Shortlisting putative candidate genes underlying qDTY$_{1.1}$, a major effect drought tolerant QTL in rice (Oryza sativa L.)

Krishna Sai Karnatam, Deepa Jaganathan, Kambale Rohit Dilip, Manikanda Boopathi N and Raveendran Muthurajan*

Department of Plant Biotechnology, Centre for Plant Molecular Biology and Biotechnology, Tamil Nadu Agricultural University, Coimbatore - 641 003, Tamil Nadu, India
*E-Mail: raveendrantnau@gmail.com

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$_{1.1}$ 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$_{1.1}$ 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$_{1.1}$ 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; Suiji 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$_{1.1}$, 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$_{1.1}$ 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 salt 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$_{1.1}$, 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$_{1.1}$, was selected based on literature survey (Table 1) and its physical location was delineated from Gramene database (http://archive.gramene.org/qtl/). 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$_{1.1}$**

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/) available with Rice Annotation Project.

**Identification of drought responsive genes in qDTY1.1**

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$_{1.1}$ 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$_{1.1}$ with drought tolerance in rice

| S.no | Population         | Marker interval | Explained phenotypic variation (%) | Reported by                        |
|------|--------------------|-----------------|------------------------------------|------------------------------------|
| 1.   | N22/Swarna         | RM11943-RM431   | 13.4                               | (Vikram et al., 2011)              |
| 2.   | N22/IR64           | RM11943-RM431   | 16.9                               | (Vikram et al., 2011)              |
| 3.   | N22/MTU1010        | RM11943-RM431   | 12.6                               | (Vikram et al., 2011)              |
| 4.   | Apo/IR64           | RM486-RM472     | -                                  | (Venuprasad et al., 2012)          |
| 5.   | Dhagaddeshi/Swarna | RM431-RM104     | 22.6                               | (Ghimire et al., 2012)             |
| 6.   | Dhagaddeshi/IR64   | RM104-RM12091   | 9.2                                | (Ghimire et al., 2012)             |
To analyze the presence of drought responsive cis regulatory elements in promoter region, 1000 bp up stream 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

qDTY1.1, a major effect drought tolerance related QTL located on rice chromosome 1 (flanked between RM 11943 to RM 431and 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. qDTY1.1 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 | Cis-acting elements | Conserved cis motif sequence | Reference |
|------|---------------------|-----------------------------|-----------|
| 1.   | ABRE                | ACCTGTC, ACCTGTC            | (Uno et al., 2000) |
| 2.   | DRE/CRT             | A/GCCGAC                    | (Yamaguchi-Shinozaki and Shinozaki, 2005) |
| 3.   | MYB                 | C/TAACG/TG                  | (Abe et al., 1997; Abe et al., 2003) |
| 4.   | MYC                 | CACATG/CATGTG               | (Abe et al., 1997; Abe et al., 2003) |

Fig. 1. Number of drought responsive genes of qDTY1.1 in various tissues
### Table 3. List of drought responsive genes found in qDTY1.1

