In silico identification of genes for combined drought and salinity stress in rice (*Oryza sativa* L.)

ABSTRACT:

The salinity and water stress are the important abiotic stresses which severely affect the rice growth, development and yield depending on degree of stress. Moreover, this stresses are inter-related and includes many crosstalk at genetically and physiologically. The identification of genes controlling both the stress would mean a lot in understanding molecular mechanism of tolerance, which in turn assist in development of stress resilient genotypes. In the present study, an *in silico* approach was used to identify genes commonly expressed under combined drought and salt stress using microarray data retrieved from NCBIGEO database. The meta-analysis of this transcriptome data revealed 35 candidate genes expressed under combined stress with 82.8 % of the genes showing up regulation and 18.2 % genes with down regulation. The functional annotation of candidate genes showed the expression of diverse stress responsive proteins mainly transcription factor, UDP-glucosyl transferase, glycosyl ptransferase 8, flavanone 3-hydroxylase, plant PDR ABC transporter associated domain and dehydrins. Among the expressed proteins, transcription factors shared major part in gene regulation. The key gene Os05g48650.1 which present on chromosome five at 28.8 Mb physical position encoded for HBP-1b protein. The earlier authors proved that the over expression of this HBP-1b gene in rice plant showed improved tolerance to salt and drought stress. Two more genes Os11g26780.1 and Os11g26790.1 co-localized on chromosome 11 encodes for an important stress responsive dehydrin protein which is positively correlated with the tolerance to cold, drought, and salt stress. Finally, in conclusion the genes Os11g26780.1, Os11g26790.1, Os06g27560.1 and Os05g48650.1 were directly related with salt and drought stress tolerance. The introgression of these genes in high yielding stress susceptible genotypes could assist in developing stress tolerant cultivars.

Key words: *In silico*, Rice, Oryza sativa L. Salinity Stress, Drought Stress

Introduction:

Rice (*Oryza sativa* L.) is a staple food supporting more than three billion people and comprising 50% to 80% of daily calorie intake [1]. Rice production requires larger amount of water throughout its life cycle compared to other crops for good yield. Hence, water related stress cause severe threat to rice production across the world. Approximately 23 million

hectares of rainfed rice cultivated across the world was affected by severe drought [2]. The global climate change resulted in 32% of rice yield variability from year-to-year with 53% of rice harvesting regions experiencing rapid climatic change [3]. The increased frequency of dry spells in many regions of the world forced the farmer to use water from dam, canal and bore well etc. The irrigation water used from these canals contains large amount of dissolved salts. Approximately 20% of irrigated areas suffer from salinization problems by various degrees. The drought and salinity stress are often inter-related and obstruct the water uptake adversely, affecting plant growth and productivity.

These stress causes a wide range of genetically, physiological and biochemical responses in plants. Understanding plant responses to abiotic stresses at the transcriptomic level provides an essential foundation for future breeding and genetic engineering efforts. A significant number of QTLs were discovered in rice under drought and salinity, but a QTL would be more useful only when it contains functional candidate gene. With the advancement of molecular technology, the interest on gene expression studies increased enormously. A large number of genes have been identified under salinity and drought stress [4,5]. Among the available methods, DNA microarrays have been devised as standard strategy for the global analysis of plant gene expression [4,6]. Several important traits of rice have been analyzed using microarray which helped in monitoring the gene expression pattern under multiple abiotic stress like drought, and high salinity stresses at temporal and spatial level [4,7]. But, different microarray studies reported different set of genes for a particular stress due to varied reasons. With this view, the present study was designed to carry out meta-analysis to identify the genes responsible for combined drought and salinity stress tolerance in rice. The study will helps in pointing out the genes responsible for combined stress tolerance and assist in understanding the molecular mechanisms underlying these complex traits in rice. The data pertaining to meta-analysis was downloaded from NCBIGEO database, were rice transcriptomic data pertaining to drought and salinity stress deposited by various authors.

