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## Research Article

### Shortlisting putative candidate genes underlying qDTY<sub>1.1</sub>, a major effect drought tolerant QTL in rice (*Oryza sativa* L.)

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#### Abstract

Drought is one of the major constraints affecting rice (*Oryza sativa* L.) productivity and production. Conventional breeding is leading to slow progress in developing drought tolerant varieties. In this context, mapping consistent major effect QTLs and unraveling molecular basis of those QTLs will pave way for deployment of those QTLs into breeding applications. Out of more than 2500 drought tolerance related QTLs, hardly 5 to 10 QTLs have been put into breeding applications due to larger QTL window, lack of tightly linked markers and lack of knowledge on candidate genes underlying QTLs. In this present study, *in silico* analysis was carried out to characterize a major effect QTL qDTY<sub>1.1</sub> region on chromosome 1 for its gene content, drought responsiveness of genes in the QTL window and metabolic functions. Out of 352 genes residing in the qDTY<sub>1.1</sub> window, 50% of the genes were found to be drought responsive. Bioinformatics search identified precise drought responsive expression pattern of 100 genes in the QTL window. Fifty seven genes were found to be up regulated and forty three genes were found to be down regulated. The qDTY<sub>1.1</sub> region is found to contain 23 drought responsive transcription factors such as *WRKY*, *bZIP*, *NAC*, *AP2* and *C2H2*. These drought responsive genes are confirmed for the possession of drought responsive *cis* elements like DRE, ABRE, MYB and MYC.

#### Key words

Rice, drought tolerance, QTL, *in silico* analysis, Transcription Factors.

#### INTRODUCTION

Increasing global population demands at least 50% of increase in food production by 2050 (Muthu, 2019). But, prevailing growth rate of yields in major crops is not satisfactory (Ray *et al.*, 2013). Rice is one of the important staple cereals in India whose yield is frequently affected by abiotic stresses including drought, salinity, heat and submergence (Dixit *et al.*, 2017). Further, changing climate increases the frequency such occurrences and thus posing serious threat for reaching the targeted production of 160 million tonnes during 2050. Among all abiotic stresses, drought remains at the top in limiting rice production and thus causing major threat to food security (Zhang, 2018).

Progress through conventional breeding approaches in developing drought tolerant rice genotypes is slow due to the complexity of tolerance mechanisms. Recent progress in genetic mapping of drought tolerance traits in rice paved the way for accelerated development of drought tolerant rice genotypes through molecular breeding (Kamoshita *et al.*, 2008; Khowaja *et al.*, 2009; Suji *et al.*, 2012; Mai *et al.*, 2014; Swamy *et al.*, 2017). Approximately 2137 QTLs have been reported to be associated with drought tolerance related traits in rice until 2009 (Courtois *et al.*, 2009) and currently it would have crossed 2500. Only limited number of QTLs have been put into breeding applications because of their

consistency across environments, effect on yield during stress and availability of tightly linked markers.

Among the various drought tolerance related QTLs, a major effect loci qDTY<sub>1.1</sub> flanked by RM11943 and RM431 on Chromosome 1 has been reported by several authors (Vikram *et al.*, 2011). This QTL showed a consistent effect in high yielding multiple elite genetic backgrounds viz., Swarna, IR64, and MTU1010 (Vikram *et al.*, 2011). However, qDTY<sub>1.1</sub> was reported to be tightly linked to sd1 loci controlling plant height in rice. (Vikram *et al.*, 2015) demonstrated that such linkage between drought tolerance and height was successfully overcome by increasing population size and by use of dense markers in the target region. This indicated that such larger QTLs require fine mapping and QTL cloning which will allow us to develop gene based markers for precise introgression of QTLs with minimum linkage drag. Fine mapping is time consuming and labor intensive process as it involves development and screening a large number of back cross progenies, use of large number of markers in the QTL window and extensive phenotyping. Availability of genomic information including whole genome sequence information, expression data across diverse growth/environmental conditions and sophisticated bioinformatics allowed molecular dissection of QTLs so as to identify putative candidate genes underlying QTLs. *In silico* analysis of a major effect drought tolerance QTL on chromosome 9 spanning 22 cM (73 cM to 95 cM) revealed the presence of 145 genes and further exploration of expression data shortlisted potential candidate genes like Aquaporin (OsPIP2; 7) and cytochromeP450 (OsCYP78A3) (Ramya *et al.*, 2010). Similar studies have been undertaken to shortlist genes underlying QTLs for salinity tolerance (Abhayawickrama *et al.*, 2020), and grain protein content (Indurkar *et al.*, 2015).

