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# Computational Characterization of MIPS in *Camellia sinensis* and it's Phylogenetic Implication

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**Abstract**

Myo-inositol-1-phosphate synthase (MIPS) is a fundamental enzyme in sugar metabolism pathway, related to inositol in all living system. Inositol and its derivative compounds are involved in different signaling pathways and stress tolerance. Plant MIPS are dynamic in signal transduction, growth regulation, osmo regulation, abiotic and biotic stress response etc. Present study targets an *in silico* characterization of the MIPS from tea and estimate its phylogenetic status. The physicochemical characters and predicted quaternary structure of the protein had been analyzed using different bioinformatics tools. Predictive sub cellular localization is in line with the earlier works. All sequences were 80-90% identical among themselves, however MIPS3 isoform from *Arabidopsis thaliana* had larger similarity with tea MIPS than those of MIPS1 and 2. In a number of sites tea MIPS and ATMIPS3 had similar residue and different from other two, whereas in few sites tea had unique residue composition as well. More detailed analysis would help to understand the impact of single residue changes at specific site of differential function of the protein and its role in basic or stress compatibility of tea plant.

**Keywords:** *Camellia sinensis*; MIPS; Phylogenetic analysis; Physicochemical properties; Quaternary structure

## Introduction

Myo-inositol-1-phosphate synthase (MIPS) is key enzyme in the synthesis of inositol related sugar metabolism in all living system. Inositol and its derived compounds are involved in different signaling pathways like, auxin transport, stress tolerance [1], oligosaccharide synthesis [2-4], cell death regulation, cell wall biosynthesis [5-9]. In plants, they play active role in signal transduction, growth regulation, osmoregulation, abiotic and biotic stress response etc [10-12]. Especially, they possess salt tolerance ability through protection of cellular structures from reactive oxygen species and control cellular turgor pressure [13,14]. However crystallographic structure of MIPS from higher plants, particularly in angiosperms, is still yet to be conclude about the structre-function relationship and its variation among different members. Recently *in silico* methods to determine structural and functional characteristics of a gene, transcript or protein is being accelerated to avoid of the wet lab experimental limitations and it works too [15-19]. Tea plant, *Camellia sinensis* (L.) O. Kuntze, is the potential source of health beverage and most popular non-alcoholic drink across the world and being an evergreen shrub it grows well in diverse habitat and wide range of environment [20].The potential of its existing stress combating ability in some verities is the point of interest of the scientist to understand its mechanism and in progress in future cultivars. Present study aims to characterize *in silico* an important stress related protein, MIPS from tea and its phylogenetic status.

## Methods

Complete amino acid sequence of *Camellia sinensis* MIPS (Accession No. AJO70149.1) was retrieved through BLASTp program [21] at NCBI using a previously reported homolog from *Porteratia coarctata* [10] as query sequence. *Camellia sinensis* MIPS was computationally predicted via homology modelling with the python based modeler 9.12 tool [22] after selecting the most suitable template at protein data bank (Template id- 1LA2, Source - *Saccharomyces cerevisiae*). Essential ligand NAD+ was allowed to bind at each chain of the homotetrameric protein. The resultant pdb model was analysed for it's *in silico* quality checking and physicochemical property estimation. Using different bioinformatics tools Ramachandran plot [23], Z-score [24], subcellular localization [25], half-life, instability and aliphatic index [26] were estimated.

For a phylogenetic insight corresponding MIPS sequence was compared with the all annotated MIPS variety of *Arabidopsis thaliana* available at TAIR10 [27]. Multiple sequence alignment file was observed with the Boxshade server and represented accordingly. Multiple sequence alignment among the selected varieties was manually examined and a neighbor joining tree was drawn with the help of MEGA 7.0 software [28] to know about its isoform similarity. Relative divergent time was also calculated within the same tool.

