

Molecular characterization and differential expression of β -1,3-glucanase during ripening in banana fruit in response to ethylene, auxin, ABA, wounding, cold and light–dark cycles

Swarup Roy Choudhury · Sujit Roy ·
Sanjay Kumar Singh · Dibyendu N. Sengupta

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Abstract β -1,3-Glucanases (E.C. 3.2.1.39) are widely distributed enzyme among bacteria, fungi, and higher plants. Analyses of accumulation levels of β -1,3-glucanase protein in various tissues in banana have clearly indicated abundance of β -1,3-glucanase protein accumulation in ripe pulp tissue. After cloning of β -1,3-glucanase from banana pulp (cultivar Cavendish), we have carried out an in silico analysis to investigate the sequential, structural, and phylogenetic characteristics of the putative banana β -1,3-glucanase protein. As like other ripening specific genes, β -1,3-glucanase is regulated in response to a wide variety of factors. Therefore, we have analyzed the transcript accumulation pattern and protein levels of β -1,3-glucanase in response to ethylene, auxin, ABA, wounding and, low temperature in preclimacteric banana fruit. Expression profile analyses have indicated that whereas exogenous application of ethylene strongly stimulated β -1,3-glucanase transcript accumulation, ABA partially induced the expression of the gene. On the other hand, wound treatment did not induce β -1,3-glucanase expression. Conversely,

auxin and cold treatment negatively regulated β -1,3-glucanase gene expression and thus inhibited glucanase activity. In addition, β -1,3-glucanase transcript level was markedly decreased by constant exposure to white light. Protein level and enzymatic activity of β -1,3-glucanase were substantially increased with considerable decrease in fruit firmness by ethylene treatment and reduced exposure to white light conditions as compared with other treatments. Together, the overall study of β -1,3-glucanase expression pattern, glucanase activity, and changes in fruit firmness during ripening in various conditions suggest the possible physiological function of β -1,3-glucanase in fruit pulp softening.

Keywords ABA, auxin, β -1,3-glucanase · Ethylene · Firmness · Glucohydrolase

Abbreviations

ABA	Absciscic acid
β -1,3-gluc	β -1,3-Glucanase
BSA	Bovine serum albumin
cDNA	Complementary DNA
DTT	1,4-Dithiothreitol
IAA	Indole acetic acid
ORF	Open reading frame
RT-PCR	Reverse transcriptase-polymerase chain reaction

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S. Roy Choudhury (✉) · S. K. Singh · D. N. Sengupta
Division of Plant Biology, Bose Institute,
93/1, Acharya Prafulla Chandra Road, Kolkata,
West Bengal 700 009, India
e-mail: src.bose@gmail.com

S. Roy
Department of Chemistry, Bose Institute,
93/1, Acharya Prafulla Chandra Road, Kolkata,
West Bengal 700 009, India

Introduction

The β -1,3-glucanases (β -1,3-glucs) are abundant proteins also found in all higher plants (Stone and Clarke 1992). Besides its well-known role in antimicrobial activity,

β -1,3-gluc plays various physiological functions in different organs like bud (Krabel et al. 1993), style of flower (Ori et al. 1990), anther (Hird et al. 1993), seed (Bewley and Black 1994), and also in fruit (Dong and Dunstan 1997). β -1,3-gluc hydrolyze β -D glucosidic linkages in β -1,3-glucan and are likely to target the glucan backbone of xyloglucan, the hemicellulose that bridges between cellulose microfibrils in tomatoes and many other fruits (Bennett and Labavitch 2008). In peach fruit, disassembly of the hemicellulose matrix is most closely correlated with fruit softening (Brummell et al. 2004).

Different hydrolases like β -1,3-gluc have been shown to be differentially expressed in different response like seed maturation, fungal infection, wounding, and stress in pea (Mauch and Staehelin 1989). β -1,3-gluc has been found to be upregulated by treatment with ethylene, microbial infection and down regulated by treatment with combination of auxin and cytokinin in cultured tobacco tissue (Fellx and Meins 1986). Ethylene treatment increases the promoter activity of tobacco class I β -1,3-gluc that ultimately responsible for high level of transcript abundance of the gene (Ohme-Takagi and Shinshi 1995). Earlier studies have also indicated that ABA could modulate fruit ripening in banana (Jiang et al. 2000). However, little is known about the ethylene, auxin, ABA-regulated expression of β -1,3-gluc in the climacteric fruit like banana during ripening.

Wounding acts as an inducer of several ripening genes like cellulase, polygalacturonase, and ACC synthase in avocado fruit during ripening (Starrett and Laties 1993). In banana, several genes are up and down regulated in response to wounding (Liu et al. 1999; Roy Choudhury et al. 2008b). Besides wound, cold treatment also controls several genes during ripening in banana fruit (Roy Choudhury et al. 2008b). It has been shown that low temperature generally suppresses ripening by inhibiting binding of ethylene to the receptor and thus subsequently blocking the ethylene-mediated inductive signal of ripening (Jiang 2004).

Light has been shown to play an important regulatory role for accumulation of β -1,3-gluc in tobacco seeds at the time of radicle emergence (Vögeli-Lange et al. 1994). The role of light in regulating pigment accumulation has been studied elaborately during tomato fruit ripening (Giovannoni 2001). On the other hand, previous studies in *Arabidopsis* have demonstrated that ethylene forming and cell wall degrading enzymes are down regulated in presence of light (Wang et al. 2001). However, the effect of light and dark phases on the regulation of expression of β -1,3-gluc has not been elucidated in detail in banana.

In this report, first of all we have detected the protein accumulation pattern of β -1,3-gluc in different tissues of banana. Since β -1,3-gluc protein level is increased during fruit ripening in banana pulp, knowledge about the

structure–function relationship of the enzyme is of great interest to gain further knowledge about the enzyme. Therefore, after cloning of β -1,3-gluc from banana pulp (cultivar Cavendish), we have illustrated the sequential, structural, and phylogenetic features of putative banana β -1,3-glucanase protein. In addition, to date a more detailed investigation of regulation of β -1,3-gluc by phytohormones and other abiotic factors is limited in banana. Therefore, the objectives of the present study were to investigate the expression profile of banana β -1,3-gluc gene in response to various phytohormones like ethylene, auxin, ABA, and abiotic factors including wounding, low temperature, and different photoperiods during ripening. Enzymatic activity and fruit firmness were examined to analyze their interrelationships.

Materials and methods

Plant material and treatments

Preclimacteric banana fruits (*Musa* sp., AAA group, subgroup Cavendish, cv. Giant governor) were collected from the banana farm at 75 days post-anthesis (West Bengal State Council of Science and Technology; Saltlake, West Bengal, India). Each banana hand was separated into individual fingers (unripe stage) and ripened naturally at 22°C unless stated otherwise. All treatments (except experiments with light/dark phases) were given to the preclimacteric banana fruit at 5 days after harvest (DAH) during ripening. For each experiment, banana fingers from a same hand representing the similar developmental stage were used as a sample group to rule out difference in ripening behaviors of fingers among different hands (Inaba and Nakamura 1986). For tissue-specific expression studies, fruits of different developmental stages were collected from the banana plant.

For ethylene treatment, fingers were treated with 100 μ l l⁻¹ ethylene at 22°C in dark (Trivedi and Nath 2004). For IAA treatment, banana fingers were dipped in a solution containing 100 μ M IAA in 0.2% teepol as described by Lohani et al. (2004). For ABA treatment, as like auxin treatment, banana fingers were dipped in a solution containing 100 μ M ABA. For wound induction, banana pulp was cut into cubes of 2–3 cm thickness and kept at 22°C under humidified conditions. Low temperature treatment was given by keeping the banana fingers at 10°C in dark. The hormonal, wounding and cold treatments were given for 24, 48, and 72 h, respectively.

Pre-climacteric banana fingers (day 0 after harvest, 75 days post-anthesis) were exposed to systematic light and dark phases including 8 days of constant white light (8L), 6 days of light/2 days of darkness (6L), 4 days of light/

4 days of darkness (4L), 2 days of light/6 days of darkness (2L) and 8 days of constant dark (8D) periods, respectively. White light intensity used was $100 \mu\text{mol m}^{-2} \text{s}^{-1}$.

