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L- *Myo*-Inositol 1-Phosphate Synthase (MIPS) in Chickpea: Gene Duplication and Functional Divergence

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1. Introduction

Gene duplication is one of the key driving forces in the evolution of genes and important features of genomic architecture of living organisms including plants. Moreover, much of the plant diversity may have arisen largely due to duplication, followed by divergence and adaptive specialization of the pre existing genes (Ohno,1970; Zhang, 2003; Flagel & Wendel,2009). Current impetus on genomic sequence data provides substantial evidence for the profusion of duplicated genes in all organisms surveyed. Functional divergence after gene duplication can possibly result in two alternative evolutionary fates: i) neofunctionalization where one copy acquires an entirely new function whereas the other copy maintains the original function. ii) Subfunctionalization, in which each copy adopts part of the task of their parental gene (Ohno,1970; Nowak et al., 1997; Jenesen,1976; Orgel,1977;Hughes,1994). However, subfunctionalization is reported as a more prevalent outcome than neofunctionalization in nature. In any case, functional divergence of such paralogous proteins is found to be the key force shaping molecular network in organisms (Ohno, 1970). Recent studies also suggest that duplicate genes diverge mostly through the partitioning of gene expression as in subfunctionalization (Force et al.,1999; Wagner,2000; Gu et al.,2002). In addition, subfunctionalization can also take place at the protein function level leading to functional specialization, when one of the duplicated genes becomes better at performing one of the original functions of the progenitor gene (Hughes, 1994; Gu et al.,2002; Conant &Wolfe, 2008; Hughes, 1999; Zhang et al., 2002).

Myo-inositol-1-phosphate synthase (MIPS;EC5.5.1.4) is an evolutionary conserved enzyme which catalyzes the rate limiting step in well conserved inositol biosynthetic pathway and is extremely widespread in living organisms including plants (Loewus & Murthy, 2000; Majumder et al., 2003). The evolution of MIPS gene/ protein among the prokaryotes seems to be more divergent and complex than amongst the eukaryotes, however they preserve a conserve core catalytic domain among the MIPS proteins (Majumder et al., 2003).

Many of the plant species are known to contain more than one copy of gene encoding MIPS and are hypothesized to arise through gene duplication. Expression studies of multiple gene encoding MIPS have revealed the possibility of specialized role for individual enzyme isoforms. Previously, two genes encoding MIPS have been identified and characterized from chickpea by Kaur *et al.* A comparative study of two divergent genes (*CaMIPS1* & *CaMIPS2*)

reveals features of both functional redundancy and diversification (Kaur et al., 2008). This chapter explores how a possible gene duplication of MIPS gene in chickpea lead to a functional diversification that perhaps contributed adaptive evolution to the plant.

2. Gene duplication and functional divergence of MIPS in chickpea

2.1 Evolution and diversification of MIPS

The inositols are the nine isomeric forms of cyclohexane hexitols and *myo*-inositol is the most abundant and physiologically favored molecule in the biological system.

The biosynthesis of *myo*-inositol has been acknowledged as an evolutionary conserved pathway and its importance across biological organisms from different domains of life has been recognized for long time. The first and rate limiting step of this pathway is catalyzed by an evolutionary conserved enzyme named as MIPS.

MIPS particularly catalyzes the conversion of glucose 6- phosphate (Glc6P) to *myo* inositol 1-phosphate (Ins1P) through an internal oxidoreduction reaction involving NAD^+. Subsequently inositol 1- phosphate is dephosphorylated to produce free inositol by Mg^{2+} dependent *myo*- inositol 1- phosphate phosphatase (IMP: EC 3.1.3.25) (Loewus & Loewus,1983;Loewus,1990). This free *myo* inositol occupies the central position in inositol metabolism since this free inositol can be chanellized to various metabolic routes and produce different inositol derivatives (Fig-1) (Loewus & Murthy, 2000; Loewus,1990).

