A general increase in the fiber content of the shoots was observed for both 1 and 20°C storages (Fig. 4, lower part). Although the fiber developments of the shoots under these conditions followed almost the same patterns, the breaking strain, indicating the toughness of the shoots, was higher during storage at 20°C than that at 1°C.

2. GS activity and ammonia accumulation

GS activities began to decrease during the first 24 h of storage at both 1 and 20°C (Fig. 2, upper part). After that, the activity at 20°C continued to decrease until day 3 and remained almost unchanged thereafter. At 1°C, on the other hand, the activity increased on day 3 of storage and then decreased on day 6. It again increased slightly at the end of the storage period. GS activity was significantly higher in the shoots stored at 1°C than in those stored at 20°C. A general increase in the ammonia content of the shoots stored at 20°C was observed throughout the experimental period but no significant ammonia accumulation was observed in the shoots stored at 1°C (Fig. 2, lower part). The ammonia content for 20°C storage increased to approximately 6-fold the initial level at the end of the 9-day storage period.

3. ACC synthase and ACC oxidase activities and ethylene production

The ACC synthase activity of the bamboo shoots began to increase during the first 24 h of storage at 20°C until day 6 except for a transient decrease.
During storage at 20°C, the ethylene production in bamboo shoots started to increase after harvest reaching a peak at 24 h (Fig. 3, lower part). In this period, the ethylene production was observed to be approximately 6-fold that of the initial level. After that, the production declined on day 6 and almost remained unchanged until day 9. On the other hand, the ethylene production in the bamboo shoots stored at 1°C sharply reached a peak on day 3 and suddenly decreased until day 6. The ethylene production at 1°C was significantly higher than that at 20°C on day 3.

4. PAL activity

The PAL activity of the bamboo shoots stored at 20°C increased after harvest reaching a peak on day 3 (Fig. 4, upper part). In this period, the PAL activity increased to approximately 2.5-fold that of the initial level. After that, the activity sharply decreased until day 6. On the other hand, the PAL activity of the bamboo shoots stored at 1°C slightly increased after day 1 and was almost unchanged thereafter.

![Fig. 3](image-url) Changes in ACC synthase and ACC oxidase activities and ethylene production during storage of moso bamboo shoots at 1 and 20°C

Each point represents the mean of three replications. Vertical bars indicate SE.

![Fig. 4](image-url) Changes in phenylalanine ammonia-lyase activity and breaking strain during storage of moso bamboo shoot at 1 and 20°C

Each point represents the mean of three replications. Vertical bars indicate SE.
Discussion

Ammonia is thought to be toxic to plants in excessive levels. Plant tissues are detoxified from ammonia by glutamine synthetase. In a recent report, it has been shown that ammonia accumulation is a factor in the perishability of asparagus, which occurs due to changes in GS activity. We have measured the ammonia content and changes in the GS activity of stored bamboo shoots and found almost the same trend as that of asparagus. The accumulation of ammonia in senescing leaves has been shown to coincide with the almost complete disappearance of GS. Recent results followed the same trend in which ammonia accumulation increased to approximately 6-fold the initial content after a 9-day storage period with a corresponding decline in the GS activity of the shoots stored at 20°C. HURST et al. reported that the postharvest inhibition of GS using phosphinothricin (PPT) at 40 and 200 ppm reduced GS activities by 50 and 80%, respectively, but did not increase ammonia content. Ammonia content was only increased when more than 95% of GS was inhibited. Thus, it seems that there is a critical level of GS activity important for the postharvest life of perishable vegetables and that under normal postharvest conditions, this critical level is exceeded and surpasses the required GS activity for ammonia salvage.

The ethylene production of sliced bamboo shoots reached the maximum 16 h after harvest, whereas that in the excised ones occurred only after days 1 and 3 at 20 and 1°C, respectively. This finding suggests that a severely wounded vegetable produces wound-induced ethylene at a faster rate and at a higher level. Although ACC oxidase and ACC synthase showed higher activities in the thinly sliced bamboo shoots than in the excised ones, the activities followed almost the same trends. ACC synthase is the rate-limiting enzyme in ethylene biosynthesis: hence, it is considered to regulate the ACC oxidase called the ethylene-forming enzyme. The results of this experiment are in agreement with those of previous reports that although ethylene production is dependent on ACC oxidase activity, the highest ACC synthase activity is delayed for 8 h or more compared with ACC oxidase activity. This suggests that in addition to ACC synthase, other enzymes may be involved in the regulation of ethylene production. Hence, the role of ACC synthase in the regulation of ethylene biosynthesis remains unclear at this time.

During storage, bamboo shoots, similar to asparagus spears, undergo textural changes because of changes in PAL activity. There was an increase in the PAL activity of the shoot which continued until day 3 at 20°C. After that, the PAL activity started to decrease and it followed almost the same pattern as that of asparagus spears. The PAL activity was higher at 20°C storage than 1°C storage until day 3. Although we did not measure lignin content in this experiment, our report on asparagus and the report of CHEN et al. clearly demonstrated that lignin production is induced to some extent by increased PAL activity. In spite of a higher ethylene production at 1°C storage, the lower activity of PAL under this storage condition may suggest that endogenous ethylene evolved is not directly related to wound-induced PAL in bamboo shoots as in asparagus. When bamboo shoots were stored for more than 3 days at 20°C, the color lightness decreased suddenly due to severe browning caused by PAL and polyphenol oxidase in the bamboo shoots. According to KOZUKUE et al., this phenomenon might be due to an increase in phenolic compounds during storage.

In conclusion, low-temperature storage is effective in decreasing the respiration rate, ammonia accumulation, PAL, ACC synthase and ACC oxidase activities of harvested moso bamboo shoots.

References
5) KOZUKUE, E., KOZUKUE, N. and TSUCHIDA, H.: Changes in several enzyme activities accompanying the pulp browning of bamboo


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本報告は，1℃と20℃に収穫後9日目まで貯蔵された孟宗竹のアンモニア含量，エチレン生成，呼吸速度，テクスチャ，色変化との関連で，グルタミン合成酵素（GS）と1-アミノシクロプロパン-1-カルボン酸（ACC）合成酵素，ACC酸化酵素，フェニルアラニンアノミアリアーゼ（PAL）活性の収穫後の変化について検討した。

GS活性は，20℃貯蔵のタケノコで，貯蔵9日目で初期の約5倍までアンモニアの蓄積が増大するに伴って減少した。

ACC酸化酵素の最大活性は最大のエチレン生成に一致したが，ACC合成酵素の最大活性は，前報*1と比較すると8時間かそれ以上の時間遅れた。PAL活性の発現は20℃で3日目まで破断歪率の増大と関係していた。

1℃の低温貯蔵が，孟宗竹収穫後の呼吸速度とアンモニアの蓄積，ACC合成酵素，ACC酸化酵素，PAL活性の減少に効果的であり，その結果，色や品質を保持した。

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