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14-3-3 PROTEIN; PHYLOGENETIC AND PROTEIN INTERACTION, AN *INSILICO* ANALYSIS

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ABSTRACT: 14-3-3 proteins are a family of conserved regulatory molecules that have the ability to bind a multitude of functionally diverse signaling proteins, including kinases, phosphatases, and transmembrane receptors. 14-3-3 proteins are conserved among many eukaryotic organisms and interact with other target proteins to trigger the transcriptional regulation of genes that generate specific modifications. 14-3-3 proteins affect multiple plant functions by mediating protein-protein interactions through post-translational modification via phosphorylation. This protein family is involved in a wide range of cellular functions including the regulation of hormonal induction in response to stress stimuli, mediation of symbiotic relationships between the plant host and symbionts, as well as the mediation of functions between cellular components and enzymes. 14-3-3 proteins are an important component in biological pathways involved in the mitigation of biotic and abiotic stresses. This study has been undertaken to study the phylogenetic relationship of the 14-3-3 protein in different plants like *Vitis vinifera* (Grape), *Solanum tuberosum* (Potato), *Populus trichocarpa* (Poplar) and *Glycine max* (Soyabean). It was observed that 14-3-3 protein is highly conserved across all the different plant species and that the 14-3-3 protein of *Solanum tuberosum* is closest to *Vitis vinifera*. The minimum evolutionary distance was observed for *Glycine* that shows that the 14-3-3 protein sequence of *Glycine* has least diverged from its ancestor. We also studied the protein-protein interactions of the 14-3-3 protein from *Solanum tuberosum*. The protein is interacting with protein kinases, ATP binding proteins and elongation factors. These interactions are probably helping the protein play a role in combating stress.

KEYWORDS: Phosphorylation, Kinases, Phosphatases, Stress.

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1.INTRODUCTION

Protein phosphorylation is the key to the regulation of many proteins. Altered protein activity often requires the interaction of the phosphorylated protein with a class of 'adapters' known as 14-3-3 protein. 14-3-3 proteins were identified as abundant, acidic, soluble brain proteins with a molecular weight of 25 ± 32 KDa [1]. Their enigmatic name was assigned according to fractionation on DEAE cellulose and electrophoretic mobility on starch gel electrophoresis during the purification of a number of bovine brain proteins of unknown function. 14-3-3 proteins are a family of conserved regulatory molecules that have the ability to bind a multitude of functionally diverse signaling proteins, including kinases, phosphatases, and transmembrane receptors [2,14]. 14-3-3 proteins are conserved among many eukaryotic organisms and interact with other target proteins to trigger the transcriptional regulation of genes that generate specific modifications [3,10,15]. 14-3-3 proteins are important components in signal transduction pathways affecting multiple plant functions by mediating protein-protein interactions through post-translational modification via phosphorylation [12,16,17]. This protein family is involved in a wide range of cellular functions including the regulation of hormonal induction in response to stress stimuli, mediation of symbiotic relationships between the plant host and symbionts, as well as the mediation of functions between cellular components and enzymes [18]. 14-3-3 proteins are an important component in biological pathways involved in the mitigation of biotic and abiotic stresses [3]. Plant 14-3-3 isoforms, like their highly conserved homologues in mammals, function by binding to phosphorylated client proteins to modulate their function [22]. Through the regulation of a diverse range of proteins including kinases, transcription factors, structural proteins, ion channels and pathogen defense-related proteins, they are being implicated in an expanding catalogue of physiological functions in plants [20,21]. 14-3-3 are affected, both transcriptionally and functionally, by the extracellular and intracellular environment of the plant [19]. They can modulate signaling pathways that transduce inputs from the environment and also the downstream proteins that elicit the physiological response [13]. The key emerging roles for plant 14-3-3s includes role in the response to the plant extracellular environment, particularly environmental stress, pathogens and light conditions. It also plays potential key roles in primary metabolism, hormone signaling, growth and cell division. There is involvement of 14-3-3 protein in signal transduction pathways with emphasis on the regulation of plant metabolism. Originally 14-3-3 proteins were thought to be found only in brain tissue [4]. However, it became clear that 14-3-3 proteins belong to a family of proteins present in all the eukaryotic organisms investigated. 14-3-3 proteins mainly exist as saddle-shaped homo- or hetero dimers in which a broad central groove is able to bind to target proteins [5,11]. Thus, 14-3-3 proteins can function as 'adapters', leading specific target proteins to each other or to a specific location.

2. MATERIALS AND METHODS

Protein sequence: Protein sequences of 14-3-3 protein of *Solanum tuberosum* having accession no. P93785 was retrieved from Uniprot (www.uniprot.org) [6].