Based on the above facts, current study was undertaken to unravel the molecular basis of a major effect QTL qDTY<sub>1.1</sub> linked to grain yield under drought stress in rice. Detailed bioinformatics analyses were carried out to identify the stress responsive genes, their possible roles in metabolic pathways related to drought tolerance and also to analyze the presence of *cis* elements in the promoter region(s).

## MATERIALS AND METHODS

### Details of the target QTL

A drought tolerant QTL, qDTY<sub>1.1</sub> was selected based on literature survey (table 1) and its physical location was delineated from Gramene database (<https://archive.gramene.org/ql/>). Two overlapping loci viz., loci flanked by RM 486 and RM 472 (34.95 – 37.88 Mb) as reported by (Venuprasad *et al.*, 2012) and nearby region flanked by RM 11943 and RM 431 (37.85 – 38.89 Mb) as reported by (Vikram *et al.*, 2011) were chosen for the analysis.

### *In silico* analysis of qDTY<sub>1.1</sub>

Genes located in the QTL window (34.95 Mb - 38.89 Mb) were identified from TIGR Rice pseudomolecules version 7.0 ([http://rice.plantbiology.msu.edu/pub/data/Eukaryotic\\_Projects/o\\_sativa/annotation\\_dbs/pseudomolecules/version\\_7.0/](http://rice.plantbiology.msu.edu/pub/data/Eukaryotic_Projects/o_sativa/annotation_dbs/pseudomolecules/version_7.0/)) available with Rice Annotation Project.

### Identification of drought responsive genes in qDTY<sub>1.1</sub>

Putative drought responsive genes in the QTL window and their level of expression were picked up from the publicly available expression database namely Rice Metasys (<http://14.139.229.201/RiceMetaSys/index.php>). Drought responsive expression pattern of the genes located in qDTY<sub>1.1</sub> was also assigned using the same database. Drought responsive expression pattern (Log Fold Change) of these genes in tissues like leaves, panicle and root were identified.

### Pathway mapping of drought responsive genes

Identified drought responsive genes were mapped onto metabolic pathways based on literature survey and gene ontology database. The locus ID of the drought responsive genes was converted to RAP ID using RAP DB (<https://rapdb.dna.affrc.go.jp/index.html>) and used for search in the KEGG and TIGR database.

### Identification of transcription factors modulating drought responses

Transcription factors putatively modulating drought responses were identified. Using PlantPAN 3.0 database (<http://plantpan.itps.ncku.edu.tw/>). Individual drought responsive transcription factors in these regions were downloaded and annotated for their function using literatures.

**Table 1. Details of studies reporting association of qDTY<sub>1.1</sub> with drought tolerance in rice**

S.no	Population	Marker interval	Explained phenotypic variation (%)	Reported by
1.	N22/Swarna	RM11943-RM431	13.4	(Vikram <i>et al.</i> , 2011)
2.	N22/IR64	RM11943-RM431	16.9	(Vikram <i>et al.</i> , 2011)
3.	N22/MTU1010	RM11943-RM431	12.6	(Vikram <i>et al.</i> , 2011)
4.	Apo/IR64	RM486-RM472	-	(Venuprasad <i>et al.</i> , 2012)
5.	Dhagaddeshi/Swarna	RM431-RM104	22.6	(Ghimire <i>et al.</i> , 2012)
6.	Dhagaddeshi/IR64	RM104-RM12091	9.2	(Ghimire <i>et al.</i> , 2012)

### Analysis of cis elements in the promoter region of drought responsive genes

To analyze the presence of drought responsive *cis* regulatory elements in promoter region, 1000 bp upstream sequences of all 100 drought responsive genes were individually downloaded from RAP DB and TIGR database. These 1 kb upstream sequences were analyzed in the PLACE database (<https://www.dna.affrc.go.jp/PLACE/?action=newplace>). Position and frequency in the occurrence of drought responsive *cis* regulatory elements like ABRE/CRT, DRE, MYB and MYC were analyzed (Table 2.)

### RESULTS AND DISCUSSION

*qDTY<sub>1.1</sub>*, a major effect drought tolerance related QTL located on rice chromosome 1 (flanked between RM 11943 to RM 431 and RM 486 to 472) was reported to

be responsible for increased grain yield under drought stress and found to be consistent across different genetic backgrounds (Vikram *et al.*, 2011; Venuprasad *et al.*, 2012). Bioinformatics analysis using TIGR database identified around 352 putative protein encoding genes in the QTL region. *qDTY<sub>1.1</sub>* was found to harbor This region was found to harbour several drought tolerance related genes namely, WRKY, serine/threonine protein-kinase, MYB, etc. Systematic search using Rice Metasys database identified 140 drought responsive genes (Table 3.). Subsequent analysis for picking up the level of drought responsive changes exhibited by these genes using Metasys database revealed that expression data was available only for 100 genes which were used for further analysis. Out of the 100 drought responsive genes available with expression data, 57 genes were found to be up-regulated and 43 were found to be down regulated (Fig. 1).

