OPEN ACCESS

Int. J. of Life Sciences, 2013 Vol.1 (1): 42-45 ISSN: 2320-7817

International Journal of Life Sciences

www.ijlsci.in

Research Article

Divergence analysis of Phosphoglucomutase from two members of family *Asteraceae* by applying computational tools

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ABSTRACT:

Phylogenetic relationships of Phosphoglucomutase enzyme sequences was performed in two members i.e. *Helianthus annus* L. and *Lactuca sativa* L. of family *Asteraceae* by applying CLUSTAL-W programme. Ten sequences of phosphoglucomutase (PGM) from each species those are already deposited on NCBI were subjected to Multiple Sequence Alignment (MSA). Dendrogram reveals that 20 phosphoglucomutase sequence divided into two distinct clusters. The cluster-I consist of 8 phosphoglucomutase sequences from *Lactuca sativa*, whereas cluster-II consist of 12 phosphoglucomutase sequences belonging to *Helianthus annus* L. and *Lactuca sativa* L.

KEY WORDS

Asteraceae, CLUSTAL-W, Phylogenetic analysis, Phosphoglucomutase (PGM).

INTRODUCTION

Asteraceae is the second largest family of plants, with over 20,000 species, distributed in the world members of this family are found throughout the world (Bremer, 1994). The significance of this family is noteworthy as it contain many economically and medicinally important plants like Sunflower, Lettuce. The typical members of family *Asteraceae* viz. *Helianthus annus* (Sunflower) is native of Western America (Peru) (Almeida, 2001) and *Lactuca sativa* (Lettuce) is native of Persia, South Europe and West Asia (Gupta *et al.,* 2010). For the past two decades, numerous phylogenetic studies using chloroplast DNA sequence data and non coding DNA have contributed to our understanding of the evolutionary relationships within this family. These include comparisons of the chloroplast genes *rbcL* and *ndhF,* as well as noncoding DNA from the *trnL* intron plus the *trnL-trnF* intergenic spacer, *matK* and with lesser resolution, *psbA-trnH* (Kim *et al.,* 1992; Kim and Jansen 1995; Bayer and Starr 1998; Denda *et al.,* 1999). Panero and Funk (2002) used over 13,000 bp per taxon for the largest family-wide classification revision of *Asteraceae* in over a hundred years. Still many uncertainties remain with regards to species, generic, and tribal level relationships.

Phosphoglucomutase (PGM) is a key enzyme in glycolysis. It is a ubiquitous metallo-enzyme that is expressed in all organisms from bacteria to plants and animals. The plant possessing the enzyme Phosphoglucomutase (PGM) catalyses the interconversion of glucose 1-phosphste and glucose 6-phosphate that enters the glycolysis pathway while glucose 1-phosphate serves as a precursor for the generation of sugar nucleotides used in biosynthesis (Ben-Zvi *et al.,*1961). Early genetic studies of PGM revealed that the human isozymes are encoded by three gene loci, PGM1, PGM2 and PGM3 on separate human chromosomes and other studies have demonstrated multiple loci for PGM and PGM like enzymes in several eukaryotic and prokaryotic species (Hopkinson and Harris, 1968; Rattazzi *et al.,* 1983). Bioinformatics is a new and advanced field of this century and has revolutionized Biological science. It utilizes raw sequencing information of protein and nucleic acid for analysis and finds their similarities and differences with the help of computing tools from NCBI (National Centre For Biotechnology Information, http://www.ncbi.nlm.nin.gov).

The protein sequence prediction is very much important in bioinformatics for studying the phylogenetic origin by compairing with successive divergence in many species sequences (Rastogi *et al.*, 2003). Among the various software available, CLUSTAL-W is a programe that perform multiple sequence alignments (MSA). It is mostly important for finding similar domains in a set of sequence and also a tool to determine level of homology for studying phylogenetic analysis. Considering the role of bioinformatics tools in determination of phylogenetic relationships, present investigation was under taken to study the relationships and similarities of Phosphoglucomutase enzyme from two members i.e. *Helianthus annus* and *Lactuca sativa* of family *Asteraceae.*

MATERIALS AND METHODS

Software's: Windows operating system XP/2007, CLUSTAL-W (Online software), Ms-Word.

Methods:

Collection of Sequences Data:

- Ten Protein sequences, of enzyme Phosphoglucomutase (PGM) from two members of family *Asteraceae* (Table 1) were downloaded from NCBI (National Centre for Biotechnology Information).
- Protein sequences were saved in FASTA format.