Material and Method:

Data mining:

The current study is a bioinformatics approach to identify the genes that commonly expressed under salinity and drought stress in rice. Rice is the one of the crop which had large amount of genomic, transcriptomic and proteomic data deposited in various repositories. Likely,

NCBI GEO has large amount of microarray data deposited by various author [http://www.ncbi.nlm.nih.gov/geo/]. NCBI GEO is the platforms were gene expression data generated from microarray studies was deposited, which is easily downloadable and can be processed to find new insights using meta analysis [8]. To identify the genes commonly expressed under different stress, it is mandatory to use uniform microarray platform which has large number of genes spotted and having more number of series and samples [8]. To have consistency in results, the platform GPL2025 [Affymetrix Rice Genome Array] having 51,279 probes used in present investigation. This array (GPL2025) was mostly used for gene expression studies under different abiotic stresses in rice. The GPL2025 platform has 3096 samples and 191 series, out of which the data regarding differential gene expression [DEGs] for salinity and drought stress belonging to six series [GSE24048, GSE6901, GSE41647, GSE3053, GSE4438 and GSE16108]. In this study, the differential gene expression among tolerant genotypes constituting 9 samples belonging to above mentioned six series was selected manually for further study. The identification of genes in tolerant genotypes helps in better understanding the molecular mechanism involved in stress tolerance [9, 10].

Data processing:

The raw data retrieved from NCBI GEO was subjected to GEO2R to obtain the log2 fold change [logFC] values [http://www.ncbi.nlm.nih.gov/geo/geo2r/]. The so obtained logFC values were used to identify the DEGs in respective stress. The logFC value of ±1 [two fold change] was set as threshold level to discriminate the DEGs from the total genes. The genes which met the set criteria under each stress among multiple samples were sorted and saved as separate files. Later, the genes expressed commonly under both the stresses were identified using excel with various function. To view the expression of the genes diagrammatically, a tab-delimited file was created with logFC values for salinity and drought stress having corresponding spot IDs. Using MultiExperimental Viewer [MeV] software heat map of gene expressed under combined stress was viewed [11]. The software is freely downloadable from http://mev.tm4.org/. Later in progress of analysis, the gene loci associated with spot ID were retrieved from ricechip.org [http://www.ricechip.org/]. The retrieved gene loci were used has input in orygenesdb.cirad.fr to know the further information regarding the gene position on different chromosome and functional annotation [http://orygenesdb.cirad.fr/tools.html].

Results and Discussion:

Plant responses to drought and salinity are complexes and involve morphological, physiological and molecular changes which may lead plant to adaptive advantage and/or deleterious effects. The both salinity and drought stress has similar effect on plant growth by obstructing the water uptake and finally decreasing the water potential [12]. The decrease in the water potential occurred in both abiotic stresses results in reduced cell growth, root growth and shoot growth and also causes inhibition of cell expansion and reduction in cell wall synthesis [13]. [4] in his study found that among multiple abiotic stresses encountered by rice plant, there would be more cross talks between drought and salinity stress. In the present study, the comprehensive analysis of trancriptome data of salinity and drought stress retrieved from multiple experiments found that a total of 1261 and 849 genes were differentially expressed at log fold change value of ± 1[logFC] i,e. 2 fold change [14]. Majority of the authors used logFC ±1 has a criteria to distinguish the DEGs . The further insights into the results showed more number of up regulated genes [936] compared to down regulation [325] under drought stress. Earlier reports proved the expression of more number of up regulated genes in tolerant genotypes [15, 16]. These up regulated genes may contribute to adaptive mechanism of tolerant genotypes under stress condition. Similarly, more or less equal number of genes were expressed under salinity stress [Fig. 1]. This fact proves that both up regulation and down regulation of genes play major role in salinity stress tolerance in rice. The analysis for combined stress revealed 35 genes commonly expressed with more number of genes showing up regulation. Among these, 82.8 % of the genes showed up regulation and in contrast only 18.2 % of the genes showed down regulation. The differential expression pattern of the common genes under both the stress can be viewed in fig.2. The gene loci associated with affymetrix probe ID were traced out using ricechip.org web site. All the gene loci associated with the probe ID were retrieved and further used for functional annotation of the genes. The retrieved gene loci were used as input in orygenesdb.cirad.fr under locus search option to map the position of the gene on to respective chromosome [Fig. 3]. The genes Os05g48650.1, Os08g36920.1, Os02g46030.1, Os06g27560.1, Os05g25920, Os02g33380.1, Os11g26780.1, os06g04940.1, Os07g46950.1, Os07g48830.1, Os10g21590.2, Os04g12960.1, Os11g26790, Os11g26790.1 and Os02g41510 showed strong up regulation under both the stresses. Similarly among all the DEGs, the genes Os12g12390.1, Os01g44390.1, Os01g63180.1, Os07g03870.1 and Os10g31720.1 showed high down regulation. The integration of gene on to chromosomes revealed that chromosome 2 and 8 had more number of candidate genes under combined stress. In present analysis chromosome 9 did not show any genes for combined drought and salinity stress. The