Table 2. Details of drought responsive *cis* elements searched in this study

S.no	<i>Cis</i> -acting elements	Conserved <i>cis</i> motif sequence	Reference
1.	ABRE	ACGTGGC, ACGTGTC	(Uno <i>et al.</i> , 2000)
2.	DRE/CRT	A/GCCGAC	(Yamaguchi-Shinozaki and Shinozaki, 2005)
3.	MYB	C/TAACG/TG	(Abe <i>et al.</i> , 1997; Abe <i>et al.</i> , 2003)
4.	MYC	CACATG/CATGTG	(Abe <i>et al.</i> , 1997; Abe <i>et al.</i> , 2003)

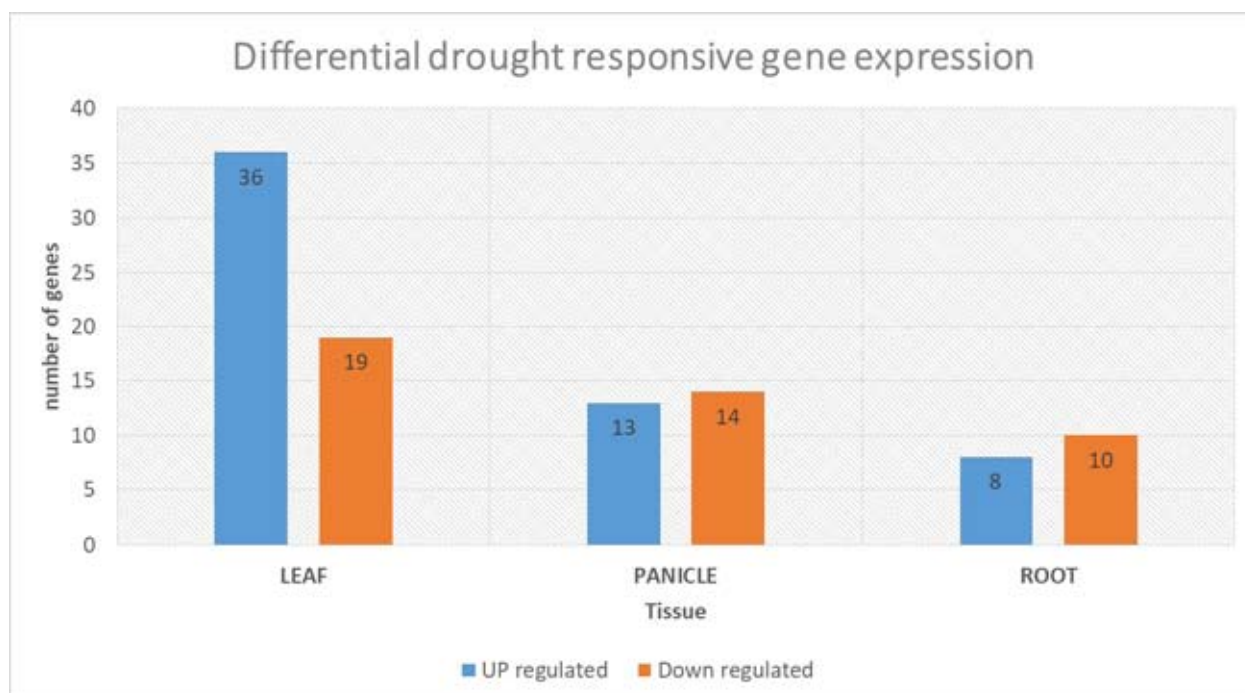


Fig. 1. Number of drought responsive genes of *qDTY<sub>1.1</sub>* in various tissues

