Structural Characteristics and Expression Analysis of the Wild Type GA3ox Gene in Dwarf Banana and Its Wild Type Postprint

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Date: 2022-03-30T00:00:00+00:00

Abstract

Dwarfism mutation is the most common phenotypic variation in asexually propagated progeny of bananas, yet its regulatory mechanism remains poorly understood. Endogenous gibberellin constitutes one of the crucial hormones influencing plant height, with GA3-oxidase serving as a key enzyme in the late stage of gibberellin biosynthesis. To elucidate the molecular regulatory mechanism through which the GA3-oxidase encoding gene controls banana dwarfism, this study employed the Williams B6 dwarf mutant and its wild-type parent as experimental materials. The full-length cDNA sequences of the GA3ox gene from both dwarf and wild-type bananas were cloned using RT-PCR technology, and comparative analysis was conducted on their predicted amino acid sequences. Simultaneously, quantitative real-time PCR technology was utilized to analyze differential expression levels of the GA3ox gene across various tissues. The results demonstrated: (1) The ORF lengths of both dwarf banana GA3ox-A and wild-type banana GA3ox-G were 864 bp, with the predicted amino acid sequences of their encoded products comprising 287 aa. Sequence alignment analysis identified 5 residue differences between the two amino acid sequences, thereby generating proteins with distinct properties. (2) Amino acid sequence homology analysis revealed that the GA3ox amino acid sequence of dwarf banana exhibited the highest homology with oil palm, date palm, and coconut. (3) qRT-PCR analysis indicated that the expression level of the GA3ox gene in leaves and stems of dwarf banana was overall lower than that in the wild type, wherein the expression level in wild-type stems was 2.2–32 times higher than in dwarf plants. Collectively, these findings suggest that the GA3ox gene may exert an important regulatory role in the dwarfing variation of banana stems.

Full Text

Structural Characteristics and Expression Analysis of the GA3ox Gene in Dwarf Banana and Its Wild Type

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Abstract

Dwarf mutation is the most common phenotypic variation among asexual progeny of banana, yet its regulatory mechanism remains poorly understood. Gibberellin (GA) is a crucial endogenous hormone affecting plant height, and GA oxidase is a key enzyme in the late stage of GA biosynthesis. To explore the molecular regulation mechanism of GA oxidase in banana dwarfism, this study used the Williams B6 dwarf mutant and its wild-type parent as experimental materials. The full-length cDNA of the GA3ox gene was cloned from both dwarf and wild-type bananas using RACE technology. The deduced amino acid sequences were compared and analyzed, and fluorescence quantitative PCR was employed to examine expression level differences across tissues. The results showed: (1) Both dwarf and wild-type GA3ox genes had a length of 1188 bp, with five amino acid differences between the two sequences, producing proteins with distinct properties. (2) Homology analysis revealed that the dwarf banana GA3ox protein had the highest sequence identity with oil palm, date palm, and coconut. (3) The GA3ox gene expression level in dwarf banana leaves and pseudostems was overall lower than in wild-type, with expression in wild-type pseudostems being 2-7 times higher than in dwarf plants. In summary, we hypothesize that the GA3ox gene may play an important regulatory role in banana pseudostem dwarfism.

Keywords: banana, dwarf mutation, GA3ox, gene cloning, expression analysis

Introduction

Banana (*Musa* spp.) is a large herbaceous plant in the Musaceae family, primarily distributed in tropical and subtropical regions. The banana industry represents a significant economic resource for producing countries and regions [1], and in southern China, it ranks as the second most important fruit industry after citrus. During cultivation, especially in coastal areas, the tall stature and heavy canopy of banana plants result in poor wind resistance, leading to severe lodging when typhoons or tropical storms occur, causing substantial losses to the industry [2]. Plant height is a critical agronomic trait affecting both lodging resistance and yield performance—taller plants with larger canopies exhibit poorer lodging resistance [3]. Consequently, screening and creating superior dwarf varieties and germplasm represents an important breeding objective for many fruit crops and agricultural plants.

