Comprehensive Genome-Wide Analysis of The Catalase Enzyme Toolbox In Potato (*Solanum Tuberosum L.*)

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Research Article

Abstract

As antioxidant enzymes, catalase (CAT) protects organisms from oxidative stress *via the* production of reactive oxygen species (ROS). These enzymes play important roles in diverse biological processes. However, little is known about the *CAT* genes in potato plants despite its important economical rank of this crop in the world. Yet, abiotic and biotic stresses severely hinder growth and development of the plants which affects the production and quality of the crop. To define the possible roles of *CAT* genes under various stresses, a genome-wide analysis of *CAT* gene family has been performed in potato plant.

In this study, the *StCAT* gene's structure, secondary and 3D protein structure, physicochemical properties, synteny analysis, phylogenetic tree and also expression profiling under various developmental and environmental cues were predicted using bioinformatics tools. The expression analysis by RT-PCR was performed using commercial potato cultivar.

Three genes encoding *StCAT* that code for three proteins each of size 492 aa, interrupted by seven introns have been identified in potatoes. StCAT proteins were found to be localized in the peroxisome which is judged as the main H_2O_2 cell production site during different processes. Many regulating cis-elements related to stress responses and plant hormones signaling were found in the promoter sequence of each gene. The analysis of motifs and phylogenetic trees showed that *StCAT* are closer to their homologous in *S. lycopersicum* and share a 41% − 95% identity with other plants' CATs. Expression profiling revealed that *StCAT1* is the constitutively expressive member; while *StCAT2* and *StCAT3* are the stress-responsive members.

Key Message

The genome-wide analysis of the *catalase* genes in *S. tuberosum* provides new information that can help characterize the molecular mechanisms, and that *StCAT1* would be an interesting candidate for potato breeding technologies.

Introduction

The yield of a culture is influenced not only by genetic factors but also by the intervention of environmental factors that could significantly affect crops until more than 50% due to morphological and physiological alterations in plants (Tuteja 2007). Abiotic and biotic stresses negatively influence survival, biomass production and crop yield (Hirayama and Shinozaki 2010).

In a general view, stress leads to a secondary stress that is oxidative stress (Iqbal et al. 2014). The reactive oxygen species (ROS) are produced during aerobic cellular processes (Kim et al. 2005) leading to an imbalance between the production and the elimination of ROS that increases free radicals (Apel and Hirt 2004; Vellosillo et al. 2010). In fact, ROS are continually produced by different metabolic pathways at different cellular compartments especially the chloroplasts, mitochondria, peroxisomes and the endoplasmic reticulum. Indeed, about 1 to 2% of the oxygen consumed by the plant leads to the formation of ROS (Bhattacharjee 2005; Sharma et al. 2012; Bose et al. 2014).

At high concentrations, ROS accumulation causes damage to cells, whereas at low concentrations, they essentially serve as second messengers in intracellular signaling cascades of stress responses (Karuppanapandian et al. 2011; Bhattacharjee 2012).

Plants grown under optimal conditions can control the ROS content via antioxidant systems action that control the basic production of ROS resulting from normal aerobic metabolism.

Under various stress conditions, this redox balance is upset by excessive levels of ROS or depletion of antioxidant defense systems or both, leading to the collapse and cell death (necrosis) of plants (Karuppanapandian et al. 2011). When the level of ROS exceeds the defense capacity, the resulting oxidative stress status can cause damage to biomolecules such as DNA, proteins and lipids (Sharma et al. 2012).

The activation of a variety of enzymatic and non enzymatic antioxidants constitutes an important plant adaptation strategy to overcome oxidative stress (Parida and Das 2005; Noctor et al. 2012). Indeed, a wide range of enzymes is induced to minimize the effects of oxidative stress. These are Superoxide dismutase (SOD), catalase (CAT) and enzymes of the glutathione and ascorbate cycle.

Catalase action in plant and animal tissues was first observed in 1818 by Thenard, who noted that such tissues readily degraded hydrogen peroxide, a substance he had also discovered some years earlier (Aebi and Suter 1971).

Catalase, as an antioxidant enzyme, is found in practically all aerobic organisms. It dissolves hydrogen peroxide (H_2O_2) into water and oxygen in peroxisomes (Shangari and O'Brien 2006).

H_2O_2 symbolizes a two-electron reduction position of molecular oxygen and instigates primarily from the enzymatic dismutation catalyzed by SOD isoforms. Notwithstanding its small reactivity, H_2O_2 can simply spread across biological membranes and generate hydroxyl radicals (•OH), that can reply with biomolecules and cause damage (Novo and Parola 2008; Freire et al. 2017; Palma et al. 2020).

Catalases have been purified and structurally characterized from various microbial (Borges et al. 2014; Loewen et al. 2015) and animal sources (Gouet et al. 1995). However, limited understanding of catalases function from rice (Alam and Ghosh 2018), wheat (Matsumura et al. 2002), rapeseed (Raza et

al. 2021), cucumber (Hu et al. 2016), cotton (Wang et al. 2019b) and *Phyllanthus emblica* (Sharma and Hooda 2018) is known using structural and functional data.

A number of studies reported that the overexpression of genes encoding antioxidant enzymes such as catalases in plants improved their salt tolerance (M'Hamdi et al. 2009; Chaudhry et al. 2021), as well as other abiotic (Noctor et al. 2014; Su et al. 2018a; Alam and Ghosh 2018) and biotics stresses (Morkunas et al. 2013; Su et al. 2014).

Plant adaptation to abiotic stress might be positively correlated with *CAT* transcriptional activation in maize (Scandalios et al. 2000; Zang et al. 2018a), cucumber (Gao et al. 2009) olive (Cansev et al. 2011), broccoli (Lin et al. 2010), banana (Figueroa-Yáñez et al. 2011) and potatoes (Jbir-Koubaa et al. 2015, 2019).

Potato (*Solanum tuberosum* L.), originated from the Andean regions of Peru and Bolivia (Spooner et al. 2005), is actually grown worldwide as the third most consumed food crop plant after wheat and rice (FAO 2019). Potato is vulnerable to all kinds of biotic and abiotic stresses in its growth and development periods, often resulting in lower quality and yields of tubers (Handayani et al. 2019; Hill et al. 2020; Chourasia et al. 2021). Therefore, potato research involving mining and exploitation of its stress response genes has acquired a new urgency in enhancing production and breeding.

Considering the potential value of catalase in improving stress tolerance, we screened the annotation of *Solanum tuberosum* group phureja DM1-3 version 2.1.10 (http://potato.plantbiology.msu.edu/cgi-bin/gbrowse/potato/) and identified 3 CAT genes. Detailed analyses of catalase in potato including gene classification, multiple alignment, gene phylogeny, conserved motif composition and synteny analysis have been performed based on sequence similarity with their Arabidopsis and tomato counterparts. Moreover, the structural properties and functional components of potato catalase were examined using computational tools. Finally, PCR expression analysis was carried out for the potato catalase genes under salt stresses.

Our results from the genome-wide survey of potato catalase family offer a useful basis for further and targeted molecular analysis and functional characterization of potential catalase candidate genes.

Materials And Methods

1. Database search and identification of genes encoding catalase enzymes in potato

For the identification of putative genes encoding catalase enzymes in *Solanum tuberosum*, a BLASTp search was performed in the database of the potato genome (http://phytozome.jgi.doe.gov/pz/portal.html). The *Arabidopsis thaliana* proteins corresponding to these enzymes were used as queries collected from the database TAIR (https://www.arabidopsis.org/) to identify their counterparts in potato by BLAST keeping the sequence similarity cut off of >70% and E-value cut off of 0.001. To further verify the reliability of these candidate sequences, the Pfam database (http://pfam.sanger.ac.uk/search) was used to confirm the presence of conserved domains. All the confirmed potato antioxidant enzyme proteins were named as prefix "St" for *Solanum tuberosum* followed by sub-class identifiers (*StCAT*) and they were numbered progressively (*StCAT*1 to *StCAT3*).