Multiple Sequence Alignment:

- CLUSTAL-W (Fig. 1) was used for sequence alignment.
- FASTA format sequences were loaded into CLUSTAL-W.
- The alignment results were saved as 'clustalw.aln

Phylogenetic Analysis:

- Phylogenetic analysis was performed using software CLUSTAL-W (Paula A. *et al*., 2000)
- The new evolutionary method using dendrogram. The phylogenetic clusters can be visualized along with closer clades and accessions genetically nearer.

Result Determine From CLUSTAL-W:

CLUSTAL-W is sequence prediction software which available online on web address. www.genome.jp.ad.

- After opening the desired web page, the protein sequences in FASTA format was imported in a particular CLUSTAL-W window and submitted to CLUSTAL-W server.
- The result gets appeared with the tree form.

RESULTS AND DISCUSSION

The result of present study are based on comparison of amino acid sequences of enzyme Phos- phoglucomutase from two species viz. *Lactuca sativa* and *Helianthus annus* belonging to family *Asteraceae*. The 10 sequences of phosphoglucomutase (PGM) from each species were collected from NCBI and converted in to FASTA format as it is prerequisite of CLUSTAL-W (Dangi *et al*. 2011, Tang *et al.,*2011).

In Multiple Alignment Sequence (MSA) the 20 phos- phoglucomutase sequence of two species belonging to family *Asteraceae* divided into two distinct clusters (Fig. 2), the upper cluster-I consist of 8 phosphoglucomutase sequences of *Lactuca sativa,* whereas lower cluster-II consist of 12 phosphoglucomutase sequences belonging to *Helianthus annus* and *Lactuca sativa*. Multiple sequence alignment (MSA) is the fundamentally important tools for DNA and protein sequence analysis and it is important for finding similar domains in a set of sequences and for doing Phylogenetic analysis (Duret and Abdeddaim, 2000).

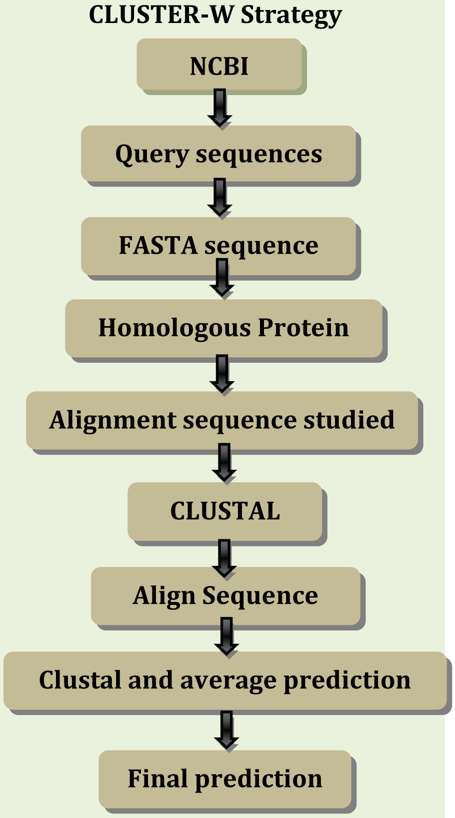
Cluster-I further sub divided into six subcluster showing gi 85691207sp Q32539.2 NU5C and gi 122212030 sp Q332X5.1 NDHJ as most closely related phosphoglucomutase sequences, while gi 85691207sp Q32539.2 NU5C and gi 75220947 sp Q40251.1 VDE L as most divergent phosphoglucomutase sequences of *Lactuca sativa.* In case of cluster-II, though two sequences of *Lactuca sativa* showing homology with *Helianthus annus*; they still showing their distinctness by keeping them outgroup from *Helianthus annus* and showing relatedness with *Lactuca sativa* gi 75220947 sp Q40251.1 VDE L sequences. This close affinity of phosphoglucomutase sequences of *Lactuca sativa* and *Helianthus annus* in cluster –II is well proved based on rDNA sequences (Tang *et al.,* 2011) showing *Helianthus annus* and *Lactuca sativa* are closest genera from family *Asteraceae*.