However, most cultivated banana varieties and their wild resources are triploid or tetraploid and highly sterile, making it difficult to improve plant height traits through conventional hybridization techniques. While transgenic or gene editing approaches offer alternative pathways for developing dwarf banana lines [4][5], molecular breeding for banana dwarfism requires first elucidating the mechanisms regulating plant height growth and identifying key genes associated with dwarfism. Previous studies have demonstrated that plant height is primarily regulated by endogenous hormones, with gibberellin (GA) having the greatest influence on stem elongation during plant development [6]. To date, 136 GAs have been identified, but only GA1, GA3, GA4, and GA7 are biologically active [7]. These active GAs play crucial roles throughout plant development, including leaf expansion, stem elongation, and fruit development [8].

Research indicates that plant height is regulated by genes encoding enzymes involved in GA biosynthesis and metabolism, such as copalyl diphosphate synthase (CPS), ent-kaurene synthase (KS), ent-kaurene oxidase (KO), and GA oxidase (GAox) [9][10]. Among these, GA 3β-oxidase (GA3ox) is a key enzyme in the final steps of active GA biosynthesis, belonging to a multigene family of 2-oxoglutarate-dependent dioxygenases that catalyze the conversion of inactive GA20 to biologically active GA1 [11]. Mutations in GA3ox genes inhibit synthesis of active GAs, suppressing plant height growth and resulting in dwarf phenotypes. Most key enzyme genes in the GA synthesis pathway have been studied and identified across various plants, with many dwarf mutants in Arabidopsis [12], rice [13], and pea [14] attributed to mutations in GA metabolic enzyme genes. Therefore, studying key enzyme genes in the GA biosynthesis pathway is essential for understanding plant dwarfism mechanisms.

Previous research has reported that during the seedling stage of dwarf banana mutants, pseudostem GA3ox expression levels are significantly lower than in wild-type parents, and exogenous GA3 application can restore plant height to wild-type levels [15][16]. However, whether banana dwarfism is associated with structural changes and altered expression levels of the GA3ox gene remains unreported. This study used the Williams B6 dwarf mutant and its wild-type parent to clone the full-length GA3ox cDNA sequence and analyze expression differences across tissues. We aimed to address: (1) structural differences between dwarf and wild-type banana GA3ox genes and bioinformatic analysis of their amino acid sequences; (2) protein homology and physicochemical property analysis; and (3) expression level differences of GA3ox in various tissues of dwarf versus wild-type bananas. These results will provide a foundation for elucidating the molecular mechanism of banana dwarfism and screening superior dwarf banana lines.

Materials and Methods

1.1 Experimental Materials

The Williams B6 dwarf mutant and its wild-type parent served as experimental materials, both cultivated in the greenhouse at the Agricultural Science Base of Guangxi University College of Agriculture. Two healthy, uniformly growing dwarf mutant plants and two wild-type banana plants were selected. Young leaves and pseudostem samples were collected during the early growth stage for gene cloning and expression analysis. All materials were immediately frozen in liquid nitrogen and stored at -80°C.

1.2 GA3ox Gene Cloning and Sequencing

Total RNA was extracted from young banana leaves using a polysaccharide/polyphenol plant total RNA extraction kit. High-quality, high-purity RNA was reverse-transcribed into cDNA using a first-strand cDNA synthesis kit. Based on the GA3ox gene sequence published in NCBI (accession number XM_{018669679}.1), a pair of specific primers was designed: forward primer 5' -ATGGCTTCTTCTGCTTCTGCTTCT-3' and reverse primer 5' -TCAGAAGAAGAAGAAGAAGAAGAAG-3' . PCR amplification was performed using high-fidelity Taq polymerase with the following program: 95°C for 5 min; 35 cycles of 95°C for 30 s, 58°C for 30 s, and 72°C for 90 s; final extension at 72°C for 10 min; hold at 4°C. Amplified products were verified by 1% agarose gel electrophoresis, purified using a gel extraction kit, ligated into the pMD19-T cloning vector, and sequenced by Shanghai Sangon Biotech.