**Table 3. List of drought responsive genes found in qDTY<sub>1.1</sub>**

S.No	Locus ID	Annotation	Tissue	Fold Change (LogFC)	Expression of gene identified (*)
1	LOC_Os01g60660	methionyl-tRNA synthetase, putative, expressed	Leaf	1.036	*
2	LOC_Os01g60700	serine/threonine-protein kinase NAK, putative, expressed	Leaf	1.369	*
3	LOC_Os01g61030	VHS and GAT domain containing protein, expressed	Leaf	1.097	*
4	LOC_Os01g61070	heavy metal-associated domain containing protein, expressed	Leaf	1.204	*
5	LOC_Os01g61500	BAG domain containing protein, expressed	Leaf	1.042	*
6	LOC_Os01g61830	zinc finger C-x8-C-x5-C-x3-H type family protein, expressed	Leaf	1.411	*
7	LOC_Os01g62190	ZOS1-15 - C2H2 zinc finger protein, expressed	Leaf	6.084	*
8	LOC_Os01g62210	50S ribosomal protein L17, putative, expressed	Leaf	1.179	*
9	LOC_Os01g62290	DnaK family protein, putative, expressed	Leaf	1.058	*
10	LOC_Os01g62300	SLT1 protein, putative, expressed	Leaf	1.187	*
11	LOC_Os01g62410	MYB family transcription factor, putative, expressed	Leaf	2.093	*
12	LOC_Os01g62610	peptidyl-prolyl cis-trans isomerase, FKBP-type, putative, expressed	Leaf	1.854	*
13	LOC_Os01g62650	pumilio-family RNA binding protein, putative, expressed	Leaf	1.217	*
14	LOC_Os01g62760	protein phosphatase 2C, putative, expressed	Leaf	2.306	*
15	LOC_Os01g62900	amino acid kinase, putative, expressed	Leaf	2.274	*
16	LOC_Os01g63060	phosphatidic acid phosphatase-related, putative, expressed	Leaf	1.675	*
17	LOC_Os01g63230	growth regulator related protein, putative, expressed	Leaf	1.295	*
18	LOC_Os01g63354	ELM2 domain containing protein, putative, expressed	Leaf	1.436	*
19	LOC_Os01g64280	sorting nexin 1, putative, expressed	Leaf	1.213	*
20	LOC_Os01g64300	DUF584 domain containing protein, putative, expressed	Leaf	2.449	*
21	LOC_Os01g64360	MYB family transcription factor, putative, expressed	Leaf	2.784	*
22	LOC_Os01g64520	uricase, putative, expressed	Leaf	2.233	*
23	LOC_Os01g64700	OsFBT3 - F-box and tubby domain containing protein, expressed	Leaf	1.396	*
24	LOC_Os01g64730	bZIP transcription factor domain containing protein, expressed	Leaf	2.661	*
25	LOC_Os01g64750	sterol 3-beta-glucosyltransferase, putative, expressed	Leaf	1.113	*
26	LOC_Os01g64970	CAMK_CAMK_like.11 - CAMK includes calcium/calmodulin dependent protein kinases, expressed	Leaf	2.012	*
27	LOC_Os01g65200	proton-dependent oligopeptide transport, putative, expressed	Leaf	1.47	*
28	LOC_Os01g65310	DUF803 domain containing, putative, expressed	Leaf	1.539	*
29	LOC_Os01g65380	patellin protein, putative, expressed	Leaf	1.051	*
30	LOC_Os01g65520	Sad1 / UNC-like C-terminal domain containing protein, putative, expressed	Leaf	3.468	*
31	LOC_Os01g65780	glycosyl transferase, putative, expressed	Leaf	1.357	*
32	LOC_Os01g65986	DUF803 domain containing, putative, expressed	Leaf	1.146	*
33	LOC_Os01g66030	OsMADS2 - MADS-box family gene with MIKCC type-box, expressed	Leaf	1.443	*
34	LOC_Os01g66120	No apical meristem protein, putative, expressed	Leaf	1.071	*
35	LOC_Os01g66520	serine/threonine-protein kinase RIO-like, putative, expressed	Leaf	1.037	*
36	LOC_Os01g66850	pectinacylesterase domain containing protein, expressed	Leaf	1.055	*
37	LOC_Os01g60730	RING-H2 finger protein, putative, expressed	Panicle	1.028	*
38	LOC_Os01g60920	OsFBX30 - F-box domain containing protein, expressed	Panicle	1.107	*
39	LOC_Os01g61430	HIT zinc finger domain containing protein, expressed	Panicle	1.695	*
40	LOC_Os01g61890	AT hook motif family protein, expressed	Panicle	1.195	*
41	LOC_Os01g62630	aspartic proteinase nepenthesin precursor, putative, expressed	Panicle	1.553	*
42	LOC_Os01g64630	actin, putative, expressed	Panicle	1.234	*
43	LOC_Os01g65080	ZOS1-18 - C2H2 zinc finger protein, expressed	Panicle	1.535	*
44	LOC_Os01g65200	proton-dependent oligopeptide transport, putative, expressed	Panicle	1.535	*
45	LOC_Os01g65900	chitin-inducible gibberellin-responsive protein, putative, expressed	Panicle	1.287	*

