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Cloning and differential expression of *QM like protein* homologue from tea [*Camellia sinensis* (L.) O. Kuntze]

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Abstract The *QM like protein* gene encodes for ribosomal protein L10, which is implicated in tumor suppression, transcription factor regulation, and ribosome stability in yeast and mammals. Present study describes cloning of a full-length *QM* cDNA (*CsQM*) from tea leaves using differential display of mRNA followed by rapid amplification of cDNA ends. Expression of *CsQM* was studied in leaves of different stages of development and under various external cues. *CsQM* contained an open reading frame of 651 bases, encoding 216 amino acids. *CsQM* shared 71–87% and 85–91% identity at nucleotide and amino acid sequences, respectively with *QM* genes isolated from other plant species. During active-growth period of tea, higher expression was observed in apical buds that decreased gradually with increasing age of the leaf. During dormancy season, the expression of *CsQM* gene was severely down-regulated in all the leaves studied. *CsQM* transcript was found to be down regulated in response to drought stress and abscisic acid treatment but up-regulated by gibberellic acid treatment. A positive association of *CsQM* transcript abundance with active cellular growth suggested its role in plant growth and development.

Keywords *Camellia sinensis* · Development · Gene expression · Stress · Tea · *QM like protein*

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Introduction

QM family protein was originally identified as a putative suppressor of Wilms' tumor [1]. The higher expression of the *QM* gene in non-tumorigenic Wilms' microcell hybrid cells as compared to tumorigenic parental cell line suggested *QM* gene product to be a putative tumor suppressor. The *QM* gene encodes a ribosomal protein L10. L10 is associated with 60S sub unit and is a key factor in joining of 40S and 60S ribosomal subunits to yield a functional 80S ribosome [2–4]. Increasing evidences suggested that besides housekeeping roles in protein synthesis, *QM*s have multiple extra-ribosomal functions [5, 6]. QMs are basic and hydrophilic protein with molecular weights ranging between 24 and 26 kDa. These proteins have two basic domains possibly to bind to DNA; and an acidic domain involved in activation of transcription [7]. Highly conserved sequences of *QMs* suggested fundamental and critical functions of *QM* across species.

Besides human non-tumourigenic cells, *QM* homologues have been identified in other animals, protozoan and fungi. *QM* gene has been cloned from arabidopsis [8] and rice [9] but physiological functions is yet to be understood. Chen et al. [10] showed that the expression of tomato QM-like protein in *Saccharomyces cerevisiae* protected yeast cells against oxidative stress caused by hydrogen peroxide, paraquat and heat by regulating the intracellular levels of proline.

Tea [*Camellia sinensis* (L.) O. Kuntze] is an important commercial perennial woody plant and gaining importance not only as most popular beverage but also as a source of secondary metabolites with immense medicinal properties [11]. In the present study, *QM* was cloned from tea plant through differential display of mRNA (DD) followed by *R*apid *A*mplification of *c*DNA *E*nds (RACE). Expression

analysis of *CsQM* in response to environmental cues and developmental stages of the leaf during dormant and non-dormant season of tea growth suggested its high expression in actively growing tissues.

Materials and methods

Plant materials and growth conditions

UPASI-10, a prevalent Chinary type of tea clone [12], growing well in the tea garden of the Institute (latitude 32°06′32″ N, longitude 76°33′43″ E and altitude 1,300 m above mean sea level) was selected in the present study. Various experiments were performed on apical bud (AB; youngest), and on the leaves at positions 1st, 2nd, 3rd and 4th (position with reference to the apical bud). Leaves at different node positions represent different stages of development, apical bud being the youngest whereas leaf at 4th node position is the oldest in text to the present experiment. Leaves were collected during the period of active growth (July, average temperature 22.5°C) and during winter dormancy (December, average temperature 5°C) when the growth of apical bud and adjoining leaves ceases almost completely. These were harvested between 9 and 10 a.m., stored immediately in liquid nitrogen and later transferred to a −80°C freezer till further use.

In a separate experiment, study was also carried out in vegetatively propagated tea plants (2 years old), raised from the same mother plant (UPASI-10). The plants were grown in plastic sleeves and were housed in a plant growth chamber (temperature, 25 ± 1°C; light intensity, 200 μE m^{-2} s^{-1}; RH, 70–80%; Saveer Biotech, India). Drought stress (DS) was created by withholding water during the entire period of experimentation. Abscisic acid (ABA, 100 μM) and gibberellic acid (GA_3, 50 μM) were sprayed separately and also applied to the soil [13]. Leaf tissues were harvested on 0, 2nd, 4th, 6th and 8th day of the treatments. Frozen samples were stored at −80°C and used for various analyses. Relative water content (RWC) was measured as described by Lazacano-Ferrat and Lovatt [14].

