Transcriptomic responses under combined bacterial blight and drought stress in rice reveal potential genes to improve multi-stress tolerance

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Abstract

Background: The unprecedented drought and frequent occurrence of pathogen infection in rice is becoming more due to climate change. Simultaneous occurrence of stresses lead to more crop loss. To cope up multiple stresses, the durable resistant cultivars needs to be developed, by identifying relevant genes from combined biotic and abiotic stress exposed plants.

Results: We studied the effect of drought stress, bacterial leaf blight disease causing Xanthomonas oryzae pv. oryzae (Xoo) pathogen infection and combined stress in contrasting BPT5204 and TN1 rice genotypes. Mild drought stress increased Xoo infection irrespective of the genotype. To identify relevant genes that could be used to develop multi-stress tolerant rice, RNA sequencing from individual drought, pathogen and combined stresses in contrasting genotypes has been developed. Many important genes are identified from resistant genotype and diverse group of genes are differentially expressed in contrasting genotypes under combined stress. Further, a meta-analysis from individual drought and Xoo pathogen stress from public domain data sets narrowed down candidate differentially expressed genes. Many translation associated genes are differentially expressed suggesting their extra-ribosomal function in multi-stress adaptation. Overexpression of many of these genes showed their relevance in improving stress tolerance in rice by different scientific groups. In combined stress, many downregulated genes also showed their relevance in stress adaptation when they were over-expressed.

Conclusions: Our study identifies many important genes, which can be used as molecular markers and targets for genetic manipulation to develop durable resistant rice cultivars. Strategies should be developed to activate down-regulated genes, to improve multi-stress tolerance in plants.

Keywords: Drought, Xanthomonas, Rice, Combined stress, Transcriptome, Meta-analysis, Translation, Climate change, Transcription factor, Kinases

Introduction

Plants are sessile, exposed to diverse biotic and abiotic stresses leads to reduction in yields of many agricultural economically important crops [1]. Rice is one of the most important staple food which feeds more than half of the population globally. Due to climate change, the frequency
of uneven rainfall and severe drought stresses are common, which threaten the crop production [2]. During drastically changing climatic conditions, many bacterial pathogens can infect plants and reduce the yield. Rice being grown in the puddled condition is more sensitive to uneven rainfall and drought stress. Besides its direct effect on the crop, drought stress alters plant-pathogen interaction and disease development [3]. The occurrence and severity of combined abiotic and abiotic stresses, depend on host resistance or susceptibility, duration of stress exposure and pathogen race [4]. Evidences suggest that, plant responses overlap for drought and bacterial stresses in many crops like Arabidopsis, rice, chickpea, sunflower and several cross-talk mechanisms have been identified [1, 5–7]. Transcriptomic and meta-analysis approaches using expression profile between biotic and abiotic stresses have revealed unique genes which perform similarly across different stress stimuli [8, 9]. Interestingly phytohormone cross-talk mechanisms share many common responsive genes in combined multiple stresses [10–12].

Multiple QTLs for drought resistance and resistance against bacterial blight caused by Xanthomonas oryzae pv. oryzae (Xoo) bacteria has been identified [3, 13]. The introgression of Xa21, Xa5, Xa13 conferred broad spectrum resistance in different rice cultivars against bacterial infection [14]. Studies have shown genotype dependent pathogen infection in rice under drought-induced conditions [3]. Genotypes with suitable Xa genes provide resistance against bacterial blight under drought conditions. The introgression of R genes Xa4 and Xa7 in near isogenic lines confers resistance against bacterial blight under high temperature [15]. Combined stress of high temperature and bacterial blight, drought stress and bacterial blight at seedling stage found multiple Xa genes, which can be introgressed to improve resistance [7]. Combined stress tolerance was improved by the introgression of four resistance genes (Xa4, xa5, xa13 and Xa21) with submergence (Sub1), salinity (Saltol), blast (Pi2, Pi9) and gall midge (Gm1, Gm4) [16]. Evidences suggest that, introgression of multiple drought QTLs along with many R genes in an elite genotype can provide tolerance against combined stress in rice [3].

Multiple stress tolerance is governed by several genes, to develop durable resistant genotypes, evaluating rice varieties under combined stresses is the best strategy [3]. The candidate genes which are involved in multi-stress tolerance may be identified in plants exposed to combined stress. Existing reports suggested that, several overlapping genes in Xoo and drought stress play role in improving tolerance. Transcriptome data of drought and Xoo infection showed 2276 overlapping genes which were differentially expressed [8]. Meta-analysis study of transcriptome from drought and bacterial blight combined stress, 5084 and 1618 differentially expressed genes (DEGs) were identified in rice and Arabidopsis respectively [17]. Meta-analysis of sunflower transcriptome revealed 526 upregulated and 4440 downregulated genes in combined stress of drought and pathogen along with NaCl, cold and oxidative stress [5]. These studies identified the genes by comparing the individual stress transcriptome data. Comparative study for drought and Xoo, in resistance rice line H471 and its recurrent parent HHZ identified 306 and 840 DEGs, and 178 genes were common among both stresses [18].