| S.No | Locus ID          | Annotation                                                                 | Tissue | Fold Change (LogFC) | Expression of gene identified (*) |
|------|-------------------|---------------------------------------------------------------------------|--------|---------------------|---------------------------------|
| 1    | LOC_Os01g60660    | methionyl-RNA 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_Os01g63090    | phosphatidic acid phosphatase-related, putative, expressed              | Leaf   | 1.675               | *                                |
| 17   | LOC_Os01g63320    | 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 depedent 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    | pectinacetylesterase domain containing protein, expressed               | Leaf   | 1.055               | *                                |
| 37   | LOC_Os01g67300    | RING-H2 finger protein, putative, expressed                             | Panicle| 1.028               | *                                |
| 38   | LOC_Os01g69020    | 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_Os01g62830    | 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               | *                                |
| LOC_Os01g66120 | No apical meristem protein, putative, expressed | Panicle 1.601 |
| LOC_Os01g66180 | cytochrome c, putative, expressed | Panicle 1.46 |
| LOC_Os01g66420 | PHD finger protein, putative, expressed | Panicle 1.499 |
| LOC_Os01g66520 | serine/threonine-protein kinase RIO-like, putative, expressed | Panicle 1.843 |
| LOC_Os01g60730 | RING-H2 finger protein, putative, expressed | Root 2.48 |
| LOC_Os01g62390 | growth regulator related protein, putative, expressed | Root 1.004 |
| LOC_Os01g62980 | LTP101 - Protease inhibitor/seed storage/LTP family protein precursor, expressed | Root 2.143 |
| LOC_Os01g63190 | laccase precursor protein, putative, expressed | Root 2.143 |
| LOC_Os01g64730 | bZIP transcription factor domain containing protein, expressed | Root 1.734 |
| LOC_Os01g66170 | SNARE associated Golgi protein, putative, expressed | Root 1.086 |
| LOC_Os01g66500 | phosphoribosylformylglycinamidine synthase, putative, expressed | Root 1.195 |
| LOC_Os01g66520 | serine/threonine-protein kinase RIO-like, putative, expressed | Root 1.495 |
| LOC_Os01g61010 | nodulin, putative, expressed | Leaf -2.17 |
| LOC_Os01g61170 | prenylated rab acceptor, putative, expressed | Leaf -1.22 |
| LOC_Os01g61320 | thioredoxin, putative, expressed | Leaf -1.57 |
| LOC_Os01g61590 | CAMK_CAMK_like.1 - CAMK includes calcium/calmodulin dependent protein kinases, expressed | Leaf -1.01 |
| LOC_Os01g61880 | respiratory burst oxidase, putative, expressed | Leaf -2.28 |
| LOC_Os01g62440 | ribonuclease protein, putative, expressed | Leaf -1.47 |
| LOC_Os01g63190 | laccase precursor protein, putative, expressed | Leaf -2.73 |
| LOC_Os01g63480 | transferase family protein, putative, expressed | Leaf -1.3 |
| LOC_Os01g63770 | transmembrane amino acid transporter protein, putative, expressed | Leaf -1.54 |
| LOC_Os01g64170 | glycosyl hydrolases family 17, putative, expressed | Leaf -1.11 |
| LOC_Os01g64540 | anthocyanidin 3-O-glucosyltransferase, putative, expressed | Leaf -3.04 |
| LOC_Os01g64670 | soluble inorganic pyrophosphatase, putative, expressed | Leaf -1.17 |
| LOC_Os01g64960 | chlorophyll A-B binding protein, putative, expressed | Leaf -1.64 |
| LOC_Os01g65100 | peptide transporter, putative, expressed | Leaf -1.14 |
| LOC_Os01g65350 | deoxynucleoside kinase family, putative, expressed | Leaf -1.61 |
| LOC_Os01g65560 | snRK1-interacting protein 1, putative, expressed | Leaf -1.12 |
| LOC_Os01g65880 | nodulin MIN3 family protein, putative, expressed | Leaf -1.09 |
| LOC_Os01g66000 | NADH dehydrogenase I subunit N, putative, expressed | Leaf -4.5 |
| LOC_Os01g66970 | zinc finger, C3HC4 type domain containing protein, expressed | Leaf -1.2 |
| LOC_Os01g60860 | spotted leaf 1, putative, expressed | Panicle -1.17 |
| LOC_Os01g61170 | prenylated rab acceptor, putative, expressed | Panicle -1.08 |
| LOC_Os01g61500 | BAG domain containing protein, expressed | Panicle -1.35 |
| LOC_Os01g61760 | myosin heavy chain-related, putative, expressed | Panicle -1.06 |
| LOC_Os01g62020 | NAD dependent epimerase/dehydratase family domain containing protein, expressed | Panicle -1.38 |
| LOC_Os01g62850 | membrane associated DUF588 domain containing protein, putative, expressed | Panicle -1.51 |
| LOC_Os01g62900 | amino acid kinase, putative, expressed | Panicle -1.18 |
| LOC_Os01g64170 | glycosyl hydrolases family 17, putative, expressed | Panicle -1.45 |
| LOC_Os01g64300 | DUF584 domain containing protein, putative, expressed | Panicle -1 |
| LOC_Os01g64760 | Yip1 domain containing protein, expressed | Panicle -1.77 |
| LOC_Os01g64860 | OsSub11 - Putative Subtilisin homologue, expressed | Panicle -2.67 |
| LOC_Os01g65680 | 4,5-DOPA dioxygenase extradiol, putative, expressed | Panicle -1.02 |
| LOC_Os01g66110 | methyltransferase, putative, expressed | Panicle -1.19 |
| LOC_Os01g66590 | DUF260 domain containing protein, putative, expressed | Panicle -1.52 |
| LOC_Os01g62190 | ZOS1-15 - C2H2 zinc finger protein, expressed | Root -2.17 |
| LOC_Os01g62420 | trisepoxide isomerase, cytosolic, putative, expressed | Root -1.69 |
