Sequence analysis and biochemical studies on Catalase (CAT) in potato (*Solanum tuberosum* L.)

A

Dissertation

Submitted In the partial fulfillment of the requirement for the award degree of

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Submitted by

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CANDIDATE'S DECLARATION

I, hereby declare that the work which is being presented in the thesis entitled, "**Sequence analysis and biochemical studies on Catalase (CAT) in potato (*Solanum tuberosum* L.)**" in the partial fulfillment of the requirement for the award of degree of Master of Science in Biochemistry, Thapar University, Patiala, is an authentic record of my own research work carried out under the guidance and supervision of Dr. N. Das, professor, Department of Biotechnology, Thapar University, Patiala, India. The matter embodied in this dissertation has not been submitted to any other university or institute for award of other degree.

Shweta Sharma

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CERTIFICATE

This is to certify that dissertation entitled "**Sequence analysis and biochemical studies on Catalase (CAT) in potato (*Solanum tuberosum* L.)**"Submitted by Shweta Sharma (Regd. No. 301507008) in partial fulfillment of requirement for the award of the degree of Master of Science in Biochemistry, to Thapar University is a record of student's work carried out by her under our guidance and supervision. The report has not been submitted for the award of any other degree or certificate in this or any other university or institute.

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(Shweta Sharma)

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LIST OF ABBREVIATIONS

Abbreviation	Name
µg	Microgram
µL	Microlitre
BLAST	Basic Local Alignment Search Tool
BLASTp	BLAST for Proteins
Bp	Base-Pair
DNA	De-oxy Ribo nucleic acid
IU/ml	International unit per mL
Kb	Kilo Base
kDa	Kilo-Daltons
M	Molar
mM	Mili-molar
NCBI	National Centre for Biotechnology Information
Nm	Nanometer
O.D.	Optical density
ORF	Open reading frame
pH	Potential of Hydrogen
Pmoles	Pico moles
ROS	Reactive oxygen species
SDS	Sodium Dodecyl sulphate
TAE	Tris acetate EDTA
TBE	Tris borate EDTA
TE	Tris EDTA

ABSTRACT

In plants, supra-optimal light, salinity, insufficient supply of water and mineral nutrients, atmospheric and soil pollutants, attack by microbial pathogens and aerobic metabolic activities are some of the major contributing factors towards stress conditions which lead to the accumulation of reactive oxygen species (ROS). H_2O_2 is considered to be a potential signal molecule involved in various metabolic processes involved in plant development. Although several type of H_2O_2 - metabolizing proteins are present in plant systems, catalases are very distinct from other proteins as they catalyze dismutase reaction primarily and have low affinity for H_2O_2, play an important role in stress-linked H_2O_2 signaling. Potato is most important non-cereal cash crop ranked fourth after maize in terms of global food production. Plant growth and tuberization are important aspects of potato life cycle which involve generation of stress mainly due to oxidative metabolism. This study focused on some of the commercially important Indian potato cultivars namely Kufri Chipsona-1, Kufri Chipsona-2, Kufri Chandramukhi, Kufri Ashoka, Kufri Pukhraj, and Kufri Jyoti. They represent early, medium and late maturing varieties. The exotic cultivar Desiree was taken as reference. All these plants were grown under field condition, and different tissues like tuber stem and leaves, were collected at different stages of growth. The purpose was to carry out catalase assay in different tissues of the individual cultivars. Tissues were frozen in liquid nitrogen and homogenized in phosphate buffer (pH 7.0). The clarified crude extracts were used for both catalase assay and protein estimation. Catalase activities were found to be significantly different between growing and mature tubers within and between the potato cultivars. The growing tubers showed significantly higher catalase activity as compared to the mature tubers. Leaf tissues showed considerable activities but it was very low in the stem tissues. Efforts were also made for purification of catalase from the tubers and other tissues. *In silico* analysis was done by retrieving the sequences from the databases. Biochemical attributes, predicting motifs and structures, 3-D modeling and construction of Phylogenetic tree were done and reported in the study. Some of the attributes were not reported earlier. In conclusion, this study on catalase is quite important with regard to the Indian potato cultivars.

Keywords: *Solanum tuberosum* L., Catalase, Motif search, Enzyme assay, Potato tissues, Enzyme assays.

CHAPTER-1 INTRODUCTION

1.1 About Potato

Potato (*Solanum tuberosum* L.) is one of the most important food crops after wheat, maize and rice, contributing to food and nutritional security in the world. It is a non-grain food crop belonging to family *Solanaceae*. The word potato may refer to the plant itself as well as the edible tuber (**Fig.1**). Around 8000 years ago, potato originated in the highlands of the Peruvian Andes-mountains in South America on the border between Bolivia and Peru (Bachem et al. 2000) .In 16th century, through Spanish conquerors potato was introduced to Europe where it developed as temperate crop and with colonial expansion of European countries it got distributed throughout the world. Either through British missionaries or Portuguese traders, potato first came to India in 17th century (Averyanov and Lapikova 1989). Soon it became an important staple food and field crop and became the third largest food crop after rice and wheat.

Potato crop is cultivated in around 1.2 million hectare of land in our country with an approximate production of 23.5 million tonnes. Potato is an annual, herbaceous, dicotyledonous and vegetatively propagated plant (Percy 1984). However, in some environments it can grow as perennial and can be propagated through seeds, which are called true potato seeds (TPS). It is a nightshade plant and is susceptible to frost and freezing. Potato plant produces a fibrous root system arising from the underground portion of stem. It has dark green, broad and compound leaves with oval leaflets. They bear white, pink, blue, or purple flowers with yellow stamens (Craighead et al. 1998). It could have three kinds of stems including sprouts (leafy stems), (Nejat and Mantri 2017). Tuber is swollen underground stem used in commercial propagation. The tubers bear lateral buds (eyes) which grow into a new plant under favorable conditions. Potato tubers are major sink organs.

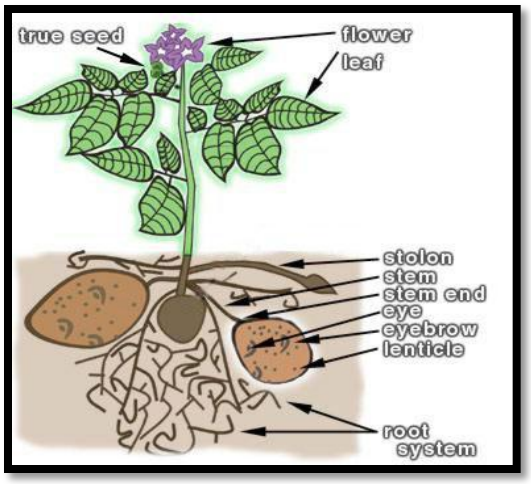


Fig. 1: Schematic view of potato plant

1.2 Biotic and abiotic stresses in plants

Plants are persistently confronted to both biotic and abiotic stresses that decreases their productivity. Numerous molecular, physiological, cellular adaptations are involved in response to these stresses and hence are complex. Biotic stress is a stress that occurs due to bacteria, fungi, parasites, weeds which results in damage to plants (Gill and Tuteja 2010). Most of the diseases in plants are caused due to fungi. Morever, abiotic stress is an important characteristic which has a major effect on growth and development of plant. Abiotic stress is responsible for major losses in the field (Teng et al. 1984). When plants are exposed to abiotic and/or biotic stresses, the kinase cascades, reactive oxygen species (ROS) and specific ion channels get activated, phytohormones like ethylene, abscisic acid (ABA), and salicylic acid accumulate. The re-modeling of the genetic machinery results in appropriate defense reactions and the plant tolerance increases so as to reduce the biological damage which is caused by stress. Latest evidence shows that a combination of biotic and abiotic stress can cause a positive effect on plant productivity by decreasing the sensitivity towards biotic stress. Certain metabolites get accumulated under stress, and influence positive effects on plant's response to both stresses and thus multiple aggressors are protected(Mhamdi et al. 2010) .Accumulation of callose is the first responses i.e. changes in ions fluxes, ROS, and phytohormones persuade to combat the stress and the signal transduction to activate the metabolic reprogramming towards defense.

1.3 Reactive oxygen species (ROS), site of production, and their effects

The examples of stress condition are insufficient supply of water, supra-optimal light, pathogen attack, soil and atmospheric pollutants. A plant often experiences multiple adverse conditions simultaneously in the field (Hardesty). Depending upon variety, individual plants have inherent capacity to develop resistance against stresses. The aerobic metabolism such as photosynthesis and respiration results in generation of reactive oxygen species (ROS). The damage to proteins, DNA, lipids is caused by ROS(Foyer and Noctor 2005). It comprises free radicals such as hydroxyl radical, non-radical molecules like hydrogen peroxide and superoxide anion. The deposition of reactive oxygen species (ROS) and ROS-induced changes in redox state are the central theme in many stress responses.

The grouping of free radicals, ions which are more sensitive atoms and are derived from O_2 are called ROS.

It will be accepted that 1% of O_2 used by plants may be directed to produce ROS on numerous subcellular loci; for example, mitochondria, chloroplasts, peroxisomes. In order to avoid oxidative stress, the production and removal of ROS must be strictly controlled (Monaghan et al. 2009). The cell is said to be in state of "oxidative stress" when the level of ROS exceeds the requirement of defence mechanisms. At high concentration ROS can cause damage to various biomolecules such as DNA, proteins, lipids. These reactions also alter the instrinsic properties of membrane like ion transport, fluidity, loss of enzyme activity, protein synthesis inhibition, DNA damage which ultimately results in cell death (Bhattacharjee 2005) . However at low concentration it also act as second messenger in intracellular singnaling cascades which mediate responses in plant cells that includes programmed cell death, stomatal closure.

The ROS reactivity may naturally cause some changes to cell membrane (Averyanov et al. 1989) Various molecular and biochemical processes such as the activation of ROS-driven gene expression, calcium signatures, specific protein kinases, the programmed production of ROS by NADPH oxidases, the need of specific type of gene modulation for ROS-induced cell death, ROS-mediated hormonal signal transduction are important parameters for sustainable cell growth (Iwata et al. 2013) . The specific effects caused by different ROS, changes in roles of homeostatic components i.e. glutathione, in transmitting ROS signals and the importance of physiological and environmental factors in determining the effect of ROS-associated redox signaling.

1.4 Antioxidative defense mechanism in plants

There is a complex antioxidative defense system in plants which comprises of enzymatic and nonenzymatic components. The antioxidative defense system scavenges the ROS. Specific ROS producing and scavenging systems are found in different organelles of plant cell like peroxisome, chloroplast and mitochondria (Yan et al. 2000). The toxic metabolites i.e. oxygen are produced at low level under normal conditions and maintain correct balance between the production and quenching of ROS. But this balance can be affected by various adverse environmental factors resulting in increase of intracellular ROS levels. Thus induces oxidative damage to lipids, proteins

and nucleic acids. The higher plants increase the level of endogenous antioxidant defense to prevent the oxidative damage.