Differential display of mRNA

As a part of our ongoing programme on understanding the trancriptome response of tea to various environmental cues, differential display of mRNA (DD) was performed using total RNA isolated from the shoots of control, drought stress (DS) and GA_3 treated tissues (day 8 of the treatment). ABA treatment was omitted as DS and ABA might modulate same type of genes [15]. Various steps of DD namely, isolation of RNA, removal of contaminating DNA, cDNA synthesis, radioactive PCR, separation of PCR products on denaturating polyacrylamide gel, elution of cDNA bands and cloning of PCR products was performed essentially as described by Lal et al. [16] and Sharma and Kumar [13]. The only difference was that while performing radioactive PCR reactions, a range of arbitrary primers supplied by M/S GenHunter Corporation, USA were used. The kits from M/S GenHunter Corporation, USA was used to perform various reactions in DD as follows: Message-Clean® to remove contaminating DNA, RNAimage® for performing differential display, PCR-TRAP® vector to clone PCR products after eluting and amplifying from the polyacrylamide gel and AidSeq™ primer set C for performing sequencing. Manufacturer's instructions were followed as and where needed. *Taq* polymerase was purchased from M/s. Qiagen, GmbH and α-^{33}P-dATP (Specific activity, 2,500 Ci/mmol, 10 mCi/ml) was purchased from Bhabha Atomic Research Centre, Bombay, India. Those cDNA bands that exhibited differential regulation in response to control and treated tissues were selected, cloned and analysed. Overall 133 bands were analysed and submitted to Genbank at EST database (accession numbers DN976085 to DN976213, DR397420, AY694187, AY694189 and AY694190).

5′ and 3′ RACE to clone full-length *CsQM*

Anchored and arbitrary primer pair (Left primer: 5′-AAGC TTGATTGCC-3′; Right primer: 5′-AAGCTTTTTTTTTT TTTA-3′) generated a fragment of 454 base pairs (dbEST accession number DN976112) that was down-regulated under DS and sequencing and BLAST analysis showed it to be homologous to *QM* gene. 3′ and 5′ RACE (SMART RACE cDNA amplification Kit, BD Biosciences, USA) was performed to clone 3′ and 5′ ends of *CsQM* cDNA using primers 5′-CAGGAAGTGGGGATTCACCAAGTA CA-3′ for 3′ RACE and 5′-AAAGCAGGAGTCCTTC AGTTCCAGAGAC-3′ for 5′ RACE. The amplified fragments were cloned into pGEM-T easy vector (Promega) and sequenced.

Bioinformatics analysis of cloned gene

Homology search of sequences in databases was conducted using the BLAST algorithm (http://www.ncbi.nlm.nih.gov/). Deduced amino acid sequence was used to analyze protein families and domains using various tools available at PROSITE database at ExPASy proteomics server (http://ca.expasy.org/) and SMART (simple modular architecture research tool) (http://smart.embl-heidelberg.de/). The deduced *CsQM* and other known *QM* sequences retrieved from GenBank were aligned with CLUSTALW (www.ebi.ac.uk/) at default setting. Secondary structures of

deduced amino acids sequences were predicted by SOPMA (http://npsa-pbil.ibcp.fr/).

Gene expression analysis

Expression of *CsQM* was studied in response to different stages of development of leaves and under different environmental cues. Total RNA (15 μg) was separated on 1% (w/v) denaturing formaldehyde agarose gel and transferred onto Hybond XL membranes from Amersham Biosciences, USA as detailed by Sambrook et al. [17]. The cDNA probes were radiolabelled with α-^{32}P-dATP using klenow DNA polymerase (Hot prime labeling kit, GenHunter Corporation, USA). Hybridization was performed using ExpressHyb Hybridization solution (BD Biosciences, USA) as per manufacturer's instruction. Membrane was exposed to phosphorimager screen (Kodak ID 3.5, USA) and analyzed using Molecular Imager FX (Bio-Rad, USA). Blots were scanned using a gel documentation system (Alpha DigiDoc^{TM}, Alpha Innotech, USA) and integrated density values (IDV) were calculated using Alpha Digidoc-1000 software. Expression of *26S rRNA* was used as a control to check equal loading [18].