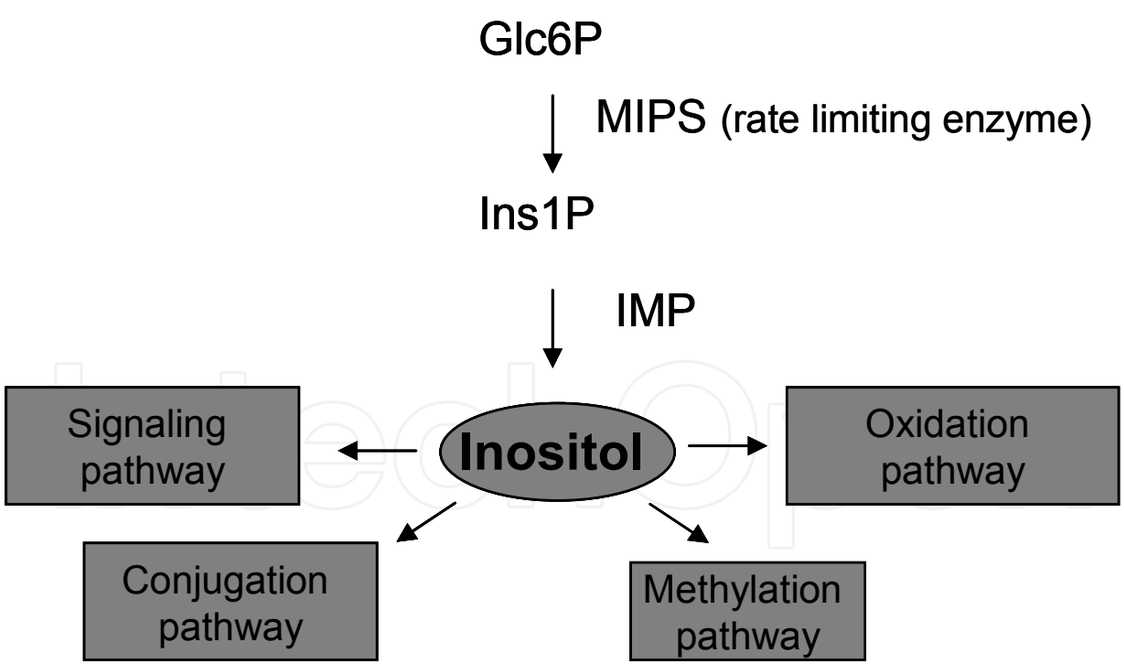


Fig. 1. Inositol biosynthesis and its consumption in other pathway.

This free inositol and its derivatives have acquired diverse functions over the course of evolution. As for example, inositol containing phospholipids are the important constituents of many archaea. Few thermophilic archaea also use inositol phosphodiester as thermo protective solutes. Then with the emergence and diversification of eukaryotes, function of

inositol and its derivatives proliferated dramatically. So far, inositol and its derivatives have been shown to be involved in growth regulation, membrane biogenesis, hormone regulation, signal transduction, pathogen resistance and stress adaptation in higher plants (Loewus & Murthy, 2000; Stevenson et al., 2000; Michell,2008).

Since the usage and distribution of inositol and inositol derivatives are reported in all domains of life, it is imperative to contain MIPS enzyme in diverse organisms such as archaea, eubacteria, parasites, animals, higher plants and many others. Few higher plants and algae are reported to have both cytosolic and chloroplastic isoforms of MIPS. However, the biochemical and enzymatic properties of these two forms do not differ significantly between each other. Recent studies suggest that rice chloroplastic MIPS is coded by *OsINO1-1* gene located on chromosome 3 (RayChaudhuri et al.1997;Ray et al., 2010).

The structural gene coding (*INO1*) for this ancient enzyme was first identified and cloned in *Saccharomyces cerevisiae* (Donahue & Henry,1981). Subsequently more than 80 *INO1* genes were reported from various sources including both prokaryotes and eukaryotes.

Evolution and diversification of MIPS has been highlighted by Majumder et al. (2003) and a clear difference between prokaryotic and eukaryotic MIPS protein sequences was observed when compared among each other. The MIPS protein sequences of prokaryotes are quite divergent among themselves and significantly distinct than any other known eukaryotic sequences. In contrast, the eukaryotic MIPS sequences show remarkable similarities among each other. A phylogenetic tree constructed to include few representative MIPS sequences from diverse organisms present an overall evolutionary divergence of this enzyme in the biological kingdom. The higher plants constitute one close subgroup, while the higher animals, protozoa, fungi form the other subgroups in the eukaryotic cluster (Fig-2). In Archeoglobus, MIPS shows more sequences similarity to the eukaryotic MIPS than the other known prokaryotic ones and thereby all eukaryotic MIPS seems to have evolved from one common stock, probably from the fusion of an archaebacterial and eubacterial MIPS genes (Fig-2 & 3).

Four stretches of amino acid residues (GWGGNG, LWTANTERY, NGSPQNTFVPGL and SYNHLGNNDG) are found to be conserved in MIPS proteins of all eukaryotes and among them; SYNHLGNNDG is identified as highly conserved. Interestingly among higher plants, MIPS enzyme shows greater conservation in addition to these four domains (Fig-3). Many of the plant species also possess multiple genes encoding MIPS and are thought to arise through gene duplication in course of time.

Subsequent analysis of crystal structures of various MIPS proteins provide ample evidence towards the presence of conserved "core structure" in all MIPS proteins throughout evolution. Moreover, some of the important amino acid residues are identified in the active site of the yeast MIPS and are shown to be highly conserved in all eukaryotic MIPS. These amino acids are considered to be the part of a "eukaryotic core structure" which has remained largely the same during evolution, despite the divergence in rest of the sequences over time (Fig-3) (Stein & Geiger, 2002; Norman et al.,2002).

Crystal structure analysis of MIPS from *Saccharomyces cerevisiae* also revealed that each monomer of the homo-tetrameric MIPS has three functionally important structural domains namely the NAD binding Rossman fold, the catalytic binding site and the core domain. This study also exemplifies a case of induced fit model for binding of the substrate with the catalytic domain of the enzyme. (Stein & Geiger, 2002)