1.5 Various enzymatic systems to overcome ROS in plants

The antioxidative system have components which includes many antioxidant enzymes i.e. superoxide dismutase, catalase, guaiacol peroxidase (APX), glutathione reductase (GR), dehydroascorbate reductase. These enzymes work in compartments which are subcellular and respond to the cell's exposure to oxidative stress.

1.5.1 Superoxide Dismutase:

In aerobic organisms superoxide dismutase (SOD, EC. 1.15.1.1) plays an important role in defense against oxidative stress. They belong to a group of metalloenzymes which catalyzes the dismutation of $O_2^{\cdot-}$ to oxygen and hydrogen peroxide (Yan et al. 2000). It is present in subcellular compartments which generate activated oxygen. In plants basically, three isozymes of superoxide dismutase are reported which are copper/zinc SOD (CU/Zn-SOD), manganese SOD (Mn-SOD) and iron SOD (Fe-SOD). They are encoded by nuclear genes and targeted to specific subcellular compartments by amino-terminal transit peptide. SOD is present in the mitochondria whereas Fe-SOD is present in the chloroplasts.

1.5.2 Catalase

Catalase is an important antioxidant enzyme in plants. Considerable progress has been made on catalase research. For details, see the review of literature section.

1.6 ROS overproduction in stressful conditions

In normal conditions the production of ROS is low in plants. Plants have high ROS which disturb their balance of $O_2^{\cdot-}$, $^{\cdot}OH$ and H_2O_2 in intracellular environment, due to changes in external environment (Yan et al. 2011). Production of reactive species is increasing due to environmental stress i.e. metal toxicity, pathogenic attack, salinity and chilling.

1.6 Generation and metabolism of H_2O_2 in plants

Hydrogen peroxide generated after dismutation of $O_2^{\cdot -}$ also acts as a moderately reactive species and excess of H_2O_2 in cells leads to the occurrence of oxidative stress.

H_2O_2 is also produced due to reduction of $O_2^{\cdot -}$ by reducing agents present inside the cells such as ascorbate, thiols, ferredoxins etc (Bergamini et al. 2004) To prevent the oxidative damage in plants, CAT is frequently used by cells that rapidly decompose H_2O_2 into less reactive O_2 and H_2O molecules (Noctor and Foyer 1998). Therefore, CAT plays an important role in plant defense, ageing and senescence.

Source and sink tissues are predominant sites of oxidative stress and the transporting tissues also bears some oxidative stress. The present study deals with the assay of catalase activity along protein estimation from different parts of potato tissues such as tubers (sink), leaves (source) and stems (transport) at different stages of growth. Keeping in view, review of literature deals mainly with the recent advances on catalase in plants including the members of the *Solanaceae* family.

CHAPTER-2 REVIEW OF LITERATURE

2.1 Brief introduction of catalases

The catalase (CAT, EC 1.11.1.6) is an enzyme which was first discovered and characterized among antioxidant enzymes. It is a ubiquitous tetrameric enzyme which contains heme group, catalyzes to form water and oxygen by the dismutation of two molecules of H_2O_2. Catalase shows weak activity against organic peroxides but it is highly specific for H_2O_2 (Krishnamurthy and Wadhwani 2012) .

The H_2O_2 production site is peroxisome. Catalase scavenges the H_2O_2 produced in the peroxisome during the photorespiratory oxidation, β -oxidation of fatty acids. Catalases are unique because they do not require cellular reducing equivalents. It has low affinity for H_2O_2 than APX (ascorbate peroxidase) but has very fast turnover rate. The major sites of H_2O_2 production is peroxisomes. All angiosperms species studied till date contain three CAT genes. Based on expression profile of tobacco genes, (Willekens et al. 1994) introduced a classification of CAT. In photosynthetic tissues, the Class I CATs are expressed and regulated by the light (Shi et al. 2013). In seeds and young seedlings, the Class III CATs are present; whereas, in vascular tissues high level of Class II CATs are expressed. The H_2O_2 is produced through catabolic processes when cells are stressed for energy. Depending upon the type of stress, duration and intensity of the stress, CAT activity could be increased or decreased.

The action of catalase in plants and animals was first observed by (Bailey 1917) . Hydrogen peroxide was found to be quickly degraded in these tissues. (Dounce 1983) also made similar observation. (Frugoli et al. 1996) proposed that catalase was an enzyme containing iron group. This explains why cyanide inhibits catalase. The catalase enzyme was purified, crystallized and characterized from beef liver (Scandalios 1968) Russian biologist (Scandalios 1968) studied catalases at genetic level in blood samples of many animals species.

2.2 Catalase characterization

Catalase is an enzyme which contain tetrameric heme group and it degrade H_2O_2 very rapidly which is found in aerobic organism. It is one of the most active catalysts produced by nature. It decomposes H_2O_2 at very fast rate. It exhibits a dual function depending on the concentration of H_2O_2.

It acts "peroxidatically" at low concentration of H_2O_2, ethanol and ascorbic acid are donors of hydrogen atom which can be oxidized in different ways.

$$H_2O_2+RH_2 \longrightarrow R+2H_2O$$

When substrate is present at higher concentration, then the toxic H_2O_2 was decomposed by catalase at a very high rate using catalytic reaction in which both donor and acceptor is H_2O.

$$2H_2O_2 \rightarrow 2H_20+O_2$$

A catalase is a mechanism of two-steps in both the peroxidatic and catalytic reactions which is suggested by spectrophotometric and kinetic evidence (Bajji et al. 2007) .

First, oxygen-rich iron peroxide is formed by the interaction of heme iron of catalase with H_2O_2.

$$CAT\text{-}Fe\text{-}OH+H_2O_2 \longrightarrow CAT\text{-}FE\text{-}OOH + H_2O$$

Compound I was referred to iron peroxide (CAT-Fe-OOH) which is easily detected by a cell system because it changes the property of catalase heme (Lee et al. 2008). Compound I is used as an indicator of H_2O_2 concentrations in vivo because of special kinetic properties of catalase. At very low concentration of H_2O_2, the compound I can be reduced by hydrogen donors peroxidatically.

$$CAT\text{-}Fe\text{-}OOH+C_2H_5OH \rightarrow CAT\text{-}Fe\text{-}OH+H_2O+CH_3CHO \text{ (Peroxidatic Rxn)}$$

At very high concentration of H_2O_2, compound I reacts with a second H_2O_2 to produce water and molecular O_2.

$$CAT\text{-}Fe\text{-}OOH + H_2O_2 \rightarrow CAT\text{-}Fe\text{-}OH + H_2O+O_2 \text{ (Catalytic Rx)}$$

Catalase degrades H_2O_2 without using cellular reducing equivalents. Therefore, catalase provides a mechanism which is energy-efficient for removing H_2O_2 in the cell. Through a catabolic emergency processes there is generation of H_2O_2 because cells are under stressed condition. This results in net gain of reducing equivalents and therefore, cellular energy .It has been proposed that catalase helps to maintain the homeostasis of the H_2O_2 in a cell (Sakajo et al. 1987). The catalytic mode in which catalase had high Michaelis constant which is not easily saturated with the substrate.

With the increasing H_2O_2 concentrations there is an increase in enzyme activity and thus control a concentration of H_2O_2 inside the cell. There is circumstantial evidence that in mammalian systems the catalase may function in this manner. An organ such as (liver, kidney) has high concentration of catalase as well as has high levels of H_2O_2. In liver if there is a rise in H_2O_2 concentration then the working capacity of catalase is stopped.

The catalase can either catalyze directly the dismutation of H_2O_2 into H_2O and O_2 or use H_2O_2 to oxidize the substrates i.e. methanol, ethanol, formate or nitrite. In the mesophyll leaf and bundle-sheath of maize have low peroxidatic activity and but on the other hand contain high catalytic activity. *In vivo* as homotetramer, CAT-3 isozyme is found only when its temporal and tissue specific expression coincide with CAT-1 and CAT-2 isozymes. The specific targeting position of CAT-3 protein suggests that catalase is encoded by nuclear genes.

2.3 Multiple molecular forms of catalase in plants

Catalase have different enzymatic forms in plants which is absent in animals. In maize, it was first demonstrated that the different isozymes of catalase were products of distinct, unlinked genes (Diehl et al. 2008). However, in other plant species, multiple forms of catalase have been proved to be the rule rather than exception (Adare et al. 2014). The different species of plants such as *Nicotina tobacco*, cotton, *Nicotiana plumbaginfolia*, *Pinus taeda*, sunflower, pumpkin and tomato were found to contain different isozymes of catalase. The single forms of catalase were initially reported in few species of plants like castor bean, but when examined multiple forms were identified. However, few species possess only one kind of form, but in some cases such as, tobacco and *Arabidopsi* there are three genetically different catalases. The presence of multiple catalase isozymes suggests that the enzyme performs different functions at various stages of development.

2.4. Studies on catalase in potato till date

Biochemical and molecular studies on catalase: purified catalase from potato tubers. Its molecular weight was found to be 224 kDa in native PAGE, and 56 kDa in SDS-PAGE. Studies showed that the specific activity was low i.e, approx.3000 units per mg of protein. (Taneja and Das 2014) studied the role of alternative oxidases and catalase in suppression of mitochondrial breakdown. studied the possible relationship between the ROS metabolism and dormancy release of potato tuber. (Pence and Naylor 1990) investigated the link between catalase activity and salt tolerance *in vivo* and *in vitro* in

kansgeni lines of cultivar Desiree and noticed the catalase contribution to salt tolerance in potato studied the involvement of H_2O_2 and catalase activity during dormancy and sprouting of potato. It was also found that H_2O_2 was maximal and catalase activity was minimal at the time of dormancy. At the time of sprouting higher activity of H_2O_2 is reported.

(Demirelli et al. 2013) cloned a 1900-bp catalase cDNA consisting of 1476-bp ORF, and compared the deduced amino acid sequences with mammalian catalase and found dissimilarities especially in N-terminal (1-17) and C-terminal (369-492) regions. It was found that some amino acids were well conserved and responsible for catalytic activity. The highly positively charged amino acids at C-terminus play an important role in delivery of proteins into microbodies. first reported potato catalase gene of 1772-bp from mature root. investigated around 100 proteins associated with tuberisation involved in various diverse functions such as protein biogenesis, storage and metabolism. This study proposed that the catalase activity was enhanced in the early stages of tuberisation, and then decreased after maturation.

2.5 Origin of the problem

Activity of catalase enzyme is triggered under various stresses. In potato, this enzyme plays a vital role during the process of tuberisation. Catalase also plays a significant role in potato dormancy. It has been studied that catalase inhibitors helps in release of dormancy. Usually, during the early stages of tuberization elevated levels of catalase is observed. It is likely that a coordinated balance between the generation and scavenging of the ROS plays a crucial role in tuber development and maturation. With regard to the Indian potato cultivars, no reports are available in the literature on catalase at biochemical and molecular level.

