Differential expression in *Oryza sativa* due to stress condition

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ABSTRACT

Rice is s staple food for most of the people across the globe. It requires high heat and humidity for growth and temperature range should be 20-30^{o}C. Any change in the conditions causes impairment of growth. Under stress conditions agricultural productivity decreases. According to reports environmental stress is going to increase and thus it is very important to produce stress tolerant plants. Differential gene expression (DEG) of *Oryza sativa* due to stress condition helps to understand and identify the genes responsible for such responses. Upon identification we can up-regulate or down regulate these genes as per need. Sometimes gene also shows different expressions (protein products) at multiple stress conditions. Thus it becomes very important to study DEG's of rice. This study mainly focuses on identifying and studying differentially expressed genes of *Oryza sativa (japonica)* under different stress conditions.

Keywords- Differential gene expression; *Oryza sativa*; Abiotic stress; Biotic stress; RNA-Seq.

INTRODUCTION

Rice (*Oryza sativa*) is one of the major food crops in the world. Around 40% of the world population consumes rice as the major staple food along with maize and wheat. Asia consumes the rice the most. China and India are the leaders of rice cultivation. Rice makes of 60% of the total food intake of the people of Southeast Asia and around 35% in South Asia and East Asia. *O. sativa* is nutrition source of many people. It has many nutritional values. Rice is low in sodium and fat content and also is free of cholesterol. As population is increasing exponentially demand of rice is also increasing. According to a report to feed the estimated world population of around 9.1 billion by the year 2050 the overall food production rate has to be increased by 70% of the existing one (Vijay D., Bidhan R.,2013). *O. sativa* crop production gets affected due to different biotic and abiotic stresses. Biotic stress includes disease causing microbes like fungi and bacteria. Abiotic stress includes temperatures change, flood, drought and salinity etc. These stress factors reduces the yield of the crop and also deteriorates the quality of the crop. As it is staple food for many people, less yield of the crop can affect many lives. (C. B. Sruthilaxmi1, Subramanian Babu1, 2018).

Differential gene expression analysis (DGE) is performed in order to find gene or transcript differences between conditions, treatments and developmental stages, etc. In simple language it is done to study the given

gene's expression variation between two (or more) conditions based upon the information gained from two or three replicates per condition**.**Understanding differential expression of genes of *O. sativa* due to stress condition can help to improve the yield of the crop by over expression or under expression of the genes as required.

It has been observed that rice plants under stress conditions tend to adapt by regulating its molecular and cellular responses .When it faces multiple stress, it usually tackles the situation by switching its responses by using cross-talking signaling pathway. In this condition the responses sometimes causes to tolerance by overlapping genes and their protein products. According t some studies due to stress, rice plants often shows up- regulation of lectins and heme activator proteins, ribosome-inactivating proteins, hormone responsive proteins, transcription factor etc. Based upon the threat level and severity of the stress rice plant tends to regulate their molecular responses by either switching on or off of the cross-talking genes (C. B. Sruthilaxmi Subramanian Babu,2018).