RNA isolation, molecular cloning of banana β -1,3-glucanase gene, and transcript analysis by semi-quantitative RT-PCR

Total RNA was isolated from pulp tissue of banana fruits as described previously (Roy Choudhury et al. 2008a). DNaseI-treated RNA was used for amplification of first strand cDNA by reverse transcription following the manufacturer's instruction (Invitrogen, USA). The full-length ORF of β -1,3-glucanase cDNA was obtained from ripe pulp tissue of Cavendish (15 DAH) by PCR amplification with the gene-specific oligos (Glu5: 5'-GTACGAGCTCAT GGCAACAAAAGCTTC-3' and Glu3: 5'-GTACGAGCTCCTAAAAGCTTATTTGGTA-3') made from public data base sequence of banana β -1,3-glucanase (AF001523). The reaction for RT-PCR was subjected to 30 cycles of 94°C for 1 min, 42°C for 1 min, and 72°C for 2 min. The amplified fragment was cloned into pBluescript (Stratagene) vector, sequenced, and sequences of β -1,3-glucanase from Cavendish banana were subsequently submitted to GenBank (EU014210). For semi-quantitative RT-PCR analysis, a first cycle of 5 min at 94°C, 45 s at 43°C, and 1 min at 72°C was followed by 45 s at 94°C, 45 s at 43°C, and 1 min at 72°C for 24 cycles. The conditions were chosen so that none of the RNAs analyzed reached a plateau at the end of the amplification protocol. We also performed a negative control containing RNA instead of cDNA to rule out genomic DNA contamination in each set of reactions. Equal amounts of RT-PCR products were loaded in agarose gel for analysis of the transcript profile. All experiments were repeated at least three times in each case.

Protein data mining, sequence analysis, and phylogenetic inference

We performed maximum likelihood analysis using 28 β -1,3-gluc protein sequences from various plant and fungi species to analyze their relationship and those sequences were obtained from GenBank hosted on National Centre for Biotechnological Information (NCBI) webpage (<http://www.ncbi.nlm.nih.gov/>, validated 22 April 2008). Blast and PSI-Blast programs (Altschul et al. 1997) were used for initial analysis and comparison. Domain positions were confirmed using simple modular architecture research tool (SMART, <http://smart.embl-heidelberg.de/>, validated 22 April 2008) (Schultz et al. 1998) and Conserved Domain Database v2.11 (CDD, <http://www.ncbi.nlm.nih.gov/>

[Structure/cdd/cdd.shtml](http://Structure.cdd/cdd.shtml), validated 22 May 2007) (Marchler-Bauer et al. 2003).

Multiple sequence alignments were performed with the amino acid sequences in the ClustalW 1.83 programme (Thompson et al. 1994). The following alignment parameters were used: gap open penalty 10.00; gap extension 0.20; sequence >10% diverged delayed; BLOSSUM 62 matrix; residue specific penalties on; and hydrophobic penalties on. Phylogenetic analysis was performed using MEGA version 3.1 (Tamura et al. 2007) and Neighbor-joining method was used for phylogenetic tree searching and inference. The phylogenetic tree was tested by bootstrap analysis with 1,000 replication and protein distances were calculated by using the algorithm of p-distance. The complete deletion option was used in handling gaps or missing data obtained from alignment.

Residues of structural and evolutionary importance of banana β -1,3-gluc were calculated by ConSeq server (Berezin et al. 2004). This program enabled us to calculate the conservation score in every alignment position, taking into account the relationships between the sequences and the physicochemical similarity between the amino acids. The conservation scores were represented on a 10-point scale and can be used to determine functional relevance of a residue in evolution.

Genomic DNA isolation and Southern blotting

Genomic DNA was isolated from the leaves of banana plants by following the method of Henry (1997) with some modifications. Banana genomic DNA (12 μg per lane) was digested with *Bam*HI, *Hind*III, and *Eco*RI, and digested DNA was separated by electrophoresis on a 0.8% agarose gel and then blotted onto a Hybond-N⁺ nylon membrane filter (Amersham, UK). The filter was hybridized to ³²P-labeled β -1,3-gluc cDNA as described by Sambrook et al. (1989).

Preparation of buffer soluble protein extracts from banana pulps

All steps were carried out at 4°C. Protein extracts were prepared by homogenizing 5 g of pulp tissues in a pre-chilled mortar and pestle with three volume of ice-cold buffer A containing 50 mM Tris-Cl (pH 7.5), 250 mM sucrose, 25 mM KCl, 5 mM MgCl₂, 3 mM EDTA, 1 mM PMSF, and 1 mM DTT. The homogenate was passed through two layers of muslin cloth and then centrifuged at 5,000g for 5 min at 4°C to eliminate cellular debris. The supernatant was again centrifuged at 10,000g for 10 min. The supernatant was taken and used in subsequent experiments. Total protein content of each sample was estimated by the Bradford (1976) method, using BSA (Fraction V) as standard.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis and Western blotting

A 10% discontinuous SDS-PAGE was performed using a vertical mini-gel system (Bangalore Genei, India) with 1 mm thickness. The gel was prepared according to Laemmli (1970). Equal amounts ($\sim 25 \mu\text{g}$) of protein extracts from control and treated banana pulps were separated in 10% SDS-PAGE. Immuno-blotting was carried out using anti-tobacco class I β -1,3-glucanase polyclonal antibody (1:1,000 dilution) as described previously (Roy Choudhury et al. 2008b). All experiments were repeated at least three times in each case.

Immunoprecipitation assay

Approximately 50 μg of banana unripe (0 DAH) and ripe (15 DAH) protein extracts were incubated with either β -1,3-glucanase polyclonal antibody (3 μg of protein) or similar amounts of pre-immune serum (used as control) in the presence of binding buffer (50 mM Hepes–KOH, pH 8.0, 10 mM MgCl_2 , 1 mM DTT, 1 mM PMSF and 5 $\mu\text{g ml}^{-1}$ leupeptin) for 4 h at 4°C with rotation. The immunocomplex was adsorbed into binding buffer equilibrated with protein A agarose. The mixture was incubated for overnight at 4°C with rotation. The immunocomplex was pelleted by centrifugation at 2,000g for 10 min. The experiment was repeated three times in each sample.

Detection of glucanase activity and measurement of fruit firmness

Glucanase activity was determined by using laminarin (sigma) as a substrate (Abeles et al. 1970). One grams of pulp tissue was extracted with 3 ml of 50 mM K-acetate buffer (pH 5.0) in a mortar and pestle and then homogenate was centrifuged at 10,000g at 4°C for 10 min. Supernatant fraction (0.1 ml) was added to 0.1 ml of 2% laminarin and incubated for 1 h at 50°C. The reaction was terminated by addition of 3 ml of DNS reagent (dinitro salicylic acid) and then heating the tubes for 5 min in boiling water bath. The glucanase activity was measured spectrophotometrically at 510 nm, and the enzyme activity was expressed as nmol glucose equivalent released $\text{min}^{-1} \text{mg protein}^{-1}$ of original fresh weight of the tissue. All experiments were repeated at least three times with five replicates in each case.

To estimate fruit firmness, peel from one side of the banana finger was removed and measurement was carried out at three different places using penetrometer and recorded as force in Newton (N). Average of three readings was taken as measure of firmness of individual fruit. Both experiments were repeated at least three times with five replicates in each case.

Results

Steady-state level of β -1,3-glucanase protein and its enzymatic activity in different tissues of banana