2.6 Objectives of the study

Keeping in view the biological importance of catalase during the process of tuberization the following objectives were framed for this study:

- Assay of catalase activity in various potato tissues and partial purification
- Sequence analysis, motif search, Phylogenetic tree and 3-D modeling of catalase

CHAPTER-3 MATERIALS AND METHODS

3.1 Catalase estimation

3.1.1 Procurement of plant and other materials

For the study, high-yielding, commercially important Indian potato cultivators such as Kufri Chipsona-1(CS-1), Kufri Chipsona-2 (CS-2), Kufri Chandramukhi (KCM), Kufri Jyoti (KJ), Kufri Ashoka (AS), Kufri Pukhraj (PR) and Desiree (De) procured from CPRI Shimla, and maintained in tissue culture room, laboratory 4, Thapar University, Patiala were chosen. For convenience, short name of each cultivator is shown within parenthesis. These cultivars were used as they, differ in their genetic make-up, time of maturation and belong to different agro-climatic zones of the Indian subcontinent. KCM and AS cultivars are early maturing whereas other cultivars were medium maturing.

After acclimatization these plants were grown into field in the starting month of November, which is best suited for cold temperature and short day length light conditions. As potato is a cold weather crop, so optimum conditions were taken care of while their growth till maturity. For the study the tissues were collected at young stage (January) and at maturity (March).

Other materials: Various chemicals, enzymes, molecular biology items were procured from different sources. The chemicals were purchased from Sigma-aldrich India Pvt.Ltd, and HiMedia Laboratories Mumbai. Various enzymes used were purchased from Bangalore Genei Pvt.Ltd., Bangalore and Amersham Biosciences Ltd., Hongkong. Glassware's and Plasticwares were purchased from Borosil and Tarsons Products Pvt. Ltd.

3.1.2 Protein extraction from different plant materials

Approx. 3g of potato tissue sample (tissue cultured plantlets, leaves and tubers) was crushed to fine powder using liquid nitrogen. 10 ml of protein extraction buffer (50 mM phosphate buffer, 1%cysteine, and 1% PVP) was mixed well and transferred to the microfuge tube (Taneja and Das 2014). Centrifugation was carried out at 10,000 rpm for 30 minutes at 4°C till the pellet settle down and clear supernatant was transferred to fresh microfuge tube , was used as crude enzyme and stored at -80°C for further use.

3.1.3 Protein estimation

Protein content was estimated by Folin- Lowry method. The divalent ion of copper form a complex with the peptide bonds which gets reduced to a monovalent ion under alkaline conditions. Monovalent ions of copper and the radical groups of cysteine, tryptophan, tyrosine, produced unstable product by reacting with the Folin reagent which gets reduced to molybdenum/tungsten blue. The following solutions was used: Solution A(Alkaline sodium carbonate solution) – 2% Sodium carbonate in 0.1 N NaOH; Solution B (Copper sulphate-sodium potassium tartrate in 1% copper sulphate; ,Folin-ciocalteu reagent(diluted with water in 1:1). 5.0 mM of alkaline solution (mixture of 50mM solution A and 1.0 mM of solution B freshly prepared) was added to 1.0 mL of test solution (protein sample and volume made up with water), mixed well and kept at room temperature for 10 min, then 0.5 ml of diluted Folin-ciocalteu reagent was added and mixed immediately.

Samples were kept at room temperature for 30 min and absorbance was taken at 750 nm. Standard curve was made by using different concentrations of BSA protein .

3.1.4 Catalase assay

The catalase activity was determined by measuring a decrease in the absorbance at 240nm. 30µL enzyme was taken in a reaction mixture and 10mM 30% H_2O_2 in 50mMpH 7 phosphate buffer in the total volume of about 3mL.(Aebi 1984)

One unit of activity is defined as the amount of enzyme which catalyzes the decomposition of 1 µmol H_2O_2 as calculated from the extinction coefficient for H_2O_2 at 240nm of 39.4M^{-1} cm^{-1}.

3.1.5 Partial purification of catalase by Three Phase Partitioning method (TPP)

Some efforts were made on catalase purification by TPP a simple, convient method for purification of protein.The crude extract was saturated with 30% (w/v) ammonium sulphate at 25ºC followed by addition of t-butanol in ratio1:1 (v/v) to crude extract.`The mixture was vortexed and incubated at 35ºC for 60 minutes. After incubation it was centrifuged at 5,000rpm for 5 min at 4ºC to form the three phases i.e. the upper phase of organic followed by interfacial precipitate phase and lower phase of aqueous layer which were carefully separated. Then the middle interfacial precipitate were taken out carefully in a separate tube and was dissolved in 50mM phosphate buffer of 7 pH.

The middle interfacial phase and the lower phase of aqueous was analyzed to check the catalase activity and total protein content. The interfacial phase which contains catalase was collected (Duman and Kaya 2013).

3.1.6 Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE)

Proteins were separated through SDS-PAGE according to their electrophoresis mobility (a function of length of polypeptide chain or molecular weight). SDS-PAGE gel have two parts one stacking gel and other is resolving gel as shown in Table (**1**) and (**2**). For this study 10% stacking gel was used as reported in literature (Taneja and Das 2014). The components of the resolving gel was mixed in a test tube and poured in the casting apparatus followed by over layering with water saturated isobutanol.

After 30 min when the gel was polymerized, isobutanol was decanted and gel was washed with water. Stacking gel was poured over the resolving gel and comb was placed 1X PAGE running buffer. After polymerization, the comb was removed and the gel was submerged in 1X PAGE running buffer. The protein samples were mixed with equal volume of 2X sample and boiled for 3-5 min.

Gel was run at 100 volts for 2 hrs. (1X PAGE running buffer 3.03 g Tris base, 14.4 g glycine, 1.0 g SDS and made up the volume to one litre). Gel was stained in staining solution for 1 hour. After destaining in destaining solution for 2-3 hours, the protein bands were visualized and photographed.

Table 1: Composition of stacking gel (5.0 mL)

COMPONENTS	**4%**
Water	3.0 mL
30%Acrylamide/Bisacrylamide (30%/0.8%w/v)	0.8 mL
4X Stacking gel buffer (0.5M Tris-HCL, Ph 6.8)	1.25 mL
10%SDS	
10%APS	25µL
TEMED	50µL
	2.5µL

Table 2: Composition of resolving gel (5.0 mL)

COMPONENTS	7%	10%
Water	5.1 *mL*	4.0 *mL*
30%Acrylamide/Bisacrylamide (30%/0.8%w/v)	2 .3 *mL*	3.4 *mL*
4X Stacking gel buffer (0.5M Tris-HCL, Ph 8.8)	2.5 *mL*	2.5*mL*
10%SDS	100µL	100µL
10%APS	50µL	50µL
TEMED	5.0µL	5.0µL

Staining solution -0.15% Coomassie Brilliant Blue-R250, 30% Methanol, 10% glacial acetic acid and made final volume with water.
Destaining solution -30% Methanol, 10% glacial acetic acid and made final volume with water.

3.2 *In-silico* analysis:

Stream line/outline for Insilico analysis

A flow-charts of strategies used for the identification and study of catalase isoforms of *Solanum tuberosum* is as follows:

Accession number encoding potato catalase from the literature

↓

BLASTp at NCBI (www.ncbi.nlm.nih.gov)

↓

The sequences were retrieved and selected

↓

Used for *In-silico* characterization

3.2.1 Sequence Analysis: The nucleotide sequence of Catalase cDNA was analyzed by NCBI Blast tools. The amino acid sequence was predicted by the open reading frame (ORF) finder available at the National Center for Biotechnology Information website (http://www.ncbi.nlm.nih.gov).
They are discussed as below:-

3.2.2 *Biochemical characterization*: The theoretical pI, amino acid composition and molecular weight (Bjellqvist et al. 1994) was determined by using EXPASY server (http://web.expasy.org/protparam/).
Insilico characterization:- ProtScale tools of ExPASy were used for prediction of various secondary structures such as α-helix, β-sheet,β-turn and random coil. Phyre (http://www.sbg.bio.ic.ac.uk/~phyre/) was used for insilico characterization for predicting secondary structure and solvent accessibility.

3.2.3 Identification of conserved domains: The catalase protein sequence from *Solanum tuberosum* were then uploaded to MY HITS (http://myhits.isb-sib.ch/cgi-bin/motif_scan) and different sites were predicted.

3.2.4 3-D protein modeling: Raptor X (raptorx.uchicago.edu/ StructurePrediction/predict/) was used for homology protein modeling. The protein models obtained were further analyzed by Prosa web server (https://prosa.services.came.sbg.ac.at/prosa.php).

3.2.5 Phylogenetic Distance tree: Distance tree of results was constructed by NCBI (National centre of Biotechnology information).Using pair wise neighbor joining methods with main sequence difference 0.85 at girish protein distance using all collapse modes. The tree was retrieved using MEGA7 software (http://www.megasoftware.net/).

4.1 Catalase Assay in potato

4.1.1. Plant growth and harvesting of the potato tissues

For the tissue procurement, tissue cultured plants were first acclimatized and hardened and then grown in field till maturity. Tissues at young and mature stage were collected and frozen in liquid nitrogen and stored at -80ºC for further use. The steps are shown in Fig. 2 & 3.

Fig. 2: Steps of plant grown from lab to field condition. (a) Culture maintained in MS medium in lab (b) and (c) Acclimatization and hardening of plantlets (d)field growth (e) Collection of plants

Fig. 3(a) **Fig.3 (b)**

Fig. 3 : (a) Young and growing tissues (Stem, Leaf, Tuber) collected in the month of January 2017.
(b) Mature tissues (Stem, Leaf, tuber) collected in the month of march 2017.

After harvesting, each tissue was collected separately, manually and kept in a plastic bag under ambient conditions. Followed by, weighing their weight, tissues were separately pulverized in liquid nitrogen.

4.1.2 Catalase activity and protein estimation in different cultivators and tissue

Catalase was estimated from the source, transport and sink tissues i.e. leaf, stem, and tuber respectively collected from the field grown potato cultivars. (See section 3 of 'Materials & Methods'). The tissues were pulverized in phosphate buffer and were used for protein and catalase activity estimation. Standard curve for proteins was made using standard protein BSA (Bovine serum albumin) from Sigma at different concentrations and absorbance was taken at 750 nm as shown in **Table 3** and **Fig 4**. We have considered both specific activity (per mg protein) and activity per gram of tissues as it is the biological characteristic of potato.

