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Research paper

Sugars in postharvest lotus seeds were modified by 6-benzylaminopurine treatment through altering related enzymes involved in starch-sucrose metabolism

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ABSTRACT

To investigate the effect of 6-benzylaminopurine (6-BA) treatment on starch-sucrose metabolism in postharvest lotus seeds, the lotus pods were immersed with 20 mg L^{-1} 6-BA and stored at 25 ± 1 °C for 8 days. Our results revealed that 6-BA treatment not only delayed the browning of lotus pods and seeds, but also inhibited the respiration rate of lotus pods. Compared with controls, 6-BA treatment enhanced the deposition of starch, which was supported by the decreased amylase level. Contrary to the alteration of starch content, glucose and fructose contents in lotus seeds were reduced by 6-BA treatment through inhibiting the production of hexose from sucrose and starch degradations. However, the sucrose content in 6-BA treated group was higher than that in control groups until 6th day of storage, which was resulted from enhanced sucrose phosphate synthase (SPS) level and inhibited activities of synthase-cleavage (SS-cleavage) and invertase by 6-BA treatment. These results indicated that 6-BA treatment could maintain the starch and sucrose contents of lotus seeds by affecting related-synthetic or degrading enzymes involved in starch-sucrose metabolism, and alleviate postharvest senescence of lotus pods and seeds.

1. Introduction

Lotus (*Nelumbo nucifera Gaertn.*), a perennial aquatic plant, has been cultivated extensively in Asia (India, China and Japan) for more than 2000 years (Miao et al., 2013). The seeds of lotus pods, which are rich in carbohydrates (mostly starch), proteins, amino acids and alkaloids, are sold as nutritious food and employed in medical procedures to treat tissue inflammation, arrhythmia, cancer, diuretics, and skin diseases (Bhat and Sridhar, 2011; Guo et al., 2015). In recent years, evaluation of nutritional and functional components as well as antioxidant properties of lotus pods have been established (Liu et al., 2013; Lv et al., 2012; Yen et al., 2005).

Lotus pods are harvested mainly from July to September in China. At the ambient temperature of this season, fresh lotus pods are very susceptible to microbial contamination and undergo rapid senescence such as browning. Generally, their quality markedly decreased at 25 °C for about three to four days after harvest (Bhat et al., 2010; Gao et al., 2016). The use of postharvest low temperature storage for delaying senescence or inhibiting respiration rate along with maintaining nutritional quality can be beneficial for lotus pods industry as well as consumers. But, for prolonged storage, the cost of cold-chain transportation is so high. Recently, some measures were adopted to retain nutritional qualities and decontaminate microbial load of dry, such as electron beams and gamma irradiation (Bhat and Sridhar, 2008, 2011). Despite the use of 1-methylcyclopropene (1-MCP) for delaying the senescence of postharvest lotus pods and seeds in our previous study (Li et al., 2016), few information has been reported as to extending shelf life of lotus pods and seeds after harvest.

6-Benzylaminopurine (6-BA) is a good inhibitor of chlorophyll degradation (Shewfelt et al., 1983; Gomez-Lobato et al., 2012). It has been broadly employed for delaying the senescence of various harvested commodities, such as fresh-cut *zizania latifolia* (Luo et al., 2013), summer squash (Massolo et al., 2014), and cauliflower (Siddiqui et al., 2015), litchi (Jiang and Fu, 1998) and peach fruit (Zhang et al., 2015). 6-BA regulates *P. aquilinum* senescence through multiple metabolic pathway, one of which is carbohydrate metabolism (Huang et al., 2016). More recently, the study of Miyazawa et al., 2002 also indicated that cytokinins play an important role of in starch metabolism. In addition, it has been demonstrated that 6-BA was a competitive inhibitor of glycolytic kinases in storage tissue (Tuli et al., 1964), indicating that 6-BA

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might participate in sugar metabolism of some species. However, whether or not 6-BA has impact on starch-sucrose metabolism of lotus seeds was unclear.

In our study, the effect of 6-BA immersing treatment on visual evaluation, respiration rate of lotus pods, and the contents of starch, soluble sugar, and individual sugars in lotus seeds were determined; Additionally, the activities of enzymes involved in starch-sucrose metabolism were evaluated. Furthermore, we investigated endogenous 6-BA content of lotus seeds to understand its absorption after treatment. Our objective was to check the function of 6-BA on starch-sucrose metabolism of lotus seeds and postharvest senescence of lotus pods.

2. Material and methods

2.1. Lotus pods materials and experimental design

Lotus (*Nelumbo nucifera Gaertn.*) pods were harvested from a lotus base named 'Heshenglianye' at Jinhu contry, Huaian district (Jiangsu Province, China) on August 10, 2015. Harvested lotus pods were immediately transported to laboratory. Then the lotus pods of uniform size and maturity with absence of physical injuries or diseases were selected for subsequent treatments.