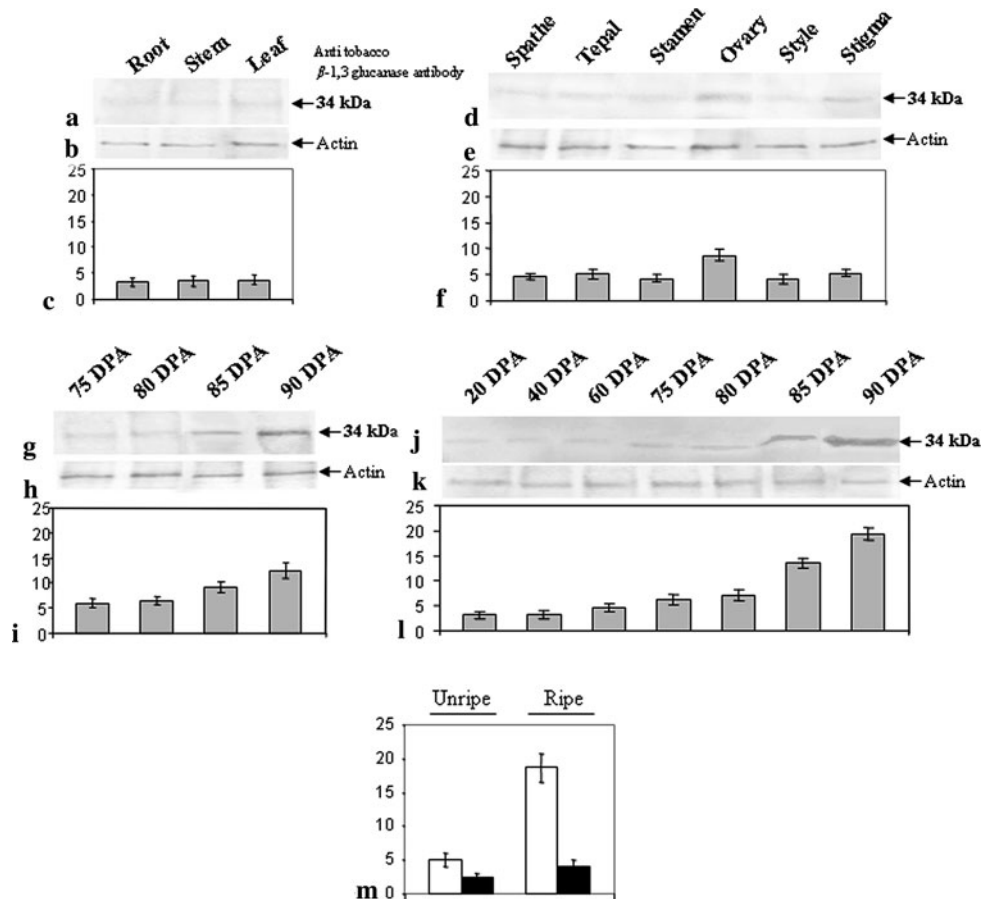
To investigate the expression pattern of β -1,3-glucanase (β -1,3-gluc) in various organs of banana plants, total protein was isolated from different tissues of banana (subgroup Cavendish cv. Giant governor), and immunoblotting was performed by taking equal amount of protein from each tissue type by using tobacco class I β -1,3-glucanase antibody (Fig. 1). Immunoblotting experiments revealed a significant variation in the accumulation profile of β -1,3-gluc protein in different tissues. A low level of this protein was found to accumulate in root, stem, and leaf, whereas a moderate level of expression was detected in flowering parts (Fig. 1a, d). In the reproductive parts, expression level of β -1,3-gluc protein was comparatively higher in ovary tissue than in sepal, petal, stamen, style, and stigma. Although the β -1,3-gluc protein was accumulated at low level during unripe stage (75 DPA or 0 day after harvest) in both peel and pulp tissues of banana (Fig. 1g, j), the amount of this 34-kDa β -1,3-gluc protein was increased distinctly during ripening in peel and pulp tissues at 80 and 85 DPA or 5 and 10 DAH. Maximum level of β -1,3-gluc protein accumulation was observed in ripe pulp tissue at 90 DPA or 15 DAH (Fig. 1g, j). During early fruit developmental stages, β -1,3-gluc protein was expressed at a low level in the pulp tissues (Fig. 1j). Enzymatic activity of β -1,3-gluc in the different tissues were also consistent with the higher expression level of β -1,3-gluc protein in ripe banana peel and pulp tissues (Fig. 1c, f, i, l). Similar set of proteins was probed with actin antibody (Fig. 1b, e, h, k) which was used as an internal standard.

Furthermore, analysis of enzymatic activity of glucanase in the unripe and ripe fruit extracts before and after immunoprecipitation with class I β -1,3-glucanase antibody revealed complete precipitation of enzymatic activity (Fig. 1m) both in unripe and ripe banana pulp tissue. Conversely, similar experiment with pre-immune serum showed no activity in immunoprecipitate (data not shown). Moreover, consistent with the expression level, the enzymatic activity of glucanase was considerably low in protein extracts from preclimacteric unripe fruit as compared with those prepared from ripe fruit (Fig. 1m). Taken together, these results indicated that tobacco class I β -1,3-glucanase antibody was able to specifically recognize and immunoprecipitate the native form of β -1,3-glucanase in banana fruit extracts.

β -1,3-Glucanase sequence analysis

To analyze the β -1,3-gluc at the gene level, we have cloned the cDNA of banana (cultivar Cavendish) β -1,3-gluc. The

Fig. 1 Immunodetection of β -1,3-glucanase protein level with total protein extracts (25 μ g) prepared from root, stem, leaf (a) different flower parts (d), different stages of banana fruit development and ripening of peel (g) and pulp (j) tissues by using anti-tobacco class I β -1,3-glucanase polyclonal antibody. Position of immunoreactive band is indicated. b, e, h, k Similar analysis with anti-actin antibody used as control. c, f, i, l In vitro enzymatic activity of banana glucanase activity from the indicated tissues. Error bar indicates the ± 1 SE of the five independent replicates. m Analysis of enzymatic glucanase activity in the unripe and ripe fruit extracts (50 μ g) before (white bar) and after (black bar) immunoprecipitation with class I β -1,3-glucanase antibody



cDNA of banana (cultivar Cavendish) β -1,3-gluc contained a coding sequence of 1,026 bp and encoding a sequence of 341 amino acids. The β -1,3-gluc cDNA was rich in G/C content with a (G + C) value of 58.87%. Alanine was found to be most abundant (11.44%), followed by Val (9.97%), Ser (9.38%), Gly (8.21%), Leu (8.21%), Asn (7.33%), and Pro (6.74%). Low abundance amino acids were Cys (0.29%) and His (0.29%). The acidic and basic amino acids constituted 5.57 and 7.03% of the polypeptide, respectively.

The β -1,3-gluc protein has a molecular mass of 37.5-kDa; however, a putative cleavage site for a signal peptidase was found to be located between Ser-28 and Ile-29 (SignalP-NN program, <http://www.us.expasy.org>) (Von Heijne 1986) residues resulting in mature protein of ~ 34 kDa instead of 37 kDa. The protein is basic (pI 9.04) and cytosolic in nature. Furthermore, the protein does not contain a C-terminal extension sequence for vacuolar targeting.

Comparison of β -1,3-gluc sequences from different plant species revealed more or less similar length of proteins comprising of approximately 306–370 amino acid residues. CDD analysis detected the main domain from this β -1,3-gluc (50–431: glucosyl hydrolase family). Multiple sequence analysis (ClustalW 1.83 programme) of the

β -1,3-gluc available in GenBank revealed β -1,3-gluc as conserved protein (Supplementary Fig. 1).

The biological importance of a residue often correlates with its level of evolutionary conservation within the protein family. We analyzed a broad range of β -1,3-gluc sequences and determined highly conserved residues that are potentially necessary for either structural integrity or biological functionality. Different degrees of darkness and the color of amino acid residues illustrate the degree of conservation and this conservation was mapped onto the sequence of β -1,3-gluc (Fig. 2a). The most highly conserved residues of functional importance were found to be exposed while the structurally important highly conserved residues were buried. Among the whole regions, $\sim 56.89\%$ residues were predicted to be exposed (hydrophilic) and $\sim 43.11\%$ residues were buried (hydrophobic). The putative β -1,3-gluc polypeptide comprising of a highly hydrophilic carboxyl terminus end showed the characteristics of cytosolic protein, which was detected in Pep plot, GCG 10.0 (Gribskov et al. 1986). The two predicted *N*-glycosylated residues were detected at Asn93 and Asn166 by OGPET program (<http://ogpet.utep.edu/OGPET/>), which was different from other fruit specific β -1,3-gluc sequences. The presence of *O*-glycosylated residues is probably

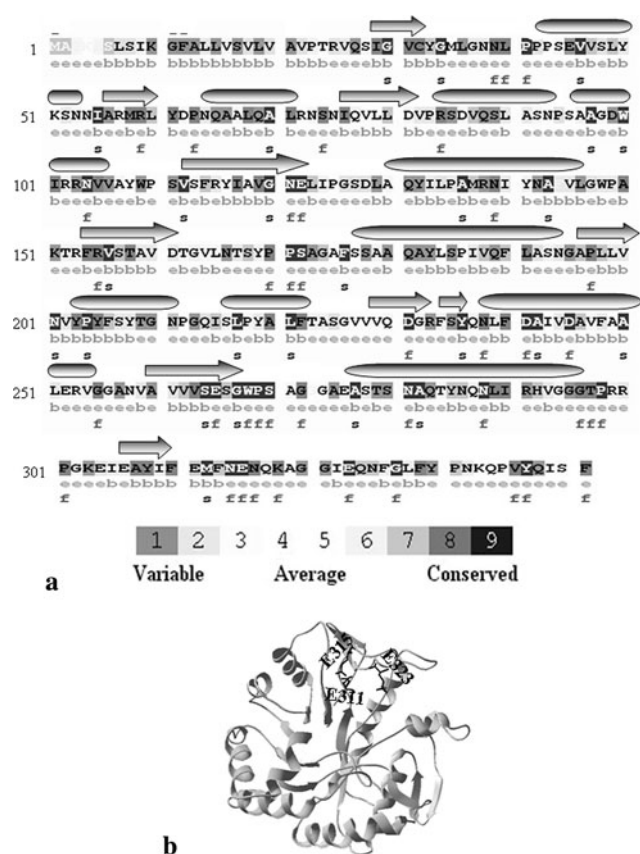


Fig. 2 **a** Prediction of residue conservation and location based on ConSeq. Different secondary structural elements are depicted above the sequence. Different conserved residues are shown in boxes. Residues that are more conserved are shown in increasingly darker background with white text; more variable sequences are shown in black text. Below the sequence, *e* suggests a residue predicted to be exposed (surface) and *b* indicates a buried residue. *s*, *f* represents ConSeq's prediction of residues of "structural" or "functional" importance. α -Helices and β -sheets are represented by α and β , respectively. **b** Schematic representation of calcium ion binding site in three-dimensional structure of banana β -1,3-glucanase; calcium ion binding site was predicted by CHED. Amino acid residues putatively interacting with calcium are labeled and represented as sticks

responsible for intercellular transportation or maintaining the protein folding.

