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# Proteomic profiling of 24-epibrassinolide-induced chilling tolerance in harvested banana fruit

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ABSTRACT

The mechanism of 24-epibrassinolide (EBR)-induced chilling tolerance in harvested banana fruit was investigated. Results showed that EBR pretreatment remarkably suppressed the development of chilling injury (CI) in harvested banana fruit during 12 days of cold storage at 8 °C, as indicated by lower CI index in treated fruit. Physiological measurements exhibited that EBR treatment reduced the relative electrolyte leakage and malondialdehyde (MDA) content while increased the chlorophyll fluorescence (*Fv/Fm*), total soluble solids (TSS) and ratio of TSS and titratable acidity. Furthermore, the differentially accumulated proteins of banana fruit in response to EBR and cold treatment were investigated by employing gel-based proteomic in combination with MALDI-TOF-TOF MS and LC-ESI-MS/MS analyses. There were fifty five protein spots to be successfully identified. Notably, most of up-regulated proteins by EBR treatment were related to energy biosynthesis, stress response and cell wall modification. In contrast, proteins involved in protein degradation and energy consumption were down-regulated by EBR treatment. These results suggest that EBR treatment could enhance the defense ability, promote the synthesis and utilization of energy, as well as maintain the protein function via enhancing protein biosynthesis and inhibiting protein degradation, consequently contributing to improvement of cold tolerance in harvested banana fruit.

*Significance:* To extend our understanding of chilling injury (CI) of harvested banana fruit, we reported the effect of 24-epibrassinolide (EBR) on CI of banana fruit when stored at 8 °C. It was the first report on the comprehensive proteomic analysis of banana fruit in response to EBR treatment at low temperature. EBR pretreatment significantly reduced CI in harvested banana fruit. Fifty five protein spots were successfully identified. Notably, the most of up-regulated proteins by EBR treatment were related to energy biosynthesis, stress response and cell wall modification. In contrast, proteins involved in protein degradation and energy consumption were down-regulated. These results suggest that exogenous EBR treatment could enhance the defense ability and maintain high energy status. Meanwhile, EBR treatment maintained protein function via enhancing protein biosynthesis and inhibiting protein degradation. These results may help us to understand the molecular mechanism of the chilling tolerance induced by EBR treatment and broaden the current knowledge of the mechanism of CI of harvested banana fruit.

## 1. Introduction

Banana is an important fruit worldwide with short shelf life due to its climacteric characteristic [1]. Low temperature storage has been considered an effective strategy to prolong storage life of harvested crops. However, banana fruit is susceptible to chilling injury (CI) when storage temperature is below 13 °C. The CI symptoms of banana fruit include delay of yellow color development, skin spotting and failure of fruit softening, which lead to serious quality deterioration and huge economic loss [2].

A number of strategies for alleviating CI of banana fruit have been examined, in which exogenous application including ethylene [2],

*Abbreviations:* CI, Chilling injury; EBR, 24-Epibrassinolide; MDA, Malondialdehyde; PPI, Protein–protein interaction; SAM, *S*-adenosylmethionine; NDPKs, Nucleoside diphosphate kinases; RbohD, Respiratory burst oxidase homolog D; SOD, superoxide dismutase; CAT, catalase; APX, Ascorbate peroxidase; UXS, UDP-glucuronic acid decarboxylase

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nitric oxide [3], hydrogen sulfide [4], malic acid [5], and heat treatment [6] were evidenced to be effective.

Plant hormones are deemed to be involved in regulating response defense and adaptation to environment in plant [7]. Recent studies have showed that some phytohormones, including ethylene, salicylic acid (SA), methyl-jasmonate (MeJA), brassinosteroids (BRs), and gibberellins (GAs) were related to fruit response to cold stress [8]. Among these plant hormones, BRs are comprehensive functional steroidal phytohormones that participate numerous physiological processes, particularly, in improvement of plant tolerance to abiotic and biotic stresses [9]. In postharvest crops, exogenous BRs application markedly improves tolerance to chilling stress as reported in mango [10], green pepper [11], oilseed rape [12], tomato [13] and grapevines [14]. However, effects of BRs on CI of banana fruit and its possible mechanism are less documented.

Proteomics approach has been widely used to reveal the essence of CI occurrence in cold-sensitive fruit [15–17]. In previous study, we investigated the ethylene-induced cold tolerance of banana fruit based on proteome change, which involves in modulation of ATP synthesis, ROS scavenging, protein refolding and degradation, etc. [18]. However, the differently accumulated proteins in response to chilling stress after BRs treatment were still unknown.

The objective of this study was to investigate the role of BR in the regulation of CI. 24-Epibrassinolide (EBR) is one of the most active forms of BRs available commercially, and has a favorable safety profile [19].For this purpose, comparative proteomic analysis was performed to investigate the differently accumulated proteins of banana fruits with and without EBR pretreatment during cold storage. The study offered new insight into BR-induced chilling tolerance of banana fruit.