Table 3: BSA at different concentration and absorbance at 750 nm

BSA standard (µg)	Absorbance at 750 nm
Blank	0
0	0.037
40	0.067
60	0.123
80	0.146
100	0.184
120	0.223
140	0.249
160	0.303
180	0.353
200	0.378

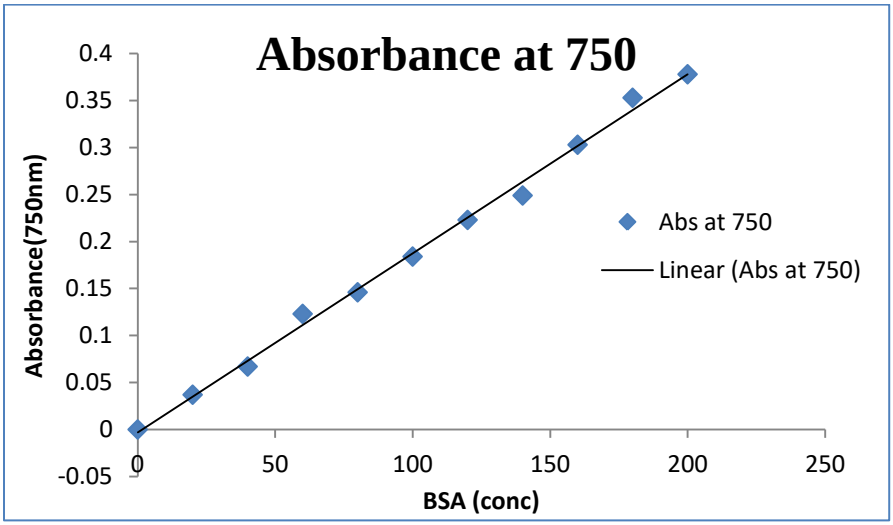


Fig 4: BSA standard curve

4.1.3 Estimation of Catalase activity in different potato cultivars

The initial weight of young tuber was approx 7 gm in all the cultivars and that of mature tuber it was 18-20 gm. For the enzyme assay 3 gm of sliced tuber was taken in each cultivar to maintain homogeneity. Similarly, every tissue was 3 gm for assay and other studies for better correlation.

Catalase estimation from samples of the above mentioned tissues and cultivars at different stages was done at 240 nm. H_2O_2 degradation was noted at every 30 seconds for three minutes. Specific catalase activity was expressed in U/mg of protein.

Catalase activity in tuber

We have observed inter-varietal differences of catalase activity in growing and mature tubers as shown in **Fig. 5** and **Table. 4**. Catalase activity was found to be highest in young AS cultivar and lowest in young cultivar of KJ. In case of CS-1 and PR, activity of catalase is higher in mature tuber as compared to young tuber. Whereas in CS-2, KCM, AS, De, it was found to be high in young tuber as compared to mature tuber .The differences could be due to different stress conditions with regard to genetic makeup.

Table 4: Unit enzymes and specific activity of Tuber extract

Cultivar	Young		Mature	
	Enzyme units/g	specific activity (units/mg protein)	Enzyme units/g	Specific activity (units/mg protein)
CS-1	112.00	8.00×10^2	125.80	9.51×10^2
CS-2	45.00	4.62×10^2	38.60	3.73×10^2
PR	105.60	6.66×10^2	99.00	7.09×10^2
KCM	107.46	7.08×10^2	62.14	5.59×10^2
KJ	165.00	2.93×10^2	70.52	5.86×10^2
De	157.00	9.37×10^2	68.00	7.58×10^2
As	181.00	13.99×10^2	94.03	8.18×10^2

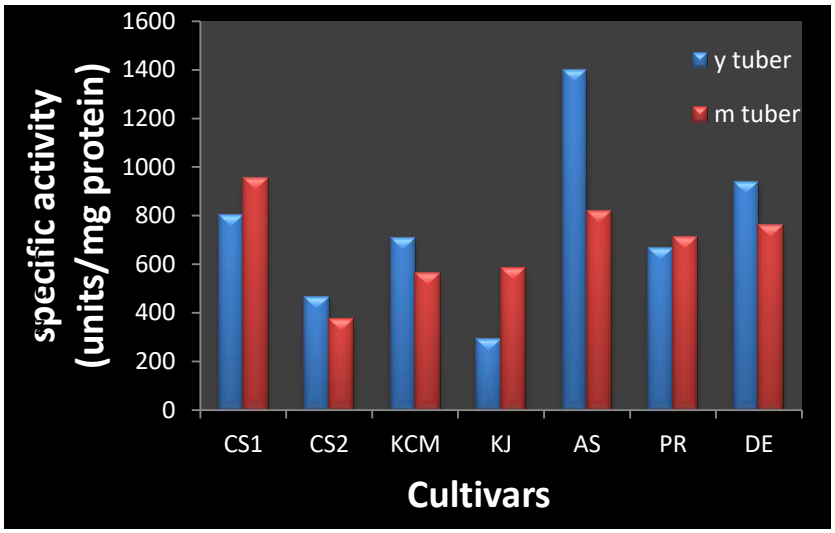


Fig. 5: Bar graph showing catalase activity in young and mature tuber in different potato cultivars

Catalase activity in leaf

The varietal differences were noticed with regard to catalase activity in both growing and mature leaves. Catalase activity was found to be highest in mature CS-1 cultivar and lowest in young leaf of PR. In case of De, KCM, KJ it was found to be high in young leaf as compared to mature leaf. Whereas in CS-1, AS it was found to be high in mature leaf as compared to young leaf. The differences may be due to overall genetic makeup and metabolic status of the individual cultivars.

Table 5: Unit enzymes and specific activity of Leaf samples

Cultivar	**Young**		**Mature**	
	Enzyme units/g	specific activity (units/mg protein)	Enzyme units/g	Specific activity (units/mg protein)
CS-1	16.792	8.46×10^{2}	29.66	9.51×10^{2}
CS-2	7.27	2.36×10^{2}	15.90	3.7×310^{2}
PR	7.836	2.20×10^{2}	11.754	7.09×10^{2}
KCM	17.35	5.04×10^{2}	6.157	5.59×10^{2}
KJ	10.05	4.54×10^{2}	16.23	5.86×10^{2}
De	21.27	9.45×10^{2}	22.39	5.01×10^{2}
As	8.956	3.11×10^{2}	24.06	5.74×10^{2}

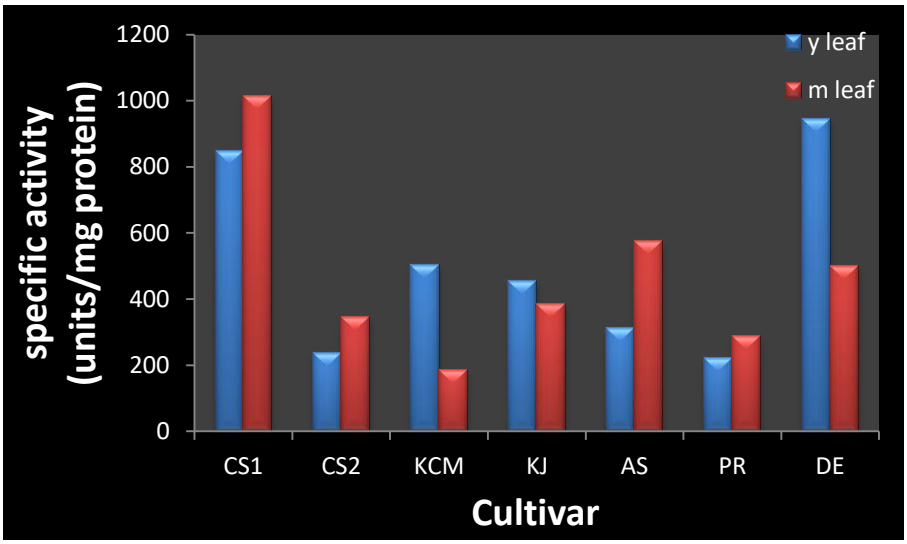


Fig. 6: Bar graph showing catalase activity in young and mature leaves of the different potato cultivars

Catalase activity in stem

Intervarietal differences in catalase activity were observed in growing and mature stem. Catalase activity was found to be highest in young stem of KCM and lowest in young stem of CS-2. In case of CS-1, KCM, KJ, De it was found to be high in young stem as compared to mature stem. Whereas in CS-2, AS, PR it was found to be high in mature stem as compared to young stem. However, enzyme units/gram found to be the lowest in the case of stem which is in correlation with the literature as lowest catalase activity is found in transport tissues.

Table 6: Enzyme units and specific activities of catalase in the stem extract

Cultivar	**Young**		**Mature**	
	Enzyme units/g	specific activity (units/mg protein)	Enzyme units/g	Specific activity (units/mg protein)
CS-1	25.00	2.54×10^{2}	6.71	1.70×10^{2}
CS-2	3.35	1.37×10^{2}	4.40	1.89×10^{2}
PR	8.956	3.39×10^{2}	8.396	4.11×10^{2}
KCM	8.00	4.99×10^{2}	6.71	2.48×10^{2}
KJ	7.80	4.27×10^{2}	4.40	2.64×10^{2}
De	8.7	4.39×10^{2}	8.3	3.35×10^{2}
As	8.39	3.12×10^{2}	11.75	4.18×10^{2}

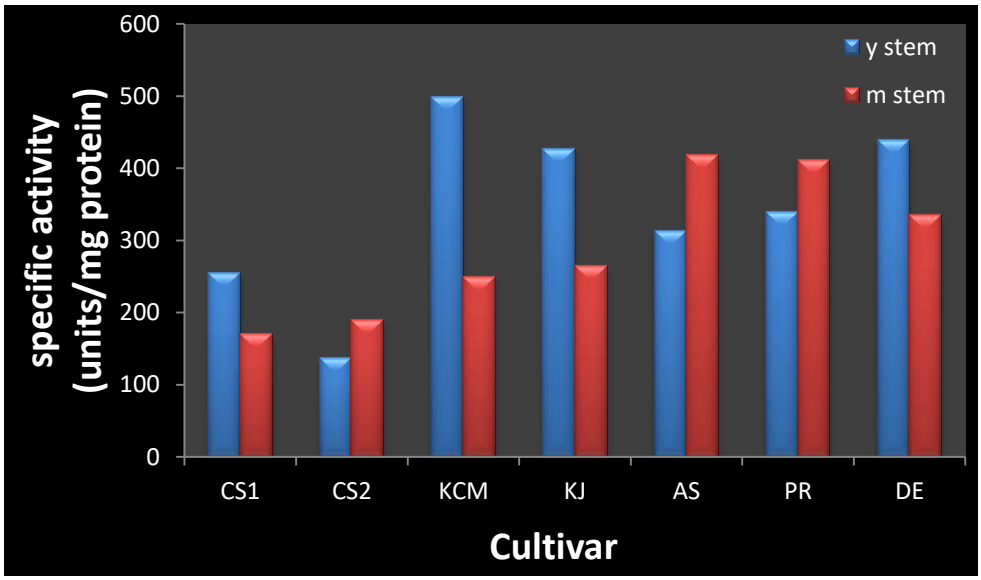


Fig. 7: Bar graph showing catalase activity in the young and mature stem of different cultivars

Catalase activity in young tissues (leaf, stem, tuber)

We have observed inter varietal differences in catalase activity in young tissues of leaf, stem and tuber. From the comparison study it has been observed that in young tissues (Leaf, stem and tuber), catalase activity was found to be lowest in case of young stem. However, a significant value was observed in KJ whereas the activity was lowest in young tuber. In case of AS catalase activity was remarkable high in young tuber.