functional annotation of candidate genes showed that most of the genes encodes for transcription factor [TF] followed by different stress related proteins [table.1]. Transcription factors are early responsive genes important candidates for expression of large number of downstream stress responsive genes by binding to the specific *cis*-acting elements of the genes to access tolerance mechanism [17]. Some of the prominent protein that expressed under combined stress include MYB family transcription factor, ethylene-responsive transcription factor, UDP-glucoronosy, WRKY53, glycosyl ptransferase 8, plant PDR ABC transporter associated domain and dehydrin. The tolerance mechanisms to drought and salinity stress include changes at genetic, transcriptomic and metabolomic level [18]. The gene Os05g48650.1 which present on chromosome five at 28.8 Mb physical position encodes HBP-1b [histone gene binding protein-1b] transcription factor showed strong up regulation. The HBP1b falls under bZIP family of TFs. These proteins are present throughout the plant kingdom and plays important role in plant response to biotic and abiotic stresses [19, 20 and 21]. The over expression of OsHBP1b dramatically increases salinity as well as drought tolerance of tobacco suggests that, further analysis of this gene will have the potential to greatly improve stress tolerance in other crop species like rice [22]. through altering the activation of ROS scavenging system and the levels of protective compounds, such as MDA, sugars and proline. This may serve as a useful 'candidate gene' for improving multiple stress tolerance in crop plant. Similary, the TF AP2 encoded by gene Os08g36920.1 which present on chromosome 8 has multiple functions under biotic and abiotic stress [23]. These TFs involves in regulation of CBF/DREB factors involved in abiotic stress responses. The over expression of DREB1A TF isolatd from rice showed enhanced tolerance to drought, salt and cold stress in transgenenic Arabidopsis [24]. The genes [Os02g41510 and Os02g46030.1] present on chromosome 2 showed their association with MYB family transcription factor. The MYB gene family comprises one of the richest groups of transcription factors in plants. Plant MYB proteins are characterized by a highly conserved MYB DNA-binding domain. MYB transcription factors are involved in plant development, secondary metabolism, hormone signal transduction, disease resistance and abiotic stress tolerance [25]. Three Os04g12960.1, Os06g27560.1 and Os07g48830.1 which located on chromosome 4, 6 and 7 respectively encodes for glucosyltransferase nad glycosyltransferase enzymes. Earlier authors reported the function of glycosyltransferase under different abiotic stress [26]. In Arabidopsis thaliana, UDP-glucosyltransferase showed improved tolerance against drought and salinity stress by enhancing the rooting capacity through regulating IBA and NAA concentrations [27]. Glycosyl transferase mainly function in to the biosynthesis of plant cell walls [28]. In

our study, we identified transporters encoded by genes Os06g03700.1 and Os10g21590.2 on chromosome 6 [1.4 Mb] and 10 [11 Mb] respectively. Different types of transporters were reported in plant kingdom, in general many of them are required for plant growth, development, nutrition, and response to abiotic stresses by manipulating the concentration of toxic ions [29]. The two genes Os11g26780.1 and Os11g26790.1 locolised on chromosome 11 encoded for dehydrin a stress responsive gene. Many studies reported that the expression of dehydrin is positively correlated with the tolerance to cold, drought, and salt stress [30,31]. The dehyrin works as a molecular chaperone under stress situation and helps in maintaining the structural and functional integrity of the proteins, enzyme activities, nucleic acids and membrane structure. The over-expression of dehydrin gene [OsDhn1] improved drought and salt stress tolerance through scavenging of reactive oxygen species in rice [32].