Results and discussion

QM like protein is gaining importance as a transcription factor and its role in stability of ribosomes [4, 7]. It is identified as a key role player in cell signaling and development [19]. In plants, though in its infancy, role of QM like proteins in growth and development has started to gain understanding [9, 10]. In the present communication, *QM* gene was cloned from tea through DD and showed its relationship with plant growth and development.

Plants were exposed to DS, ABA and GA_3 treatments. RWC kept on decreasing with increase in the time period of DS and reduced by more than 50% during the 8th day of stress. However, in other treatments, RWC remained the same as compared to the control (data not shown). In order to understand the trancriptome response of tea to various environmental cues that affect growth and developmental processes, DD was performed using total RNA isolated from the shoots of control, DS and GA_3 treated tissues (day 8 of the treatment). Analysis of 133 fragments lead to identification of a 454 bp fragment (dbEST accession number DN976112) showing high similarity to *QM like protein* gene. The *QM* sequences obtained after 5′ and 3′ RACE were aligned to generate full length cDNA of *CsQM*

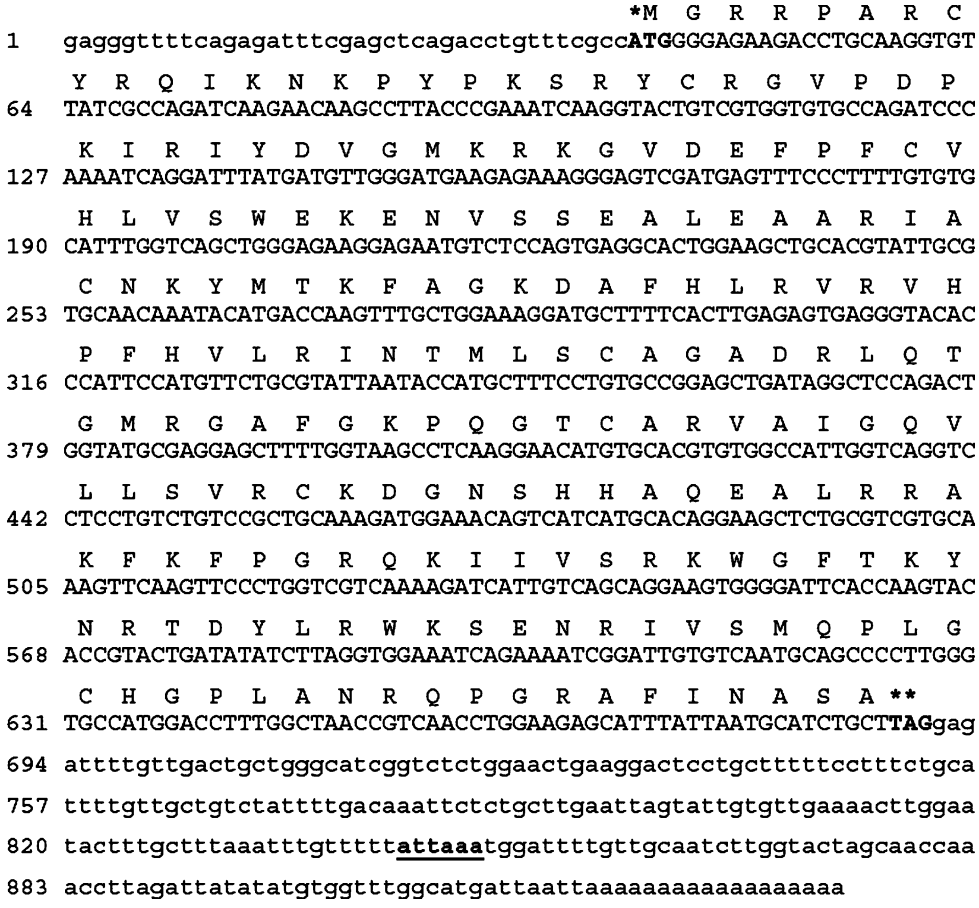

Fig. 1 Nucleotide and deduced amino acid sequence of *CsQM* isolated from tea. Start and stop codons are indicated by * and **, respectively. Nucleotides in small letters represent 5′ and 3′ untranslated regions. Poly(A) signal is underlined