| LOC_Os01g62460 | ZOS1-16 - C2H2 zinc finger protein, expressed | Root -1.28 |
| LOC_Os01g63690 | hs1, putative, expressed | Root -2.44 |
| Gene ID          | Description                                      | Expression | Fold Change |
|-----------------|--------------------------------------------------|------------|-------------|
| LOC_Os01g64520  | uricase, putative, expressed                      | Root       | -2.32       |
| LOC_Os01g65100  | peptide transporter, putative, expressed         | Root       | -1.68       |
| LOC_Os01g65110  | POT family protein, expressed                    | Root       | -5.18       |
| LOC_Os01g65650  | receptor-like protein kinase HAIKU2 precursor, putative, expressed | Root       | -1.37       |
| LOC_Os01g65880  | nodulin Mn3 family protein, putative, expressed  | Root       | -2.95       |
| LOC_Os01g66940  | kinase, pfkB family, putative, expressed         | Root       | -1.24       |
| LOC_Os01g60640  | non-green plastid inner envelope membrane protein, putative, expressed | -          | -           |
| LOC_Os01g60910  | CAMK_KIN1/SNF1/Nim1_like.12 - CAMK includes calcium/calmodulin dependent protein kinases, expressed | -          | -           |
| LOC_Os01g61044  | transmembrane amino acid transporter protein, putative, expressed | -          | -           |
| LOC_Os01g61620  | protein kinase family protein, putative, expressed | -          | -           |
| LOC_Os01g61670  | ureidoglycolate hydrolase, putative, expressed   | -          | -           |
| LOC_Os01g61780  | vacuolar ATP synthase 98 kDa subunit, putative, expressed | -          | -           |
| LOC_Os01g61910  | KIP1, putative, expressed                        | -          | -           |
| LOC_Os01g62040  | ruvB-like, putative, expressed                   | -          | -           |
| LOC_Os01g62060  | plant-specific domain TIGR01589 family protein, expressed | -          | -           |
| LOC_Os01g62070  | cation efflux family protein, putative, expressed | -          | -           |
| LOC_Os01g62350  | 60S ribosomal protein L36-2, putative, expressed | -          | -           |
| LOC_Os01g62480  | laccase precursor protein, putative, expressed   | -          | -           |
| LOC_Os01g62660  | MYB family transcription factor, putative, expressed | -          | -           |
| LOC_Os01g62840  | mannose-1-phosphate guanytransferase, putative, expressed | -          | -           |
| LOC_Os01g62950  | ras-related protein, putative, expressed         | -          | -           |
| LOC_Os01g63010  |universal stress protein domain containing protein, putative, expressed | -          | -           |
| LOC_Os01g63210  | SOUL heme-binding protein, putative, expressed   | -          | -           |
| LOC_Os01g63220  | kinase, pfkB family, putative, expressed         | -          | -           |
| LOC_Os01g63270  |alpha-glucan phosphorylast isozyme, putative, expressed | -          | -           |
| LOC_Os01g63290  |transporter, major facilitator family, putative, expressed | -          | -           |
| LOC_Os01g63580  |glycerol-3-phosphate acyltransferase, putative, expressed | -          | -           |
| LOC_Os01g63870  |nucleobase-ascorbate transporter, putative, expressed | -          | -           |
| LOC_Os01g63890  |G10 protein, putative, expressed                  | -          | -           |
| LOC_Os01g63930  |cytochrome P450, putative, expressed              | -          | -           |
| LOC_Os01g63990  |hydrolase, alpha/beta fold family protein, putative, expressed | -          | -           |
| LOC_Os01g64010  |specificity protein phosphatase, putative, expressed | -          | -           |
| LOC_Os01g64100  |glycosyl hydrolase, putative, expressed           | -          | -           |
| LOC_Os01g64120  |2Fe-2S iron-sulfur cluster binding domain containing protein, expressed | -          | -           |
| LOC_Os01g65110  |POT family protein, expressed                    | -          | -           |
| LOC_Os01g65200  |proton-dependent oligopeptide transport, putative, expressed | -          | -           |
| LOC_Os01g65230  |AGC_AGC_other_PDK1_Pk61C.1 - AGC kinases include homologs to PKA, PKG and PKC, expressed | -          | -           |
| LOC_Os01g65400  |DNA polymerase I, putative, expressed             | -          | -           |
| LOC_Os01g65410  |serine hydroxymethyltransferase, mitochondrial precursor, putative, expressed | -          | -           |
| LOC_Os01g65902  |apocytochrome f precursor, putative, expressed    | -          | -           |
| LOC_Os01g65920  |F-box/LRR-repeat protein 2, putative, expressed   | -          | -           |
| LOC_Os01g66600  |rhodanese-like, putative, expressed               | -          | -           |
| LOC_Os01g66680  |S-domain receptor-like protein kinase, putative, expressed | -          | -           |
| LOC_Os01g66700  |beta-hexosaminidase precursor, putative, expressed | -          | -           |
| 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 DUFS84 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 qDTY1.1 region. These signaling molecules are reported to regulate stress responsive gene expression in rice (Xiong et al., 2002; Diédiou 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 myb, 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).