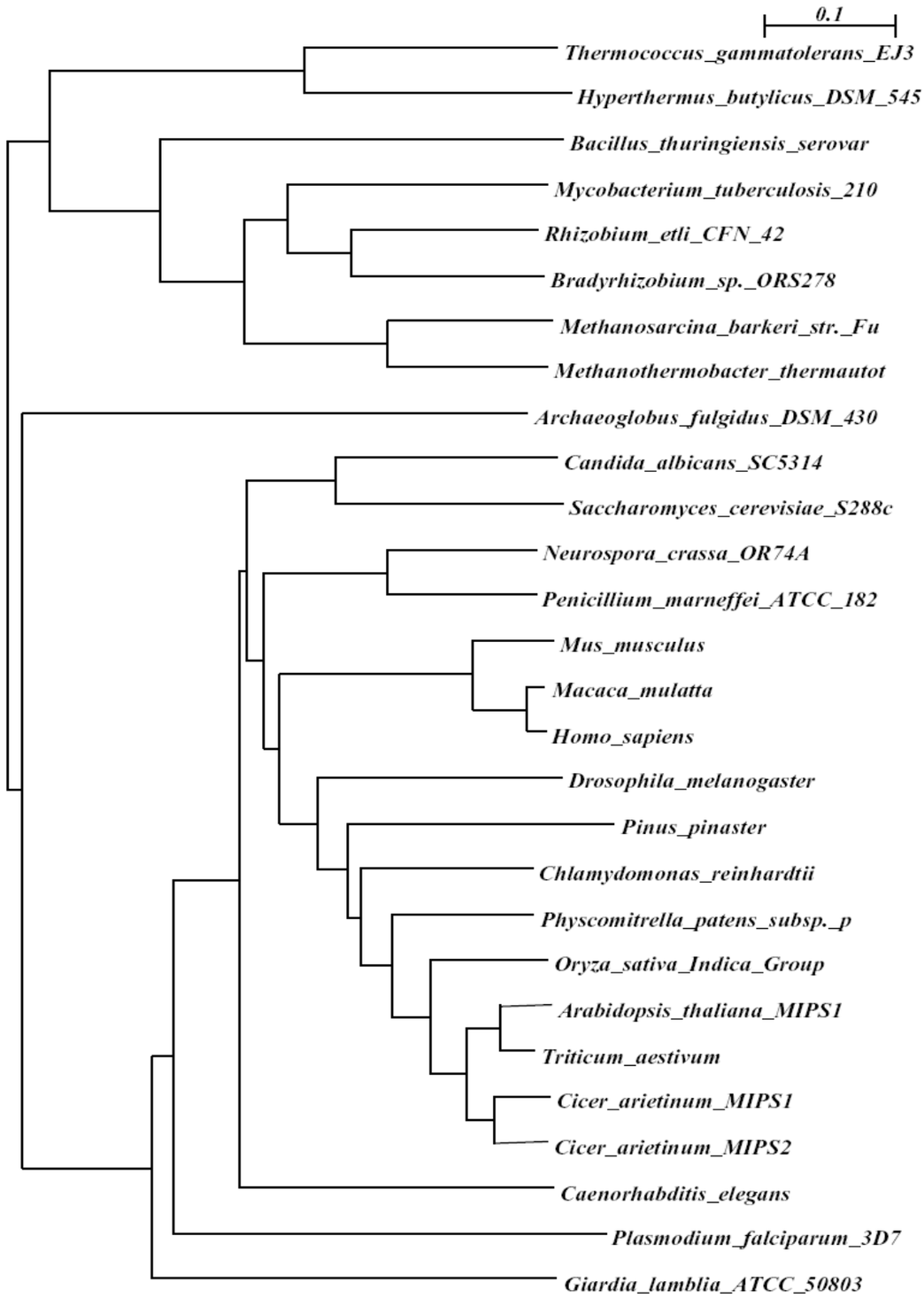


Fig. 2. A phylogenetic tree of few representative MIPS amino acid sequences from various domains of living organisms. Neighbour-Joining algorithm was used to construct tree from the distance matrix using Clustal X. Thousand rounds of bootstrapping were performed to ensure the validity of the tree.

```
CaMIPS1         ------MFIENFKVDSPNVKYTETEIQSVYNYETTELVHENRNGTYQWIVKPKTVKYEFK
CaMIPS2         ------MFIESFKVESPNVKYTDTEIQSVYSYETTELVHENRNNTYQWVVKPKTIKYEFK
OsMIPS          ------MFIESFRVESPHVRYGAAEIESDYQYDTTELVHESHDGASRWIVRPKSVRYNFR
HsMIPS          -----MEAAAQFFVESPDVVYGPEAIEAQYEYRTTRVSREG----GVLKVHPTSTRFTFR
ScMIPS          MTEDNIAPITSVKVVTDKCTYKDNELLTKYSYENAVVTKTAS---GRFDVTPTVQDYVFK
PfMIPS          ------------------------------------------------------------
MtMIPS          ------------------------------------------------------MSEHQS
AfMIPS          ------------------------------------------------------------

CaMIPS1         TDTHVP-KLGVMLVGWGGNNGSTLTGGVIANREGISWATKDNIQQANYFGSLTQASATRV
CaMIPS2         TQTHVP-KLGVMLVGWGGNNGSTLTGGVIANREGISWATKDKIQQSNYFGSLTQASAIRV
OsMIPS          TTTTVP-KLGVMLVGWGGNNGSTLTAGVIANREGISWATKDKVQQANYYGSLTQASTIRV
HsMIPS          TARQVP-RLGVMLVGWGGNNGSTLTAAVLANRLRLSWPTRSGRKEANYYGSLTQAGTVSL
ScMIPS          LDLKKPEKLGIMLIGLGGNNGSTLVASVLANKHNVEFQTKEGVKQPNYFGSMTQCSTLKL
PfMIPS          --------MVRVAIIGQGYVASIFAVGLERIKE----------GELGYYG----------
MtMIPS          LPAPEASTEVRVAIVGVGNCASSLVQGVEYYYN--------ADDTSTVPG----------
AfMIPS          --------MKVWLVGAYGIVSTTAMVGARAIERGIAPKIGLVSELPHFEG----------
                                :    .                      *

CaMIPS1         GSFQ-GEEIYAPFKSLLPMVNPDDIVFGGWDISDMNLADAMARA-RVFDIDLQKQLRPYM
CaMIPS2         GSFQ-GEEIYAPFKSLLPMVNPEDIVWGGWDINNMNLADAMGRA-RVFDIDLQKQLRPYM
OsMIPS          GSYN-GEEIYAPFKSLLPMVNPDDLVFGGWDISNMNLADAMTRA-KVLDIDLQKQLRPYM
HsMIPS          GLDAEGQEVFVPFSAVLPMVAPNDLVFDGWDISSLNLAEAMRRA-KVLDWGLQEQLWPHM
ScMIPS          GIDAEGNDVYAPFNSLLPMVSPNDFVVSGWDINNADLYEAMQRS-QVLEYDLQQRLKAKM
PfMIPS          ----------IPLANELPIKVEDIKIVASYDVDKTKIGLPLSEI-VQRYWKGNVPESLQE
MtMIPS          ----------LMHVRFGPYHVRDVKFVAAFDVDAKKVGFDLSDA-IFASENNTIKIADVA
AfMIPS          ------------IEKYAPFSFEFGGHEIRLLSNAYEAAKEHWELNRHFDREILEAVKSDL
                                  *              .  .