Table 7: Specific activity of Young leaf, Young stem, and Young tuber

Cultivar	specific activity(units/mg protein)		
	Young leaf	Young stem	Young tuber
CS-1	8.46×10^2	2.54×10^2	8.00×10^2
CS-2	2.36×10^2	1.37×10^2	4.62×10^2
PR	2.20×10^2	3.39×10^2	6.66×10^2
KCM	5.04×10^2	4.99×10^2	7.08×10^2
KJ	4.54×10^2	4.27×10^2	2.93×10^2
De	9.45×10^2	4.39×10^2	9.37×10^2
As	3.11×10^2	3.12×10^2	13.99×10^2

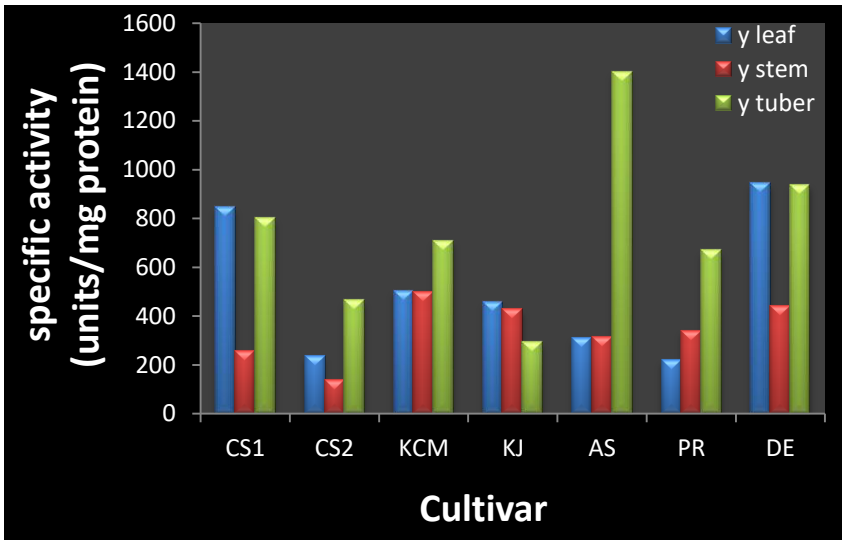


Fig. 8: Comparison study of young tissues (leaf, stem, tuber)

Catalase activity in mature tissues (leaf, stem, tuber)

From the comparison study of catalase activity between mature tissues in cultivar CS-1, the activity was found to be high in leaves. However in cultivars KCM activity in mature leaf was found to be lower than mature stem. It is significantly preliminary studies on the tissues (source, transport, sink) which would be useful for further reference.

Table 8: Specific activity of mature leaf, mature stem mature tuber

Cultivar	specific activity(units/mg protein)		
	Mature leaf	Mature stem	Mature tuber
CS-1	10.15×10^2	1.70×10^2	9.51×10^2
CS-2	3.46×10^2	1.89×10^2	3.73×10^2
PR	2.88×10^2	4.11×10^2	7.09×10^2
KCM	1.84×10^2	2.48×10^2	5.59×10^2
KJ	3.84×10^2	2.64×10^2	5.86×10^2
De	5.01×10^2	3.35×10^2	7.58×10^2
As	5.74×10^2	4.18×10^2	8.18×10^2

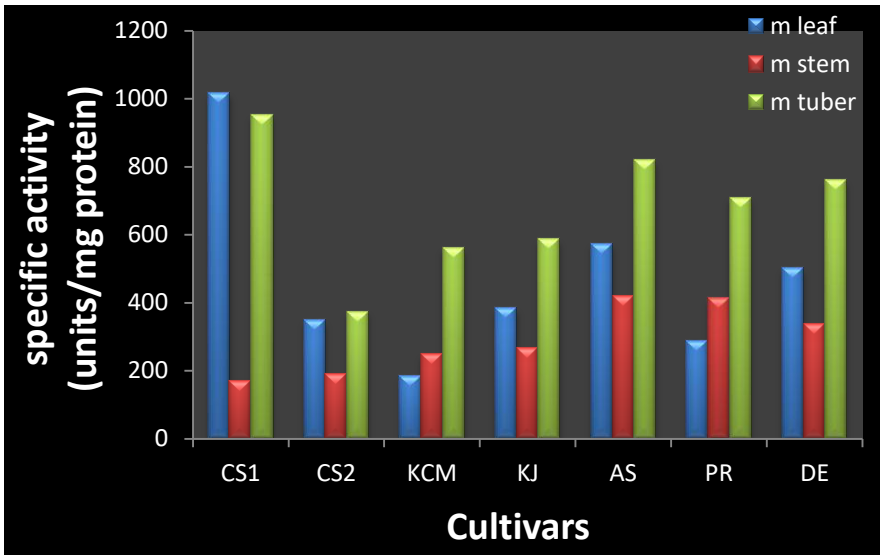


Fig. 9: Comparison study of mature tissues (leaf, stem, and tuber)

4.1.4 Three phase partitioning (TPP) method for partial purification

For partial purification, to 1.0 mL of the crude extract equal amount of t-butanol and 40 percent ammonium sulphate to desired concentration was added. The solution was mixed thoroughly by vortexing and then was kept at 35ºC for 60 minutes. After incubation it was centrifuged at 5000 rpm for 5 minutes. The interface layer (B) as shown in **Fig.10** was taken out carefully, and dissolved in 0.01M phosphate buffer. It was further used for SDS-PAGE run to check the extent of purity.

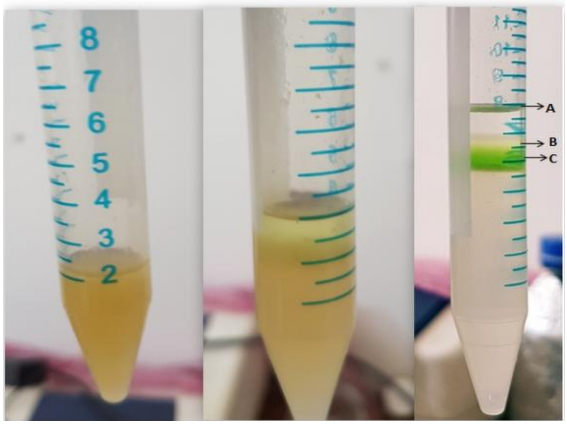


Fig.10: Three phase partitioning. Tube at left (crude extract), tube at middle(mixture of crude extract, t- butanol and ammonium sulphate) and tube at right shows phase separation(A- upper layer , B- interphase layer C-lower layer)

4.1.5 Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE)

To check the quality of isolated protein SDS-PAGE was run for crude as well as purified samples. Proteins were separated on the basis of their electrophoretic mobility.

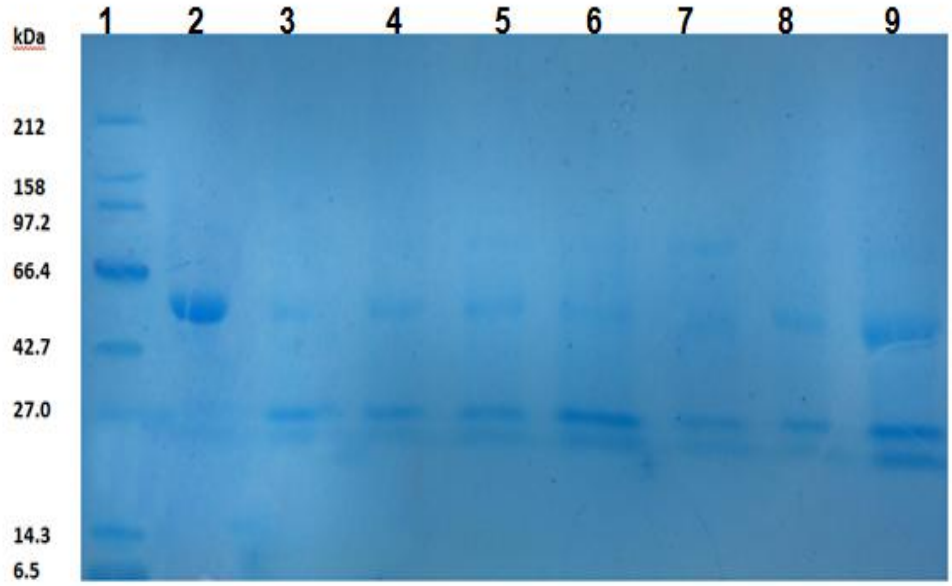


Fig. 11: SDS-PAGE of the crude protein extracts from tubers.
Lane 1- Protein ladder, Lane 2-Catalase from Sigma, Lane 3-De
Lane 4- CS1, Lane 5-CS2, Lane 6-KJ, Lane 7-KCM, Lane 8-AS, Lane 8-PR

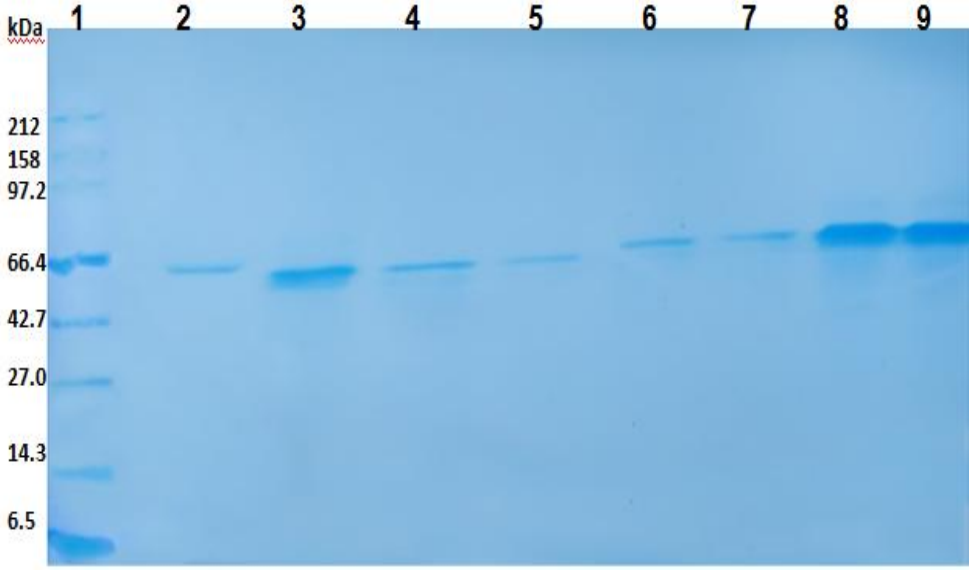


Fig. 12: SDS-PAGE of the purified catalase protein from tubers of various potato cultivars.
Lane 1- Protein ladder, Lane 2-Catalase from Sigma, Lane 3-De, Lane 4- CS1, Lane 5-CS2, Lane 6-KJ, Lane 7-KCM, Lane 8-AS, Lane 8-PR