In conclusion, our study showed only a limited number of tolerant genes common between drought and salinity stress. The genes Os11g26780.1, Os11g26790.1, Os06g27560.1 and Os05g48650.1 were directly related with salt and drought stress tolerance. The introgression of these genes in high yielding stress susceptible genotypes could assist in developing stress tolerant cultivars.

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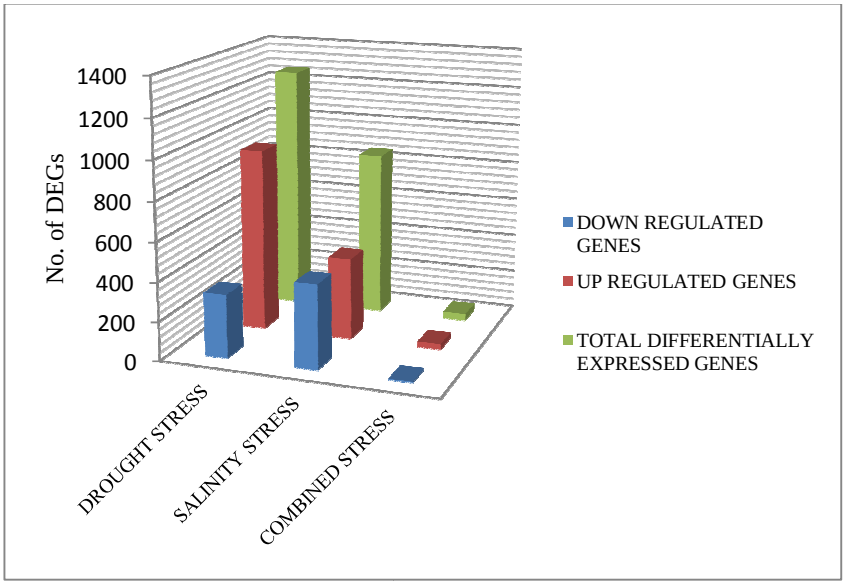

Fig. 1: differential expression of genes [DEGs] under each stress and combined stress.

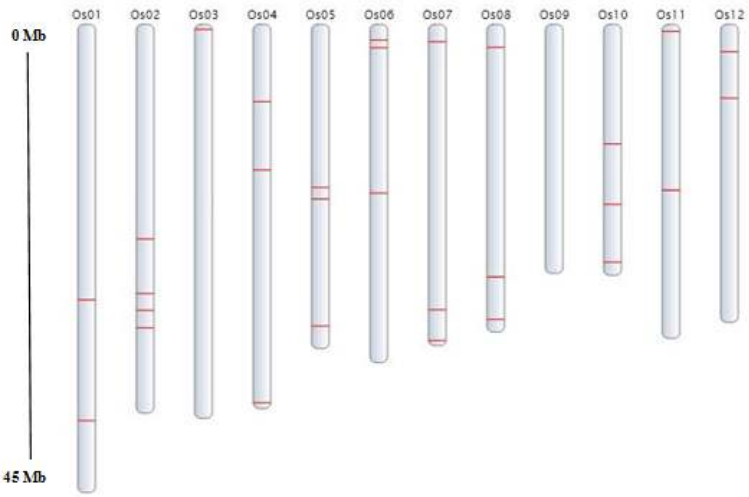

Fig. 2: Physical position of candidate gene on respective chromosome [chromosomes arranged in ascending order from 1 to 12].

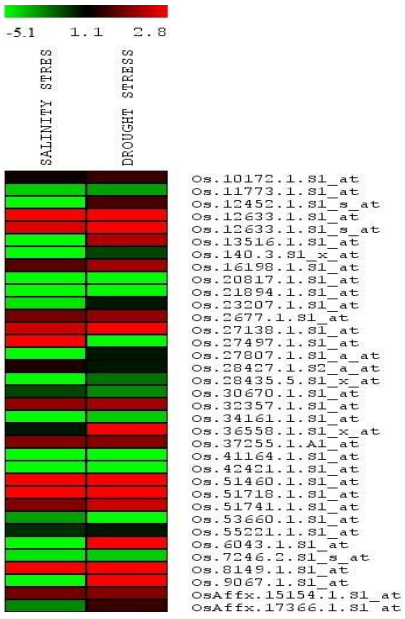

Fig. 3: MEV analyses for genes commonly expressed genes for salt and drought stress [colour gradient towards red indicates up-regulation and gradient towards green implies down-regulated genes].