We made an attempt to identify candidate genes in rice plants exposed to the combined stress of drought and bacterial blight causing pathogen infection. The major challenge in studying the multiple stresses, is imposition of combined stresses simultaneously [19]. Severe drought stress reduces the bacterial multiplication due to higher leaf water loss [20]. To overcome this, we optimized a combined stress imposition method in rice by gradually reducing the soil moisture content and subsequently infecting pathogen. A comparative transcriptomic data from contrasting BPT5204 and TN1 rice genotype was developed. Several relevant genes for individual and combined stress, regulating different pathways were identified. A meta-analysis from individual drought and Xoo infected rice was performed, using public microarray datasets. Several DEGs identified were characterized in stresses for either abiotic or biotic factors. Our results demonstrated that several genes are involved in multi-stress tolerance. The identified genes can be used as genetic markers and candidate genes for crop improvement programs.

Results

Differential response of contrasting rice genotypes to combined drought stress and pathogen infection

To study the responses of rice under drought, pathogen and combined stress, two contrasting BPT5204 and TN1 genotypes were maintained in four different sets. One set of 45-days-old plants were infected with Xoo and another set was exposed to drought stress by gradually reducing soil moisture content upto 60% field capacity (FC). For combined stress (Xoo and drought stress), the 45-days-old plants were exposed to drought stress by reducing FC to 80% for two days and infected with 0.5 × 10^8 CFU/mL of concentration of Xoo by leaf clipping method. Further, moisture level was reduced to 60% FC and plants were maintained for four days (Fig. 1A). Disease pattern and bacterial multiplication rates were assessed in resistant BPT5204 and susceptible TN1 genotypes. After 4 dpi, Xoo pathogen infection rate and lesions were measured at
different time intervals. The susceptible TN1 genotype showed higher infection under drought condition (Fig. 1B) at 6 dpi and progressed severely till 12 dpi, whereas, in BPT5204 bacterial infection progression was slow. In case of combined stress, TN1 genotype showed higher susceptibility and even BPT5204 showed higher lesions compared to individual pathogen infection. TN1 genotype showed >1-2 fold higher pathogen multiplication than BPT5204 at 4, 6, 8, 10, 12 and 14 dpi, whereas at 6 dpi >2 fold pathogen multiplication was observed in only pathogen infected plants. Increased lesions were observed in TN1, whereas, BPT5204 maintained less bacterial growth as well as disease symptoms (Fig. 1B and C). In case of combined stress, drought stress prior to pathogen infection resulted in reduced bacterial multiplication in both genotypes (Fig. 1D). At 4 dpi, no significant difference was observed in bacterial multiplication rate in both the genotypes, whereas at 6, 8, 10, 12 and 14 dpi TN1 showed higher bacterial multiplication compared to BPT5204.

The individual and combined stress effect were quantified, by measuring reactive oxygen species (ROS) such as superoxide and H$_2$O$_2$. Superoxide estimation using NBT staining showed higher level of ROS accumulation in combined as well as drought stress. In drought stress >2.5 fold levels of superoxide was accumulated in BPT5204, whereas, in pathogen infection there was no significant difference was observed in both contrasting genotypes. Similarly, in combined stress >2.5 fold accumulation was observed in BPT5204 compared to TN1 (Fig. 2A). H$_2$O$_2$ quantification using DAB was observed >2.5 fold in BPT5204 in individual stress whereas in combined stress accumulation was ~10 fold higher compared to TN1 (Fig. 2B). The effect of stress on cell membrane was quantified using Evan's blue in individual as well as combined stress (Fig. 2C). In drought stress, membrane damage was observed >3.5 fold in BPT5204 compared to TN1 whereas, in pathogen infection there was no significant difference was observed. In combined stress, accumulation of Evan's blue dye was >2 fold in BPT5204 compared to TN1 genotype (Fig. 2D).

Transcriptional profiling identifies common and unique genes in combined stress

The emphasis of the study is to identify the candidate genes which can help in improving the combined stress tolerance in rice. We followed two approaches, initially RNA sequencing data was developed from contrasting rice genotypes that are exposed to individual and combined stress to identify common and unique genes (Fig. 3A i, Additional file 1). In another approach, microarray data from individual drought and pathogen stress from public domain was analysed (Additional file 1) and common differentially expressed genes (DEGs) were identified (Fig. 3A ii).

The transcriptomic data from four different sets i.e. control, drought, pathogen and combined stress for both BPT5204 and TN1 genotypes were developed. Around 98.16% of the high-quality reads were mapped to the reference genome. A total of 3381 unique DEGs were identified across the stress treatments (Additional file 1). We identified 903 (BDP-Combined stress), 659 (BD-Drought), and 834 (BP-Pathogen) DEGs in BPT5204. In TN1 genotype, 1226 (TD-Combined Stress), 893 (TD-Drought), and 677 (TP-Pathogen) DEGs were observed (Additional file 1).