46	LOC_Os01g66120	No apical meristem protein, putative, expressed	Panicle	1.601	*
47	LOC_Os01g66180	cytochrome c, putative, expressed	Panicle	1.46	*
48	LOC_Os01g66420	PHD finger protein, putative, expressed	Panicle	1.499	*
49	LOC_Os01g66520	serine/threonine-protein kinase RIO-like, putative, expressed	Panicle	1.843	*
50	LOC_Os01g60730	RING-H2 finger protein, putative, expressed	Root	2.48	*
51	LOC_Os01g62390	growth regulator related protein, putative, expressed	Root	1.004	*
52	LOC_Os01g62980	LTPL101 - Protease inhibitor/seed storage/LTP family protein precursor, expressed	Root	2.143	*
53	LOC_Os01g63190	laccase precursor protein, putative, expressed	Root	2.143	*
54	LOC_Os01g64730	bZIP transcription factor domain containing protein, expressed	Root	1.734	*
55	LOC_Os01g66170	SNARE associated Golgi protein, putative, expressed	Root	1.086	*
56	LOC_Os01g66500	phosphoribosylformylglycinamide synthase, putative, expressed	Root	1.195	*
57	LOC_Os01g66520	serine/threonine-protein kinase RIO-like, putative, expressed	Root	1.495	*
58	LOC_Os01g61010	nodulin, putative, expressed	Leaf	-2.17	*
59	LOC_Os01g61170	prenylated rab acceptor, putative, expressed	Leaf	-1.22	*
60	LOC_Os01g61320	thioredoxin, putative, expressed	Leaf	-1.57	*
61	LOC_Os01g61590	CAMK_CAMK_like.1 - CAMK includes calcium/calmodulin dependent protein kinases, expressed	Leaf	-1.01	*
62	LOC_Os01g61880	respiratory burst oxidase, putative, expressed	Leaf	-2.28	*
63	LOC_Os01g62440	ribonuclease protein, putative, expressed	Leaf	-1.47	*
64	LOC_Os01g63190	laccase precursor protein, putative, expressed	Leaf	-2.73	*
65	LOC_Os01g63480	transferase family protein, putative, expressed	Leaf	-1.3	*
66	LOC_Os01g63770	transmembrane amino acid transporter protein, putative, expressed	Leaf	-1.54	*
67	LOC_Os01g64170	glycosyl hydrolases family 17, putative, expressed	Leaf	-1.11	*
68	LOC_Os01g64540	anthocyanidin 3-O-glucosyltransferase, putative, expressed	Leaf	-3.04	*
69	LOC_Os01g64670	soluble inorganic pyrophosphatase, putative, expressed	Leaf	-1.17	*
70	LOC_Os01g64960	chlorophyll A-B binding protein, putative, expressed	Leaf	-1.64	*
71	LOC_Os01g65100	peptide transporter, putative, expressed	Leaf	-1.14	*
72	LOC_Os01g65350	deoxynucleoside kinase family, putative, expressed	Leaf	-1.61	*
73	LOC_Os01g65560	snRK1-interacting protein 1, putative, expressed	Leaf	-1.12	*
74	LOC_Os01g65880	nodulin MtN3 family protein, putative, expressed	Leaf	-1.09	*
75	LOC_Os01g66000	NADH dehydrogenase I subunit N, putative, expressed	Leaf	-4.5	*
76	LOC_Os01g66970	zinc finger, C3HC4 type domain containing protein, expressed	Leaf	-1.2	*
77	LOC_Os01g60860	spotted leaf 11, putative, expressed	Panicle	-1.17	*
78	LOC_Os01g61170	prenylated rab acceptor, putative, expressed	Panicle	-1.08	*
79	LOC_Os01g61500	BAG domain containing protein, expressed	Panicle	-1.35	*
80	LOC_Os01g61760	myosin heavy chain-related, putative, expressed	Panicle	-1.06	*
81	LOC_Os01g62020	NAD dependent epimerase/dehydratase family domain containing protein, expressed	Panicle	-1.38	*
82	LOC_Os01g62850	membrane associated DUF588 domain containing protein, putative, expressed	Panicle	-1.51	*
83	LOC_Os01g62900	amino acid kinase, putative, expressed	Panicle	-1.18	*
84	LOC_Os01g64170	glycosyl hydrolases family 17, putative, expressed	Panicle	-1.45	*
85	LOC_Os01g64300	DUF584 domain containing protein, putative, expressed	Panicle	-1	*
86	LOC_Os01g64760	Yip1 domain containing protein, expressed	Panicle	-1.77	*
87	LOC_Os01g64860	OsSub11 - Putative Subtilisin homologue, expressed	Panicle	-2.67	*
88	LOC_Os01g65680	4,5-DOPA dioxygenase extradiol, putative, expressed	Panicle	-1.02	*
89	LOC_Os01g66110	methyltransferase, putative, expressed	Panicle	-1.19	*
90	LOC_Os01g66590	DUF260 domain containing protein, putative, expressed	Panicle	-1.52	*
91	LOC_Os01g62190	ZOS1-15 - C2H2 zinc finger protein, expressed	Root	-2.17	*
92	LOC_Os01g62420	triosephosphate isomerase, cytosolic, putative, expressed	Root	-1.69	*
93	LOC_Os01g62460	ZOS1-16 - C2H2 zinc finger protein, expressed	Root	-1.28	*
94	LOC_Os01g63690	hs1, putative, expressed	Root	-2.44	*