In the preliminary experiment, the lotus pods (n = 120) were divided into 4 groups randomly and immersed with 6-benzylaminopurine (6-BA, Sigma-Aldrich, USA) solutions with concentration of 10, 20, 30, and 40 mg L^{-1} for 10 min, respectively. Lotus pods without any treatment and treated with distilled water were used as blank control (CK_0) and CK_1, respectively. After air-dried for 2 h at room temperature (24–27 °C), the lotus pods were placed in 21-L Lock & Lock boxes with two holes (1 cm in diameter) in the diagonal position to ensure humidity, and then stored at 25 ± 1 °C with 80–90% relative humidity for 8 days. It was found that 6-BA treatments with 20 and 30 mg L^{-1} significantly maintained higher qualities of lotus pods and lower browning degree of the seeds, in comparison with other groups (see Supplementary Fig. S1 in the online version at DOI: 10.1016/j.scienta.2017.03.044). Since no significant difference was observed between these two treatments, the dose of 20 mg L^{-1} 6-BA was employed for the second set of experiments.

Then, a total of 450 lotus pods were selected for the experiment and randomly divided into 3 lots for 20 mg L^{-1} 6-BA treatment and controls (including CK_0 and CK_1) which were set as preliminary experiment. Subsequently, the dried samples were stored under the same conditions as above for 8 days. During storage, thirty lotus pods from each treatment were sampled (10 lotus pods from each replicate) every other day for physicochemical analysis. The hard lotus pod coat and seed coat were separated manually from the cotyledon, and the rest seeds whose embryo was removed with stainless steel knife were immediately frozen in liquid nitrogen and then stored at −20 °C for further analysis. The fresh lotus pods were used for analyzing respiration rate and dehydration.

2.2. Browning degree determination of lotus seeds

The frozen lotus seeds from each replicate produced by 10 lotus pods in all treatments were pulverized using a liquid nitrogen grinder (IKA A11, Germany), and 2 g sample were taken and homogenized with 10 mL of 100 mmol L^{-1} phosphate buffer (pH 6.8). After centrifuged at 10,000 × *g* for 15 min at 4 °C, the supernatants were used for browning degree analysis at 420 nm using a TU-1810 Spectrophotometer (the mixture was neutralized with 10% NaOH, and then taken up to 100 mL with distilled water.) and browning degree was expressed as $10 \times A_{420nm}$ (Jiang et al., 2014).

2.3. Visual evaluation of lotus pods and the seeds and corresponding photographs

Lotus pods were assessed by a trained panel of ten members immediately after harvest and during the storage periods. The panel members were required to score the lotus pods and seeds group immediately after samples were removed from storage. The samples were scored in terms of freshness, browning, and dehydration of lotus pods, as well as texture and browning of the seeds. Each index was divided into four scores (2, 1.5, 1, and 0.5), with higher levels mean better visual evaluation and the sum of the five indexes was the quality score of corresponding samples. After the assessment, photographs were taken.

2.4. Assay of endogenous 6-BA content in lotus seeds

The endogenous 6-BA content in lotus seeds was assayed as described by Blakesley and Constantine (1992) with some modifications. The frozen pulverized samples (2 g) were ultrasonic extracted in 8 mL of 60% (v/v) acidified methanol (50 μL of acetic acid were added in per 100 mL solution) for 30 min. The homogenate was centrifuged at 10,000 × *g* for 15 min at 4 °C and extracted repeatedly. Subsequently, 5 mL of the supernatants were evaporated to 1 mL aqueous solution under nitrogen, and then purified through a C18 Sep-Pak cartridge (Waters, Milford, MA, USA); 3 mL of distilled water were passed through the cartridge for clearing off water impurities, followed by 3 mL of methanol with the eluate for 6-BA assay.

A high performance liquid chromatography (HPLC) system (Agilent 1100, Agilent Corp., USA) was used for 6-BA assay, with an X Bridge C18 column (2.5 μm, 4.6 × 250 mm, Waters, USA) and an ultraviolet detector (Agilent 1100, Agilent Corp., USA). Methanol/acetonitrile/0.1% acetic acid (65:5:35, v/v) was used as mobile phase, operating at a flow rate of 1 mL min^{-1}, and a column temperature of 30 °C. Identification and quantification of 6-BA were performed by comparing retention time and peak area with a standard (Sigma-Aldrich, USA).

2.5. Respiration rate determination of lotus pods

At the onset of the experiment, 30 fresh lotus pods (10 lotus pods for each replicates) were collected from each treatment. The samples in each group were then sealed for 1 h in a plastic box (21 L) at 25 ± 1 °C. For respiration rate assay, 15 mL of headspace samples were taken and the concentration of carbon dioxide (CO_2) was analyzed with a gas chromatography (Agilent 7820, Agilent Corp., USA) equipped with a FID detector, The column temperature was 70 °C. The running time was 3.5 min and the carrier gas was nitrogen (N_2) with a flow rate of 25 mL min^{-1}.

2.6. Measurement of starch and total soluble sugar contents in lotus seeds

Two grams of pulverized samples were suspended in 10 mL of 80% (v/v) ethanol and extracted repeatedly for 30 min at 80 °C. After cooled at room temperature, the mixture was filtered through a Whatman No. 4 filter paper (Maidstone, UK), and residues washed with hot distilled water repeatedly. The filtrates were evaporated to dryness in vacuo and then dissolved in a volume of 100 mL with distilled water. The solution was used to determine soluble sugar content.

For starch analysis, the residues of measured soluble sugar were suspended in 30 mL of distilled water, then 2 mL of 9.2 mol L^{-1} $HClO_4$ was added, and the mixture was boiled for 1 h. After cooled at room temperature, the mixture was passed through filter papers