Title:

Mutated and wild type *Gossypium Universal Stress Protein-2* (*GUSP-2*) gene confers resistance to stresses in *Escherichia coli, Pichia pastoris* and cotton plant.

Gossypium arboreum is considered to be a rich source of stress responsive genes and EST data base revealed that mostly of its genes are uncharacterized. The full length *Gossypium universal stress protein-2 (GUSP-2)* gene (510bp) was cloned in *E.coli, Pichia pastoris* and *Gossypium hirsutum*, characterized and point mutated at three positions separately at 352-354, Lysine-60 to proline (*M1-usp-2*) & 214-216, aspartic acid-26 to serine (*M2-usp-2*) & 145-147, Lysine-3 to proline (*M3-usp-2*) to study its role in abiotic stress tolerance. It was found that heterologus expression of one mutant (*M1-usp-2)* provided enhanced tolerance against salt and osmotic stresses, recombinant cells have higher growth up to 10^{-5}dilution in spot assay as compared to *W-usp-2* (wild type *GUSP-2*), *M2-usp-2* and *M3-usp-2* genes. *M1-usp-2* in *Pichia pastoris* transcript profiling exhibited significant expression (7.1-fold) to salt and (9.7) and osmotic stresses. *M1-usp-2* gene was also found to enhance drought tolerance and significant expression (8.7) in CIM-496-*Gossypium hirsutum* transgenic plants. However, little tolerance against heat and cold stresses both in recombinant yeast and bacterial cells was observed. The results from our study concluded that activity of *GUSP-2* was enhanced in *M1-usp-2* but wipe out in *M2-usp-2* and *M3-usp-2* response remained almost parallel to W-usp-2. Further, it was predicted through *in silico* analysis that *M1-usp-2*, *W-usp-2* and M3-usp-2 may be directly involved in stress tolerance or function as signaling molecule to activate the stress adaptive mechanism.

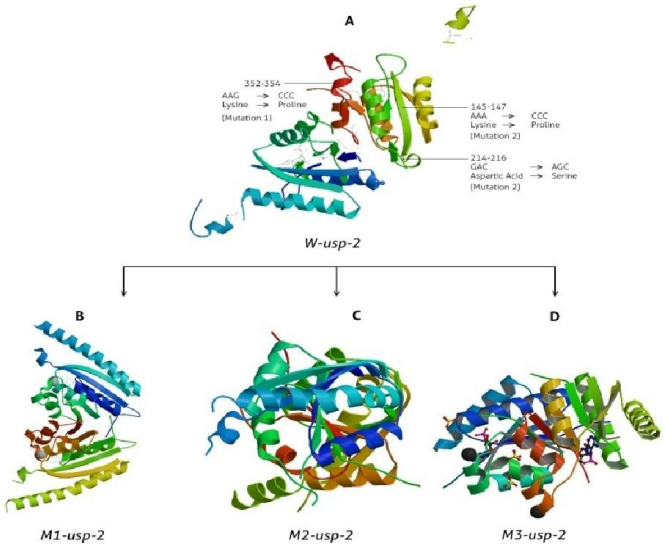


Figure: 3D SWISS-MODELS of mutated (M1-usp-2, M2-usp-2, M3-usp-2) and wild type (W-usp-2) GUSP-2-proteins

Publications

1.	Evaluation of correlation of oxidative stress with chronic kidney disease in patients with moderate to severe chronic kidney disease (2015) (Kalsoom Zaigham, Muhammad Tanveer Muneer, **Muhammad Nadeem Hafeez**, Muhammad Shahzad Iqbal, Qurban Ali) New York Science Journal 2015, 8 (3).
2.	Transformation and evaluation of Cry1Ac+Cry2A and GTGene in Gossypium hirsutum L. (2015)(Agung Nughro Puspito, **Muhammad Nadeem Hafeez**, Muhammad Shahzad Iqbal, Qurban Ali, Abdul Qayum Rao and Tayb Husnain) Frontiers of Plant Sciences, Nov, 2015, 6:708. doi: 10.3389/fpls.2015.00708 (IF: 3.9)
3.	Role of genetic studies towards solving problems of human society (2015) (Shumaila Zulfiqar, **Muhammad Nadeem Hafeez**, Muhammad Shahzad Iqbal and Qurban Ali) Nature & Science, March, 2015, 13 (4)
4.	Prediction of Host-Derived miRNAs with the potential to target PVY in Potato Plants. (2016) (Muhammad S. Iqbal, **Muhammad N. Hafeez**, Javaid I. Watto, Arfan Ali, Muhammad N. Sharif, Bushra Rashid, Bushra Tabassum and Idress A. Nasir)Frontiers in Genetics, 14 Sep, 2016, dx.doi.org/10.3389/fgene.2016.00159
5.	Physiological, Biochemical, Morphological and Molecular basis of Drought stress tolerance in Cotton. (2015)(**Hafeez MN**, Sadique S, Hassan S, Sarwar MB, Rashid B, Ali Q, Husnain T)(International Journal of Biology, Pharmacy and Allied Sciences)March, 2015, 4 (3)(IJBPAS)

Biography:

I am Nadeem Hafeez from Lahore, Pakistan. Currently, I am working as Researcher at Center for Excellence in Molecular Biology (CEMB), Lahore, Pakistan. I have expertise in gene editing, cloning and plant transformation; I have developed a first triple gene genetically engineered cotton variety of Pakistan, which confers significant resistance against biotic and abiotic stresses. I have utilized *E.coli* and yeast expression models to screen mutated genes before transformation in plants. I am currently doing a research project in abiotic tolerance of cotton to learn more about reverse genetics. In this project we are using CRISPR cas9 genome editing to study genes associated with abiotic stress tolerance.

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