BLASTP: The similar protein sequences to the query were retrieved using BLASTP [7]. (<https://blast.ncbi.nlm.nih.gov>). The 14-3-3 protein sequences of the different plants *Populus trichocarpa* (Poplar) XP 002301071.1, *Vitis vinifera* (Grape) NP 001267852.1, *Glycine max* (Soyabean) NP 001238389.1 were retrieved and multiple sequence alignment was performed using Clustal Omega.

Clustal Omega: Clustal Omega (<http://www.ebi.ac.uk/Tools/msa/clustalo/>) [8] was used to for multiple sequence alignment for all the above protein sequences mentioned. Phylogenetic tree generated was analyzed to see the evolutionary relationship between the different above mentioned protein sequences.

STRING: The interactions network of the 14-3-3 protein with other protein was found using STRING database (<http://string-db.org/>) [9]. The sequence used as a query was the 14-3-3 protein of *Solanum tuberosum* and the results were analysed.

3. RESULTS AND DISCUSSION

14-3-3 proteins are a family of conserved regulatory molecules that have the ability to bind a multitude of functionally diverse signaling proteins, including kinases, phosphatases, and trans membrane receptors. 14-3-3 proteins are conserved among many eukaryotic organisms and interact with other target proteins to trigger the transcriptional regulation of genes that generate specific modifications. 14-3-3 proteins are important components in signal transduction pathways affecting multiple plant functions by mediating protein-protein interactions through post-translational modification via phosphorylation. This protein family is involved in a wide range of cellular functions including the regulation of hormonal induction in response to stress stimuli, mediation of symbiotic relationships between the plant host and symbionts, as well as the mediation of functions between cellular components and enzymes. 14-3-3 proteins are an important component in biological pathways involved in the mitigation of biotic and abiotic stresses.

In this project the phylogenetic study 14-3-3 protein from *Solanum tuberosum* was done which is playing a role in combating the biotic and abiotic stress in plants with different plant like *Populus trichocarpa* (Poplar) *Vitis vinifera* (Grape), *Glycine max* (Soyabean) whose sequences were retrieved using BLAST P. The phylogram shows that 14-3-3 protein is highly conserved across all the different plant species and that the 14-3-3 protein of *Solanum tuberosum* is closest to *Vitis vinifera*. The minimum evolutionary distance was observed for *Glycine max* that shows that the 14-3-3 protein sequence of *Glycine max* has least diverged from its ancestor.