CaMIPS1         ESMVPLPGIYDPDFIAANQGDRANNVIKGTKR---------EQINQIIKDIKEFKEANKV
CaMIPS2         ESMVPLPGIYDPDFIAANQGDRANNVINGTKK---------EQLQQIIKDIKEFKEASKI
OsMIPS          ESMVPLPGIYDPDVIAANQGSRANNVIKGTKK---------EQMEQIIKDIREFKEKSKV
HsMIPS          EALRPRPSVYIPEFIAANQSARADNLIPGSRA---------QQLEQIRRDIRDFRSSAGL
ScMIPS          SLVKPLPSIYYPDFIAANQDERANNCINLDEKGNVTTRGKWTHLQRIRRDIQNFKEENAL
PfMIPS          VFVRKGIHLGSLRNLPIEATGLEDEMT---------------LKEAIERLVEEWKEKKVD
MtMIPS          PTNVIVQRGPTLDGIGK-----------------------------YYADTIELSDAEPV
AfMIPS          EGIVARKGTALNCGSGIKELGDIKTLEGEGLS-----------LAEMVSRIEEDIKSFAD
                                                                  :  .

CaMIPS1         DRVVVLWTANTERYSNLVVGLNDTMENLFAAVDRNE-SEISPSTLFAIACVTENVPFING
CaMIPS2         DKVVVLWTANTERYSNVVVGLNDTMENLLASVDKNE-AEISPSTLYALACVLENVPFING
OsMIPS          DKVVVLWTANTERYSNVCVGLNDTMENLLASVDKNE-AEISPSTLYAIACVMEGIPFING
HsMIPS          DKVIVLWTANTERFCEVIPGLNDTAENLLRTIELG--LEVSPSTLFAVASILEGCAFLNG
ScMIPS          DKVIVLWTANTERYVEVSPGVNDTMENLLQSIKNDH-EEIAPSTIFAAASILEGVPYING
PfMIPS          VIINVPTTEAFTPFGKLEELEKAIKDNNKERLTATQ-AYAYAAAQYAKE--VGGAAFVNA
MtMIPS          DVVQALKEAKVDVLVSYLPVGSEE-----------------ADKFYAQCAIDAGVAFVNA
AfMIPS          DETVVINVASTEPLPNYSEEYHGSLEGFERMIDEDRKEYASASMLYAYAALKLGLPYANF
                    .          .                        .   :*      . .: *

CaMIPS1         SP-QNTFVPGLIDLAIKRNTLIGGDDFK--SGQTKMKSVLVDFLVGAGIKPTSIVSYNHL
CaMIPS2         SP-QNTFVPGLIDLAIQRNSLIGGDDFK--SGQTKMKSVLVDFLVGAGIKPTSIVSYNHL
OsMIPS          SP-QNTFVPGLIDLAIKNNCLIGGDDFK--SGQTKMKSVLVDFLVGAGIKPTSIVSYNHL
HsMIPS          SP-QNTLVPGALELAWQHRVFVGGDDFK--SGQTKVKSVLVDFLIGSGLKTMSIVSYNHL
ScMIPS          SP-QNTFVPGLVQLAEHEGTFIAGDDLK--SGQTKLKSVLAQFLVDAGIKPVSIASYNHL
PfMIPS          IPTLIANDPAFVELAKESNLVIFGDDGA--TGATPLTADILGHLAQRNRHVLDIVQFNIG
MtMIPS          LPVFIASDPVWAKKFTDAGVPIVGDDIKSQVGATITHRVLAKLFEDRGVQLDRTMQLNVG
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AfMIPS          TPSPGSAIPALKELAEKKGVPHAGNDGK--TGETLVKTTLAPMFAYRNMEVVGWMSYNIL
                 *   :  *   .          *:*     * *     :   :   .       . *

CaMIPS1         GNNDGMNLSAPQTFRSKEISKSNVVDDMVNSNGILY--APGEHPDHVVVIKYVPYVGDSK
CaMIPS2         GNNDGMNLSAPQTFRSKEISKSNVVDDMVNSNAILY--QPGEHPDHVVVIKYVPYVADSK
OsMIPS          GNNDGMNLSAPQTFRSKEISKSNVVDDMVSSNAILY--ELGEHPDHVVVIKYVPYVGDSK