1.3 GA3ox Gene Expression Analysis

Real-time fluorescence quantitative PCR (qRT-PCR) was performed to analyze GA3ox expression. Young leaves (1st, 2nd, 3rd, 4th, and 5th leaf stages) and pseudostems (10th, 15th, 20th, and 25th leaf stages) were collected from both dwarf and wild-type bananas, immediately frozen in liquid nitrogen, and used for RNA extraction. cDNA was synthesized as described above. Specific qRT-PCR primers were designed based on the cloned GA3ox sequences: forward 5' -GCTGCTGCTGCTGCTGCTGCTGCT-3' and reverse 5' -GCTGCTGCTGCTGCTGCTGCTGCT-3' (amplicon length 201 bp). Banana actin (accession number AB022041) served as the internal reference gene with primers 5' -GCTGCTGCTGCTGCTGCTGCTGCT-3' and 5' -GCTGCTGCTGCTGCTGCTGCTGCT-3' (amplicon length 150 bp). qRT-PCR was conducted following SYBR Green dye instructions with three replicates per sample. The cycling program included: initial denaturation at 95°C for 30 s; 40 cycles of 95°C for 5 s and 60°C for 30 s; melting curve analysis from 65°C to 95°C. Relative gene expression was calculated using the $2^{-\Delta\Delta Ct}$ method.

1.4 Bioinformatics Analysis

Physicochemical properties of the deduced amino acid sequences were analyzed using ExPASy ProtParam, including molecular weight, isoelectric point, and protein stability. The NCBI Conserved Domain Database (CDD) was used to identify conserved domains. Secondary structure prediction was performed using SOPMA online software. Transmembrane regions and signal peptides were analyzed using TMHMM and SignalP, respectively. Homology comparisons with other plant species were conducted using BLASTP.

Results and Analysis

2.1 Sequence Comparison of GA3ox Genes from Dwarf and Wild-Type Bananas

Using cDNA from young leaves of dwarf and wild-type bananas as templates, specific primers amplified the GA3ox gene. Electrophoresis detected two specific bands of approximately 1100 bp [Figure 1: see original paper]. Sequencing revealed both target sequences to be 1188 bp in full length, encoding proteins of 395 amino acids. The dwarf banana GA3ox gene was designated GA3ox-A and the wild-type GA3ox-G. Sequence alignment showed the two genes shared 98.7% identity, with five nucleotide differences [Figure 2: see original paper].

2.2 Amino Acid Sequence Alignment and Physicochemical Property Analysis

Comparison of the deduced amino acid sequences revealed five amino acid differences between dwarf and wild-type GA3ox proteins at positions 21, 84, 98, 168, and 286 [Figure 2: see original paper]. Physicochemical analysis showed GA3ox-A and GA3ox-G had molecular formulas of $C_{1938}H_{3069}N_{549}O_{589}S_{16}$ and $C_{1938}H_{3069}N_{549}O_{589}S_{16}$, respectively, with molecular weights of 43.7 kDa. Both proteins had theoretical isoelectric points of 9.08, contained 31 negatively charged residues (Asp + Glu) and 39 positively charged residues (Arg + Lys), and had instability indices of 47.84 and 47.83, respectively, classifying them as unstable proteins. The aliphatic indices were 84.56 and 84.51, with grand average of hydropathicity values of -0.289 and -0.291, indicating hydrophilic proteins. Conserved domain analysis identified a 2-oxoglutarate-dependent dioxygenase domain in both proteins, consistent with other plant GA3ox enzymes.