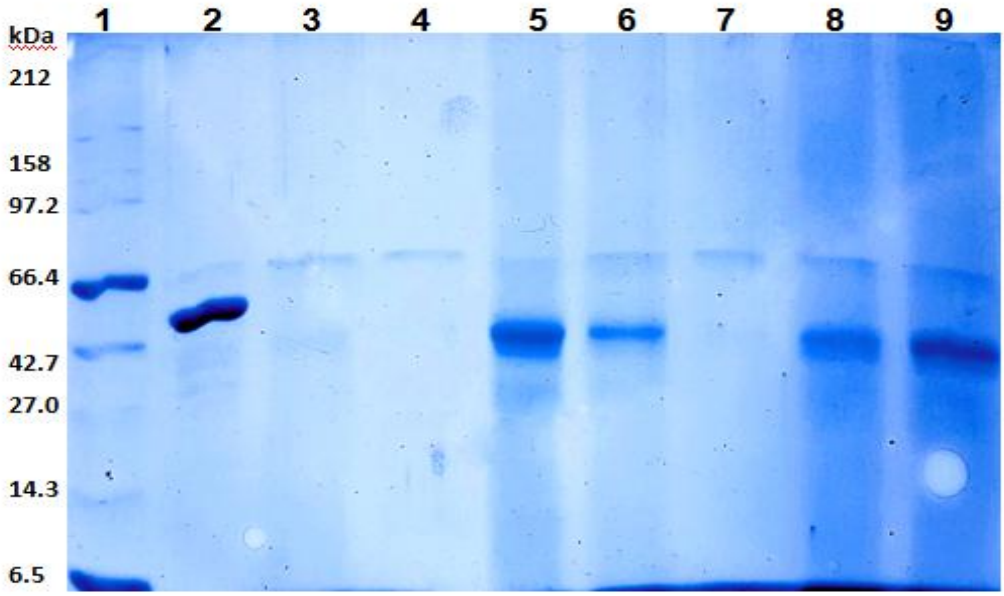


Fig. 13: SDS -PAGE of catalase protein from the leaf tissue of different potato cultivars
Lane 1-Protein ladder, Lane 2-Catalase from Sigma, Lane 3-De, Lane 4- CS1, Lane 5-CS2, Lane 6-KJ, Lane 7-KCM, Lane 8-AS, Lane 8-PR

SDS-PAGE revealed multiple bands in the crude samples as shown in **Fig. 11.** A single band of approx. 70.0 kDa was noticed in the leaf sample as shown in **Fig. 13**, whereas it was approx. 56.0 kDa from tuber sample in (**Fig. 12)**. Such differences may be due to the expression of different forms of the catalase genes in the tissues. Moreover, it may be due to differential post-translational modifications of the catalase. The findings of the study were quiet consistent with the earlier observations as reported in the literature (Duman and Kaya 2013).

4.2 *In-Silico analyses using Catalase sequence*

Catalase is an antioxidant enzyme which protects the cell from oxidative damage by ROS. It is found that very less work has been done in the literature for the *in silico* analysis. So, efforts have been made to find the structure and other biochemical attributes of catalase isoforms.

Sequence analysis, comparison and motif search in different isoforms of *Solanum tuberosum*:

The study focused on the isoform of catalase from *Solanum tuberosum.*

4.2.1 Salient sequence features

Catalase: A 1772-bp mRNA from potato (GenBank Acc.No.: U27082.1), encodes the enzyme catalase. Size of the ORF is 1476 bp consisting of 493 codons including stop codon. Therefore, catalase consists of 492 amino acids. The corresponding GenBank protein id. is AAA80650. This amino acid sequence of catalase was used as reference sequence for *in silco* analysis including protein modeling. Amino acid sequences were retrieved in FASTA format from NCBI and submitted in BLASTp server of NCBI. BLASTp gave the homologous sequences with maximum identity and query coverage, supported the presence of heme binding pocket and tetramer interface, basic properties of catalase. The homologous sequences were used for comparison, characterization and validation of protein models.

4.2.2 BLASTp analysis of catalase isoform

A BLASTp analysis was carried out using catalase GenBank protein id: (AAA80650.1) as a query. The 492 amino acids of catalase showed 98-100 % sequence identity with other catalase isoforms. In most of the cases query coverage was 100 % except *S. tuberosum* cat gene encoding catalase i.e., 99 %. The data generated by BLASTp analysis are presented in **Fig. 14** and **Table 9**

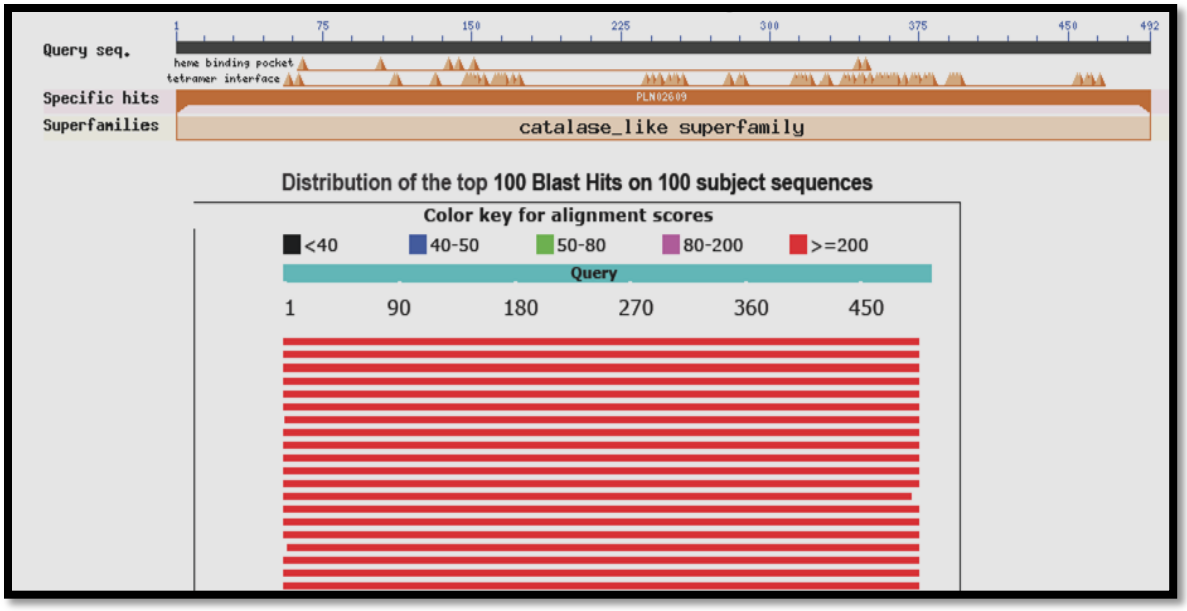


Fig. 14: BLASTp analysis of catalase (U27082)

Table 9: Details of homologous sequences of catalase

Protein name	Accession no.	ORF length	Max Score	Identity%	Query coverage %	Amino acid
Solanum tuberosum catalase (CAT1)	AAA80650.1	1476	3273	100	100	492
Solanum tuberosum catalase (CAT2)	NP_001274863	1476	1017	99	100	492
S.tuberosum cat gene encoding catalase	CAA85470.1	1475	1010	98	99	491
Seet potato mRNA for catalase	CAA29063	1476	3332	100	100	492

4.2.3 Multiple sequence alignment using Constraint-based Multiple Alignment Tool (COBALT)

For multiple sequence alignment (MSA), the amino acid sequences of four catalase isoforms were taken which included *Solanum tuberosum* catalase 1, Seet potato for catalase, *Solanum melongena*, *Solanum tuberosum* catalase 2 using multiple sequence alignment tool COBALT as shown in **Fig . 11** MSA reveals that most of the domains are highly conserved including the N-glycosylation sites. Comparison between the sequences shows variation at approximately 33 sites because of mutations, deletions or insertions as highlighted in the figure. The N-terminal sequences of the catalase isoforms appear to be highly conserved as compared to their variable C-terminal sequences.