## Result and Discussion

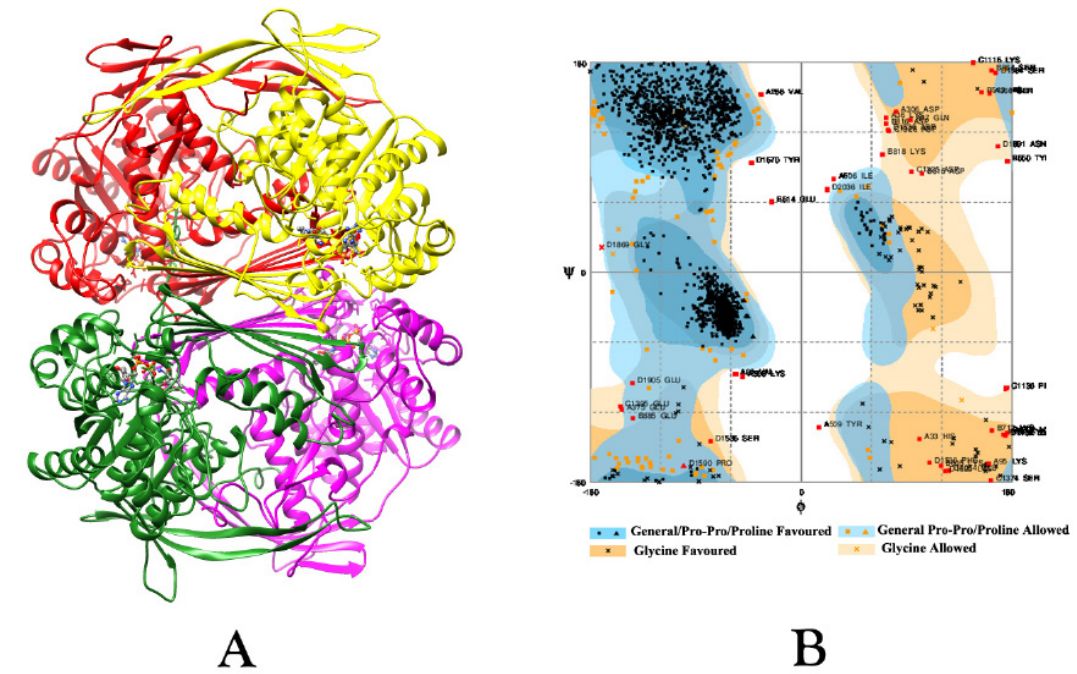


**Figure 1A-1B:** Predicted quaternary structure of MIPS from *Camellia sinensis* (A. Ribbon illustration visualized by Chimera, B. Corresponding Ramachandran Plot).

The BLAST returned homolog of MIPS isoform in tea was composed of 510 amino acid residues as found in others. Such polypeptide chain has an estimated half-life of 30 hours (mammalian reticulocytes, *in vitro*), >20 hours (yeast, *in vivo*) and >10 hours (*Escherichia coli*, *in vivo*) with the extinction coefficient is 52955$M^{-1}cm^{-1}$, at 280nm measured in water, Absorbance 0.1% (=1g/l)0.942, assuming all pairs of Cys residues form cystines and extinction coefficient is 52830$M^{-1}cm^{-1}$, at 280nm measured in water, Abs 0.1% (=1g/l) 0.940, assuming all Cys residues are reduced. The instability index is computed to be 31.59 which indicates the protein is stable. Moreover, the aliphatic index 90.92 and Grand average of hydropathicity (GRAVY) was calculated as -0.163. Quaternary structure of the protein was predicted using ligand modelling in Modeller 9.12 and visualized in Chimera [29] (Figure 1A). Ramachandran Plot analysis (Figure 1B) shown 93.4% of total number of residues in favored region and 4.4% in allowed region. Same trend of scores was found in earlier study conducted with modelled MIPS protein among different group of plants [30]. The z-score indicates overall model quality is displayed in a plot that contains the z-scores of all experimentally determined protein structures from different sources (X-ray, NMR) [24]. In predicted tea MIPS the value is calculated as -7.9 which also within the range of scores typically found for native proteins of similar size (Figure 2). According to CELLO prediction the protein was predicted to be localized in Periplasmic and Cytoplasmic regions which is similar with earlier report of its localization [4,14].