2. Phylogenetic Relationships And Identification Of Conserved Protein Motifs

The protein sequences of catalase enzymes identified in potato (St) were aligned with those of Arabidopsis (At) and tomato (Sl) by using the Clustal W software (Larkin et al. 2007). Gene structure analysis was performed using Gene Structure Display Server GSDS 2.0 (http://gsds.cbi.pku. edu.cn/; Hu et al. 2015) by aligning the mRNA sequences with the corresponding genomic sequences. Phylogenetic and molecular evolutionary analysis of AtCAT, StCAT and SlCAT protein sequences were conducted by Jones-Taylor Thornton (JTT) and Neighbor-Joining (NJ) (bootstrap =1000). The evolutionary distance was counted using this method (Jones et al. 1992). To identify the structural divergence of CAT family, the conserved motifs in the encoded proteins were achieved with Multiple Expectation Maximization for Motif Elicitation (MEME version 4.10.2) (http://meme.sdsc.edu/meme/intro.html; Bailey et al. 2009). The evolutionary correlations between potato and other species of CAT proteins were studied through phylogenetic analyses using the NJ Tree method in MEGA6 and the JTT method (Tamura et al. 2013).

3. Chromosomal localization and synteny analysis of potato CATs

The information on the chromosomal localization of *StCAT*, *AtCAT* and *SlCAT* was retrieved from the Phytozome database. Syntenic blocks between potato, Arabidopsis and tomato genomes were downloaded from the Plant Genome Duplication Database (http://chibba.agtec.uga.edu/duplication/). Syntenic blocks were drawn using Circos Tool (Krzywinski et al. 2009).

4. In silico analysis of promoter sequences

The search for putative cis-regulatory elements in the putative promoter sequence of each *StCAT* genes (1000 bp upstream of ATG start codon) was performed by the PLACE software (http://www.dna.affrc.go.jp/PLACE/signalscan.html; Higo et al. 1999). Prevalence of motif signal was visualized as a word cloud at R core (v4.0.4).

5. Structural Features Characterization

The isoelectric point, the molecular weight and the composition of amino acids of potato CAT deduced proteins were calculated using the ProtParam tool (http://us.expasy.org/tools/protparam.html). The location of the subcellular compartment was performed using the TargetP software (http://www.psort.nibb.ac.jp/form.html). The identity and the similarity between these proteins were calculated with Sequence Identity And Similarity (SIAS) (http://imed.med.ucm.es/Tools/sias.html).

The secondary structure of the CAT proteins was predicted by NPS and GORIV Secondary Structure Prediction Method (Combet et al. 2000). The predicted 3D structures of StCATs were generated by using the PHYRE2 server (**P**rotein **H**omology/analog**Y** **R**ecognition **E**ngine Version 2.0) (http://www.sbg.bio.ic.ac.uk/~phyre2/html/page.cgi?id=index; Kelley et al. 2015). The FASTA sequences of the query proteins were entered and the intensive mode was selected to attain 3D models. Molecular graphics and analyses were performed with the PyMOL Molecular Graphics System, Version 2.0Schrodinger, LLC.

6. Clustering Analyses Of Transcriptomic Data

For analyzing the expression of potato catalase genes at different developmental stages, in varied organs and in response to biotic and abiotic stress conditions; expression values were retrieved from Spud DB: Potato Genomics Resource database (http://solanaceae.plantbiology.msu.edu/). Spud DB database provides the expression information of Locus ID's from *S. tuberosum* group phureja DM1-3 genome browser. Then, the expression values, expressed in FPKM (Fragments Per Kilobase of transcript per Million fragments mapped) were modified in logarithmic manner. Finally, the heat map was generated through the hierarchical clustering with Euclidean correlation coefficient distance measurement method using the software Multiple Array Viewer MeV v4.4.1 (Saeed et al. 2006).

7. Plant Materials And Stress Treatments

The study was conducted on a commercial potato cultivar. Potato plants cultivated *in vitro* were propagated in tubes containing 15 ml solid MS basal medium (Murashige and Skoog 1962) using 8 g/l agar supplemented with vitamins (Morel and Wetmore 1951) and 30 g/l sucrose. Plants were cultivated in a growth chamber (16 h light/8 dark, 25°C and 250 µE m−2s−1 light intensity per day). After 10 days of culture, six plantlets of each potato line were transferred to MS medium supplemented with 0 and 100 mM NaCl.

8. Expression Analyses By Semi-quantitative Rt-pcr

Total RNA was isolated from leaves and roots as described by Verwoerd et al. (1989). RNA concentration was determined by absorbance at 260nm. The primers for semi-quantitative RT-PCR analyses (Additional file 1) were designed with melting temperatures of 60° C using the Primer3 v.0.4.0 software. Semi-quantitative RT-PCR was carried out as described by Degenhardt et al. (2005).

Synthesis of the first strand cDNA was performed using the Access RT-PCR System kit (Promega) according to the supplier's recommendations using 2 µg of total RNA and 3 µl of oligo dT (10 mM). The reaction mixture of a final volume of 15 µl was incubated at 75°C for 10 min and then placed immediately on ice. Then were added 5 µl RT buffer (5x); 2 µl dNTPs 10 mM; 1 µl DTT 100 mM; 1 µl RNasin; 1 µl (200 U) AMV reverse transcriptase (Invitrogen). A first incubation at 42°C was executed for 60 minutes followed by a second incubation at 70°C for 10 min to stop the reaction. The following PCR amplification was performed in a final volume of 25µl. The reaction mixture contains the reaction buffer 5 x supplied with the enzyme; 2 mM MgCl2; 10 mM dNTP; 10 mM of each primer; 1U Taq DNA polymerase (Invitrogen) and 1 µl of cDNA. The amplification is carried out in a thermocycler device in 2700 (Applied Biosystems). The program used included initial denaturation of 5 min at 95 ° C, followed by 35 cycles of amplification (30 sec denaturation at 95°C, 1 min 30 sec annealing at 60°C, 1 min elongation at 72°C) and ends by an elongation 10 min at 72°C.

Results And Discussion

I. Genome-Wide identification of catalase genes family in the potato

1. Identification of genes coding catalase enzymes in the potato

To identify the potato genes encoding catalase antioxidant enzymes, we conducted a BlastP of the potato genome database using catalase genes of *Arabidopsis thaliana* as queries. We then used the Pfam and NCBI-CDD databases to verify the presence of conserved domains. Based on sequence homology, we confirmed the identification of 3 putative non-redundant genes encoding the different CAT enzymes (Table 1). All these genes consist of 8 exons. The full size of *StCAT* genes is ranging from 3471 to 5368 pb, encoding proteins of 492 aa. Still, their CDS and mRNA size showed the same profile (Figure 1).

2. Chromosomal localization and synteny analysis of catalase genes

Chromosomal positions of the different genes encoding catalase enzymes were determined from the database Phytozome. Their genes *StCAT* are distributed on three different potato chromosomes (Figure 2). The *StCAT2, StCAT3* and *StCAT1* are localized to chromosome II, IV and XII, respectively. They are phylogenetically distinct making it possible to conclude that they do not correspond to a duplicate.

To explore more the phylogenetic relationships within catalase gene family, a comparative synteny procedure was carried out wherein the physically mapped *StCATs* were compared with those of Arabidopsis and tomato on their respective chromosomes. A synteny block involving 3 potato, 3 Arabidopsis and 3 tomato CATs genes was generated (Figure 2). Almost all potato *StCAT* orthologs displayed the same synteny location with Arabidopsis or tomato.

3. Cis elements involved in transcriptional activity regulation

After having identified and selected a region of 1500 pb upstream the *StCATs* ATG for searching putative regulatory motif, several cis-acting regulatory elements associated with cellular growth, tissue-specific expression, transcription factors, hormonal and light regulation, abiotic and biotic stresses response elements were found (Table 2).