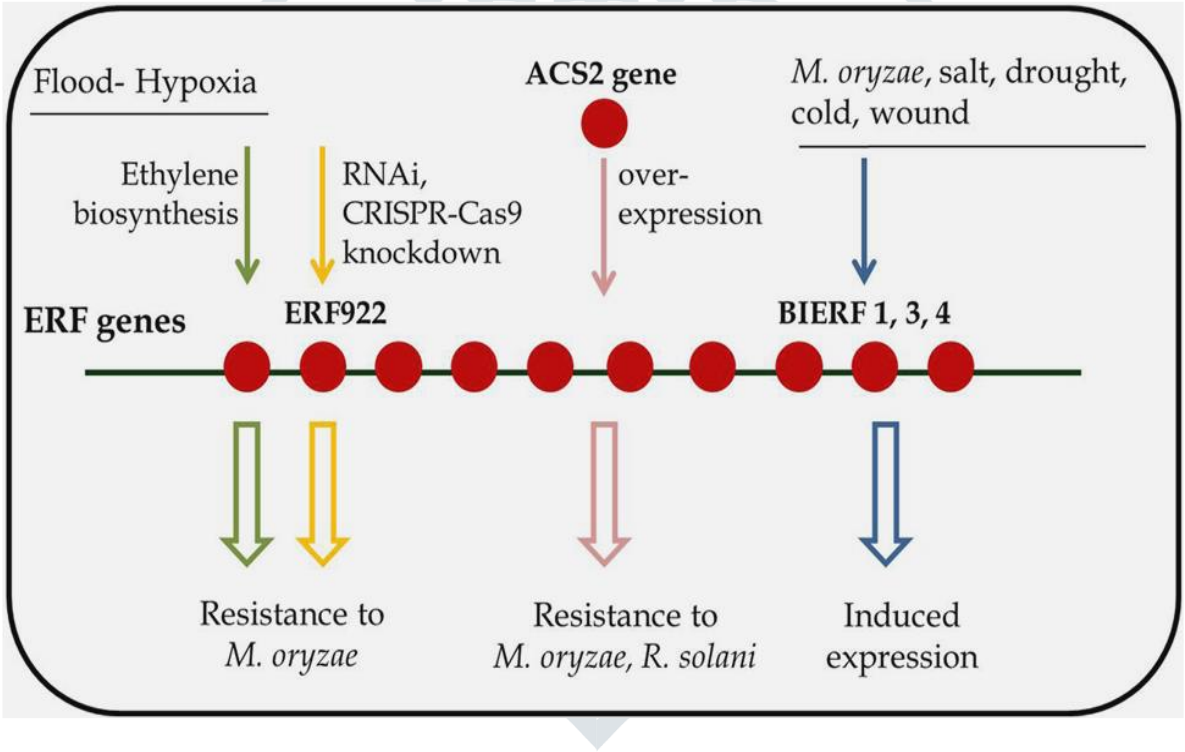

Figure 1: This represents the role of ethylene responsive factor (ERF) genes while rice is under stresses. Abiotic stresses such as flood and hypoxia induces ethylene biosynthesis pathway resulting into resistance to blast fungus. Also silencing of ERF922 induces resistance to blast fungus .Overexpression of ACS2, an important gene in ethylene biosynthesis causes resistance to both the blast and the sheath blight fungi. BIEF1, 3, 4 gets induced by both fungal infection and abiotic stress (Courtesy: C. B. Sruthilaxmi, Subramanian Babu, 2018).

Rice plants behave distinctly at different stress conditions. Abiotic stress affects the whole plant. Wide range of biological processes are down regulated and plants enters into an energy saving and protective mode under this condition. Whereas biotic stress are mostly localized and plants exhibits array of defense response at molecular level to exhibit systemic acquired resistance. Some studies suggest various different plant hormones plays the central role in various types of stress responses. In biotic stress, the salicylic acid (SA)-dependent defense

response is activated by biotrophic (hemi) pathogens, whereas jasmonic acid (JA) - and ET-dependent signaling pathways activates necrotrophic pathogens activate (Sharma et al., 2013). In case of abiotic stress ABA is activated. ABA is known to negatively regulate plant immunity under biotic stress. Due to this it was proposed that plants prefer abiotic stress tolerance over the biotic stress response and used ABA as a molecular to switch in between these two responses in order to have minimum damage (Lee and Luan, 2012).