HsMIPS          GNNDGENLSAPLQFRSKEVSKSNVVDDMVQSNPVLY--TPGEEPDHCVVIKYVPYVGDSK
ScMIPS          GNNDGYNLSAPKQFRSKEISKSSVIDDIIASNDILYNDKLGKKVDHCIVIKYMKPVGDSK
PfMIPS          GNTDFLALTDKERNKSKEYTKSSVVEDILG----------YDAPHFIKPTGYLEPLGDKK
MtMIPS          GNMDFLNMLERERLESKKISKTQAVTSNLKR-------EFKTKDVHIGPSDHVGWLDDRK
AfMIPS          GDYDGKVLSARDNKESKVLSKDKVLEKMLG----------YSPYSITEIQYFPSLVDNK
                *: *   :      .**  :* . : . :                    :.  : * *

CaMIPS1         RAMDEYTSEIFMGGKSTIVLHNTCEDSLLAAPIILDLVLLAELSTRIQFKSEA-----EN
CaMIPS2         RAMDEYISEIFMGGKNTIVLHNTCEDSLLAAPIILDLVLLAELSTRIQFKSQH-----ED
OsMIPS          RAMDEYTSEIFMGGKSTIVLHNTCEDSLLAAPIILDLVLLAELSTRIQLKAEG-----EE
HsMIPS          RALDEYTSELMLGGTNTLVLHNTCEDSLLAAPIMLDLALLTELCQRVSFCTDM-----DP
ScMIPS          VAMDEYYSELMLGGHNRISIHNVCEDSLLATPLIIDLLVMTEFCTRVSYKKVDPVKEDAG
PfMIPS          FIAMHIEYISFNGARDELIIAGRINDSPALAGLLVDLARLGKIAVDK------------K
MtMIPS          WAYVRLEGRAFGDVPLNLEYKLEVWDSPNSAGVIIDAVRAAKIAKDRGIG----------
AfMIPS          TAFDFVHFKGFLGKLMKFYFIWDAIDAIVAAPLILDIARFLLFAKKKGVKG
                          : .    :       *:     :::*      :.

CaMIPS1         KFHTFHPVATILSYLTKAPLVPPGTPVVNALSKQRAMLENIMRACVGLAPENNMILEYK-
CaMIPS2         KFHSFHPVATILSYLTKAPLVPPGTPVVNALSKQRAMLENILRACVGLAPENNMILEYK-
OsMIPS          KFHSFHPVATILSYLTKAPLVPPGTPVVNALAKQRAMLENIMRACVGLAPENNMILEYK-
HsMIPS          EPQTFHPVLSLLSFLFKAPLVPPGSPVVNALFRQRSCIENILRACVGLPPQNHMLLEHKM
ScMIPS          KFENFYPVLTFLSYWLKAPLTRPGFHPVNGLNKQRTALENFLRLLIGLPSQNELRFEERL
PfMIPS          ---EFGTVYPVNAFYMKNPGPKEAKNIPRIIAYEKLRQWAGLPPRYL-------------
MtMIPS          -----GPVIPASAYLMKSPPEQLPDDIARAQLEEFIIG----------------------
AfMIPS          -------VVKEMAFFFKSPMDTNVINTHEQFVVLKEWYSNLK------------------


CaMIPS1         --------------------------------------------------
CaMIPS2         --------------------------------------------------
OsMIPS          --------------------------------------------------
HsMIPS          ERPGPSLKRVGPVAATYPMLNKKGPVPAATNGCTGDANGHLQEEPPMPTT
ScMIPS          L-------------------------------------------------
PfMIPS          --------------------------------------------------
MtMIPS          --------------------------------------------------
AfMIPS          --------------------------------------------------
```