To explore, *in fine,* the dispersion of the cis-regulatory elements in the *StCATs* promoters, 'Word cloud' scheme was included (Figure 3) accentuating the most frequent motifs such as DOFCOREZM, TATABOX and CAATBOX. Overall, ten main motifs were checked counting the DOFCOREZM (DOF transcription factor), GT1CONSENSUS (Consensus GT-1 binding site in many light-regulated genes), CAAT box (CAAT promoter consensus sequence), ROOTMOTIFTAPOX1 (root expression motif), POLLEN1LELAT52 (regulatory elements responsible for pollen specific activation of tomato), GATABOX, ARR1AT (responsible for cytokinin expression), CACTFTPPCA1 (responsible for mesophyll expression), ARR1AT (responsible for cytokinin expression), CACTFTPPCA1 (responsible for mesophyll expression) and EBOXBNNAPA (responsible for seed expression) (Figure 3).

Various phytohormone responsive elements were also found in the *StCATs* putative promoter sequence, including ABA and cytokinin. The latter, being the most abundant with 4 to 8 duplications in each catalase gene (Table 2), implies that *StCAT* expression might be strongly induced and regulated by cytokinin in potato plants. In particular, the DOF (DOFCOREZM) motif known for enhancing transcription activity was the most abundant in all *StCAT* gene promoters. The Dof proteins are a family of plant specific transcription factors that includes Dof1, Dof2, Dof3, and PBF (Yanagisawa 2000). Maize Dof1 was suggested to be a regulator of the expression of the C4 photosynthetic phosphoenolpyruvate carboxylase (*C4PEPC*) gene (Yanagisawa 2004). Dof1 also enhances the transcription of the cytosolic orthophosphate dikinase (*cyPPDK*) genes and the non-photosynthetic *PEPC* gene (Yanagisawa 2000).

The putative recognition site for MYC, functioning as transcriptional activator upon dehydration, was identified in most *StCAT* genes with 1 to 6 copies in each. Other stress regulator motifs, such as salt-responsive element (Park 2004), light responsive elements (Terzaghi and Cashmore 2003), disease resistance response (Luo et al. 2005) and ABA responsive (Kaplan et al. 2006) were present as well in the *StCAT* promoters suggesting the probable implication of catalase family in response to these stresses in potato through ABA signaling pathway. However, no ABRE (ABA responsive elements) was detected within the *StCAT3* promoter, presuming other regulatory mechanisms than ABA responsiveness. Also, GATABOX is entailed for light-dependent and nitrate-dependent control of transcription in plants (Reyes et al. 2004). The GATA motif has been found in the promoter of the *Cab22* gene that encodes the *Petunia* chlorophyll a/b binding protein; this motif is the specific binding site of activating sequence factor-2 (ASF-2; (Lam and Chua 1989)

All of the herein mentioned putative cis-regulatory elements suggest that *StCAT* family members are implicated in varied cellular processes and they might reply to environmental stresses via different phytohormones signaling mediation.

II. Phylogenetic relationships and motif analysis of catalase potato enzymes

The analysis of phylogenetic tree elaborate from the catalase protein sequence from *S. tuberosum*, *A.thaliana* and *S. lycopersicum* (Figure 4) shows that all the StCAT proteins are closer to their homologous in S. *lycopersicum* (SlCAT) than those of Arabidopsis.

Furthermore, in order to identify conserved motifs and consensus domains constituting the CAT proteins, the online MEME Suite (v4.8.2) program was used (Figure 4a). The sequence details of each motif are shown in additional file 2. Analogous motifs were shared between the 3 members of StCAT, suggesting common conserved functions inside the catalase family.

In addition, a phylogenetic tree of 39 protein sequences including several CATs from different origins showed two main groups that seem to be associated according to their family, species, and systematic method (Figure 4b).

III. Structural Characterization of StCAT proteins

1. Primary Structure

The size of the identified StCAT proteins had the same size of 492 amino acids (aa), and the corresponding predicted molecular masses ranged from 44,9 to 59,9 kDa, (Table 1). The computed PI of these proteins was ~6.54 on average, indicating that they are likely to precipitate in either acidic or basic buffers and can be maintained within a neutral buffer.

The amino acid sequence analyses showed that the protein sequence of CAT from *S. tuberosum*, *A.thaliana* and S. *lycopersicum* exhibit a high level of identity between each other (Additional file 3). Similarity percentages within members of the CAT family ranged between 82.55 and 100 %, whereas the identity percentages varied from 75.25 to 99.39%. StCAT2 showed the highest similarity and identity level (100 and 99.39% respectively) with SlCAT2 of

S.lycopersium. The lowest similarity and identity percentages were observed between SlCAT1 and AtCAT3, the values being 82.55 and 75.5% respectively.

Main represented amino acids (Figure 5) of the StCAT members are proline (7.3%), arginine (7.1%), aspartate (6.9%), leucine (7.7%), valine (6.5%) and alanine (6.1%). The least common amino acids residues were cysteine, methionine and tryptophan which accounted for ~1% of the protein's primary structure. The low amounts of cysteine residues indicated that the chance of disulfide bond formation is low. Leucine, alanine and valine are hydrophobic, aliphatic and non polar amino acids and are thus expected to be found inside the protein or within lipidic membranes.

2. Secondary and tertiary Structure

The secondary structure features as predicted by NPS and GORIV Secondary Structure Prediction Method (Combet et al. 2000) shows that random coils (50%) dominated among secondary structure elements followed by the alpha helix (25%) and extended strand (15%). The predominance of coils points to the fact that catalase from *S. tuberosum* might not be a very stable enzyme (Perticaroli et al. 2014).

The homology search of the tertiary structure of StCAT was predicted based on template-based modeling by PHYRE2 (Kelley et al. 2015). Six templates for each StCAT were chosen based on heuristics to maximise confidence, percentage identity and alignment coverage (Table 3). The threading templates were selected by the PHYRE2 server from the PDB database on the basis of normalized Z-score of >1.0.

Furthermore, Clustal Omega (1.2.4) was used for multiple sequence alignment and active site identification. An alignment of the translated CDS of *S. tuberosum* catalase with the selected template catalases is shown in additional file 4. Conserved residues of the catalase sequence involved in the H_2O_2 binding (V2, H3, V44, D56, N76, F81, F82, F89) were identified after carefully studying the alignment. The results were found to be consistent with the experimentally determined crystallographic structures of human erythrocyte catalase (1QQW) (Putnam et al, 2006). However, few substitutions such as of isoleucine (I) by alanine (A), of methionine (M) by phenylalanine (F), of valine (V) by isoleucine (I) and of glutamine (Q) by leucine (L) were also observed in the proteins of StCAT (Additional file 4).

The quality of the 3D model was assessed on the basis of the confidence score. The validated model using various programs such as Ramachandran plot and energy plot confirmed the reliability of the model. All the parameters for validation were within the range showing the compatibility of the model with its sequence and depicting the excellent quality model (Figure 6A).

Using the Pymol software, the residues involved in the hydrogen peroxide binding are conserved among the 3 StCATs confirming the key role in hydrogen peroxide binding as identified by Prosite-ProRule annotation.

The Figure 6B shows the ligand (H_2O_2) sits in the area lined by the predicted active site residues. Since, the catalytic site is actively involved in charge transfer reactions required for formation and degradation of bonds, so it is expected to have high electron density (Vivekanand and Balakrishnan 2009).

IV. Study of the expression profile of genes encoding catalase enzymes *in silico*

1. Tissue- specific expression analysis of potato catalase genes

To check the role of the different potato CAT family members, the gene expression was analyzed, first, within different organs and tissues at various developmental stages of the *S. tuberosum* phureja variety using microarray data available in Spud DB database (Figure 7). The expression data of 3 *StCAT* genes were available and retrieved for analysis. Fluorescence intensity values were analyzed to generate a clustered heat map based on the average Euclidian's distance. As shown in figure 7, StCAT family members exhibited spatial variations in transcript abundance, with high levels of transcript abundance in one or some tissues and low transcript abundance in others.