(GenBank accession number AY641733). *CsQM* contained an open reading frame of 651 bases, encoding 216 amino acids (Fig. 1). BLAST analysis of the *CsQM* showed significant homology at the nucleotide (71–87%) as well as at protein (85–91%) level with the *QM* sequences reported from other plant species like *Vitis vinifera*, *Populus trichocarpa*, *Solanum melongena*, and *Arabidopsis thaliana*. SMART analysis of deduced amino acid sequence revealed a characteristic domain of L10 protein present between amino acids positions 1 and 176. The domain was highly conserved and showed high homology with L10 protein of vertebrates (*Homo sapiens*, 63%; *Mus musculus*, 63%), *S. cerevisiae* L10 (61%), *Arabidopsis thaliana* (87%) and *Oryza sativa* (71%). Alignment of deduced amino acids of CsQM with QM protein reported from other species showed the presence of conserved domains (Fig. 2) as indicated by Farmer et al. [7]. The first basic domain was present at N terminal having 32 amino acids and the second basic domain comprised residues 150–175. Immediately following the first basic domain was clustered charged

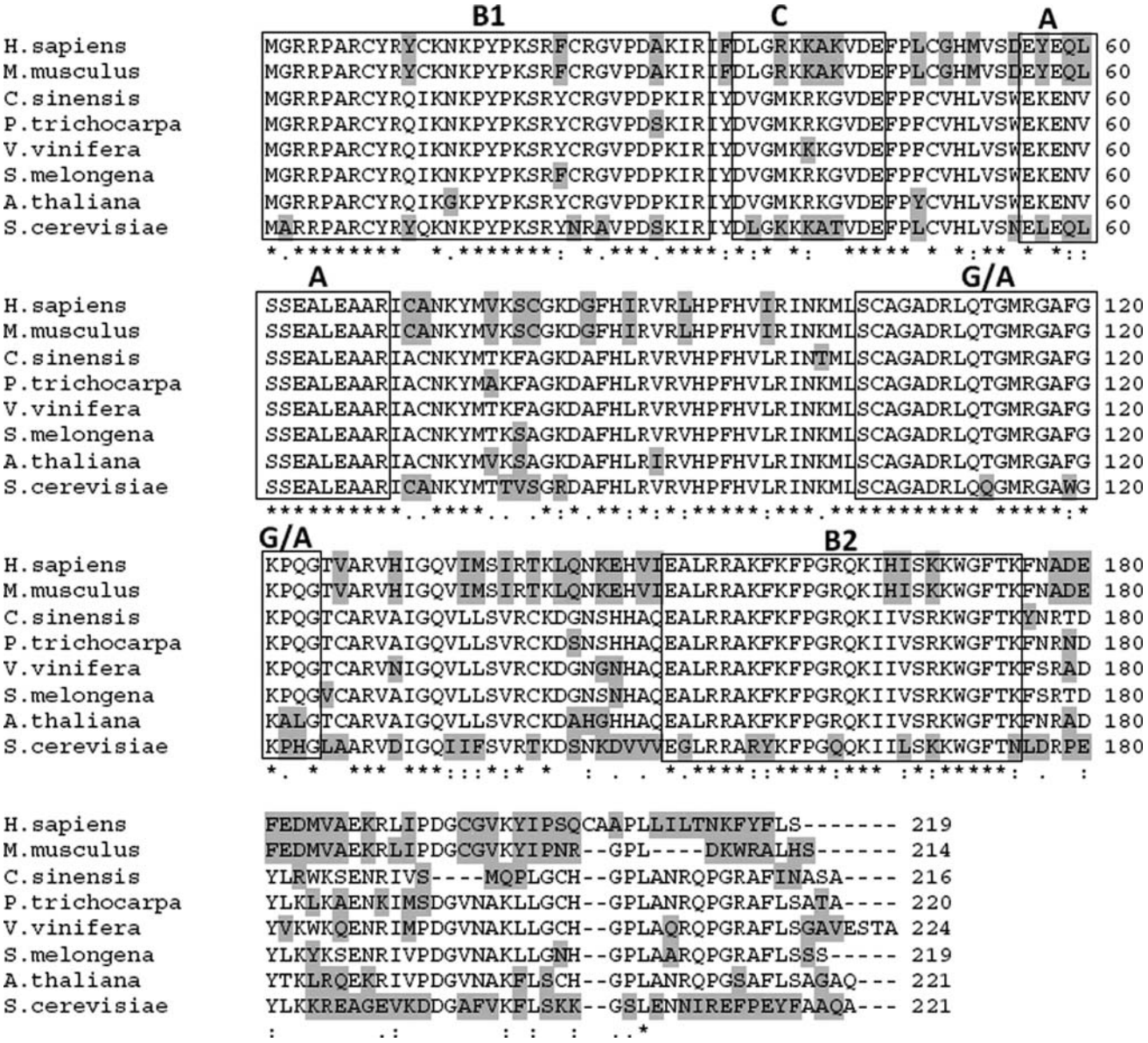

Fig. 2 Alignment of deuced amino acid sequence of QM from *C. sinensis* with that of QM from *Homo sapiens* (EAW72738), *Mus musculus* (NP_443067), *Populus trichocarpa* (ABK95468), *Vitis vinifera* (CAO68740), *Solanum melongena* (BAA19462), *Arabidopsis thaliana* (NP_174013) and *Saccharomyces cerevisiae* (NP_013176). Residues varying between sequences are indicated with grey background. Gaps are represented as -; *, : and . indicates identical amino acid residues, conserved substitutions and semi-conserved substitutions in all sequences used in the alignment, respectively. B1 and B2 represent basic domains, A represents acidic domain, C represents charged cluster and G/A represents glycine alanine loop