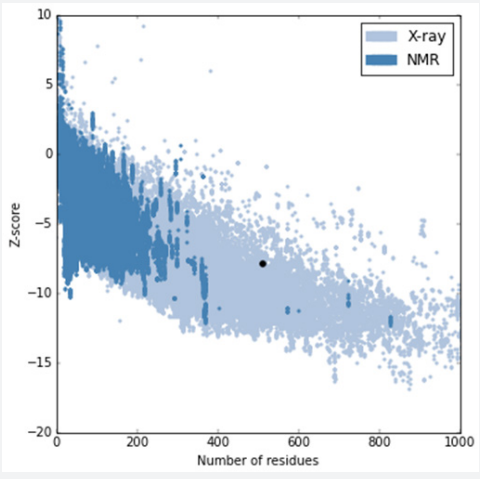


**Figure 2:** Screenshot of ProSA-web z-score plot.


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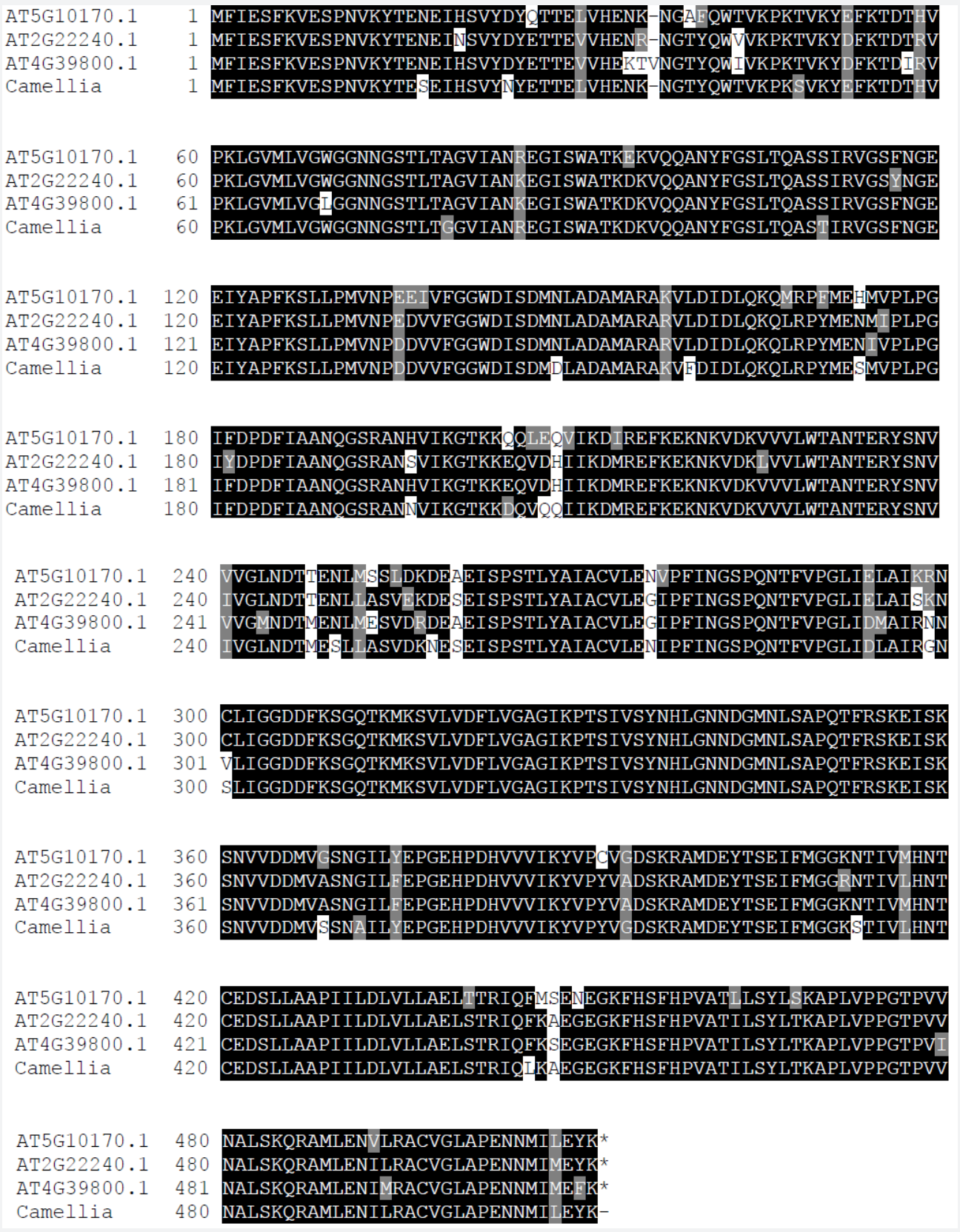


**Figure 3:** Multiple sequence alignment among *Arabidopsis thaliana* MIPS1, MIPS2,MIPS3 and *Camellia sinensis* MIPS visualized with the help of Boxshadeserver.



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Multiple sequence alignment of *Arabidopsis thaliana* MIPS varieties with the model protein in present study (Figure 3) revealed some important findings. All sequences were 80-90% identical among themselves, however MIPS3 isoform had larger similarity with tea MIPS than other two. In a number of sites tea MIPS and ATMIPS3 had similar residue which largely differ from other two, whereas in few sites tea had unique residue composition too. It is also reflected in the phylogenetic tree (Figure 4) where tea MIPS and ATMIPS3 belonged to same clade and has common ancestor to the divergent MIPS1 and MIPS2 isoforms. According to relative divergence time scale MIPS1 and MIPS2 clade took higher time to evolve than that of MIPS3 clade and tea MIPS is much primitive than MIPS3 itself. More detailed analysis would be of help to understand the impact of single residue changes at specific site of differential function of the protein and its role in basic or stress induced state of the tea plant.

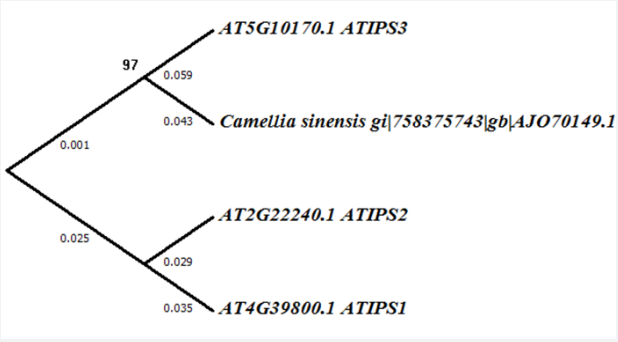


**Figure 4:** Phylogenetic tree analyzed by Maximum Likelihood method using MEGA 7.0.

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