Fig. 3. Multiple sequence alignment of MIPS from prokaryotes and eukaryotes. Proposed common active site amino acid residues for the *Mycobacterium tuberculosis* and *Saccharomyces cerevisiae* MIPS sequence are highlighted in green color and four conserved domains of eukaryotes (GWGGNG, LWTANTERY, NGSPQNTFVPGL and SYNHLGNNDG) are highlighted in yellow color. 43 variant positions between CaMIPS1 and 2 have been highlighted in blue color.

2.2 MIPS from chickpea: A case of functional divergence

Chickpea is an annual self- pollinated diploid legume crop which is mostly grown in the arid and semi arid regions of the world. Long term evolution and adaptation to harsh conditions make chickpea rich in resistant genes for environmental stresses including drought and cold. Several classes of genes controlling potential resistance have been

identified through genomic and proteomic studies (Ahmaed et al., 2005; Mantri ,2007; Bhusan et al., 2007).

In this particular plant, inositol seems to play an important role in drought tolerance besides growth and development, since inositol content and MIPS transcript was found to be significantly increased under dehydration condition (Boominathan et al.,2004). Subsequently, chickpea is reported to have two MIPS coding genes (*CaMIPS1 and CaMIPS2*) (Kaur et al. 2008) and both genes are revealed to have overall similar structure consisting of 9 introns and 10 exons (Fig-4). Sequence analysis of these two genes show high similarity (>85%) in their coding regions but their non-coding or 5' and 3' flanking regions are extremely divergent. Moreover length of each exon is similar between these two genes while the size of introns varies. Such findings suggest that these two MIPS genes most likely arose by ancestral gene duplication and have undergone considerable sequence divergence.

In spite of the remarkable resemblance in their coding sequences, some base substitutions occurs in exons leading to changes in 43 amino acids in protein sequences, however, maintaining four highly conserved functional domains and known active site amino acids of MIPS (Fig-3) (Majumder *et al.* 2003). Among these 43 amino acids, 19 amino acids differ considerably between CaMIPS1 and CaMIPS2 while rest of the amino acid substitutions are relatively insignificant, i.e. substitution between amino acids having similar physico chemical properties (Kaur et al., 2008).

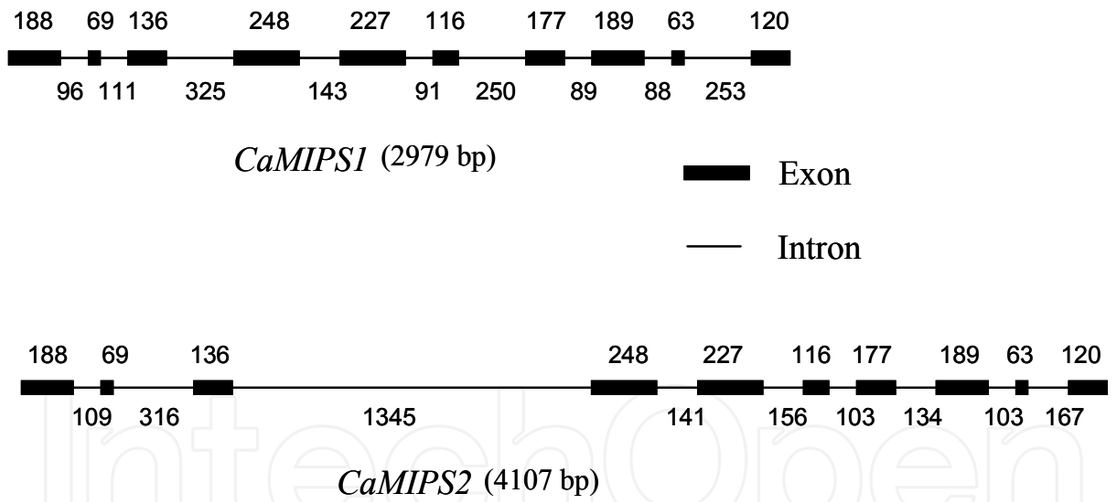


Fig. 4. Diagrammatic representation of *CaMIPS1* and *CaMIPS2* genomic structure. Length of exon and intron indicated in bp. [Modified from Kaur et al., 2008]

Functional divergence after gene duplication can result in following alternative fates: One copy acquires a novel function (neofunctionalization) or one copy loses its function completely or each copy adopts part of the task of their parental gene (subfunctionalization) (Ohno, 1970; Nowak et al., 1997; Jenesen, 1976; Orgel,1977;Hughes,1994).

Functional complementation and in-vitro enzymatic properties were analyzed to check the fate of these two genes. First to check the functional identity of these two divergent genes, a complementation experiment was carried out in natural inositol auxotroph *Schizosaccharomyces pombe* PR109 which clearly demonstrates that both *CaMIPS1* and

CaMIPS2 indeed encode functional MIPS enzymes. Subsequently, the enzymatic properties of these two enzymes were examined since CaMIPS1 and CaMIPS2 polypeptides are reported to have some differences in their amino acid sequences.

Both enzymes showed nearly same Km values for Glc6P suggesting the similar substrate specificity. For both proteins, the optimum temperature for enzyme activity is at 35°C and optimum pH is 7.0 suggesting the similar biochemical characteristics (table1).

Further the enzymatic activities of each protein under stress environment in *invitro* conditions were examined and the activities of these two enzyme proteins were shown to differ significantly in response to high temperature and salt concentration (Kaur et al., 2008). CaMIPS1 activity is considerably affected at high temperature or in presence of increasing sodium chloride concentration while the CaMIPS2 activity is less affected in similar conditions and thereby retaining higher activity than CaMIPS1 (Fig-5).

The amino acid substitutions in protein sequence as analyzed by sequence comparison and the higher enzyme activity in CaMIPS2 under stress condition also indicates that it might be evolved during the course of time to function better under stress conditions. This differential activity towards high temperature and salt of these two enzymes could be supported by the bioinformatics analysis in respect to the available yeast MIPS crystal structure and salt tolerant *PcINO1* (MIPS coding gene from Salt tolerant *Porteresia coarctata*) protein sequence (Majee et al., 2004). Based on the bioinformatics study, CaMIPS2 appears to be more stable towards destabilizing factors such as high temperature, salt, etc, thereby retains better functionality under such conditions (Kaur et al., 2008). Subsequently, growth pattern of *CaMIPS1* & *CaMIPS2* transformed *Schizosaccharomyces pombe* under stress conditions were analyzed and *CaMIPS2* transformed *S. pombe* cells were reported to grow or survive better than *CaMIPS1* transformants both at high temperature and salt environment (Fig-6) suggesting *CaMIPS2* gene product functions more efficiently under stress conditions due to its stress tolerant property and hence provide sufficient inositol to grow as compared to *CaMIPS1*.