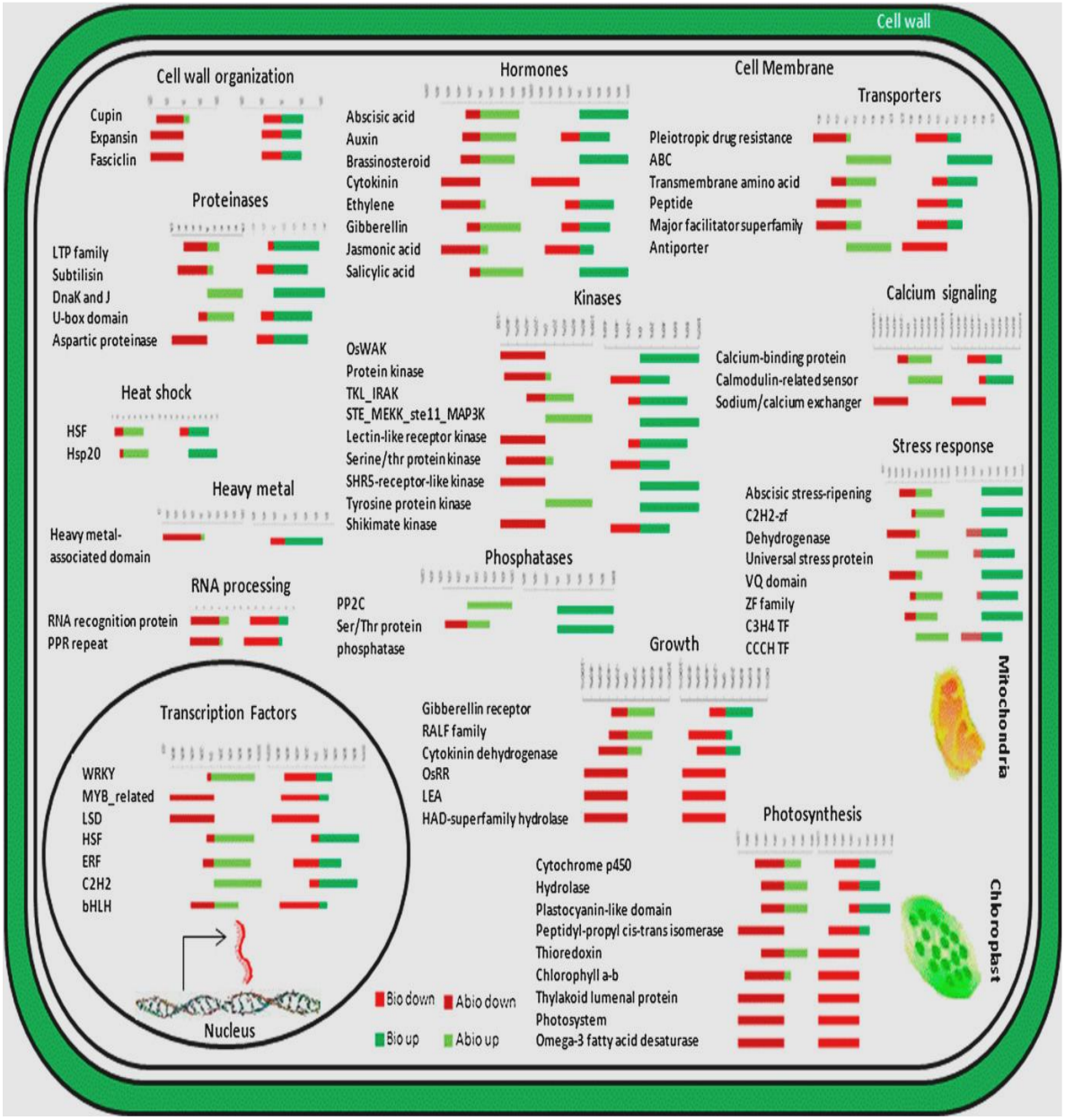

Figure 2: The visual representation of differential expression of different gene families under abiotic and biotic stresses. The stacked bars represents down-regulated genes scaled to up-regulated genes (total) scaled up to 100% respectively.(Courtesy: Rafi S. and Wusirika R.,2013)

In a study it was found that expression level of genes depends on the stress type that is whether it is single or intercrossed, it also depends on the exposure time of the stress. Under intercross stress peroxidise catalase and superoxide dismutase, and malondialdehyde activity were highly regulated. The expression levels of the *PR4*,

PAL, and *Cht-1* genes were upregulated under disease-drought intercross significant changed. (Y.P. Zhang₁,et al).

Silicon increases pathogenic resistance in rice (Oryza sativa). In an experiment conducted it was found that the relationship between silicon and Magnaporthe oryzae (rice blast pathogen) in terms of whole- genome gene expression and by assessing gene expression patterns in the rice cultivar Monko-to using microarray technology, the physiological basis for silicon-induced resistance was investigated. Silicon amendment resulted in the differential regulation of 221 genes in rice without being challenged with the pathogen. This signifies that silicon has an observable effect on metabolism in rice. Compared with control plants, silicon-amended rice differentially regulated 60% less genes, implying that silicon affects the rice response to rice blast infection at a transcriptional level. This means that silicon had an observable effect on rice metabolism, as opposed to playing a simple passive role in the resistance response of rice. Compared with control plants, silicon-amended rice differentially regulated 60% less genes, implying that silicon affects the rice response to rice blast infection at a transcriptional level.(A.M. Brunings,2009).