2. Expression analysis of *StCAT* genes under different abiotic stresses, biotic stress elicitors and hormonal treatments

The expression of *StCAT* genes was analyzed in response to a variety of stress agents, to check their specific functions. To evaluate the effect of stress on *StCAT* gene expression, various abiotic stresses such as salinity, drought, wounding and heat were evaluated on potato plants (Figure 8a). *StCAT1* showed up-regulation in all stress conditions, while *StCAT2* showed down-regulation in response to salt and mannitol stress. The *StCAT3* showed a completely opposite pattern of expression in comparison to *StCAT1*. Indeed, it was down regulated in response to almost all stresses.

Then, transcript abundance was analyzed in response to three biotic stress elicitors such as benzothiadiazole (BTH) and β-aminobutyric acid (BABA) and pathogen attack (Figure 8b). The transcript abundance of all three *StCAT* was found to be modified in response to the pathogen infection. *StCAT1* seems to be up-regulated in response to all biotic stress. *StCAT1* showed the highest activation levels in response to all hormonal treatments except for BAP However, the *StCAT2* is down regulated only in response to BABA elicitor. *StCAT3* showed down regulation in response to almost all the applied treatment stresses (Figure 8c).

V. Functional study of genes encoding catalase in the of potato variety Nicola

The expression profile of the 3 *StCAT* genes was investigated by semi-quantitative RT-PCR of RNA from the potato Nicola cultivar cultivated in the presence or in the absence of 100mM NaCl. Oligo-nucleotides were identified and used as primers in RT-PCR reactions allowing the amplification of an

internal region of each gene (Additional file 1). The RT-PCR results showed that salt stress caused activation of the expression of *StCAT1* gene in plant leaves (Figure 9). However, the expression of *StCAT2* and *StCAT3* genes seems to be not affected by salt stress. In conclusion, the investigation of *StCAT* gene expression in the leaves of Nicola potato was consistent with transcriptome data.

Discussion

An overload amount of ROS is engendered when plants are exposed to adverse environmental conditions (Kwak et al. 2006; Caverzan et al. 2016). To neutralize ROS excess, plants can activate several types of machinery. As one of the main antioxidant enzymes, the catalase (EC 1.11.1.6) serves as an efficient scavenger of ROS and can catalyze the dismutation of two molecules of hydrogen peroxide (H_2O_2) into oxygen (O_2) and water. Considering its significant role, a genome-wide analysis was performed in potato to identify and characterize the catalase gene family and to check their expression profile in response to various developmental and environmental clues using computational tools.

Previously, genome-wide studies have demonstrated that *CAT* is encoded by a small gene family which has been studied extensively in different plant species (Mhamdi et al. 2012) including *Nicotiana plumbaginifolia* (3 genes; Willekens et al. 1994); *Hordeum vulgare* (2 genes; Skadsen et al. 1995), *Zea mays* (3 genes) (Polidoros and Scandalios 1999), *Arabidopsis thaliana* (3 genes; Frugoli et al. 1998; Du et al. 2008), *Solanum lycopersicum* (3 genes; Drory and Woodson 1992), *Capsicum annuum* (3 genes; Lee and An 2005), *Oryza sativa* (4 genes; Lin et al. 2012; Alam and Ghosh 2018), *Saccharum officinarum* (2 genes; Liu et al. 2015) and *Cucumis sativus* (2 genes; Hu et al. 2016). Up now, very few works has been done on Solanaceae family members, in spite of covering whole genome sequence

The current study showed that potato plant contains three *CAT* genes (*StCAT1-StCAT3*). The analysis of corresponding protein revealed that both tomato and Arabidopsis possess a similar number of catalase proteins (three each) through alternative splicing (Table 1; Figure 1). The presence of multiple catalase genes suggested multiple functions for catalases in a variety of plant tissues at various developmental stages and under constantly changing environments from which plants can not readily escape.

The potato catalase genes are located on three different chromosomes like those of tomato, while *AtCAT1* and *AtCAT3* reside in the same chromosome for Arabidopsis (Figure 2). The orthologous gene pairs followed phylogenetic clustering in a similar class. As well, the synteny analysis suggests the conservation in loci of *StCAT* genes. In recent studies, the synteny and phylogeny analysis showed the conservation of *CAT* genes in bread wheat and cotton suggesting the role of polyploidy in the expansion of catalase gene family (Wang et al. 2019b; Tyagi et al. 2021).

The sequence data shows that each of the three *StCAT* genes are interrupted by seven introns. The numbers of introns of catalase genes, and their positions, were found to be extremely conserved among 13 monocot and dicot plants. This suggests that they come from an ancestral catalase gene common to monocots and dicots containing seven introns (Frugoli et al. 1998). Although At*CAT2* has seven introns; both At*CAT1* and At*CAT3* have six introns in positions conserved with *their homologue*, but each has lost a different intron (Figure 1). The presence of large introns in *StCAT* transcripts could improve the recombination frequency as well as maintain the counterbalance of mutation bias (Alam and Ghosh 2018). The putative promoter regions of the three *StCAT* genes don't share any sequence identity, possibly explaining the differential regulation observed for each of these genes. The exploration of up-stream promoter region of *StCAT* genes revealed the occurrence of numerous *cis*-acting elements which were broadly categorized into (i) light-responsive such as IBOX, GT1CONSENSUS, SORLIP1AT, etc (ii) stress-responsive such as ARE, LTRECOREATCOR, GT1GMSCAM4,CAATBOX, etc (iii) hormone-responsive such as ARR1AT, ABRELATERD1, ACGTATERD1, etc (iv) transcription factor binding sites such as DOFCOREZM, MYBCORE, WBOXATNPR1, etc (Figure 3). The existence of similar *cis* acting elements has been accounted in *CATs* of other plant species such as *A. thaliana* and *O.sativa* (Alam and Ghosh 2018), as well as in other antioxidant gene family of *S. tuberosum,* for instance, g*lutathione S transferase* (*StGST*) (Islam et al. 2018).

The analysis of conserved motifs and phylogenetic tree elaborated from the protein sequence of catalase from *S. tuberosum*, *A.thaliana* and *S. lycopersicum* (Figure 4) shows that all the *StCAT* are closer to their homologous in *S. lycopersicum* (*SlCAT*) than those of Arabidopsis. Also, they possess significant homology to other known plant CATs (Zang et al. 2018b). Previously, Sheoran and collaborators (Sheoran et al. 2013) reported that most of the dicot and monocot catalases are closely related with each other. This clearly revealed the divergence between dicot and monocot of catalase genes during the process of evolution. Similarly, this pattern of evolution was shown in case of glutathione peroxidase proteins (Wang et al. 2019a).

Based on the subcellular localization, the *StCAT* are predicted in peroxisome. Considering the prominent role played by catalase in the H_2O_2 metabolism, peroxisomes are judged as the main cell production for the H_2O_2 during different process (Mhamdi et al. 2012; Corpas et al. 2019). This tensed link between catalase and peroxisomes, supported by the composition of the polypeptide C-terminus sequence and the diverse number of methods used to localize this enzyme within the cell, has led the scientific community to consider catalase as the typical marker enzyme for these organelles (Palma et al. 2020).

The StCAT polypeptides have the same size of 492 aa with the average MW of ~52 kDa. Also, an average pI of 6.54 was predicted for these polypeptides. The comparable physicochemical features of catalases were reported in other plant species, for occurrence, a range of 377−492 aa and 44−56 kDa in *A.thaliana*, 285−493 aa and 32−56 kDa *in O. sativa*, 409−494 aa and 47−57 kDa in *G. hirsutum*, 459 to 601 aa and 53−67 kDa in *G. barbadense*, 458−744 aa and 53−82 kDa in *Cucumis sativus* L., etc., The length of 492 aa and 56.9 kDa is the most frequent (Hu et al. 2016; Alam and Ghosh 2018; Wang et al. 2019b).

Each of the potato catalase isozymes is structurally similar to those found in other organisms (Chandlee et al. 1983). All the catalase have a conserved residue (catalase domain) involved in the H_2O_2 binding (Alam and Ghosh 2018; Wang et al. 2019a). Hence, the 3D model constitutes a perfect active site, which attracts the H_2O_2 ligand (Additional file 5; Figure 6). Based on structural homology modelling, StCATs showed the common best PDB hit with the catalase from *E.coli* (c1p81A) and predicted ~13 α-helices (including both long and short helices) and two 4-stranded antiparallel β-sheets (Table 4; Figure 6). These results were found to be consistent with the experimentally determined crystallographic structures of *Deinococcus radiodurans* catalase (4CAB) (Borges et al. 2014).