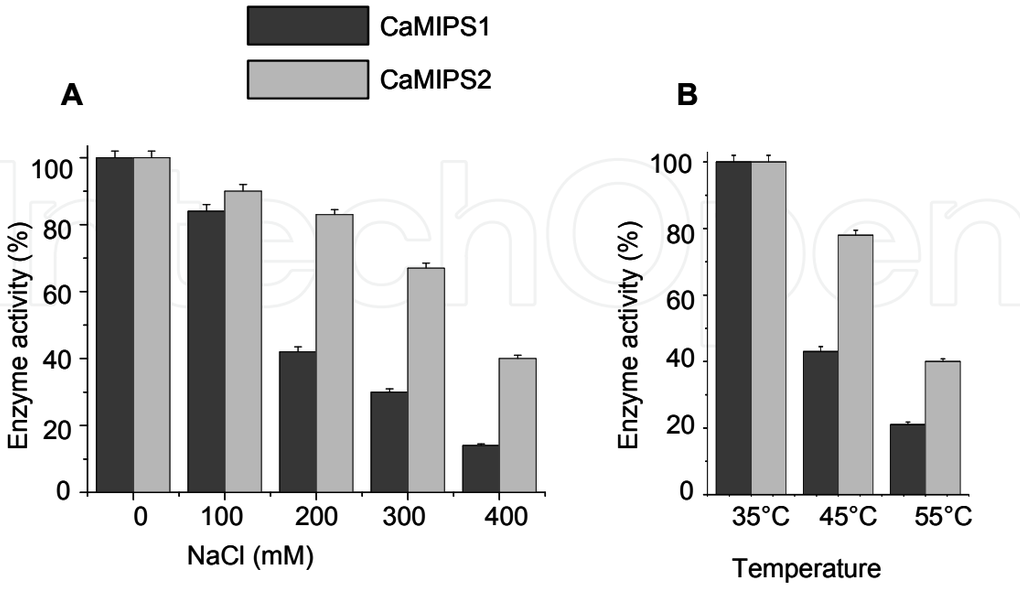


Fig. 5. Effect of salt (A) & temperature (B) on CaMIPS1 and CaMIPS2 enzyme activity. [Modified from Kaur et al., 2008]

Characters	**CaMIPS1**	**CaMIPS2**
Km		
Gluc 6-P	2.63 mM	2.70 mM
NAD^+	0.181 mM	0.192 mM
Vmax		
Gluc 6-P	0.074 μmole min^{-1}	0.075 μmole min^{-1}
NAD^+	0.069 μmole min^{-1}	0.070 μmole min^{-1}
pH optima	7.5	7.5
Temp. optima	35°C	35°C

[Modified from Kaur et al., 2008]

Table 1. Biochemical characterization of recombinant CaMIPS1 and CaMIPS2 enzymes.

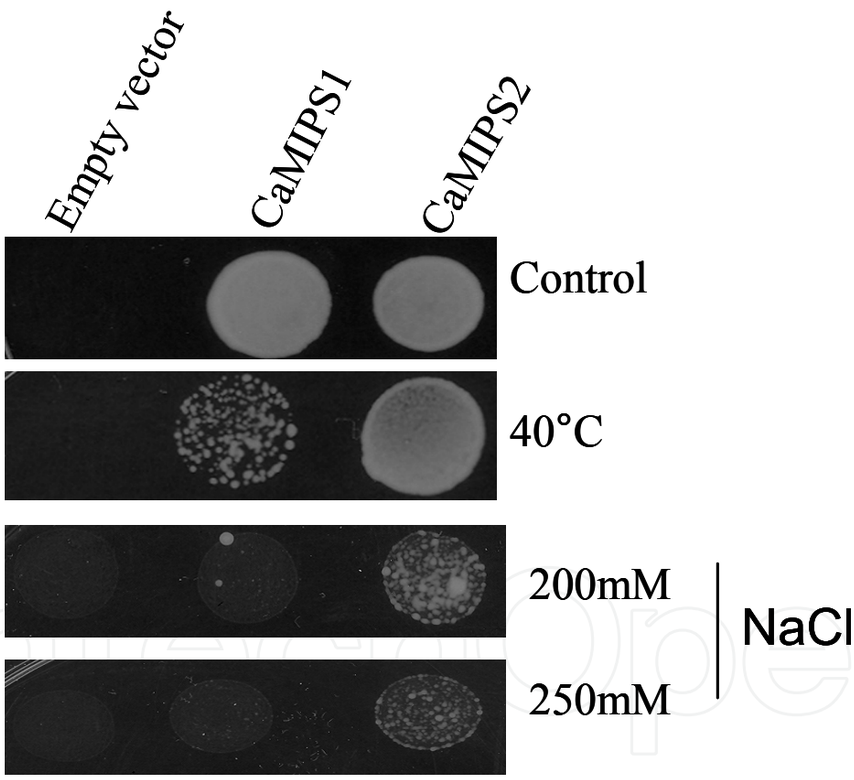


Fig. 6. Growth pattern of *Schizosaccharomyces pombe* transformed with CaMIPS1 and CaMIPS 2 at high temperature and salt environment. [Modified from Kaur et al., 2008]

Recent studies suggest that duplicate genes diverge mostly through the partitioning of gene expression as in subfunctionalization and thereby being expressed in a differential manner; redundant genes may acquire functional divergence (Force et al., 1999; Wagner, 2000; Gu et al., 2002). This hypothesis was examined on *CaMIPS1* and *2*.