Rice as a non-legume crop, it can form associations which are beneficial for both the nitrogen-fixing bacteria such as Azospirillm brasilense and itself. In a study a wild variety of rice ((*Oryza sativa* cv. Nipponbare) was used as the tested. RNA from the plants were extracted using Qiagen RNeasy® Plant Mini Kit (Cat #74904, California, USA) as described in Hiltenbrand *et al* [1].. In that study experimental system was used where the bacteria could promote plant growth by colonizing the plant roots in rice and a symbiotic relationship was established. The data collected from the experiment suggested that the genes dmi3 and pollux were not responsible for the plant root penetration and its growth promotion, but it was promoted due to the bacteria. Then colonization model was used to identify regulation of gene expression at two different time points :(a)Day 1 of post incubation- 1622 differentially expressed genes were identified in roots;and (b)Day 14 of post incubation- 1995 differentially expressed genes were identified. Comprehensive data mining was performed to classify differentially expressed genes into the categories of protein kinase (PK), transporters (TR) and transcription factors (TF). A number of these differentially expressed genes encoded for proteins that were involved in the defense, hormone signaling pathways and flavonoid biosynthetic pathway. Several other genes were also identified which were involved in sugar and nitrate transport and they also play a role in various plant-microbe interactions (Santi C, 2013).

METHODOLOGY

RNA- seq is a tool to study differential gene expression. This project aims to study the differential gene expression of *O. sativa* due to stress condition. In stress condition some genes function differently as compared to the function in normal conditions. If we are able to identify the genes which express itself differently, we can predict a treatment to make the plant resistant to that stress condition by either over expressing or under expressing of those genes as required.

We are using Galaxy tool for our research. Galaxy is a web based platform which is widely used for studying proteomics; genomics, imaging and metabolomics across the globe. We are using reference genome based approach for our research. Our model organism is *O. sativa.*

Work flow of differential gene expression analysis is mentioned below (a) Alignment and processing of sequencing reads. (i) Download files in Fastq format from NCBI SRA database: The Sequence Read Archive (SRA) is the main repository for nucleic acid sequences. It currently stores more than 25,000 tera bases. Download the desired files (multiple) in fastq formats only. Or note the accession number and fetch the data using galaxy tool through accession number. (ii) Combined multiple fastq into single file: By using galaxy tool fetch the data. After the fastq files rare downloaded and combine multiple fastq into single file. b) Alignment: Feed raw fastq into trinity for contig assembly then mapping by using TopHat, TopHat2, and HiSat tools. (c)Annotation: Annotate the de novo Transcriptome by using cDNA database which can be downloaded from Ensemble. Ensemble is provides wide range of annotated data and genomes of mostly model organisms. (d)Normalization: The main purpose of normalization is to" eliminate systematic effects that are not associated with the biological differences of interest so as not to skew exploratory analyses". (e) Differentially expressed genes analysis using DESeq2: (i) Count transcript expression level in the two individual samples. (ii) Export the results into text file onto local computer.

CONCLUSION

Oryza sativa is one of the major staple foods in many part of the world. The biotic stresses like the diseases and insect pests which leads to reduction the yield of crop if it not controlled. In 1943, India faced Bengal famine which was epidemic. Bengal famine is one of best example of the how disastrous affect of biotic stress could be.

Also environmental conditional are rapidly changing day by day mainly due to anthropogenic activities. Soil, water and air is getting polluted. Plants are exposed to these harsh conditions. They are not able to cope up with the changing environment. They are facing edaphic stresses like chilling, drought, freezing, high heat, water logging, irradiation etc. drought and water stress affects the most the productivity of the rice.

Differential gene expression of *O. sativa* due to stress will help us to identify the gene responsible for the different expression at different condition. After doing DGE analysis we can predict the cause of the disease, which tissue is affected, treatment for the disease and also propose ways to make the plant resistance to the stress condition.

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