Assumed from the prediction tissue-specific expression, the *StCAT* genes are expressed in different tissues such as the leaves, the roots and the flowers. The strongest expression was observed for *StCAT1* particularly in the tubers and leaves, whereas *StCAT3* had the lowest expression in all tissues (Figure 7). In the model plant *Arabidopsis thaliana*, *AtCAT3* showed expression in both leaves and vascular tissues, while *AtCAT1* demonstrated a spectacular change in its expression profile during floral organ and seed development and *AtCAT2* is related with photosynthetic tissues and also roots (Mhamdi et al. 2012; Su et al. 2018b). Further, three catalase genes of maize illustrated diverse tissue specificity, while *ZmCAT1* and *ZmCAT3* proved kernel specific expression, *ZmCAT2* expressed after kernel development (Wadsworth and Scandalios 1989; Guan and Scandalios 1993; Guan et al. 1996).

Besides, the expression analysis of *StCAT* genes in different biotic and abiotic stresses of potato demonstrated that *StCAT1* is the highly expressive member, followed by *StCAT2* and *StCAT3* suggesting that these genes are likely able to respond to several developmental stimuli. Consistent with the regulator motifs found in promoters of *StCAT1* gene, the expressional analyses based on transcriptome data studies and semi quantitative RT-PCR data analysis revealed constitutive and ubiquitous expression profile in response to abiotic, biotic, and hormonal stimuli and at different development stages meaning that this gene play various roles in potato developmental stages and adaptation behaviors. In advance, Jbir et al. (2018) have shown that *StCAT1* and *StCAT2* genes were the most upregulated in potato somatic hybrid lines under salt stress conditions. Similarly, the orthologue of *StCAT1*, *AtCAT3* was the highly expressive member. Besides, *AtCAT2* and *AtCAT3* display opposite circadian profiles, while *AtCAT1* scarcely varied under the circadian clock (Li et al. 2015, 2017; Su et al. 2018b). Chen et al. (2012) concluded that the expression of sweet potato catalase *SPCAT1* was in cope with H_2O_2 homeostasis in natural. *ZmCAT* was a member of the plant CAT family in *Zostera marina* and implicated in minimizing oxidative damage effects under temperature stress (Zang et al. 2018a).

The overexpression of *KatE* gene coding for *Escherichia coli* affects the multiplication rate of the *in vitro* potato plants, as well as vegetative and physiological growth parameters under salt stress conditions (M'Hamdi et al. 2009). Besides, the overexpression of *IbCAT2*, encoding gene from sweet potato cv. Xushu 18, conferred salt and drought tolerance in *Escherichia coli* and *Saccharomyces cerevisiae* (Yong et al. 2017).

These results confirm the role of these enzymes in plant tolerance to salinity. Indeed, several studies have demonstrated that the overexpression of genes encoding antioxidant enzymes in plants improved their tolerance to salt (M'Hamdi et al. 2009; Su et al. 2014; Yong et al. 2017), to abiotic (Rasoulnia et al. 2011; Zhang and Shi 2013; Noctor et al. 2014) and biotic stresses (Morkunas et al. 2013).

Conclusion

In a gist, this study offers a genome-wide annotation and nomenclature of potato *CAT* gene family as well as gene structure, the secondary and the 3D protein structure, physicochemical properties and the phylogenetic tree. The expression profiling at different developmental stage-specific, abiotic and biotic stress-responsive and hormone-inducible *CAT* genes expression was studied. Furthermore, the expression analysis effectuated with a semi-quantitative RT-PCR was carried out. These results present a novel road and starting point to distinguish the functions of CAT family members in *S.tuberosum*. Successively, this study will not only develop our knowledge on the mechanisms of stress tolerance of plants, but also will help to raise transgenic crop plants with better yield potential and stress tolerance potential.

Declarations

Compliance with Ethical Standards

Conflict of Interest: The authors declare that they have no conflict of interest

Availability of data

The datasets supporting the conclusions of this article are included within the article and its additional files.

Author Contributions: RJK, MA, and MNS designed the study. RJK, MA, SC and RGB performed bioinformatics and data analyses. RJK, MNS and ONE performed the experiments. RJK, MA, and RGB analyzed the data and wrote the manuscript. All authors read and approved the final manuscript.

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Tables

Table 1: List of different genes encoding catalase antioxidant enzymes identified in *S. tuberosum*

Identifier	Accession (phytozome)	Gene size (bp)	Chromosome, position (bp) and sens (+/-)	Exon numbers	mRNA/CDS (bp)	Protein			Predicted Subcellular localization
						Size (aa)	MW (d)	PI	
StCAT1	PGSC0003DMG400029408	3471	ch12: 57454380.....57457850 (+)	8	1479/ 1479	492	44925,6	5,97	Peroxisomal
StCAT2	PGSC0003DMG400001570	3987	ch02: 38568112.....38572098 (+)	8	1479/ 1479	492	56935,2	6,73	Peroxisomal
StCAT3	PGSC0003DMG400009906	5368	ch04: 71630248.....71635615 (+)	8	1479/ 1479	492	56813,4	6,93	Peroxisomal

Table 2: Cis-Elements Involved in Transcriptional Activity Regulation

| Function/responsive	Putative cis-element/consensus	Number of cis elements		
		StCAT1	StCAT2	StCAT3
Tissue expression specific to the roots	OSE1ROOTNODULE	2	0	0
	OSE2ROOTNODULE	4	3	2
	ROOTMOTIFTAPOX1	14	9	3
Specific expression of pollen	POLLEN1LELAT52	8	7	8
	QELEMENTZMZM13	0	1	0
Specific expression of mesophyll	CACTFTPPCA1	0	14	4
Specific expression of leaf	CIACADIANLELHC	0	1	0
	GATABOX	9	8	5
Early responsive elements in dehydration	ABRELATERD1	1	1	0
	ACGTATERD1	6	4	0
Light-responsive elements	GT1CONSENSUS	18	9	9
	SORLIP1AT	1	0	0
	SORLIP2AT	0	0	1
	IBOX	5	0	5
	IBOXCORE	5	0	4
	IBOXCORENT	0	0	1
	INRNTPSADB	5	4	2
Heat-responsive element	CAATBOX1	10	17	6
	CCAATBOX1	0	2	2
Low temperature responsive element	LTRECOREATCOR15	0	0	1
Pathogen and salt- induced gene	GT1GMSCAM4	6	5	5
Responsible for cytokinin expression	ARR1AT	8	7	4
Transcription factor binding sites	DOFCOREZM	18	18	11
	MYBCORE	1	1	6
	MYBCOREATCYCB1	0	1	2
	WBOXPCWRKY1	1	3	0
	WBOXATNPR1	2	5	0
	WBOXHVISO1	1	4	1
	RAV1AAT	2	0	1

Table 3: Details of secondary structure of StCATs proteins according to the NPS secondary structure prediction method (GORIV & SOPMA).