## 2. Material and methods

### 2.1. Plant material and treatment

Green mature banana (*Musa spp*., AAA group cultivar 'Brazil') fruit were harvested around 110 days after flowering from an orchard in Haikou city, Hainan province in China. Banana fruit fingers were dipped in 0.1% Sportak® (prochloraz, Bayer) fungicide solution for 3 min to control the decay, and followed by air-dry. Fruit with uniformity of shape, color and size were selected and divided into two groups. The first group was immersed in 40 μM aqueous EBR (Yuanye Biotechnology Co., Ltd., Shanghai, China) solution for 10 min at 25 °C. Another group immersed in water was served as control. After treatments, fruits were packed into 0.03 mm polyethylene bags (9 holes with 8 × 5 mm diameter for each bag, 3 fruits per bag) and stored at 8 °C and 85–90% RH for up to 12 days. CI index, chlorophyll fluorescence parameter, relative electrolyte leakage and malondialdehyde (MDA) content, total soluble solids (TSS) and ratio of TSS and titratable acidity (TA) were measured at 0, 4, 8 and 12 day of storage. Mixed peel tissues (the middle section) were collected, frozen in liquid nitrogen and stored at −80 °C for protein extraction and analysis. There were three replicates for each treatment at each sampling time, the CI index was investigated in 30 fruit per replicate, the other physiological parameters and protein extraction were analyzed in 6 fruit per replicate.

### 2.2. Physiological parameters measurements

CI index was calculated according to method described in our previous report [18]. Chlorophyll fluorescence of banana peel was measured using portable chlorophyll fluorimeter (PAM 2100, Walz, Germany) and *PSII* quantum yield (*Fv/Fm*) was used to express chlorophyll fluorescence parameter of the banana peel. Relative electrolyte leakage and MDA content was determined by our previous approach [20]. TSS, TA and ratio of TSS and TA (TSS/TA) in banana pulp tissue were analyzed according to method of Zhao et al [21].

### 2.3. Protein extraction and 2-D analysis

About ten grams of peel tissues of banana fruit were used for protein extraction according to our previous research [18]. Bio-Rad Protein Assay kit was used to determine the protein concentration. Then 2 mg of banana peel proteins was applied to 17 cm ReadyStrip™ IPG strips at pH 5–8 (Bio-Rad, USA) for passive hydration overnight in sample loading trays. IEF and 2-D PAGE was conducted as described by Li et al. [18]. Three independent biological replicates were conducted for each sample. After stained with freshly prepared Coomassie blue, gel images were scanned and analyzed using MagicScan V6.0 and the PDQuest™ Basic 2-D Gel Analysis Software Version 8.0.1 (Bio-Rad). The differentially accumulated spots were determined according to our previous method [18]. In detail, data were firstly normalized using the total quantity of valid spots on the corresponding gel to account for quantitative variations in the intensity of protein spots between samples. The normalized intensity of spots on three independent biological replicate 2-DE gels was averaged. Then, SPSS software 13.0 (SPSS Inc., Chicago, IL, USA) was used to determine whether the relative change was statistically significant between any two mean values by one-way analysis of variance (ANOVA) and $p < .05$ was considered to indicate significant changes in abundance. Finally, spots with more than a 3-fold differential accumulation in comparison to those from other samples were excised and used for protein identification. To reduce false positive results, we also performed a non-parametrical ANOVA evaluation and multiple comparisons.

### 2.4. In-gel protein digestion and protein identification

In-gel protein digestion was conducted according to our previous method [18]. For MALDI-TOF/TOF MSand LC-ESI-MS/MS analysis, the method described by Li et al. [22] was used with minor modification. Mascot software 2.3.02 was also used against banana genome database (http://banana-genome-hub.southgreen.fr/home). For MALDI-TOF/TOF MS, protein candidates provided by combined PMF and MS/MS search were considered as valid when the global Mascot score was greater than significance score (58) with a significance level of e-value < 0.05. For LC-ESI-MS/MS, to reduce the probability of false peptide identification, peptides with ion scores greater than 'identity' score were counted as identified. Each confident protein identification involves at least one unique peptide.

### 2.5. Informatics analysis of differentially accumulated proteins

GO analysis were conducted using Blast2Go software (version 4.0). GO enrichment was also conducted and the *Musa acuminata* DH Pahang v2 genome was set as background. The hypergeometric Fisher exact test ($P < .01$) and Benjamini and Hochberg method (FDR < 0.05) was performed to detect statistically significant enrichment of GO term. Protein–protein interaction (PPI) analysis was conducted according to our previous research [20]. Briefly, all proteins were subjected to a BLAST search against *Musa acuminate* data lodged in the STRING database (version 10.5; http://string-db.org). The matching proteins with a confidence score of at least 0.400 were used for PPI analysis. The PPI network was constructed and displayed using Cytoscape (version 3.0.2) software. Principal component analysis (PCA) was conducted using the "vegan" package in R version 3.1.0. Protein accumulation was subjected to partial least squares discriminant analysis (PLS-DA) and partial least squares (PLS) regression analysis using SIMCA (version 15.0). For PLS-DA, the value of variable importance in projection (VIP) was conducted and PLS-DA model validation was performed by permutation testing in which $p < .05$. Identification of proteins influenced by EBR treatment towards the separation in PLS-DA model was further analyzed where proteins with VIP values exceeding 1.0 were selected as cut off according to previous research [23]. For PLS, the differentially accumulated proteins were denominated by X, and the vector Y

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