The crystal structure of barley β -1,3-gluc has been determined by Varghese et al. (1994). In banana, molecular modeling of β -1,3-gluc based on barley β -1,3-gluc has indicated existence of a TIM-barrel architecture as like other endohydrolases. The active site of the enzyme was identified (Glu 122, Glu 265, Glu 323; homologous to barley Glu 94, Glu 231, Glu 288), and this region was involved in the retention mechanism of substrate hydrolysis. The contents of α -helices, β -strands, turns, and coils of banana β -1,3-glucanase constituted 35.58, 18.59, 30.45, and 15.38% of total amino acids, respectively. However, banana β -1,3-gluc is a heat stable protein as like other endoglucanases, since the recombinant enzyme activity

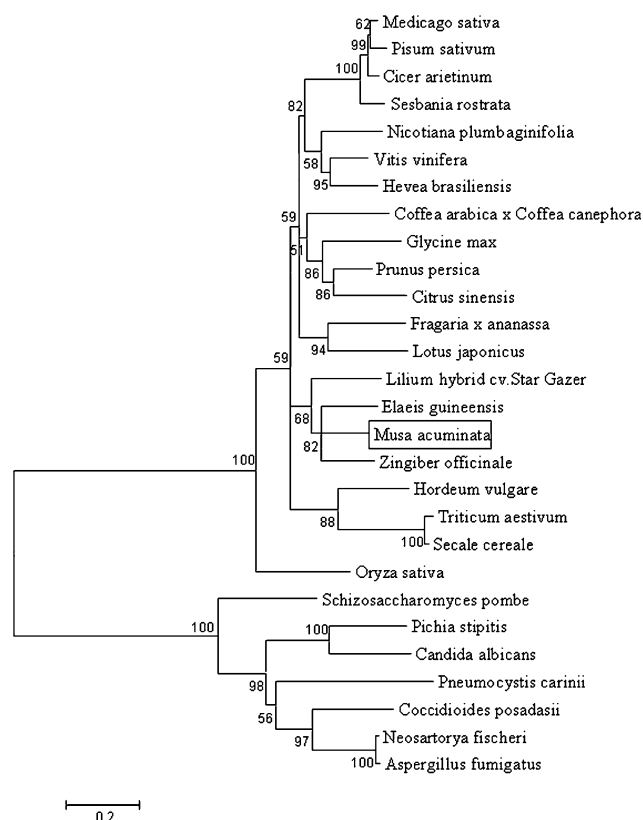


Fig. 3 A consensus bootstrap neighbor-joining tree based on the ClustalW alignments of the amino acid sequences of β -1,3-glucanase from different organisms has been constructed. Banana β -1,3-glucanase is indicated by a rectangle. Sequences with their GenBank accession number in parenthesis are as follows: *Musa acuminata* (ABU40624); *Elaeis guineensis* (EU014210); *Zingiber officinale* (ABC69706); *Lilium hybrid cv. Star Gazer* (ABD85024); *Vitis vinifera* (AAF44667); *Nicotiana plumbaginifolia* (CAA30261); *Medicago sativa* (AAV66071); *Cicer arietinum* (CAA10287); *Prunus persica* (AAL30426); *Hevea brasiliensis* (AAP87281); *Coffea arabica* $\times$ *Coffea canephora* (AAQ90286); *Sesbania rostrata* (BAE53382); *Pisum sativum* (AAB24398); *Citrus sinensis* (CAA03908); *Fragaria x ananassa* (AAX81590); *Oryza sativa* (BAC02926); *Glycine max* (AAB03501); *Hordeum vulgare* (AAU11328); *Triticum aestivum* (AAY96422); *Lotus japonicus* (BAG34628); *Secale cereale* (CAJ58514); *Schizosaccharomyces pombe* (CAA91245); *Pneumocystis carinii* (ACF21011); *Neosartorya fischeri* (XP_001265240); *Aspergillus fumigatus* (EDP55763.1); *Coccidioides posadasii* (ABH10633); *Pichia stipitis* (XP_001383750); *Candida albicans* (CAB62579)

remains stable up to 65°C (Roy Choudhury et al. 2009a). We have identified three calcium-binding residues within the three-dimensional structure of banana β -1,3-gluc (Glu 311, Glu 315, Glu 323) by CHED database (Babor et al. 2008) (Fig. 2b), which may be the basic reason of stability of this enzyme at high temperatures.

Phylogenetic analyses

An evolutionary comparison of banana β -1,3-gluc sequences across different plants and fungal species has been performed to understand phylogenetic relationship of

β -1,3-gluc with other 27 β -1,3-gluc sequences (Fig. 3). Phylogenetic analysis has revealed two main clades; in between them one clade represented the fungal protein sequences, which diverged much earlier from that of the plant β -1,3-gluc sequences. CDD analysis has detected that the main domain of fungal β -1,3-gluc belonged to the glucosyl hydrolase 81 superfamily, whereas domain of glucosyl hydrolase 17 superfamily was found to be within plant β -1,3-gluc. However, plant proteins were found to belong to a same clade and within the same clade, β -1,3-gluc of *Oryza sativa* remained in a sub-clade which was different from other plant β -1,3-gluc. Furthermore from another sub-clade, three different sub sub-clades were originated; within this, monocots were found to belong in two different sub sub-clades and all dicots remained within the third sub sub-clade. The overall phylogenetic analyses have indicated that further divergence probably occurred after the split of monocots and dicots. Despite difference in position, banana (*Musa acuminata*) β -1,3-gluc was closely related to other monocot β -1,3-gluc proteins (mainly *Zinziber officinale* and *Elaeis guineensis*). Banana β -1,3-gluc was found to be tightly clustered with β -1,3-gluc sequences from other plant species with a topology that was supported by a significantly high bootstrap value (>50%).

Amino acid compositional distances were calculated in order to study the difference in amino acid composition of the sequences used for phylogenetic analysis. Compositional distance has demonstrated the number of differences between sequences even if the substitution patterns were homogeneous among lineages. Compositional distance analysis has revealed that compositionally banana β -1,3-gluc was more closer to *Z. officinale* (0.176), while most distantly related to the *Fragaria x ananassa* (0.961) and *Citrus sinensis* (0.918) among the plants (Table 1). Amino acid identity between banana β -1,3-gluc and the fungal β -1,3-gluc was relatively low (compositional distance >1.3), which was maximum with *Pneumocystis carinii* (2.122) (Table 1). However, β -1,3-gluc of plants were derived from a common ancestor in evolution based on their conserved sequence characteristics such as amino acid homologies and conserved domain motif.

Genomic organization of the β -1,3-glucanase gene

To analyze the organization of β -1,3-gluc gene in banana genome, Southern hybridization was performed using [α - ^{32} P] dCTP labeled cDNA of β -1,3-gluc obtained in this study as probe. Such analyses have revealed a strong band of hybridization signal in *Hind*III and *Eco*RI digested DNA and two strong band of hybridization signal in *Bam*HI digested DNA due to the existence of *Bam*HI site within the cDNA sequence (Fig. 4). This result suggests the presence of a single gene homologous to β -1,3-gluc in the banana genome.

Analysis of expression of β -1,3-glucanase, glucanase activity, and fruit firmness in response to ethylene, auxin, ABA, wound, and cold treatment

To analyze the patterns of β -1,3-gluc transcript and the protein accumulation level in control and in response to various treatments in banana pulp, we next performed studied transcript accumulation profiles of β -1,3-gluc gene by semi-quantitative RT-PCR. The mature unripe banana at preclimacteric stage (day 5 after harvest) was treated with different phytohormones or exposed to various abiotic factors as described under “Materials and methods” to monitor the expression status of β -1,3-gluc. As shown in Fig. 5a, b, exogenous application of ethylene induced the β -1,3-gluc mRNA level in preclimacteric banana pulp. About 2.5-fold higher β -1,3-gluc transcript level was observed in response to ethylene as compared with control after 24 h of treatment, but relatively similar levels of β -1,3-gluc mRNA were detected in control and in wound-treated tissue (Fig. 5a, e). On the other hand, auxin and cold treatment distinctly repressed β -1,3-gluc transcript accumulation (Fig. 5c, f). β -1,3-gluc mRNA level was reduced to ~1.2 and 2.4-fold after 24 h following auxin and cold treatment. In contrast, ABA treatment partially increased (1.4-fold) β -1,3-gluc transcript level as compared with control for 24 h treatment (Fig. 5d). The constitutive expression pattern of *MA-actin* as revealed by RT-PCR analysis under various conditions was used as an internal standard (Fig. 5g–l).