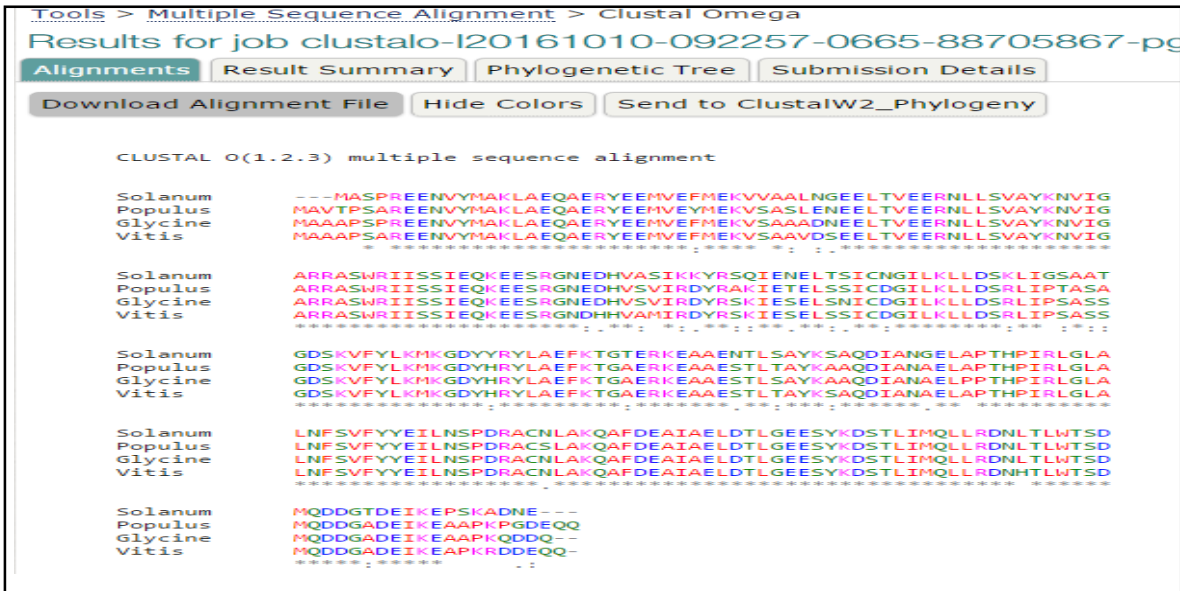


Figure 1: Multiple sequence alignment using Clustal Omega



Figure 2: Phylogram showing the evolutionary relationship of 14-3-3 protein

The interaction of 14-3-3 protein with other proteins was found using STRING database. The 14-3-3 protein sequence of *Solanum tuberosum* was used as a query. The results showed the protein interaction network with 10 different proteins that includes kinases, ATP binding proteins, Elongation factor 1-gamma, Minichromosome maintenance protein MCM etc.

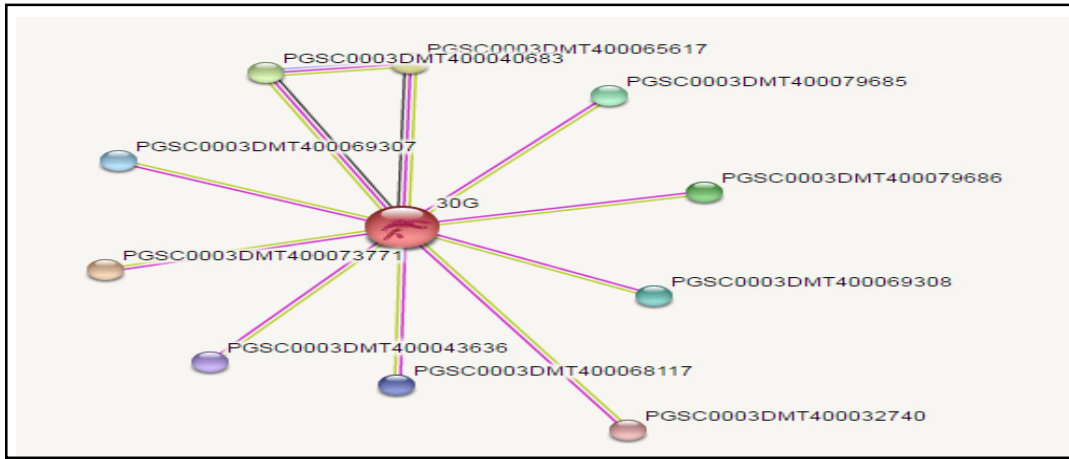


Figure 3: The interactions of 14-3-3 protein from *Solanum tuberosum*

Your Input:										
30G	14-3-3 protein (254 aa)									
Predicted Functional Partners:		Neighborhood	Gene Fusion	Cooccurrence	Coexpression	Experiments	Databases	Textmining	[Homology]	Score
PGSC0003DMT400073771	Phosphatidylinositol 4-kinase (546 aa)									0.927
PGSC0003DMT400065617	Elongation factor 1-gamma (414 aa)									0.894
PGSC0003DMT400040683	Elongation factor 1-gamma (413 aa)									0.894
PGSC0003DMT400079685	Kinase family protein (288 aa)									0.885
PGSC0003DMT400079685	ATP binding protein (873 aa)									0.885
PGSC0003DMT400069308	Protein kinase (364 aa)									0.885
PGSC0003DMT400069307	ATP binding protein (876 aa)									0.885
PGSC0003DMT400068117	Protein kinase family protein (422 aa)									0.885
PGSC0003DMT400043636	Hypothetical Ser-Thr protein kinase (654 aa)									0.885
PGSC0003DMT400032740	Minichromosome maintenance protein MCM (581 aa)									0.809
Your Current Organism:		Solanum tuberosum								

Fig 4: The interacting proteins of 14-3-3 protein using STRING

4. CONCLUSION

The study showed the evolutionary relationship of 14-3-3 protein of *Solanum tuberosum* which plays a role in combating the biotic and abiotic stress in plants with different plant like *Populus tricarpha* (Poplar) *Vitis vinifera* (Grape), *Glycine max* (Soyabean). The phylogram shows that that 14-3-3 protein is highly conserved across all the different plant species and that the 14-3-3 protein of *Solanum tuberosum* is closest to *Vitis vinifera*. The minimum evolutionary distance was observed for *Glycine max* that shows that the 14-3-3 protein sequence of *Glycine max* has least diverged from its ancestor. The 14-3-3 protein interacts with 10 different proteins that includes kinases, ATP binding proteins, Elongation factor 1-gamma, Minichromosome maintenance protein MCM etc.

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CONFLICT OF INTEREST

Authors have no conflict of interest.

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