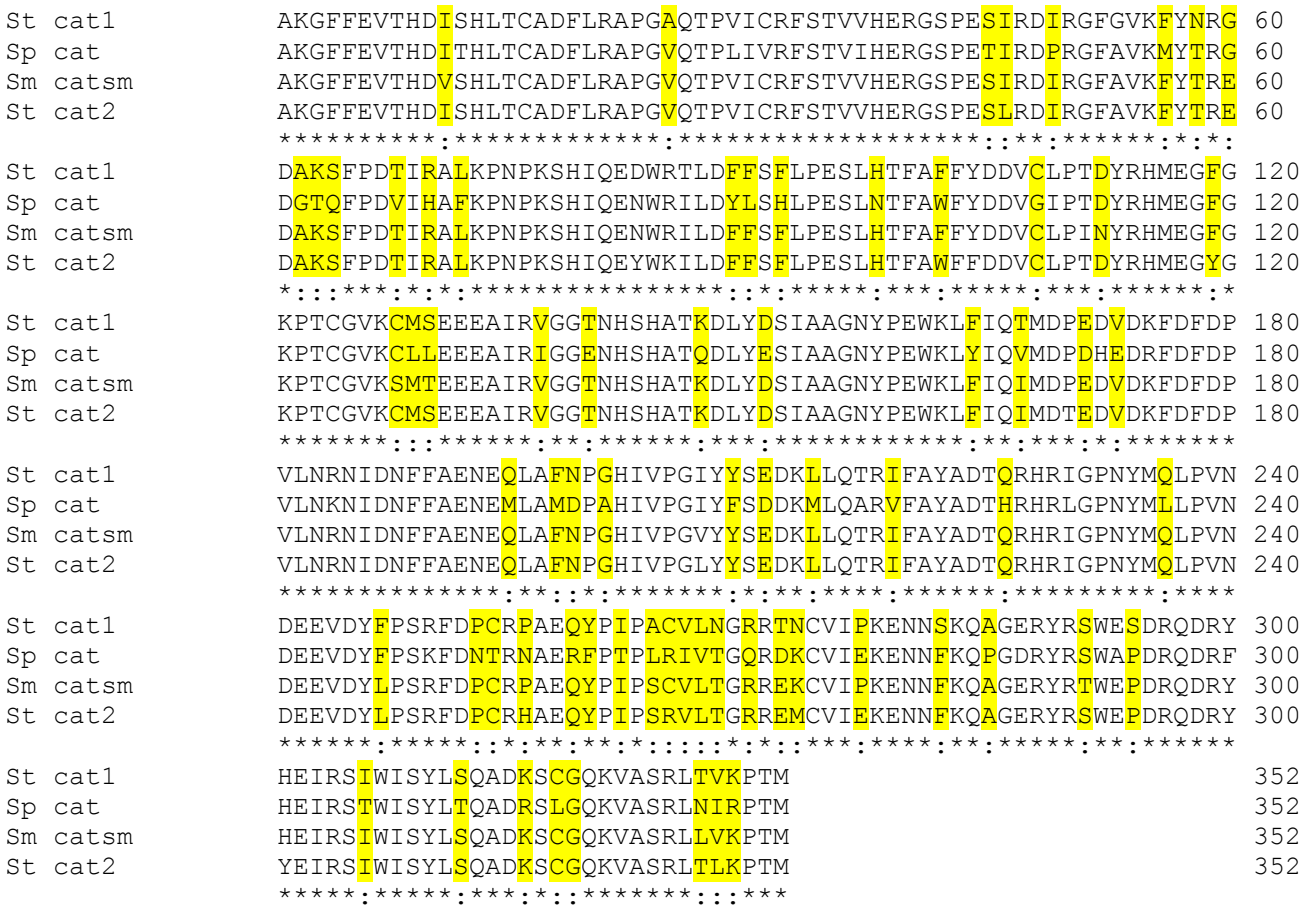


Fig. 15: Multiple sequence alignment of 4 amino acid sequences of five different forms of catalase protein.The alignment is based on COBALT tool. The asterisk (*) symbol denotes the conserved sequences amino acids between all forms; full–stop (.) denotes the amino acids which have divergence from other sequences.

4.2.4 Searching protein motifs

The amino acid sequence of catalase was analysed for prediction of some protein motifs using the tool for MY HITS as shown in **Fig. 12**

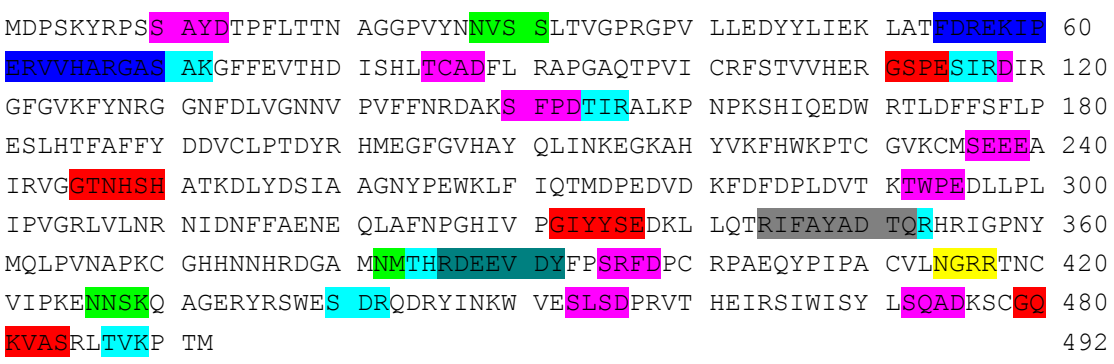


Fig. 12 Amino acid sequence of catalase (GenBank protein id: AAA80650). Some protein motifs are highlighted using different colors.

The position and amino acid sequence of the individual protein motifs are presented in **Table 10**.

Table 10: Motif scan and position of Catalase

S.NO	Site	Sequence	Amino acid sequence	Color
1	*Amidation site*	NGRR	414-417	Yellow
2	*N-glycosylation site*	NVSS NHSH NMTH NNSK	28-31 247-250 382-385 426-429	Bright green
3	*Casein kinase II phosphorylation site*	SAYD TCAD SIRD SFPD SEEE TWPE THRD SRFD SLSD SQAD	10-13 85-88 115-118 150-153 236-239 292-295 384-387 395-398 453-456 472-475	Pink
4	*N-myristoylation site*	GSPESI GTNHSH GIYYSE GQKVAS	111-116 245-250 332-337 479-484	Red
5	*Protein kinaseC Phosphorylation site*	SAK SIR TIR TQR THR SDR TVK	70-72 115-117 154-156 351-353 384-386 440-442 487-489	Turquoise
6	*Tyrosine kinase Phosphorylation site*	RDEEVDY	386-392	Teal
7	*Catalase Proximal heme-ligand*	RIFAYADTQ	344-352	Grey
8	*Catalase proximal active site signature*	FDRERIPERVVHARGAS	54-70	Blue

Likewise, the amino acid sequence of catalase was analyzed for prediction of some protein motifs using the MY HITS as shown in **Fig .10**

Amidation site has receptor reorganization, cyclic AMP Phosphorylation domain which regulates glycogen, sugar and lipid metabolism in the cell. N-glycosylation site are present in the catalase isoforms where sugar molecules could be attached at the specific sites. The types of N-glycans synthesized depends on its accessibility of different enzymes present in cellular components.

It occurs in the selective Asparagine residues. Casein kinases II catalyses the transfer of phosphate to peptide substrate. N-myristoylation site helps in the lipidation modification i.e., in attachment of amide bond to alpha-amino group of N-terminal glycine residue. It plays role in membrane targeting and signal transduction cascade and are involved to environmental stress. Proteinase kinase C enzymes are signal transduction cascade and are involved in controlling function of other proteins through Phosphorylation of hydroxyl groups of serine and threonine amino acid residues. Cell attachment sequence present in isoforms acts as receptor for cell adhesion molecules and cell-cell interaction. Further, biochemical approaches are required to validate the functionality of the individual motifs as predicted in the study.

4.2.5 3-D modeling studies

3-dimensional protein structures for catalase were predicted by using Raptor X. The amino acid FASTA sequences from NCBI were taken and upload in the tool, many pdb files were generated which were further used to find the best model. The best models are given in **Table 11**.

- **X-ray /NMR structure**

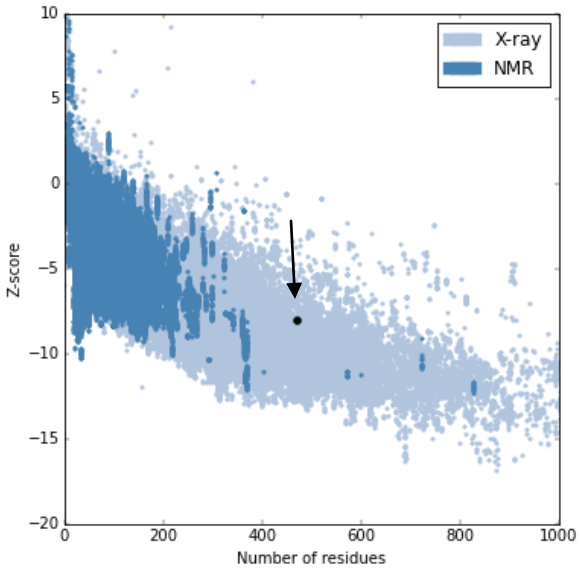
Using ProSA-web tool the protein models validated. For validation, the pdb files were upload on ProSA tool and the following data was obtained as given in Table 11: which depicts the position of protein in the X-ray/NMR region. The predicted is shown by the black colored dot (.) if the protein falls in the region then it is considered as the best model.

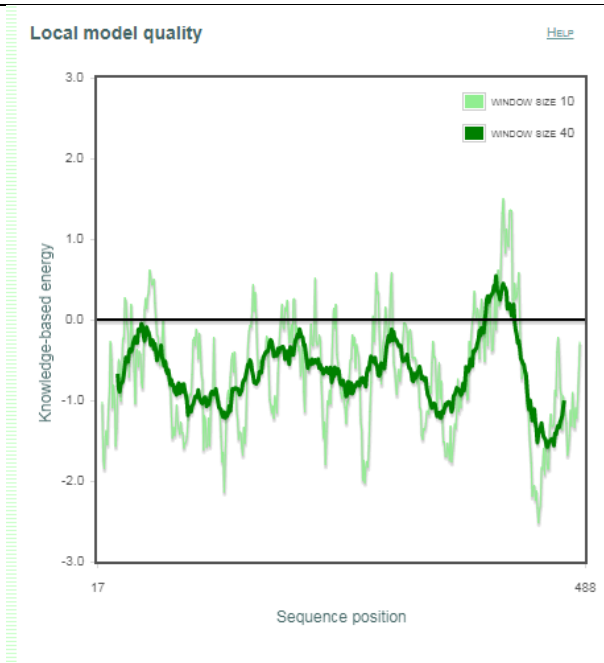
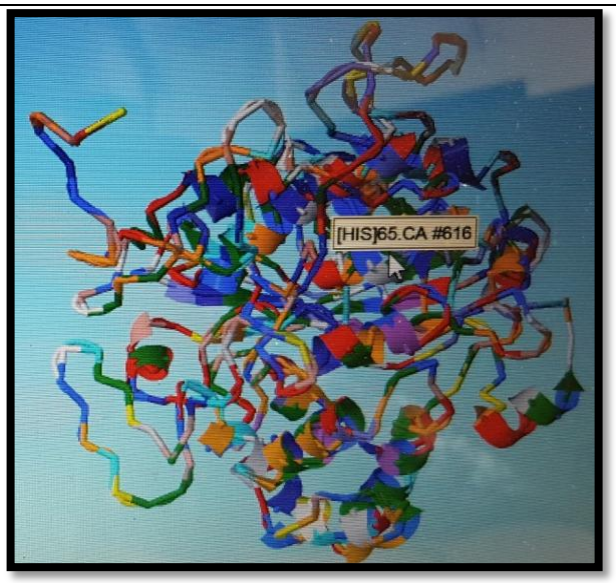
Model quality

An important way of validation of protein model is based on energy level as shown in **Table 11**.

If the energy level of the sequences is less than 0 then it is considered to be the best model. Another way of validation of the protein model is done by checking the z-score. The model with minimum negative z-score is considered to be the best model. So considering all the factors which help in prediction of best model contributes for its selection. Raptor X was used to identify the catalytic site (Histidine at 65 position).

Table 11: Model structure, X-ray NMR structure, model quality and catalytic site of catalase

Features	**Catalase**
Model structure	
X-ray/NMR	

Model quality	
Model showing catalytic site	

4.2.6 Comparison between different isoforms of catalase

4.2.6.1 (a) Biochemical attributes: Table 12 shows the comparison of biochemical attributes of different isoforms of catalase reported in literature and retrieved from SpudDb. (http://solanaceae.plantbiology.msu.edu/integrated_searches.shtml).