Table. 1: Functional annotation of candidate genes commonly expressed undet combined stress.

Probe ID	**Locus ID**	**Chr.**	**Start [bp]**	**End [bp]**	**Putative function**
Os.41164.1.S1_at	Os01g63180.1	01	36616801	36621516	laccase-6 precursor

Os.10172.1.S1_at	Os01g44390.1	01	25461991	25463691	MYB family transcription factor
Os.51460.1.S1_at	Os02g41510	02	24878775	24879934	MYB family transcription factor
Os.53660.1.S1_at	Os02g33380.1	02	19834281	19835139	pectinesterase inhibitor domain containing protein
Os.8149.1.S1_at	Os02g43790.1	02	26422182	26423485	ethylene-responsive transcription factor
Os.27807.1.S1_a_at	Os02g46030.1	02	28041115	28044149	MYB family transcription factor
Os.140.3.S1_x_at	Os03g01740.1	03	470268	471096	expressed protein
Os.16198.1.S1_at	Os04g58810.1	04	34980601	34982203	CAF1 family ribonuclease containing protein
Os.6043.1.S1_at	Os04g12960.1	04	7153701	7156100	UDP-glucoronosyl/UDP-glucosyl transferase
Os.11773.1.S1_at	Os04g23550.1	04	13466420	13468869	basic helix-loop-helix family protein
Os.51741.1.S1_at	Os05g27730	05	16150266	16152747	WRKY53, expressed
OsAffx.15154.1.S1_at	Os05g25920	05	15077666	15078358	expressed protein
Os.7246.2.S1_s_at	Os05g48650.1	05	27883503	27884410	transcription factor HBP-1b
Os.34161.1.S1_at	Os06g27560.1	06	15601591	15603932	Glycosyl transferase protein
Os.37255.1.A1_at	Os06g03700.1	06	1459192	1464339	oligopeptide transporter
Os.20817.1.S1_at	Os06g04940.1	06	2175882	2176926	early nodulin 93 ENOD93 protein
Os.2677.1.S1_at	Os07g03870.1	07	1612858	1616245	receptor like protein kinase
Os.9067.1.S1_at	Os07g48830.1	07	29220283	29221843	glycosyl transferase 8 domain containing protein
Os.55221.1.S1_at	Os07g44140.1	07	26382581	26385011	cytochrome P450 72A1
Os.12452.1.S1_s_at	Os08g43120.1	08	27268083	27277540	Plant PDR ABC transporter associated domain
Os.23207.1.S1_at	Os08g36910	08	23340676	23343533	alpha-amylase precursor
OsAffx.17366.1.S1_at	Os08g04340.1	08	2129613	2130615	plastocyanin-like domain containing protein
Os.21894.1.S1_at	Os08g36920.1	08	23353882	23355003	AP2 domain containing protein
Os.27138.1.S1_at	Os10g31720.1	10	16625819	16626573	glycine-rich cell wall structural protein 2 precursor
Os.28435.5.S1_x_at	Os10g21590.2	10	11053081	11055159	transporter family protein, putative
Os.51718.1.S1_at	Os10g40934.1	10	21988523	21993310	flavonol synthase/flavanone 3-hydroxylase
Os.12633.1.S1_s_at	Os11g26780.1	11	15336034	15337163	Dehydrin
Os.36558.1.S1_x_at	Os11g26790.1	11	15342564	15343857	Dehydrin
Os.13516.1.S1_at	Os11g02290	11	651185	652057	expressed protein
Os.27497.1.S1_at	Os12g05540.1	12	2535821	2539020	Ser/Thr protein phosphatase family protein
Os.42421.1.S1_at	Os12g12390.1	12	6821234	6822471	transposon protein, putative, CACTA

304 Chr. : chromosome, bp: basepair