qDTY1.1 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

Abe, H., Urao, T., Ito, T., Seki, M., Shinozaki, K. and Yamaguchi-Shinozaki, K., 2003. Arabidopsis AtMYC2 (bHLH) and AtMYB2 (MYB) function as transcriptional activators in abscisic acid signaling. The Plant Cell, 15(1):63-78. [Cross Ref]

Abe, H., Yamaguchi-Shinozaki, K., Urao, T., Iwasaki, T., Hosokawa, D. and Shinozaki, K., 1997. Role of Arabidopsis MYC and MYB homologs in drought- and abscisic acid-regulated gene expression. The Plant Cell, 9(10):1859-1866. [Cross Ref]

Abhayawickrama, B.P., Gimhani, D.R., Kottearachchi, N.S. and Herath, V., 2020. Utilization of SNP-based Highly Saturated Molecular Map of a RIL Population for the Detection of QTLs and Mining of Candidate Genes for Salinity Tolerance in Rice. Journal of Agricultural Sciences–Sri Lanka, 15(3). [Cross Ref]

Courtois, B., Ahmadi, N., Khowaja, F., Price, A.H., Rami, J.F., Frouin, J., Hamelin, C. and Ruiz, M., 2009. Rice root genetic architecture: meta-analysis from a drought QTL database. Rice, 2(2-3):115-128. [Cross Ref]

Diédiou, C.J., Popova, O.V., Dietz, K.J. and Golldack, D., 2008. The SNF1-type serine-threonine protein kinase SAPK4 regulates stress-responsive gene expression in rice. BMC plant biology, 8(1):1-13. [Cross Ref]

Dixit, S., Singh, A., Sandhu, N., Bhandari, A., Vikram, P. and Kumar, A., 2017. Combining drought and submergence tolerance in rice: marker-assisted breeding and QTL combination effects. Molecular breeding, 37(12): 143. [Cross Ref]

Ghimire, K.H., Quiatchon, L.A., Vikram, P., Swamy, B.M., Dixit, S., Ahmed, H., Hernandez, J.E., Borromeo, T.H. and Kumar, A., 2012. Identification and mapping of a QTL (qDTY1.1) with a consistent effect on grain yield under drought. Field Crops Research, 131:88-96. [Cross Ref]

Indurkar, A.B., Majgahe, S.K., Sahu, V.K., Vishwakarma, A., Premi, V., Shrutoffatava, P., Dubey, M. and Chandel, G., 2015. Identification, Characterization and Mapping of QTLs related to Grain Fe, Zn and Protein Contents in Rice (Oryza sativa L.). Electronic Journal of Plant Breeding, 6(4):1059-1068. [Cross Ref]

Joshi, R., Wani, S.H., Singh, B., Bohra, A., Dar, Z.A., Lone, A.A., Pareek, A. and Singla-Pareek, S.L., 2016. Transcription factors and plants response to drought stress: current understanding and future directions. Frontiers in Plant Science, 7: 1029. [Cross Ref]

Kamoshita, A., Babu, R.C., Boopathi, N.M. and Fukai, S., 2008. Phenotypic and genotypic analysis of drought-resistance traits for development of rice cultivars adapted to rained environments. Field crops research, 109(1-3):1-23. [Cross Ref]

Khowaja, F.S., Norton, G.J., Courtois, B. and Price, A.H., 2009. Improved resolution in the position of drought-related QTLs in a single mapping population of rice by meta-analysis. BMC genomics, 10(1):276. [Cross Ref]
Liu, Q., Wang, Z., Xu, X., Zhang, H. and Li, C., 2015. Genome-wide analysis of C2H2 zinc-finger family transcription factors and their responses to abiotic stresses in poplar (Populus trichocarpa). *PloS one*, 10(8): e0134753. [Cross Ref]