Catalase isoforms have some biochemical attributes which play a very important role in the functioning of the proteins.

The comparison between different isoforms of catalase with *Solanum tuberosum* (CAT1) as reference is given in Table 13. The instability index gives the estimation of protein stability in test tube. A weight value comes from this technique which tells about the stability of the protein. Proteins with instability index less than 40 are considered to be stable and the proteins with instability index above 40 are unstable. Here, catalase ranges from 38.73-41.34 telling all the catalase proteins to be stable.

The aliphatic index occupied by aliphatic side chains (A, V, I, L) is considered as positive factor and is defined as relative volume of a protein for the increase of thermal stability of globular proteins. In catalase it was found to be in the range 68.35 – 73.52.

Table 12: Biochemical attributes of different catalase isoforms

Species name	Accession number	Isoelectric point(pI)	Molecular weight (kDa)	Instability index	Aliphatic index
• ***Solanum tuberosum* catalase (CAT1)**	U27082	6.56	56	39	69.15
Solanum tuberosum catalase (CAT1)	NM_001287934.1	6.56	56	40.29	68.35
S.tuberosum cat gene encoding catalase	Z37106	6.58	56	38.13	70.29
Seet potato mRNA for catalase	X05549	6.60	56	37.06	69.35
Solanum tuberosum group Phureja DM1-3 – iTAG	Sotub12g027890.1.1	7.44	50	38.72	72.38
Solanum tuberosum group Phureja DM1-3 – iTAG	Sotub04g035910.1.1	6.93	56	38.17	73.52
Solanum tuberosum group Phureja DM1-3 – PGSC	PGSC0003DMP400017481	6.93	56	38.17	73.52
Solanum tuberosum group Phureja DM1-3 – PGSC	PGSC0003DMP400017481	6.93	56	38.17	73.52
Solanum tuberosum group Phureja DM1-3 – PGSC	PGSC0003DMP400051216	6.56	56	39.40	70.14
Solanum tuberosum group Phureja DM1-3 – iTAG	Sotub02g026140.11	6.73	56	37.73	70.53
Solanum tuberosum group Phureja DM1-3 – PGSC	PGSC0003DMP400051216	6.56	56	39.40	70.14
Solanum tuberosum group Phureja DM1-3 – iTAG	Sotub02g026140.1.1	6.73	56	37.73	70.53
Solanum tuberosum group Phureja DM1-3 – iTAG	Sotub04g035910.1.1	6.93	56	38.17	73.52
Solanum tuberosum group Phureja DM1-3 – iTAG	Sotub12g027890.1.1	7.05	57	38.72	72.38
Solanum tuberosum group Phureja DM1-3 – PGSC	PGSC0003DMP400002845	6.73	56	37.73	70.53

Average occurrence of amino acids

Amino acids which occur on an average in every protein are present in catalase isoform in varied amount. The amino acid composition data show that amino acids such as Valine (6.50), Phenylalanine (5.89), Proline (7.32), Aspartate (7.11), Arginine (6.91), Leucine (6.30) are present in high amount in catalase than they occur at average in plants. Whereas Histidine (3.86) and Glutamine (2.64), Methionine (1.63) occurred less frequently as compared to average occurence.

Table 13: Comparison of amino acids values with their average occurrence

Amino Acid	Average occurrence	Catalase
Phenylalanine	3.9	5.89
Proline	5.2	7.32
Aspartate	5.3	7.11
Leucine	9.1	6.30
Glutamic acid	5.9	2.64
Methionine	5.2	1.63
Histidine	2.3	3.86
Arginine	5.1	6.91

4.2.7. Insilco attributes of catalase isoforms: Comparison is given below

Table 13: Insilico attributes of catalase isoforms

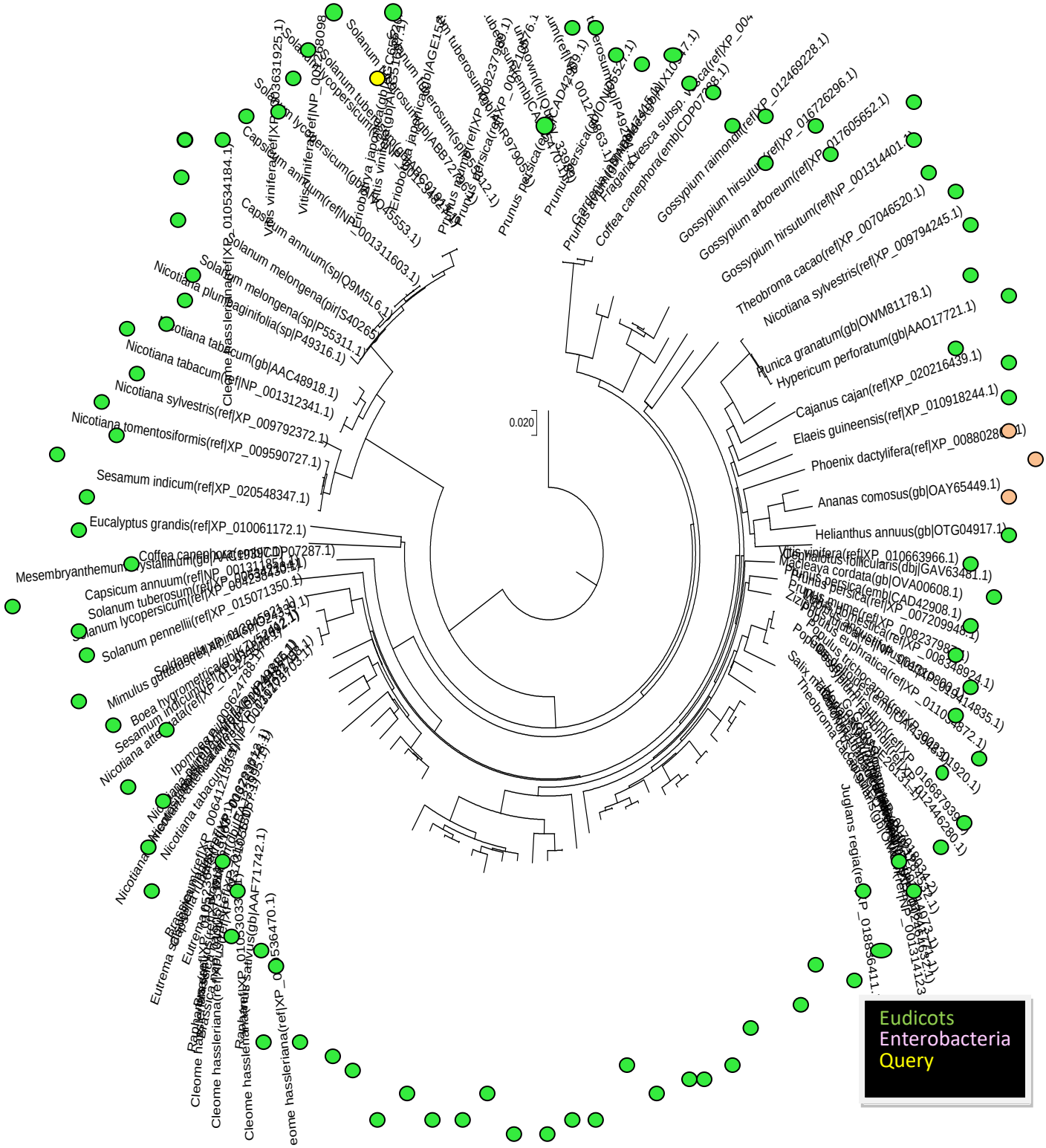
S.no.	Species name	Accession number	Secondary structure composition (%)			Solvent accessibility (%)		
			Loop	Helix	Strand	Exposed	Buried	Interm ediate
1	***Solanum tuberosum catalase (CAT1)***	U27082	63.41	22.97	13.62	40.04	49.39	10.57
2.	***Solanum tuberosum catalase (CAT1)***	NM_00128 7934.1	62.20	24.19	13.62	37.40	51.83	10.77
3.	***Seet potato mRNA for catalase***	X05549	64.02	22.76	13.21	37.80	50.20	11.99
4.	***S.melongena CATSM***	X71653	63.41	23.98	12.60	39.02	48.78	12.20
5.	***Lycopersiconesculentum catalase (cat1)***	M93719	63.82	22.76	13.41	40.04	48.58	11.38

6.	***Nicotiana tabacum Petit Havana SR1 catalase (CAT-1)***	U07627	63.21	24.39	12.40	39.23	49.59	11.18
7.	***Nicotiana tabacum SR1 salicylic acid binding catalase***	U03473	61.96	25.15	12.88	38.65	50.92	10.43
8.	***Solanum tuberosum group Phureja DM1-3 – iTAG***	Sotub12g027890.1.1	65.15	21.19	13.66	42.38	47.92	9.70

The secondary structures such as a-helix, β-sheet, were also predicted with the help of ProtScale tool, in different catalase isoform

4.2.9 Phylogenetic Tree

Phylogenetic tree also known as evolutionary tree is a branched diagram which is used to find the evolutionary relationships between homologous sequences. The sequences joined together imply similarities and common ancestors, whereas, the sequences which have evolved from different root imply divergence. The length of the branches depicts the evolutionary time. There are numerous ways to construct the Phylogenetic tree. Here, neighbour joining method by Mega 7 has been used to construct the Phylogenetic tree for different isoforms of catalase protein of *Solanum tuberosum*. A different color in the Phylogenetic tree depicts different species of the plant which show the evolutionary history. The distance tree created using 99 sequences obtained from NCBI BLASTp using reference potato catalase isoform no U27082 (GenBank ID:U27082.1)


0.020
Eudicots
Enterobacteria
Query

Concluding Remarks

Both biotic and abiotic stress generates Reactive Oxygen Species (ROS) in the living beings. To combat the harmful effects of the ROS living beings develop many mechanisms. To neutralize the hydrogen peroxide generated in side the cell, catalases are most reliable as they convert it into useful products (water and oxygen). So, to understand the mechanism and to estimate its activity becomes more interesting for the biologist. Although it is a hundred year old enzyme but its literature on plants is minimal and no comprehensive report is present on Indian potato cultivars. So, in this context this study is quite relevant. In this study, catalase was estimated from various tissues at different stages from different commercially important Indian potato cultivars, and both enzyme activity per gram and specific activity were calculated. Partial purification of protein from source and sink tissue was done. This helped not only to distinguish genotypic variations between these cultivars, but also suggested the presence of allelic variants of catalase isoforms. Potato Moreover, it helped to know about the salient sequence features. Some conserved amino acids were found by comparing the different isoforms from database. Such type of information is not only helpful in understanding the evolutionary consequences but also important with regard to gene manipulation and protein engineering. In conclusion, this report made a consolidated base for further studying the expression and activity and structure and function of catalase genes in potato.

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