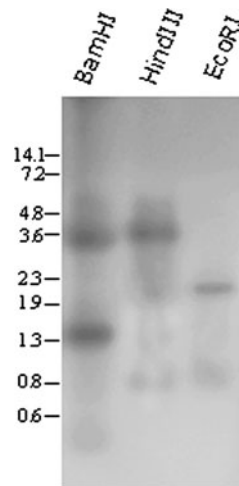
The transcript accumulation profiles of the β -1,3-gluc gene was fairly different with increasing duration of ethylene, auxin, ABA, wounding, and cold treatments. The transcript level of the gene gradually increased after 48 and 72 h (control), whereas β -1,3-gluc mRNA level was rapidly induced after 48 and 72 h of ethylene treatment (Fig. 5a, b, lanes 1–3). As indicated in Fig. 5c, the transcript abundance of β -1,3-gluc was substantially reduced after 48 and 72 h following auxin treatment. Conversely, β -1,3-gluc mRNA was increased only marginally after 48 and 72 h of ABA and wound treatment. In case of low-temperature exposure, no appreciable increase in β -1,3-gluc mRNA was detected after increasing duration of treatment (48 and 72 h) (Fig. 5c, d, e, f, lanes 1–3).

We have further verified the correlation between β -1,3-gluc transcript level and the accumulation pattern of β -1,3-gluc protein in control and in response to various treatments in preclimacteric banana pulp after 24, 48, and 72 h of treatments. As shown in Fig. 5m–r, immunoblot analysis using anti-tobacco β -1,3-glucanase antibody showed the cross-reactive band of β -1,3-gluc of 34-kDa with a considerable variation in expression level in control and in response to ethylene, auxin, ABA, wound, and cold treatments. As like transcript level, β -1,3-gluc protein level

Table 1 Estimates of base composition bias difference between sequences

	Musa	Elaeis	Zingiber	Lilium	Vitis	Nicotiana	Medicago	Cicer	Prunus	Pisum	Citrus	Fragaria	Oryza	Hordeum	Triticum	Yeast	Pneumocystis	Aspergillus	Coccidioides	Pichia
Musa																				
Elaeis	0.387																			
Zingiber	0.176	0.599																		
Lilium	0.355	0.251	0.516																	
Vitis	0.341	0.781	0.624	0.796																
Nicotiana	0.369	0.613	0.799	0.742	0.326															
Medicago	0.484	0.588	0.918	0.789	0.254	0.391														
Cicer	0.634	0.613	1.197	0.778	0.366	0.444	0.075													
Prunus	0.430	0.48	0.853	0.656	0.208	0.254	0.168	0.201												
Pisum	0.502	0.674	0.932	0.781	0.226	0.455	0.043	0.065	0.179											
Citrus	0.918	1.136	1.362	1.348	0.541	0.581	0.477	0.548	0.253	0.47										
Fragaria	0.961	0.692	1.355	0.839	0.914	1.093	0.523	0.513	0.502	0.502	0.839									
Oryza	0.538	0.867	0.925	1.075	0.71	0.71	0.516	0.613	0.681	0.491	1.158	0.695								
Hordeum	0.681	0.792	0.706	0.545	0.932	1.308	0.9	0.996	0.953	0.828	1.624	0.57	0.703							
Triticum	0.369	0.538	0.437	0.523	0.538	0.516	0.538	0.713	0.493	0.609	0.914	0.638	0.656	0.448						
Yeast	1.627	1.713	1.975	1.409	1.362	1.67	1.269	1.215	1.323	1.154	1.871	1.097	1.591	1.29	1.380					
Pneumocystis	2.122	1.971	2.939	1.953	1.556	1.57	1.452	1.222	1.434	1.437	2.294	1.423	1.753	1.986	1.864	0.986				
Aspergillus	1.358	1.509	1.767	1.358	0.914	1.186	0.989	0.968	1.014	0.928	1.57	1.032	1.079	1.004	1.179	0.878	0.720			
Coccidioides	1.609	2.004	2.000	1.839	0.903	1.556	1.072	1.104	1.222	0.971	1.749	1.247	1.287	1.244	1.462	1.043	0.903	0.294		
Pichia	1.319	1.57	1.814	1.419	1.129	1.534	0.946	0.914	1.265	0.889	1.871	0.993	0.921	0.961	1.351	0.699	0.953	0.434	0.423	

Fig. 4 Genomic Southern blot analysis of β -1,3-glucanase gene in banana (cv. Cavendish). Total DNA was digested with *Bam*HI, *Hind*III and *Eco*RI (lanes 1–3), and ^{32} P-labeled cDNA of banana β -1,3-glucanase was used as probe. The sizes of the DNA molecular mass standards are indicated



was substantially induced in response to ethylene treatments (~ 2.1 -fold high than control), and it was further increased with increasing duration of ethylene treatment. Similar to β -1,3-*gluc* transcript level, β -1,3-*gluc* protein was reduced following increasing durations of auxin treatment (Fig. 5o, lanes 1–3). On the other hand, β -1,3-*gluc* protein level was increased after 24 h of ABA treatment (~ 1.5 -fold increased than control) but did not increase appreciably further after 48 and 72 h of ABA treatment (Fig. 5p, lanes 1–3). The β -1,3-*gluc* protein accumulation level was consistent with its transcript level following increasing duration of wounding (Fig. 5q, lanes 1–3). Low temperature treatment for 24 h significantly reduced the β -1,3-*gluc* protein level (~ 2.2 -fold reduced than control). However, no further decrease was detected after 48 and 72 h of cold treatment (Fig. 5r, lanes 1–3). The mRNA level of β -1,3-*gluc* as observed following exposure to different phytohormones and abiotic factors like low temperature and wounding for various duration was in agreement with the accumulation levels of β -1,3-*gluc* protein under similar conditions in the preclimacteric banana pulp. Taken together, the results clearly suggest that the β -1,3-*gluc* mRNA levels as analyzed by semi-quantitative RT-PCR with the gene-specific primers corresponds to its specific β -1,3-*gluc* protein as detected in immunoblotting experiments using anti-tobacco- β -1,3-*gluc* antibody. These results also reflect the specificity of recognition of banana β -1,3-*gluc* protein by this antibody.

To analyze the glucanase activity and fruit firmness, we next measured the enzymatic activity of glucanase and fruit firmness under control condition and after various treatments. The enzymatic activity of glucanase was correlated well with the protein level of β -1,3-*gluc* following various treatments for 24, 48, and 72 h (Fig. 6a–f). Such analyses have indicated a substantial increase in glucanase activity following ethylene treatment to unripe banana, while ABA

only marginally stimulated glucanase activity. Wounding did not show any considerable effect on glucanase activity (Fig. 6b, d, e). Glucanase activity was rapidly increased due to increasing duration of ethylene treatment (48 and 72 h) as compared with control condition (Fig. 6a, b). Consistent with this, whereas fruit firmness decreased gradually with time in control fruit, it decreased sharply by increasing duration of ethylene treatment (48 and 72 h) (Fig. 6g, h). As shown in Fig. 6j, ABA treatment for 24, 48, and 72 h only partially reduced fruit firmness during ripening in banana fruit. Exogenous auxin treatment to some extent inhibited glucanase activity while low-temperature treatment distinctly inhibited glucanase activity (~ 1.3 and 2.5 -fold less glucanase activity as compared with control) in banana fruit during ripening. Similar to the β -1,3-*gluc* transcript and protein levels, only minor changes in glucanase activities were observed after increasing duration of auxin and low-temperature treatment (Fig. 6c, d, f). Therefore, no appreciable decrease in fruit firmness was observed after exposure to auxin and low temperature for 48 and 72 h, respectively (Fig. 6i, k, l).