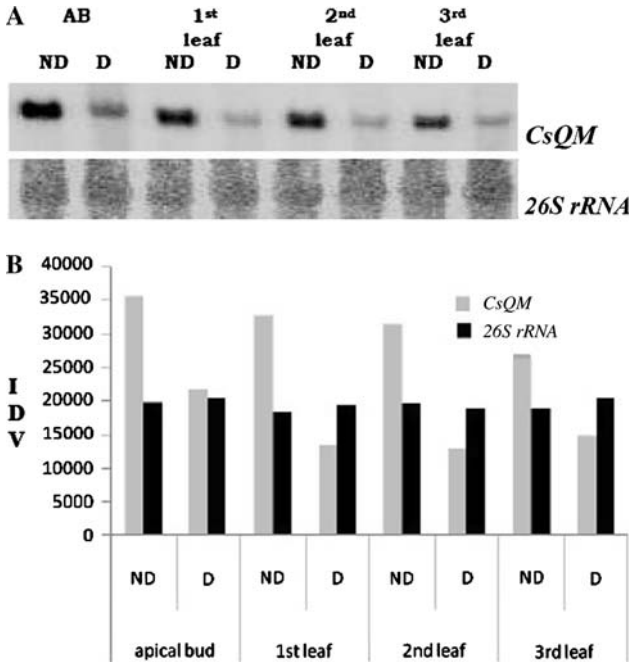

Fig. 3 (**A**) Changes in expression of *CsQM* at different stages of leaf development during active growth period and winter dormancy as revealed by northern hybridization. AB, apical bud; ND, non-dormant tissue; D, dormant tissue. 26S rRNA was used as a marker for equal loading. (**B**) Bar diagram showing integrated density values (IDV) of *CsQM* and *26S rRNA* expression of (**a**) as calculated by AD-1000 software supplied along with gel documentation system (Alpha DigiDoc™, Alpha Innotech, USA)

domain that contained a block of acidic and basic residues. The conserved acidic domain was present at positions 56–70. Glycine and alanine rich region was present at residues spanning 104–124 (Fig. 2). These domains are hallmark common to nucleotide binding proteins [7]. Theoretical pI of CsQM was found to be 10.54. Secondary structure prediction by SOPMA indicated the presence of 68 alpha helix, 41 extended strands, 18 beta turns and 89 random coils. Poly(A) signal "attaaa" [20, 21] was present at position 843–848 of *CsQM*.

Role of *QM* gene in control of cell growth and proliferation, perhaps as a tumor suppressor, and in energy metabolism has been extensively studied in human beings as well as in yeast [1, 5, 6, 22]. Although *QM* gene sequence has been reported from plant species but accessible information about the expression of *QM* like protein