Mai, C.D., Phung, N.T., To, H.T., Gonin, M., Hoang, G.T., Nguyen, K.L., Do, V.N., Courtois, B. and Gantet, P., 2014. Genes controlling root development in rice. *Rice*, 7(1):30. [Cross Ref]

Muthu, S.S. ed., 2019. *Quantification of Sustainability Indicators in the Food Sector*. Springer Singapore. [Cross Ref]

Ramegowda, V., Basu, S., Krishnan, A. and Pereira, A., 2014. Rice growth under drought kinase is required for drought tolerance and grain yield under normal and drought stress conditions. *Plant physiology*, 166(3):1634-1645. [Cross Ref]

Ramya, M., Center, K.G., Bank, G.G.R., Raveendran, M., Nadu, T., Ramalingam, J. and Nadu, T., 2010. Insilico analysis of drought tolerant genes in rice. *Int J Biol Med Res*, 1(3).

Ray, D.K., Mueller, N.D., West, P.C. and Foley, J.A., 2013. Yield trends are insufficient to double global crop production by 2050. *PloS one*, 8(6):66428. [Cross Ref]

Sheshadri, S.A., Nishanth, M.J. and Simon, B., 2016. Stress-mediated cis-element transcription factor interactions interconnecting primary and specialized metabolism in planta. *Frontiers in plant science*, 7:1725. [Cross Ref]

Suji, K.K., Prince, K.S.J., Mankhar, P.S., Kanagaraj, P., Pooirma, R., Amutha, K., Kavitha, S., Bijji, K.R., Gomez, S.M. and Babu, R.C., 2012. Evaluation of rice (*Oryza sativa L.*) near iso-genic lines with root QTLs for plant production and root traits in rainfed target populations of environment. *Field Crops Research*, 137:89-96. [Cross Ref]

Swamy, B.M., Shamsudin, N.A.A., Abd Rahman, S.N., Mauleon, R., Ratnam, W., Cruz, M.T.S. and Kumar, A., 2017. Association mapping of yield and yield-related traits under reproductive stage drought stress in rice (*Oryza sativa L.*). *Rice*, 10(1):21. [Cross Ref]

Tang, Y., Bao, X., Zhi, Y., Wu, Q., Guo, Y., Yin, X., Zeng, L., Li, J., Zhang, J., He, W. and Liu, W., 2019. Overexpression of a MYB family gene, OsMYB6, increases drought and salinity stress tolerance in transgenic rice. *Frontiers in plant science*, 10: 168. [Cross Ref]

Uno, Y., Furihata, T., Abe, H., Yoshida, R., Shinozaki, K. and Yamaguchi-Shinozaki, K., 2000. Arabidopsis basic leucine zipper transcription factors involved in an abscisic acid-dependent signal transduction pathway under drought and high-salinity conditions. *Proceedings of the National Academy of Sciences*, 97(21):11632-11637. [Cross Ref]

Venuprasad, R., Bool, M.E., Quiatchon, L., Cruz, M.S., Amante, M. and Atlin, G.N., 2012. A large-effect QTL for rice grain yield under upland drought stress on chromosome 1. *Molecular Breeding*, 30(1):535-547. [Cross Ref]

Vikram, P., Swamy, B.M., Dixit, S., Ahmed, H.U., Cruz, M.T.S., Singh, A.K. and Kumar, A., 2011. qDTY 1.1, a major QTL for rice grain yield under reproductive-stage drought stress with a consistent effect in multiple elite genetic backgrounds. *BMC genetics*, 12(1):89. [Cross Ref]

Vikram, P., Swamy, B.M., Dixit, S., Singh, B.P., Miro, B., Kohli, A., Henry, A., Singh, N.K. and Kumar, A., 2015. Drought susceptibility of modern rice varieties: an effect of linkage of drought tolerance with undesirable traits. *Scientific reports*, 5:14799. [Cross Ref]

Xiong, L., Schumaker, K.S. and Zhu, J.K., 2002. Cell signaling during cold, drought, and salt stress. *The plant cell*, 14(suppl 1): S165-S183. [Cross Ref]

Yamaguchi-Shinozaki, K. and Shinozaki, K., 2005. Organization of cis-acting regulatory elements in osmotic-and cold-stress-responsive promoters. *Trends in plant science*, 10(2):88-94. [Cross Ref]

Zhang, H., 2018. *Securing the 'Rice Bowl': China and Global Food Security*. Springer. [Cross Ref]