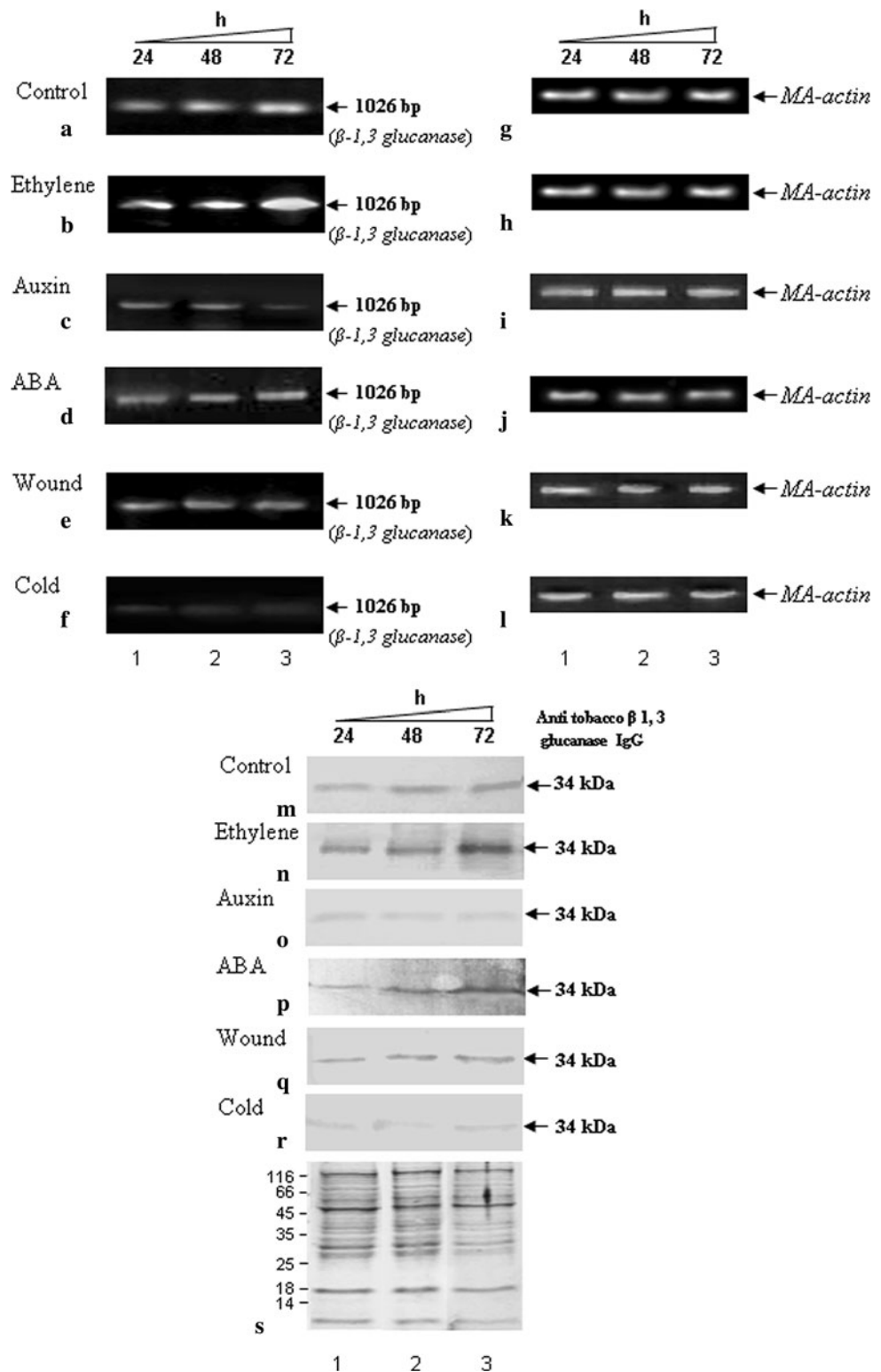
Role of ethylene in auxin, ABA, wound, and cold-treated preclimacteric banana pulp

To investigate the role of ethylene in auxin, ABA, wound and cold-treated fruit pulp, 24-, 48-, and 72-h ethylene treatments were given to 24-h auxin, wound- and cold-treated fruit separately (80 DPA or 5 DAH). Our analyses revealed that ABA alone was not able to substantially induce β -1,3-*gluc* expression, but ethylene treatments (24, 48 and 72 h) in ABA-treated fruit showed enhanced β -1,3-*gluc* expression as like ethylene-treated fruit (Fig. 7c). However, no significant change in transcript level was detected, when wound-treated fruit was exposed to ethylene (Fig. 7e). Furthermore, partial increase in β -1,3-*gluc* transcript level was observed in auxin- and cold-treated fruit after 24, 48 and, 72 h of ethylene treatment (Fig. 7a, g).

Increased duration of dark period induces expression of β -1,3-glucanase gene, glucanase activity and represses fruit firmness in banana fruit during ripening

To investigate the regulation of light and dark phases on the expression pattern of β -1,3-*gluc* during banana fruit ripening, preclimacteric (green unripe fruit) banana fingers were exposed to different photoperiodic conditions as described under “Materials and methods”. As indicated in Fig. 8a, β -1,3-*gluc* mRNA abundance was substantially increased in the fruits maintained at constant darkness for 8 days (lane 1), while the transcript was gradually decreased with increasing duration of exposure to white

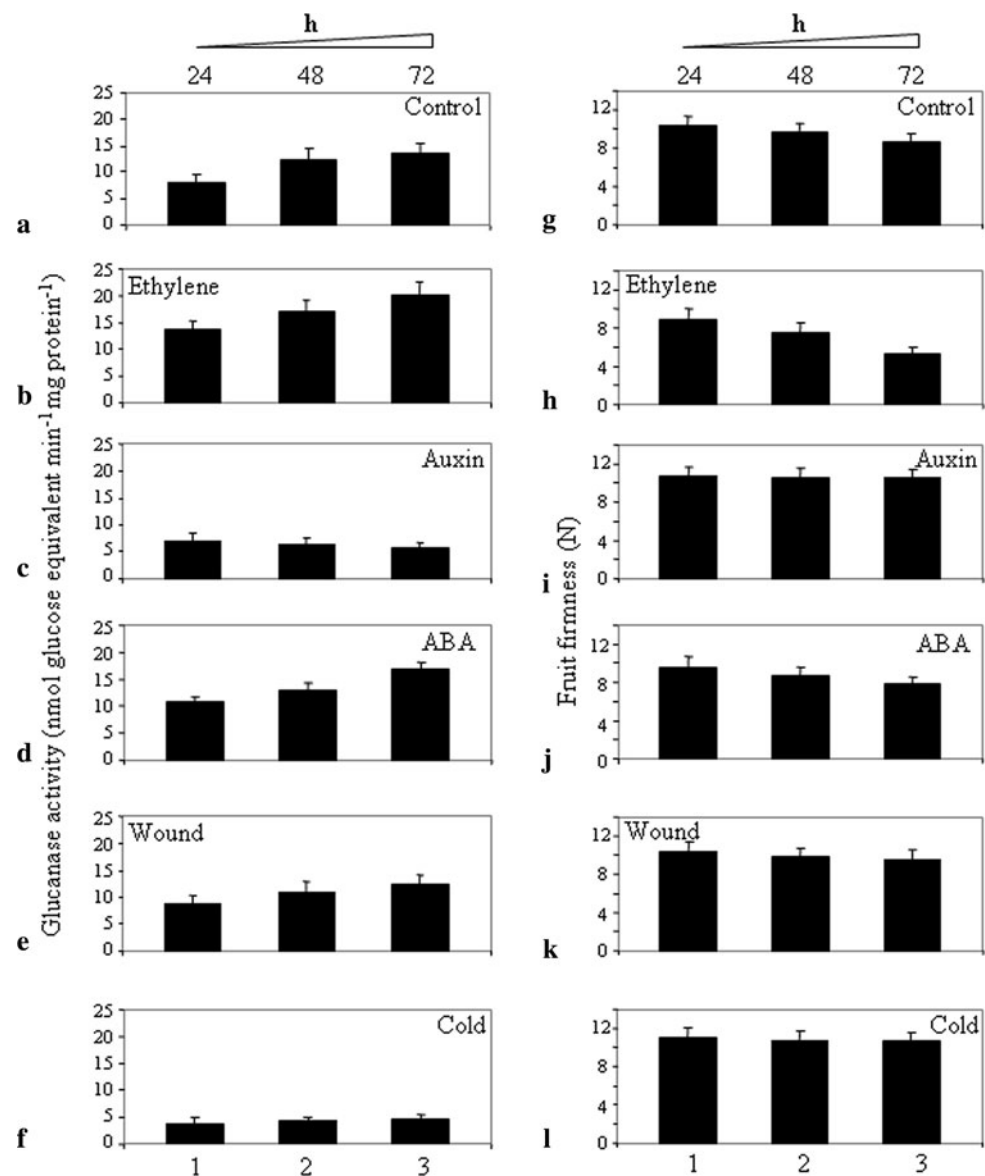
Fig. 5 a–f Changes in the abundance of β -1,3-glucanase mRNA level in banana fruit pulp in pre-climacteric stages (day 5, after harvest) in control and following ethylene, auxin, ABA, wounding, and low-temperature treatments for 24, 48, and 72 h, respectively (lanes 1–3). **g–l** mRNA levels of *MA-actin* under various conditions used as internal control. **m–r** Immunodetection of β -1,3-glucanase protein by using anti-tobacco class I β -1,3-glucanase antibody in control and following ethylene, auxin, wounding, and low-temperature treatments for 24, 48, and 72 h, respectively (lanes 1–3). **s** Buffer soluble protein profile of banana pulps after 24, 48, and 72 h of control condition shown as loading control. Protein samples were resolved on 10% SDS-PAGE and stained with Coomassie blue



light (lanes 2–5). β -1,3-gluc mRNA was reduced to ~ 1.1 -fold under 2L condition than 8D condition, while it was further decreased to ~ 1.4 , 1.7 and 1.9-fold following exposure to constant white light for 4, 6, and 8 days, respectively (Fig. 8c).