95	LOC_Os01g64520	uricase, putative, expressed	Root	-2.32	*
96	LOC_Os01g65100	peptide transporter, putative, expressed	Root	-1.68	*
97	LOC_Os01g65110	POT family protein, expressed	Root	-5.18	*
98	LOC_Os01g65650	receptor-like protein kinase HAIKU2 precursor, putative, expressed	Root	-1.37	*
99	LOC_Os01g65880	nodulin MtN3 family protein, putative, expressed	Root	-2.95	*
100	LOC_Os01g66940	kinase, pfkB family, putative, expressed	Root	-1.24	*
101	LOC_Os01g60640	WRKY21, expressed	-	-	-
102	LOC_Os01g60650	non-green plastid inner envelope membrane protein, putative, expressed	-	-	-
103	LOC_Os01g60910	CAMK_KIN1/SNF1/Nim1_like.12 - CAMK includes calcium/calmodulin dependent protein kinases, expressed	-	-	-
104	LOC_Os01g61044	transmembrane amino acid transporter protein, putative, expressed	-	-	-
105	LOC_Os01g61620	protein kinase family protein, putative, expressed	-	-	-
106	LOC_Os01g61670	ureidoglycolate hydrolase, putative, expressed	-	-	-
107	LOC_Os01g61780	vacuolar ATP synthase 98 kDa subunit, putative, expressed	-	-	-
108	LOC_Os01g61910	KIP1, putative, expressed	-	-	-
109	LOC_Os01g62040	ruvB-like, putative, expressed	-	-	-
110	LOC_Os01g62060	plant-specific domain TIGR01589 family protein, expressed	-	-	-
111	LOC_Os01g62070	cation efflux family protein, putative, expressed	-	-	-
112	LOC_Os01g62350	60S ribosomal protein L36-2, putative, expressed	-	-	-
113	LOC_Os01g62480	laccase precursor protein, putative, expressed	-	-	-
114	LOC_Os01g62660	MYB family transcription factor, putative, expressed	-	-	-
115	LOC_Os01g62840	mannose-1-phosphate guanyltransferase, putative, expressed	-	-	-
116	LOC_Os01g62950	ras-related protein, putative, expressed	-	-	-
117	LOC_Os01g63010	universal stress protein domain containing protein, putative, expressed	-	-	-
118	LOC_Os01g63210	SOUL heme-binding protein, putative, expressed	-	-	-
119	LOC_Os01g63220	kinase, pfkB family, putative, expressed	-	-	-
120	LOC_Os01g63270	alpha-glucan phosphorylase isozyme, putative, expressed	-	-	-
121	LOC_Os01g63290	transporter, major facilitator family, putative, expressed	-	-	-
122	LOC_Os01g63580	glycerol-3-phosphate acyltransferase, putative, expressed	-	-	-
123	LOC_Os01g63870	nucleobase-ascorbate transporter, putative, expressed	-	-	-
124	LOC_Os01g63890	G10 protein, putative, expressed	-	-	-
125	LOC_Os01g63930	cytochrome P450, putative, expressed	-	-	-
126	LOC_Os01g63990	hydrolase, alpha/beta fold family protein, putative, expressed	-	-	-
127	LOC_Os01g64010	dual specificity protein phosphatase, putative, expressed	-	-	-
128	LOC_Os01g64100	glycosyl hydrolase, putative, expressed	-	-	-
129	LOC_Os01g64120	2Fe-2S iron-sulfur cluster binding domain containing protein, expressed	-	-	-
130	LOC_Os01g65110	POT family protein, expressed	-	-	-
131	LOC_Os01g65200	proton-dependent oligopeptide transport, putative, expressed	-	-	-
132	LOC_Os01g65230	AGC_AGC_other_PDK1_Pk61C.1 - ACG kinases include homologs to PKA, PKG and PKC, expressed	-	-	-
133	LOC_Os01g65400	DNA polymerase I, putative, expressed	-	-	-
134	LOC_Os01g65410	serine hydroxymethyltransferase, mitochondrial precursor, putative, expressed	-	-	-
135	LOC_Os01g65902	apocytochrome f precursor, putative, expressed	-	-	-
136	LOC_Os01g65920	F-box/LRR-repeat protein 2, putative, expressed	-	-	-
137	LOC_Os01g66600	rhodanese-like, putative, expressed	-	-	-
138	LOC_Os01g66680	S-domain receptor-like protein kinase, putative, expressed	-	-	-
139	LOC_Os01g66700	beta-hexosaminidase precursor, putative, expressed	-	-	-
140	LOC_Os01g66860	serine/threonine protein kinase, putative, expressed	-	-	-