Protein	Prediction	Alpha helix	Extended strand	Random coil
StCAT1	GORIV	17,89	24,19	57,93
	SOPMA	30,28	15,45	48,78
StCAT2	GORIV	17,48	20,53	61,99
	SOPMA	27,64	15,65	50,41
StCAT3	GORIV	25,61	16,06	58,33
	SOPMA	28,25	14,23	52,64

**Table 4:** Details of tertiary structure of StCATs proteins according to the Protein data bank (PDB)

	Description	Confidence	% Identity	Model (pdb code)	Species
StCAT1	PDB header: oxidoreductase Chain: A PDB Molecule: catalase hpii;	100%	43%	c1p81A_ (1p81A)	*E. coli*
	Fold: Heme-dependent catalase-like Superfamily: Heme-dependent catalase-like	100%	43%	d1p80a2 (1p80)	*E. coli*
	PDB header: oxidoreductase Chain: D: PDB Molecule: catalase-3;	100%	40%	c3ej6D_ (3EJ6)	*Neurospora crassa*
	PDB header: oxidoreductase Chain: B: PDB Molecule: catalase 1;	100%	43%	c1sy7B_ (1SY7)	*Neurospora crassa*
	Fold: Heme-dependent catalase-like Superfamily: Heme-dependent catalase-like	100%	47%	d1m7sa_ (1m7s)	*Pseudomonas syringae*
	PDB header: oxidoreductase Chain: D: PDB Molecule: catalase	100%	47%	c2j2mD_	*Exiguobacterium oxidotolerans*
StCAT2	PDB header: oxidoreductase Chain: A PDB Molecule: catalase hpii;	100%	43%	c1p81A_ (1p81A)	*E. coli*
	Fold: Heme-dependent catalase-like Superfamily: Heme-dependent catalase-like	100%	43%	d1p80a2 (1p80)	*E. coli*
	PDB header: oxidoreductase Chain: D: PDB Molecule: catalase-3;	100%	40%	c3ej6D_ (3EJ6)	*Neurospora crassa*
	PDB header: oxidoreductase Chain: B: PDB Molecule: catalase 1;	100%	42%	c1sy7B_ (1SY7)	*Neurospora crassa*
	Fold: Heme-dependent catalase-like Superfamily: Heme-dependent catalase-like	100%	47%	d1m7sa_ (1m7s)	*Pseudomonas syringae*
	PDB header: oxidoreductase Chain: D: PDB Molecule: catalase	100%	47%	c4qoqC_	Bacillus pumilus
StCAT3	PDB header: oxidoreductase Chain: A PDB Molecule: catalase hpii;	100%	43%	c1p81A_ (1p81A)	*E. coli*
	Fold: Heme-dependent catalase-like Superfamily: Heme-dependent catalase-like	100%	43%	d1p80a2 (1p80)	*E. coli*
	PDB header: oxidoreductase Chain: D: PDB Molecule: catalase-3;	100%	41%	c3ej6D_ (3EJ6)	*Neurospora crassa*
	PDB header: oxidoreductase Chain: B: PDB Molecule: catalase 1;	100%	43%	c1sy7B_ (1SY7)	*Neurospora crassa*
	Fold: Heme-dependent catalase-like Superfamily: Heme-dependent catalase-like	100%	49%	c2j2mD_ 2J2M)	*Exiguobacterium oxidotolerans*
	PDB header: oxidoreductase Chain: D: PDB Molecule: catalase	100%	51%	c4qoqC_	*Bacillus pumilus*

Figures
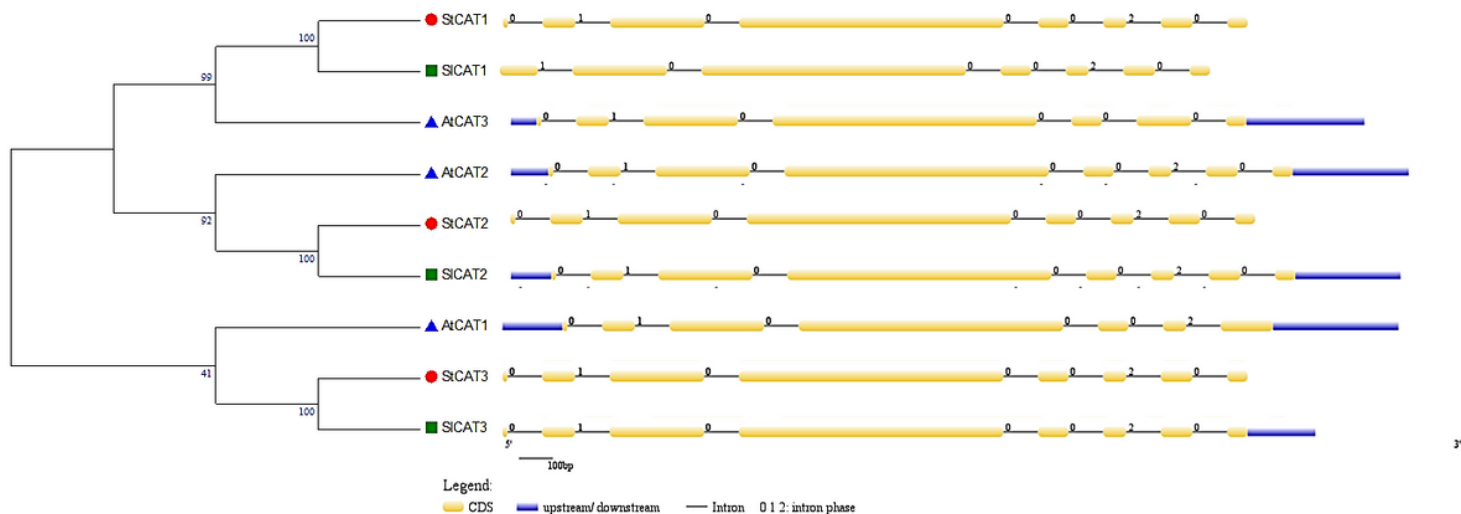
Figure 1

Genomic organization and phylogenetic tree of catalase gene of potato (St) with those of Arabidopsis thaliana (At) and tomato (Sl). The tree was constructed using the neighbor-joining algorithm of MEGA 6 (Tamura et al. 2013). The bootstrap consensus tree was inferred from 1000 replicates. Scale bar corresponds to 0.1 nucleic acid substitution per residue. The diagrams of exon−intron structures were obtained using the database Gene Structure Display Server (GSDS). Exons and introns are presented by green boxes and black lines, untranslated regions (UTRs) are indicated by blue box, respectively.

Figure 2
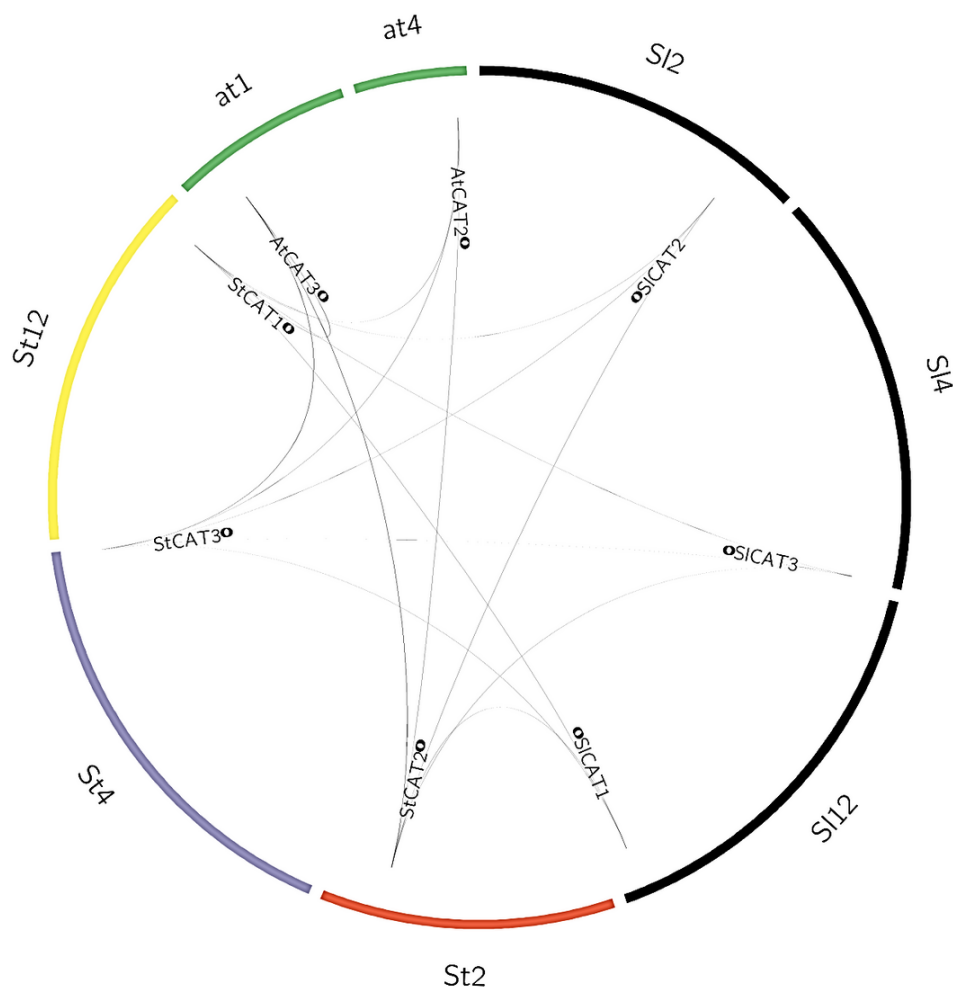
Syntenic relationships between StCAT, SlCAT and AtCAT genes. Comparative physical mapping by Circos software showing the degree of orthologous relationships of CAT gene located on chromosomes of Solanum tuberosum (St), Solanum lycopersium (Sl) and Arabidopsis thaliana (Ath). The chromosomes of S. tuberosum, A.thaliana and S. lycopersicum are depicted as a circle. Colored curves denote the details of syntenic regions between StCAT, SlCAT and AtCAT genes