CaMIPS1 gene was shown to express in root, shoot, leaves, and flower in fairly equal abundance but no transcript was observed in seed, while *CaMIPS2* transcript was observed

in all examined tissues including seed. This result proposes that *CaMIPS1* and *CaMIPS2* genes are indeed differentially regulated in different organs to coordinate inositol metabolism with cellular growth as hypothesized previously (Loweus & Murthy,2000). Subsequently, expression pattern of these two genes are examined in various environmental stresses. Interestingly, *CaMIPS2* was shown to be induced at different level in various environmental stresses while level of *CaMIPS1* transcript was found to be unaltered by such stresses (Fig-7). This differential expression is also supported by the divergence of their upstream regulatory sequences.

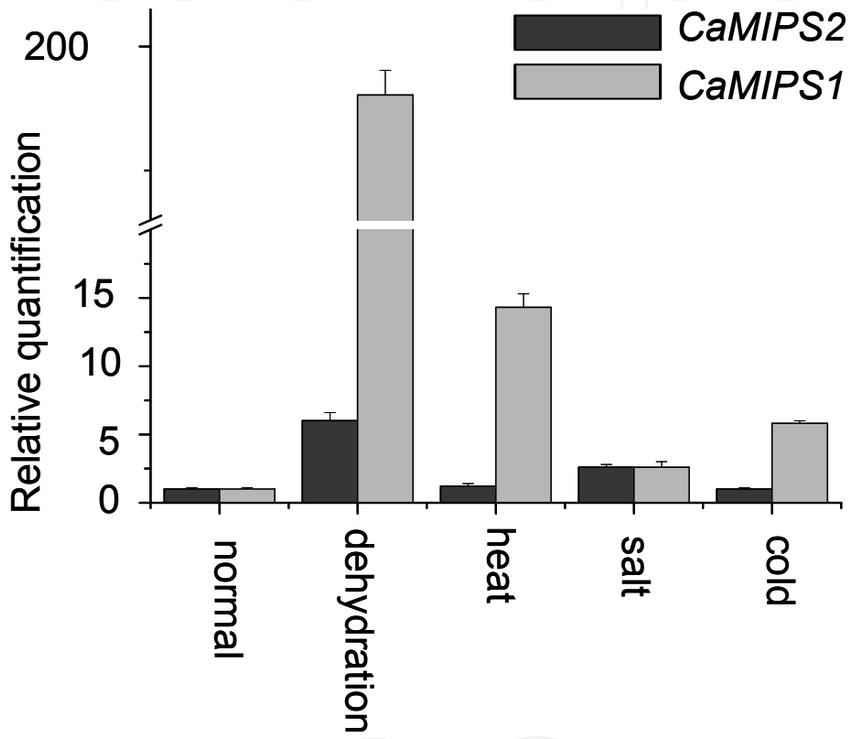


Fig. 7. Expression analysis of CaMIPS1 and CaMIPS2 through real time PCR analysis under various stresses. [Modified from Kaur et al., 2008]

3. Conclusion

Gene duplication, followed by sequence divergence leads to functional divergence of the paralogous proteins, is a major force for adaptation of living organisms. Without gene duplication, the plasticity of genome or organism in adapting to changing environment would be very limited. Chickpea plants are know to be evolved and diversified considerably over time and acquired subsequently various potential genes for their adaptation to environmental stresses. It seems that this drought tolerant legume plant requires more inositol for their adaptation particularly under drought condition and hence acquired *CaMIPS2* over time. Collectively, our results exemplified that *CaMIPS1* and *CaMIPS2* are differentially expressed in chickpea to play discrete though overlapping roles in plant;

however *CaMIPS2* is likely to be evolved through gene duplication, followed by adaptive changes in its sequences to function better under environmental stresses and thereby play a key role in environmental stress adaptation along with other aspects of inositol metabolism in chickpea.

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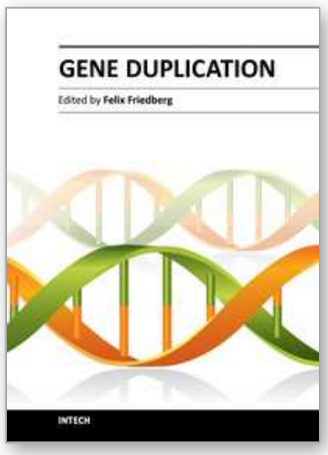
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The book Gene Duplication consists of 21 chapters divided in 3 parts: General Aspects, A Look at Some Gene Families and Examining Bundles of Genes. The importance of the study of Gene Duplication stems from the realization that the dynamic process of duplication is the "sine qua non" underlying the evolution of all living matter. Genes may be altered before or after the duplication process thereby undergoing neofunctionalization, thus creating in time new organisms which populate the Earth.

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