“\*\*” – identified from database (1 to 100)

“-” – Not available in database (101 to 140)

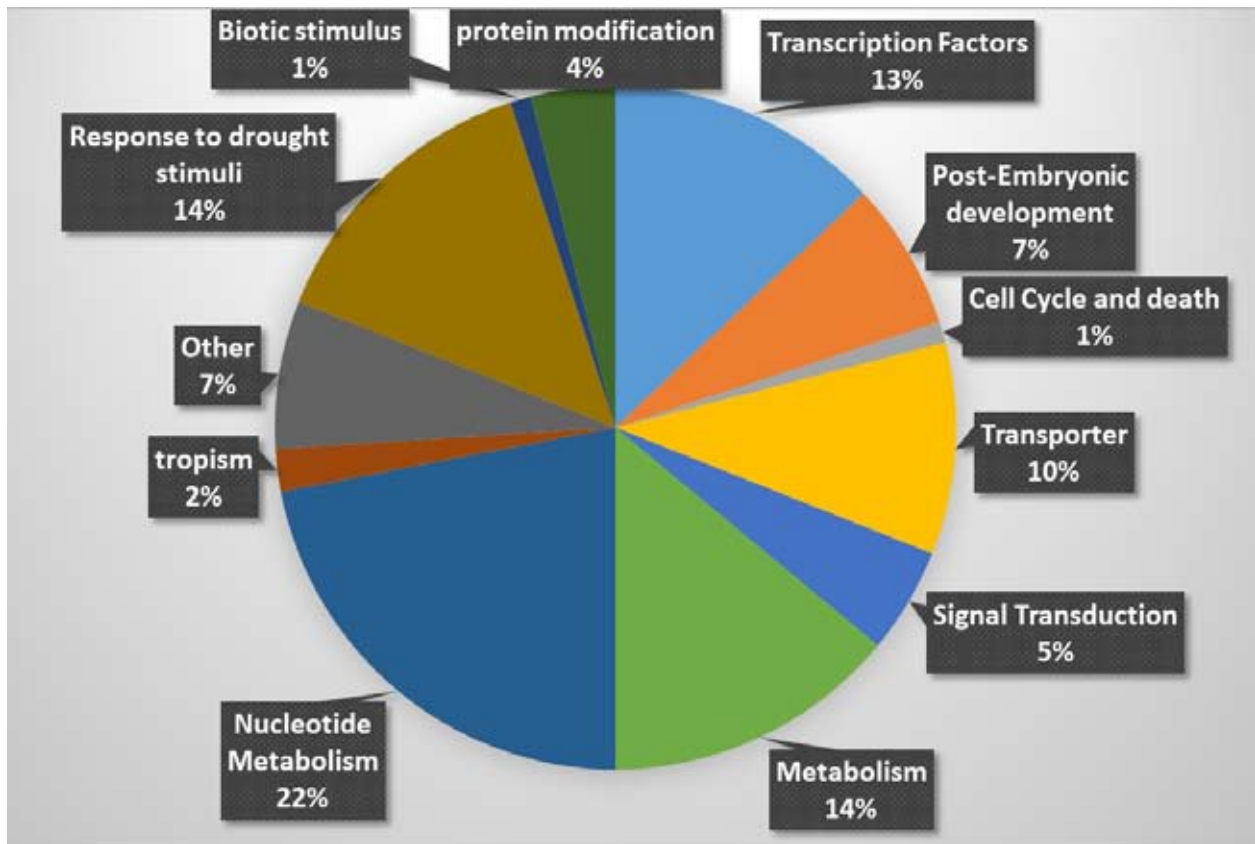


Fig. 2. Pathway mapping of drought responsive genes

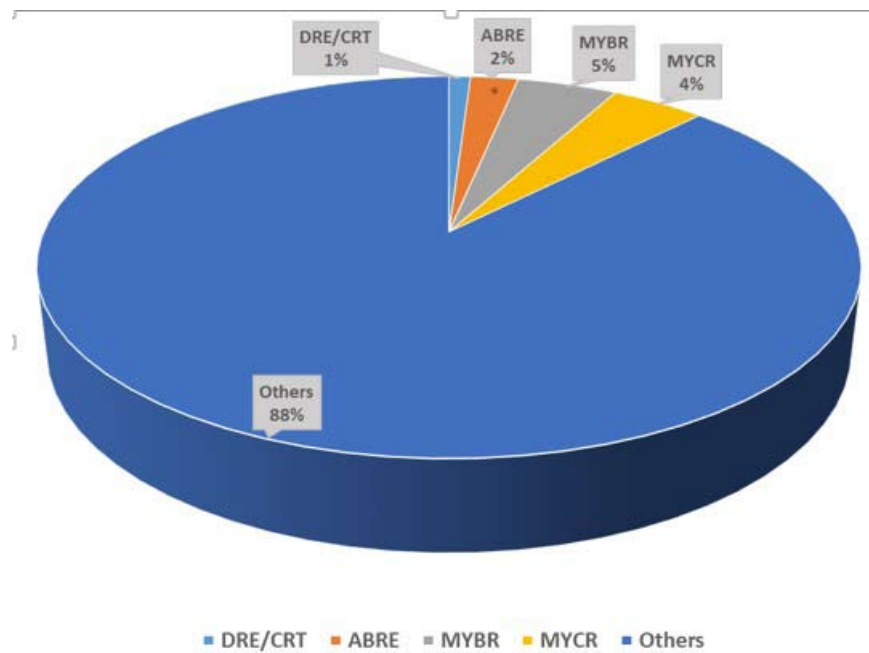


Fig. 3. Frequency in the occurrence of *cis* elements in the upstream of drought responsive genes

Among the up-regulated genes, ZOS1-15 - C2H2 zinc finger protein was found exhibit more than 6 fold change, Sad1 / UNC-like C-terminal domain containing protein was found to exhibit > 3 fold change and DUF584 domain containing protein and a MYB family transcription factor were found to be having more than 2.5 fold change under drought. These up regulated genes were found to play a key role in drought resistance (Ramegowda *et al.*, 2014; Liu *et al.*, 2015; Tang *et al.*, 2019).

Pathway mapping of all the drought responsive genes revealed that these genes were found to be involved in key metabolic functions viz., responsive to drought stimuli (14%), Transcription factors (13%), Cell cycle (1%), Transporter (10%), signal transduction (5%), Metabolism (14%), Nucleotide metabolism (22%), protein modification (4%) and tropism (2%). Bioinformatic analysis revealed the presence of several signaling genes namely calcium dependent protein kinase, serine/threonine-protein kinase, protein kinase, amino acid kinase and ACG kinase in the qDTY<sub>1,1</sub> region. These signaling molecules are reported to regulate stress responsive gene expression in rice (Xiong *et al.*, 2002; Diédhiou *et al.*, 2008).