Figure 3

Word cloud showing the identified cis-elements in StCATs promoter regions. The intensity and size of the motifs indicate their frequency
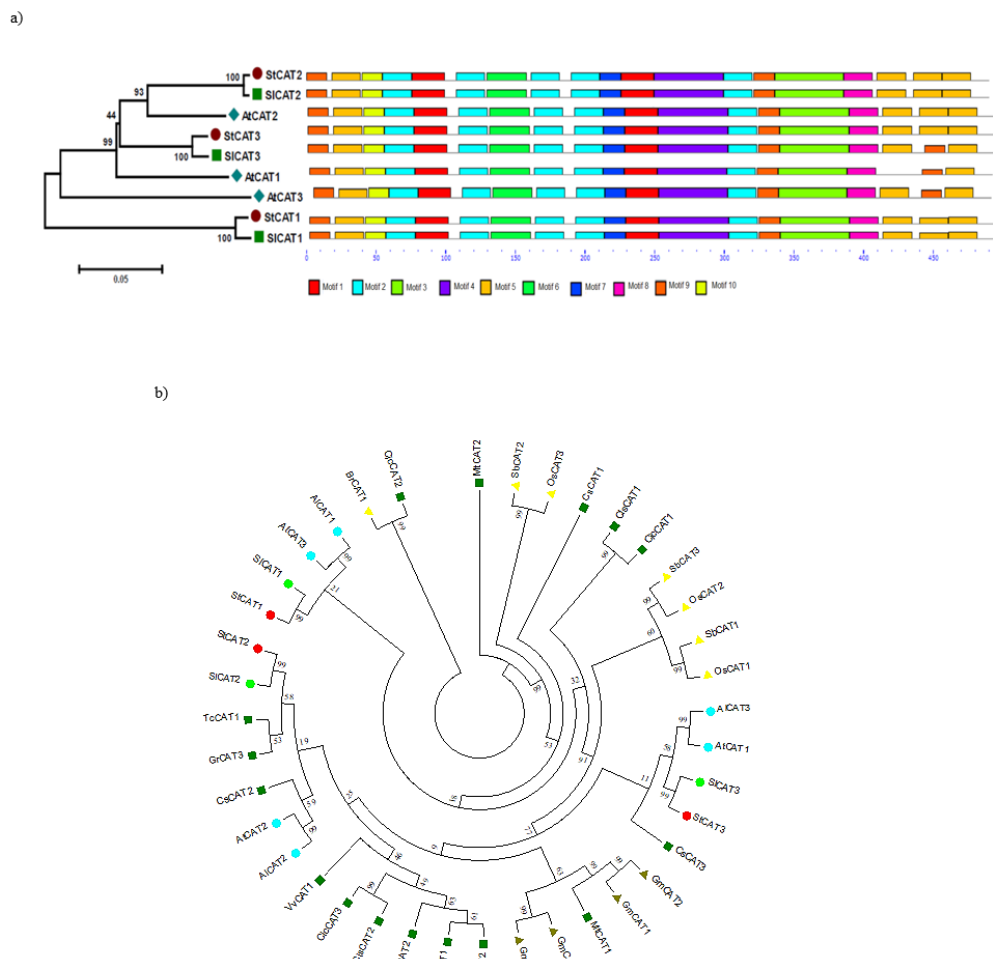
Figure 4

Phylogeny analysis of StCAT proteins a) Phylogeny and schematic representation of the 10 conserved motifs in catalase proteins of potato (St) with those of Arabidopsis thaliana (At) and tomato (Sl). The tree was constructed using the neighbor-joining algorithm of MEGA 6 (Tamura et al. 2013). The bootstrap consensus tree was inferred from 1000 replicates. Scale bar corresponds to 0.1 amino acid substitution per residue. Motif analysis was performed using Meme 4.0 software as described in the Methods. Different motifs, numbered 1–10, are displayed in different colored boxes. A detailed motif introduction for NHXs proteins is shown in additional file 2. b) Unrooted phylogenetic tree representing relationships among CAT of potato and other species. The phylogenetic tree was drawn with MEGA 6 for evolutionary analysis of xx protein sequences (Tamura et al. 2013). The amino acid sequences of all catalase proteins were obtained from Phytozome database. The abbreviations of CAT proteins are shown in additional file 3.
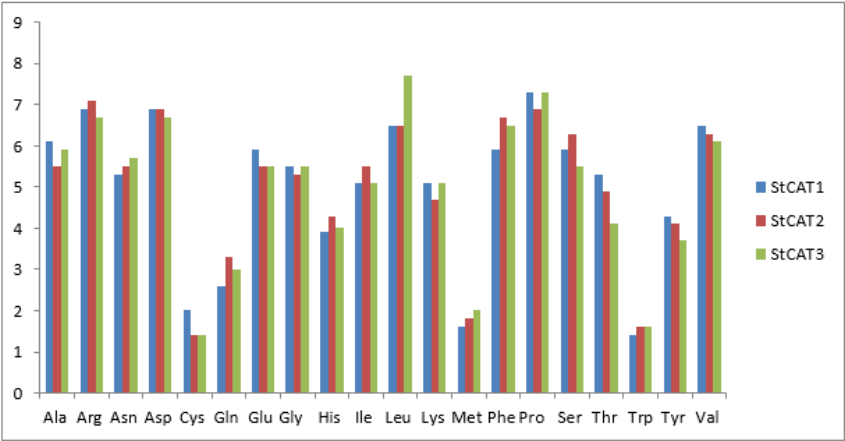
Figure 5