To correlate with the transcript abundance pattern, we then investigated the relative level of β -1,3-gluc protein in banana fruits after exposure to different duration of light/dark phases. As shown in Fig. 8d, immunoblotting with anti-tobacco class I β -1,3-glucanase antibody revealed

Fig. 6 Analysis of changes in glucanase activity (a–f) and fruit firmness (g–l) in preclimacteric banana fruits was measured in control and following 24, 48, and 72 h treatments with ethylene, auxin, ABA, wounding and cold treatments. Data points are mean of the five replications, ± 1 SE



maximum expression of β -1,3-gluc protein in the fruits kept at constant darkness for 8 days (lane 1). About 1.3, 1.5 and 1.6-fold less β -1,3-gluc protein level were detected in fruit pulp maintained under 4L, 6L, and 8L conditions as opposed to those kept at 8 days of constant darkness (Fig. 8f). Similar set of proteins was probed with rabbit-pre-immune serum, which did not detect the β -1,3-gluc protein band (data not shown).

To further substantiate the expression profile of β -1,3-gluc under various conditions, we estimated the glucanase activity and fruit firmness in banana fruits exposed to different light/dark cycles. Assay of enzymatic activity of glucanase as well as fruit firmness under various photoperiods revealed a gradual reduction of glucanase activity and enhancement of fruit firmness with increased exposure to constant white light period (Fig. 8g, h). The induction of

β -1,3-gluc gene expression along with the increased glucanase activity and decreased fruit firmness in response to darkness may suggest an important regulatory function of dark period during ripening in banana fruit.

Discussion

The *O*-glycosyl hydrolases are a widespread group of enzymes that hydrolyze the glycosidic bond between two or more carbohydrates or between a carbohydrate and a non-carbohydrate moiety. A glycosyl hydrolase includes lichenase, xyloglucan endotransglycosylase (XET), beta-agarase, kappa-carrageenase, endo- β -1,3-glucanase, endo- β -1,3-1,4-glucanase, and endo- β -galactosidase, all of which have a conserved jelly roll fold with a deep active

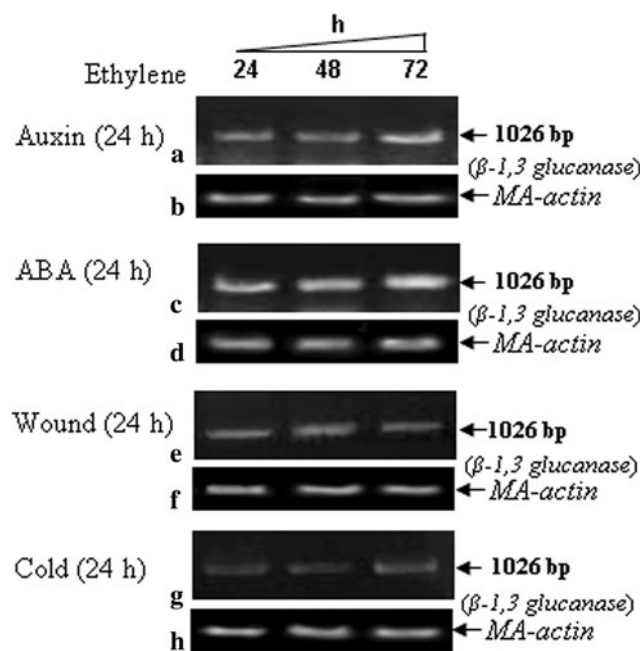


Fig. 7 Transcript level of β -1,3-glucanase in 24 h auxin, ABA, wound- and cold-treated banana pulp after different durations of ethylene treatment (**a, c, e, g**) assessed by semi-quantitative RT-PCR. mRNA levels of *MA-actin* under various conditions used as internal control (**b, d, f, h**)

site channel harboring the catalytic residues (Henrissat et al. 1995). Endo- β -1,3-glucanases hydrolyze 1,3- β -glucosyl linkages, but they usually require contiguous 1,3- β -linked glucosyl residues (Gueguen et al. 1997). As like other plant endo- β -1,3-glucanases, banana β -1,3-glucanase belongs to the glucosyl hydrolase 17 family and shown requirement for contiguous 1,3- β -linked glucosyl residues (Roy Choudhury et al. 2009a).

Sequence conservation analysis using ConSeq has showed that most of the exceptionally conserved amino acid residues fell under either α -helix region or β -sheets (Fig. 2). A homology-based model of the banana β -1,3-gluc protein was generated and used to predict the putative binding site(s) for calcium and this structural interactions potentially responsible for the stability of the protein at high temperature as like endo- β -1,3-glucanase of hyperthermophilic archaeon *Pyrococcus furiosus* (Gueguen et al. 1997).

The β -1,3-gluc exist as multiple structural isoforms, which differ in size, primary structure, cellular localization, and pattern of regulation (Leubner-Metzger and Meins 1999). The enzyme hydrolyzes laminarin, an oligomeric, water soluble β -1,3-glucan (Trudel et al. 1998). There are three classes of β -1,3-gluc such as class I, class II, and class III in tobacco. The class I β -1,3-glucs are mainly restricted in vacuole of epidermal cell and basic in nature. The class II and class III β -1,3-gluc are acidic in nature and secreted into the extracellular space (Meins et al. 1992). The basic

banana β -1,3-gluc belongs to class I β -1,3-gluc and as like other class I β -1,3-glucs, this β -1,3-gluc showed moderate expression in various parts of banana flower, indicating the role of β -1,3-gluc in banana flower formation. Comparatively weak expression pattern of β -1,3-gluc in root, stem, and leaf tissues undoubtedly interpreted the negligible role β -1,3-gluc protein in vegetative tissues. However, the enhanced expression of β -1,3-gluc in the ripe peel and pulp tissue clearly indicated that β -1,3-gluc is ripening regulated. As like banana, in strawberry fruit, two β -1,3-glucanase genes were found to be induced during ripening (Shi et al. 2006).

Previously, catalytically active forms of β -1,3-gluc have been purified and characterized from banana fruit and its probable function in fruit softening has been indicated (Peumans et al. 2000). However, based on the present knowledge, it is generally considered that β -1,3-gluc perform diverse biological functions and notable efforts are being undertaken to unravel the non-defense related functions of this enzyme in non-infected or uninduced plants (Morohashi and Matsushima 2000; Buchner et al. 2002; Akiyama et al. 2004). In addition, earlier it has been also suggested that β -1,3-gluc play a role in fruit ripening (Hinton and Pressey 1980). Recently, we have also established that banana fruit wall polysaccharide fraction can be degraded by recombinant banana β -1,3-gluc (Roy Choudhury et al. 2009a).

Breakdown of stored starch (polysaccharide) and loss of turgor pressure along with the partial disassembly of fruit cell wall is responsible for softening and change in fruit coat texture during the later stages of ripening. Previous experiments have demonstrated the involvement of expansin (Trivedi and Nath 2004) and pectate lyase (Dominguez-Puigjaner et al. 1997) in the disintegration of fruit cell wall during the ethylene-regulated ripening process in banana. Although β -1,3-gluc is the PR-related protein, their role in banana pulp is questionable, because banana pulp is well protected by peel. To address the issue of possible physiological function of β -1,3-gluc in fruit softening during banana fruit ripening, we have also measured the fruit firmness, since the enzyme has shown to be highly abundant in banana fruit and with no anti fungal activity (Peumans et al. 2000). The physiological substrate of β -1,3-gluc is also a β -1,3-linked carbohydrate component of cell wall of certain cell types during specific developmental stages of plants (Kauss 1987) and in *P. furiosus*, this β -1,3-gluc polymer was degraded by β -1,3-gluc to form smaller oligomers and finally hydrolyzed to glucose by β -galactosidase (Gueguen et al. 1997). In banana as like β -1,3-gluc, β -galactosidase activity significantly increased during fruit ripening (Ali et al. 1998).

The ripening program requires differential gene expression, which is regulated by environmental conditions

Fig. 8 a Analysis of steady-state-mRNA levels banana β -1,3-glucanase, indicated by RT-PCR following exposure of fruits to different photoperiods, including 8 days of constant white light (8L), 6 days of light/2 days of dark (6L), 4 days of light/4 days of dark (4L), 2 days of light/6 days of dark (2L) and 8 days of constant dark (8D) periods, respectively.