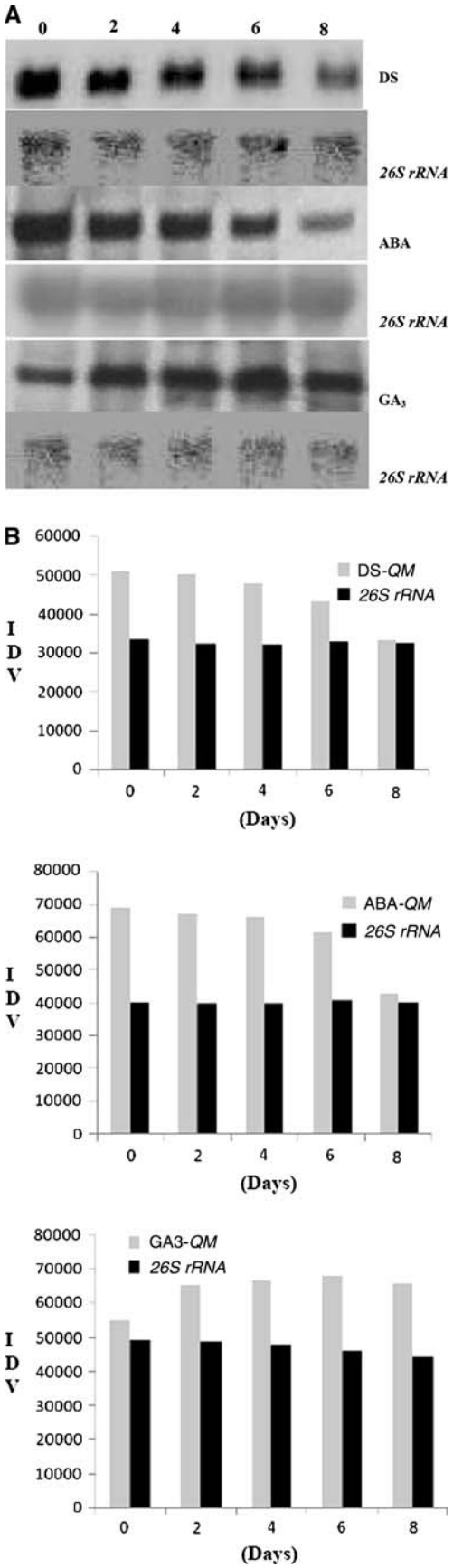

Fig. 4 (**A**) Effect of DS, ABA (100 μM) and GA_3 (50 μM) ▶ treatments on expression of *CsQM* in apical bud and first leaf combined as analyzed through northern hybridization. Numeral above lane indicates the day of the treatment. 26S rRNA was used as a marker for equal loading. (**B**) Bar diagram showing integrated density values (IDV) of *CsQM* and *26S rRNA* expression of (**a**) as calculated by AD-1000 software supplied along with gel documentation system (Alpha DigiDoc™, Alpha Innotech, USA)

genes in relation to the environmental cues and developmental stage is not available. Expression of *CsQM* was highest in the apical bud, which decreased with increase in the leaf age. As compared to the apical bud, the expression in 1st, 2nd and 3rd leaf was lower by 8, 13, and 25%, respectively. The expression reduced significantly in all the leaf positions during winter dormancy (Fig. 3).

There is limited information on physiological factors controlling expression of *QM* gene. External cue (2, 4, 6-trinitrotoluene) led to decrease in *QM* gene in *Chlamydomonas reinhardtii* [23]. The accumulation of transcripts varied significantly in response to DS, ABA and GA_3 treatments. Expression of *CsQM* was down-regulated in response to DS and ABA treatments, but up-regulated in response to GA_3 treatment (Fig. 4). During DS and ABA treatment, the expression was down-regulated by 35 and 38%, respectively on day 8th of treatment as compared to the day 0. However in GA_3 treated plants, expression was enhanced by 20% on day 8 as compared to day 0. Expression of *26S rRNA* gene was unaffected by all these treatments and served as a marker to depict the equal loading [18]. DS and ABA are known to down-regulate net photosynthesis in tea [13] and are also known to reduce growth, development and the cell division in general [13, 15]. It is likely that reduction in transcripts of *CsQM* might be the result of such a down-regulated environment. Plant hormone GA_3 is known to stimulate growth and development of plants [24, 25]. GA_3 mediated up-regulation of *QM* gene was reported in rice [9]. Although QM protein was first identified as putative tumor suppressor from the wilm's tumor cell lines [1], it is now known to have multiple roles like in transcription factor regulation, ribosome stability [2–4] and protecting the cells against oxidative damage [10]. The observed differential regulation of *CsQM* with developmental stage and the environmental cues suggested the gene to be involved with active growth of the cells and hence is envisaged to play an important role in plant growth and development.

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