The closeness between these two species reveals that they might have common ancestor in course of evolution.Cluster-II represented by 10 sequences of *Helianthus annus* and subdivided in to 7 subclusters. The PGM sequences gi 226444298gb

Table 1 PGM Sequence retrieved from NCBI data repositories

Sr. No.	Organism	Gi Numbers
1	*Helianthus annus*	gi|226444300|gb|ACO57738.1|_va22aa
2	”	gi|226444298|gb|ACO57737.1|_va 22aa
3	”	gi|226444296|gb|ACO57736.1|_va22aa
4	”	gi|226444294|gb|ACO57735.1|_va 22aa
5	”	gi|226444292|gb|ACO57734.1|_va 22aa
6	”	gi|226444290|gb|ACO57733.1|_va22aa
7	”	gi|226444288|gb|ACO57732.1|_va22aa
8	”	gi|226444286|gb|ACO57731.1|_va22aa
9	”	gi|226444284|gb|ACO57730.1|_va22aa
10	”	gi|226444282|gb|ACO57729.1|_va22aa
11	*Lactuca sativa*	gi|85691207|sp|Q32539.2|NU5C_L742aa
12	”	gi|122212029|sp|Q332X4.1|NDHK_225aa
13	”	gi|122212013|sp|Q332U7.1|CYB6_ 210aa
14	”	gi|108860829|sp|Q332S2.1|NU1C_ 175aa
15	”	gi|122212030|sp|Q332X5.1|NDHJ_ 158aa
16	”	gi|122212028|sp|Q332X3.1|NU3C_120aa
17	”	gi|110287699|sp|Q332S3.1|NDHI_166aa
18	”	gi|75220947|sp|Q40251.1|VDE_LA_473aa
19	”	gi|122212007|sp|Q332S1.1|NDHH_393aa
20	”	gi|3914604|sp|Q40250.1|RBS_LAC181a

Fig. 1: Logical strategy of CLUSTAL-W method.



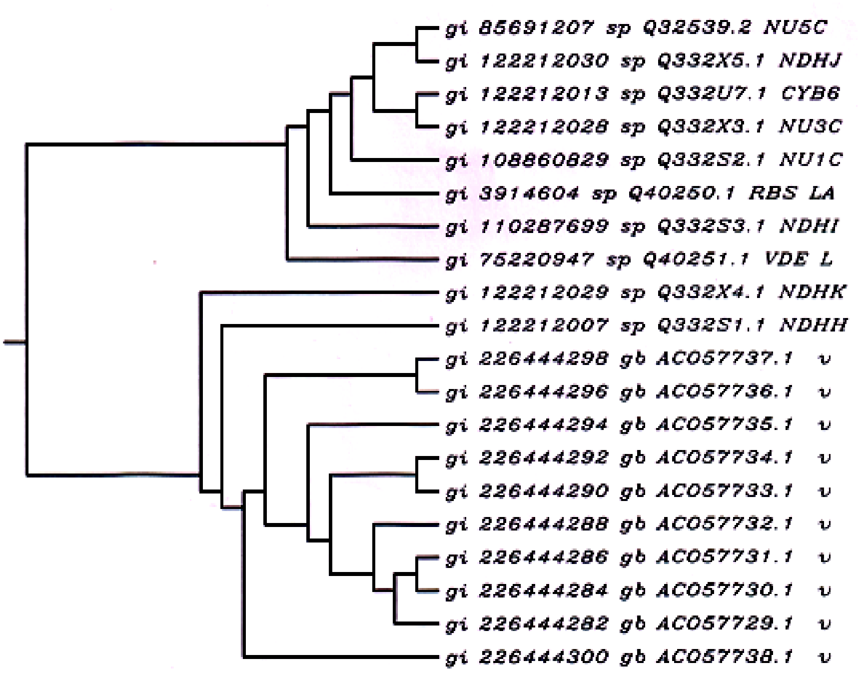


Fig. 2: Dendrogram of 20 PGM sequences

AC057737.1 v and gi 226444296 gb AC057736.1 V show highest similarity. However phosphoglucomutase gi 226444300 gb AC057738.1 V is keeping itself outgrouped. The present study shows use and applications of bioinformatics tools in explaining relationship between closely related species. Though only two species are used for this study, still it is forming a base for further investigations with more number PGM sequences and species of family *Asteraceae.*

ACKNOWLEDGEMENTS

Author thankful to Dr. S. R. Manik, Head, Department of Botany, Sant Gadge Baba Amravati University, Amravati for providing lab facilities and sincere thanks to Dr. P. A. Gawande for critically reviewing manuscript.

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