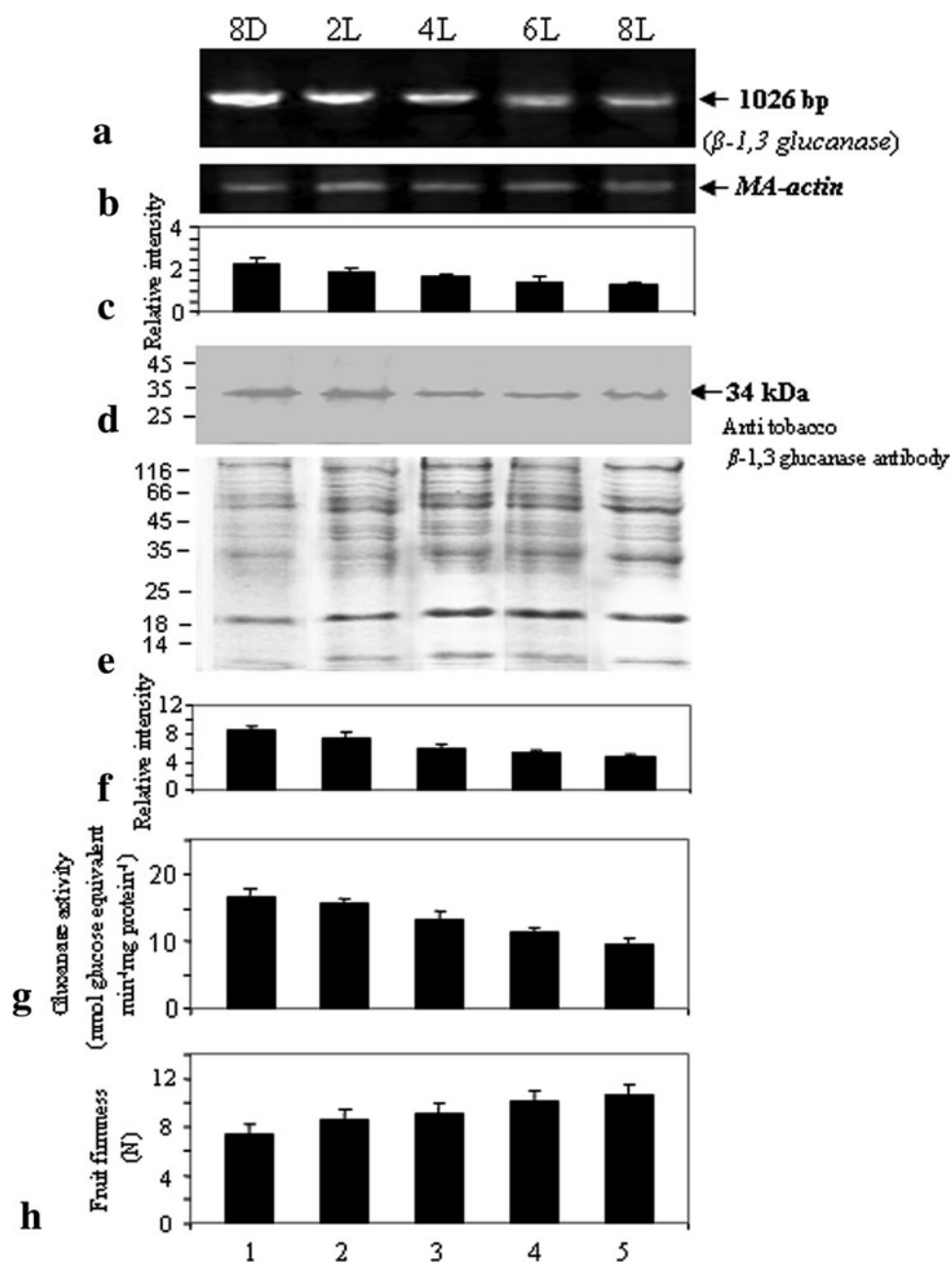
b Transcript level of *MA-actin* was used as internal control.

c Quantification of the data in **a** by densitometric analysis.

d Protein gel blot analysis using anti-tobacco class I β -1,3-glucanase antibody to detect the relative expression level of β -1,3-glucanase protein in banana fruit during ripening at the various light/dark phases (lanes 1–5). Molecular weight markers are shown on the left side of figure.

e Coomassie stained protein gel (total protein extracts) shown as loading control. **f** Quantification of the data in **d**.

g, h Enzymatic activity of glucanase and fruit firmness after exposure of the fruits to various light/dark cycles



and phytohormones. Ethylene, auxin, and cytokinin act as a regulator to control the gene expression of endohydrolases (Broglie et al. 1986; Shinshi et al. 1987). A comparative study of transcript accumulation pattern of β -1,3-gluc in the preclimacteric stages of ripening in banana fruit pulp following ethylene, auxin, ABA, wounding, and cold treatment showed a distinct profile for this gene both at mRNA and protein level. Our observations have demonstrated a strong induction of β -1,3-gluc both at mRNA and protein level in response to ethylene with a concomitant increase in glucanase activity and decrease in fruit firmness. We have detected partial increase of β -1,3-gluc both at mRNA and protein level following exogenous

application of ABA resulting in only marginal increase in glucanase activity and partial decrease of fruit firmness in ABA-treated fruit. On the other hand, in banana pulp, auxin and cold treatment reduced β -1,3-gluc expression that eventually led to decreased glucanase activity and therefore fruit firmness remained unaffected. Low temperature has been shown to down regulate the expression of gene coding for expansin in banana (Wang et al. 2006). β -1,3-gluc gene expression was also inhibited following cold treatment. Previously, we have observed that ethylene formation was repressed due to auxin and cold treatment in preclimacteric banana fruit as result of decreased ACC oxidase activity (Roy Choudhury et al. 2008b). This may also be linked to

explain the inability of ethylene to induce β -1,3-*gluc* expression in auxin and low-temperature treated fruit. ABA treatment alone partially enhanced the β -1,3-*gluc* expression in mRNA level, but when banana fruit was treated with both ABA and then ethylene, a substantial increase of β -1,3-*gluc* expression was detected, and as a result fruit firmness was significantly reduced as like ethylene-treated fruit (data not shown). These findings support the observations that the fruit firmness was decreased in banana due to either ABA or both ABA and ethylene treatment (Lohani et al. 2004). The exposure of auxin and cold-treated fruit to ethylene only partially triggered the expression of β -1,3-*gluc*, but failed to recover the β -1,3-*gluc* mRNA level as observed in ethylene-treated fruit. These observations may suggest that auxin and low temperature act negatively to nullify the ethylene-induced expression of β -1,3-*gluc* during banana fruit ripening. This was also supported by the marginal decrease in fruit firmness in banana fruit which were initially exposed to auxin or low temperature and then ethylene (data not shown).

Our results have also demonstrated a distinct pattern of regulation of expression of β -1,3-*gluc* in response to light and dark phases. Expression of β -1,3-*gluc* was significantly enhanced in response to constant dark period, while interruption of the dark phase with increasing duration of light period substantially reduced the expression. Consistent with its transcript level, β -1,3-*gluc* protein expression and glucanase activity were decreased with increased exposure to white light. Previously, we have reported that expression level of *MA-ACS1* (*M. acuminata* ACC synthase 1) and *MA-ACO1* (*M. acuminata* ACC synthase 1), two important ethylene-regulated ripening genes responsible for ethylene biosynthesis, during banana fruit ripening. Expression of both the genes and ethylene biosynthesis were considerably enhanced in response to constant dark period (Roy Choudhury et al. 2008b). On the other hand, constant exposure to white light period inhibited *MA-ACS1* and *MA-ACO1* expression and subsequently ethylene biosynthesis. The ethylene-inducible β -1,3-*gluc* expression was significantly declined with increasing duration of light period. Conversely, fruit firmness was steadily decreased due to increasing duration of dark period.

Taking into account all expression studies, it is suggested that banana β -1,3-*gluc* is ethylene regulated and probably associated with softening of the fruit. The presence of ethylene-responsive element (ERE) within the promoter of β -1,3-*gluc* of tobacco and promoter of *MA-ACS1* and *SPS* of banana has been shown to play important role in the ethylene-induced expression of these genes (Ohme-Takagi and Shinshi 1995; Roy Choudhury et al. 2008b, 2009b). Similarly, the presence of putative ERE in the promoter of banana β -1,3-*gluc* may also appear be involved in the regulation of β -1,3-*gluc* expression in

response to ethylene in banana. Therefore, characterization of various regulatory elements in banana β -1,3-*gluc* promoter will provide further important insight into the transcriptional regulatory network of β -1,3-*gluc* of banana.

Banana is considered as a model fruit crop for the study of increasing shelf life of economically valuable fruits and expression of foreign genes with therapeutic importance. In the developing countries, for many years the major area of concern in ripening research involves the post-harvest management of fruits since farmers are facing huge economic losses every year as a result of ethylene-induced senescence of fruits before they reach the consumers. Therefore, understanding the expression pattern of ripening genes and the role of various factors (both biotic and environmental) that regulate gene expression during ripening is very crucial in regulating the expression of ripening genes to control ripening under post-harvest conditions to reduce massive spoilage of fruits during shipping and storage (Zarembinski and Theologis 1994). In this scenario, the present study provides important information with wider implications for further investigation of the molecular regulatory networks impacting response during ripening in various fruit crops of agro-horticultural importance.

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