Further, this key region was found to harbor several important transcription factors like 6 *WRKY*, 3 *C2H2*, 3 *bZIP*, 2 *MADS* box, 2 *WOX*, 1 *mybs*, 1 *bHLH*, 1 *NF-YB*, 1 *LBD* 1 *NAC*, 1 *AP2* and 1 zinc finger nuclease (Joshi *et al.*, 2016). *In silico* analysis of the 1 kb up stream region revealed that most of the genes were found to contain key drought responsive elements namely ABRE, DRE, MYB and MYC. (Fig. 3.). Among these 100 genes, 44 genes identified to contain more than 10 MYB, 33 genes identified to contain more than 10 MYC in their 1 Kb up stream region. Presence of these *cis* regulatory elements may be responsible for activation of several genes during drought (Sheshadri *et al.*, 2016).

qDTY<sub>1,1</sub> was found to contain 50% drought responsive genes and these genes were found to contain high frequency of drought responsive *cis* elements in their upstream. Shortlisted genes such as ZOS1-15- C2H2 zinc finger protein (LOC\_Os01g62190), Sad / UNC-like C-terminal domain containing protein (LOC\_Os01g65520), MYB family transcription factor (LOC\_Os01g64360) and *bZIP* Transcription factors (LOC\_Os01g64730) can be considered as putative candidate genes for further validation through functional genomics studies.

## REFERENCE

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