Amino acid composition of StCATs proteins
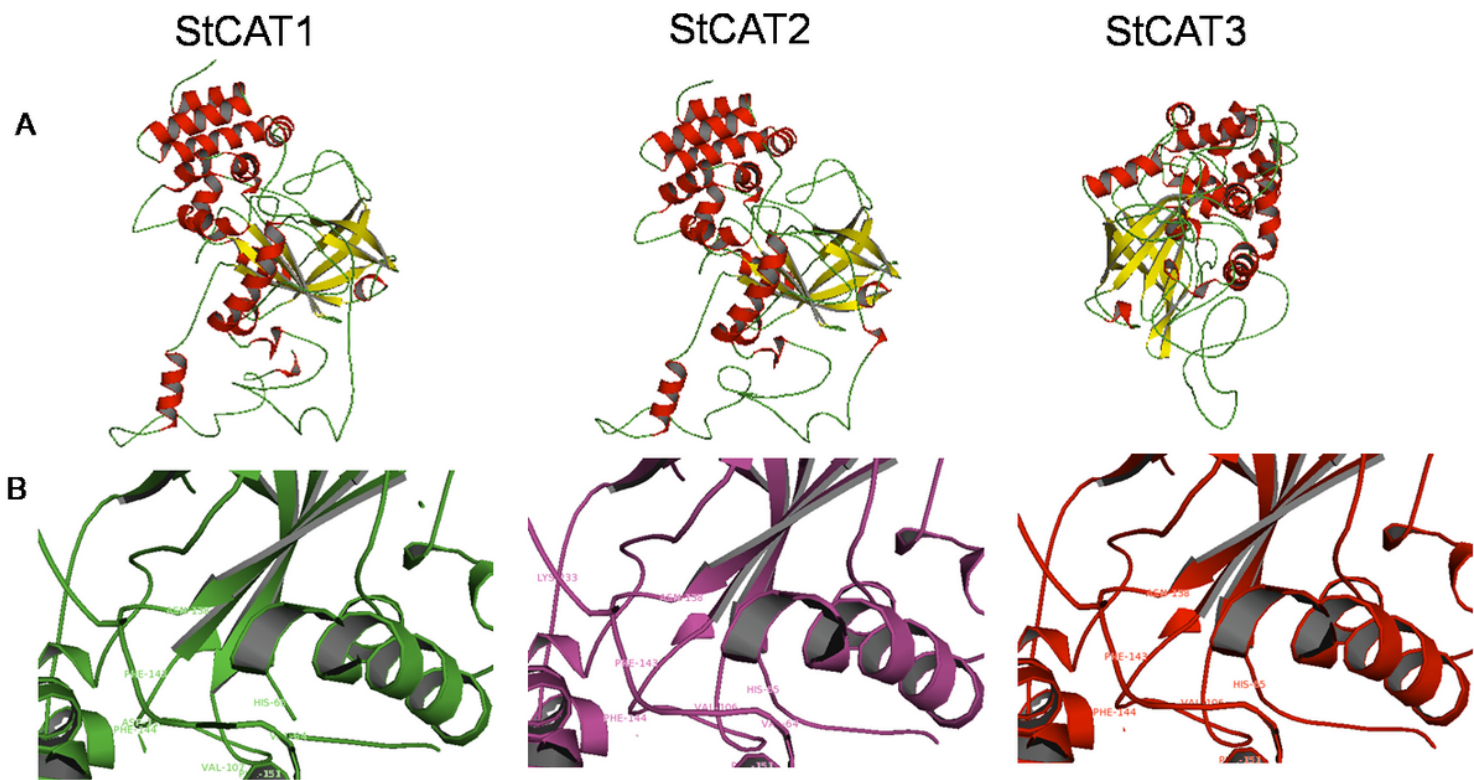
StCAT1 StCAT2 StCAT3

A

B

Figure 6

Ribbon representation of the predicted model of StCAT proteins (A) Ribbon representation of each S. tuberosum catalase protein is modeled by Phyre2 software. α-helices, β sheets and coils are colored respectively in red, yellow and green (B) 3D model of each StCAT show the labeled residues for H2O2 binding (V2, H3, V44, D56, N76, F81, F82, F89). All molecular graphics were performed with the PyMOL Molecular Graphics System.
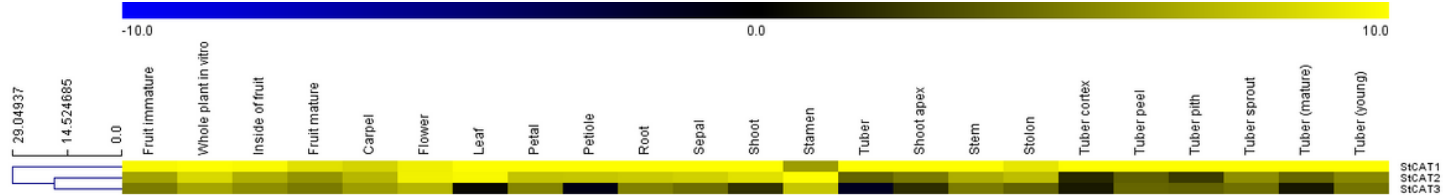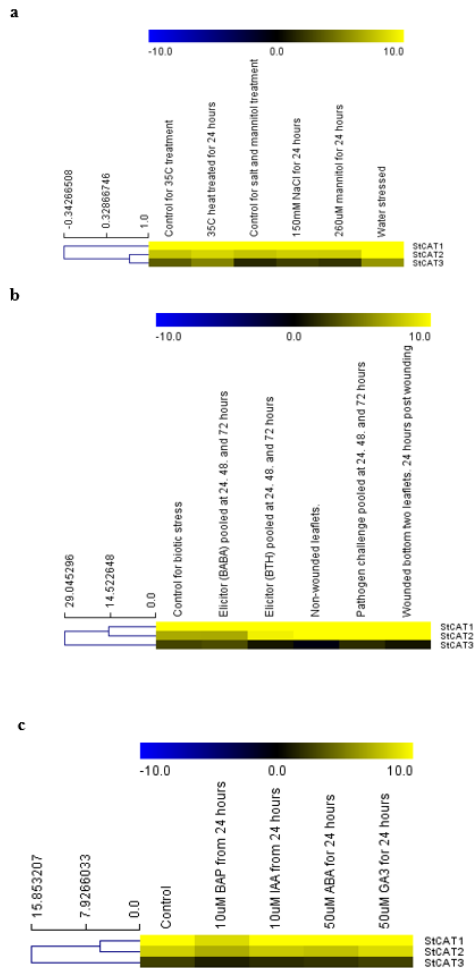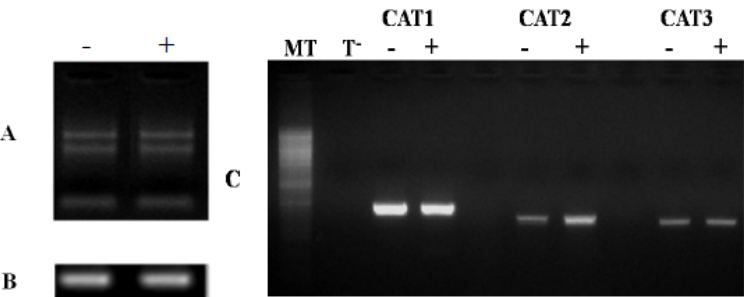

Figure 7

Clustering analyses of transcriptomic data. Expression profiling of StCATs transcripts at various anatomical tissues such as root, shoot, leaf, petiole, flower, stamen, carpel, petal, sepal, tuber, and fruit. Expression data was retrieved from Spud DB: Potato Genomics Resource database (http://solanaceae.plantbiology.msu.edu/) for S.tuberosum group phureja DM1-3. The log2 transformed data was used to generate the heatmap with hierarchical clustering of Euclidean distance correlation using MeV software package. A color scale is provided along with the heat map to recognize the differential pattern of expression. Yellow color indicates the high level of expression, black signifies medium, and blue denotes the low level of expression.

Figure 8

Analysis of StCAT genes expression against various abiotic stresses, biotic stress elicitors and hormonal treatments. Expression of the same StCAT was analyzed in response to various biotic stress elicitors (a), abiotic stresses (b) and hormone treatments (c). Expression data of S.tuberosum group phureja DM1-3 was retrieved for three biotic stress elicitors such as BABA, BTH and pathogen attack (a); four abiotic stresses such as salinity, drought, wound, and heat (b) and four plant hormonal treatments such as 6-benzylaminopurine (BAP), indole-3-acetic acid (IAA), abscisic acid (ABA), gibberellic acid (GA3) (c); along with their respective controls. The log2 transformed fold change in expression data was used to generate the heat maps with hierarchical clustering of Euclidean distance correlation using MeV software package. The color scale provided at the bottom of figure represents expression variations. The stress induced upregulation or down-regulation of StCAT transcripts is indicated by yellow or blue color, respectively.

Figure 9

Profile of electrophoresis on agarose gel of RT-PCR products of the catalase genes of in vitro plants of potato leaves (var.Nicola) cultivated in absence (-) or presence of 100 mM NaCl (+). a: Profile of electrophoresis on gel agarose (2%) of RNA extraction of potato leaves (var.Nicola) b: agarose gel (1%) of cDNA c: agarose gel (1%) of RT-PCR products of the catalase genes of potato leaves. MT: size marker; T-: negative control without RT.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Additionalfiles.docx