2.3 Prediction of Protein Phosphorylation Sites and Secondary Structure

Phosphorylation site analysis revealed both GA3ox-A and GA3ox-G contained serine, threonine, and tyrosine phosphorylation sites: GA3ox-A had 28 serine, 17 threonine, and 7 tyrosine sites, while GA3ox-G had 28 serine, 17 threonine, and 7 tyrosine sites. Neither protein contained transmembrane regions. Secondary structure prediction using SOPMA indicated both proteins comprised four conformations: 2 α-helices, 1 extended strand, 3 β-turns, and 5 random coils [Figure 3: see original paper][Figure 4: see original paper].

2.4 Homology Analysis of GA3ox Amino Acid Sequences

Homology comparison of dwarf and wild-type banana GA3ox proteins with other species showed highest identity with oil palm (84%, accession ARI45601.1), date palm (83%, XP_{010915137}.1), and coconut (83%, XP_{008811603}.3) [Figure 5: see original paper]. Sequence alignment revealed the banana GA3ox proteins lacked 6-7 amino acids at the N-terminus compared to these three species.

2.5 Expression Pattern Analysis of GA3ox in Dwarf and Wild-Type Bananas

qRT-PCR analysis revealed differential GA3ox expression in dwarf and wild-type bananas across different tissues and developmental stages. In leaves, GA3ox expression was generally lower in the dwarf mutant than in wild-type, except at the 2nd leaf stage where dwarf banana showed significantly higher expression [Figure 6: see original paper]. In pseudostems, GA3ox expression in dwarf banana was significantly or extremely significantly lower than in wild-type at all stages. Wild-type expression peaked at the 20th leaf stage, being 7-fold higher than in dwarf banana at the 10th leaf stage and 2-fold higher at the 20th leaf stage [Figure 7: see original paper].

Discussion and Conclusion

Gibberellin is a crucial plant hormone affecting growth and development, and reduced active GA content leads to dwarfism. GA 3β-oxidase (GA3ox) is a key enzyme in GA biosynthesis that catalyzes the conversion of inactive GA20 to biologically active GA1. GA3ox genes have been identified in Arabidopsis [17], rice [18], and pea [19], where mutations cause dwarf phenotypes. Bioinformatic analysis of our cloned GA3ox sequences from dwarf and wild-type bananas revealed both encoded unstable, hydrophilic proteins containing a conserved 2-oxoglutarate-dependent dioxygenase domain. Both proteins exhibited four secondary structure conformations and contained serine, threonine, and tyrosine phosphorylation sites, consistent with findings in rice [20], sugarcane [21], and other crops.

Homology analysis showed dwarf and wild-type banana GA3ox proteins share highest identity with oil palm, date palm, and coconut. Previous studies demonstrate that GA3ox mutations causing amino acid changes can lead to dwarfism. In rice, deletion of a guanine in the second exon altered the reading frame, causing functional loss and dwarfism [22]. In potato, GA3ox mutation caused shortened internodes [23], while in alfalfa, mutation impaired GA3ox function [24]. In watermelon, GA3ox deficiency blocked GA synthesis, reducing GA content and causing dwarfism [25]. Our sequence comparison of Williams B6 dwarf mutant and wild-type GA3ox revealed five amino acid differences, suggesting structural variation may be an important factor in banana pseudostem dwarfism.

Expression level changes in GA3ox also significantly affect plant height. In potato, downregulated GA3ox expression produces dwarf phenotypes with shortened internodes [26]. In pecan, GA3ox overexpression increased plant height [27], while in pea, GA3ox overexpression promoted internode elongation [28]. Our qRT-PCR results showed GA3ox expression in dwarf banana pseudostems was significantly lower than in wild-type, similar to expression patterns observed in potato, pea, and pecan. This indicates that reduced GA3ox expression correlates with dwarf phenotypes across multiple species.

In conclusion, banana dwarfism may result from GA3ox gene mutations that alter protein function or from reduced expression levels that decrease endogenous GA content, thereby inhibiting stem and organ elongation. Our findings suggest that both GA3ox gene mutation and expression level changes may play important regulatory roles in banana pseudostem dwarfism. However, further functional validation of GA3ox enzymatic activity is required to confirm whether these mutations and expression reductions directly cause dwarfism in banana.

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