In the BPT5204, 191 genes from 903 DEGs in combined stress were found to be common in drought stress, and 265 DEGs were common in pathogen infection (Fig. 3B). Similarly, in TN1, 387 and 218 of DEGs from combined stress were found to be common with drought stress and pathogen infection respectively (Fig. 3C). In all treatments, 82 and 134 genes were expressed in BPT5204 and TN1 genotypes, respectively. The transcriptomic data revealed that in BPT5204, 374 genes and in TN1, 548 genes were uniquely expressed in combined stress (Fig. 3D, Additional file 1).

Gene ontology analysis was performed to classify genes in different categories. In pathogen infection, BPT5204 and TN1 genotypes showed more number of genes in molecular function (41% and 40% respectively) followed by cellular components (35% and 36% respectively) and biological processes (24% both) (Fig. 4A, Additional file 2). Likewise in drought stress, DEGs from both BPT5204 and TN1 genotypes represented more number of genes in molecular function (42% and 40% respectively) followed by cellular components (35% and 37% respectively) and biological processes (23% both).
Fig. 1 (See legend on previous page.)
In combined stress, BPT5204 genotype represented 41% of genes in molecular functions having ATP binding, protein binding, kinase activity, zinc binding and DNA binding activity. In TN1 genotype, 41% of genes represented in molecular functions of ATP binding, electron transfer, kinase activity, DNA binding and protein binding groups. In BPT5204 and TN1, 36% and 35% of genes respectively were represented in cellular components belonging to cytoplasmic vesicle, mitochondrion, plastid, membrane, nucleus and others. 23% of genes in BPT5204 and 24% of genes in TN1 were represented in biological processes belonging to protein phosphorylation, regulation of transcription, metabolic process, oxidation–reduction process, proteolysis and others (Fig. 4C, Additional file 2).

The upregulated genes in combined stress, 22% of genes were represented in molecular function in BPT5204 genotype and 20% of genes in TN1 (Fig. 5A, Additional file 2).
Additional file 3). In downregulated genes, upon combined stress more number of genes were downregulated in TN1 belonging to molecular function (21%), whereas in BPT5204, 19% of genes were represented (Fig. 5B). Several common genes from both the genotypes were identified. To assess the role of these genes, a PubMed search was conducted to know their relevance in plant stress adaptation. Interestingly, the role of many genes in plants were validated for different stresses by many research groups across the globe (Table 1).
Fig. 4 Classification of differentially expressed genes. Differentially expressed genes from BPT5204 and TN1 were characterized based on molecular function, cellular components and biological processes under (A) Pathogen stress -P (B) Drought stress -D (C) Combined drought and pathogen (D+P) stress. GO analysis was performed using agriGO (https://agri.go.jp/).
Meta-analysis, narrowed down candidate genes for combined stress tolerance

To identify the key genes involved in combined stress, a meta-analysis was conducted using transcriptomic data from resistant and susceptible genotypes (our study) and microarray data from individual drought and pathogen infection from public domain (Additional file 4). The data was curated and analysed from both the approaches. Common and unique genes in individual and combined stress were identified which acts as candidate genes to develop multi-stress tolerant crops (Table 2, Additional file 5). In upregulated genes, under drought stress 230 genes were unique in BPT5204 and 79 genes were common in both microarray and RNA Seq data. Similarly 264 genes were unique in TN1 and 75 genes were common in microarray and RNA Seq data. This analysis identified 14 unique genes that were commonly upregulated in BPT5204 and TN1, 5 genes were found to be common in both RNA Seq and microarray data. There are 22 unique genes and 6 commonly downregulated genes were identified (Table 2, Additional file 5).

In pathogen infection, 278 genes were uniquely upregulated in BPT5204 and 51 genes were common in microarray and RNA seq data. In TN1, 194 genes were unique and 70 genes were found upregulated in both microarray and RNA Seq data. Meta-analysis with RNA Seq and microarray revealed 19 unique genes between the genotypes and 11 common genes. In downregulated genes, 36 unique and 5 common genes were identified (Table 2, Additional file 5).

In combined stress, 394 genes were uniquely upregulated in BPT5204 and 53 genes were common in microarray and RNA Seq data. 487 genes were upregulated in TN1 and 108 genes were common in microarray and RNA Seq data. From meta-analysis, 84 unique genes and 26 common upregulated genes were identified. Interestingly, no downregulated unique gene in the genotypes and 9 genes were common in both RNA Seq and microarray (Table 2, Additional file 5). Overall from our genotypes identified many genes and using meta-analysis, key important genes which may be more relevant for improving combined stresses were identified.

Differential responses of translation associated genes

Translation associated genes were differentially regulated in combined stresses. Ribosomal protein encoding genes play an important role in both biotic and abiotic stress conditions. To study the responses of translational associated mechanisms during the combined stress in both BPT5204 and TN1, the transcripts encoding ribosomal proteins (RP) were filtered from RNA seq data. In drought condition, 19 genes were commonly upregulated in both genotypes and same number of genes were downregulated (Table 3, Additional file 6). The number of genes that were up and downregulated were more in TN1 compared to BPT5204 indicating the severity of stress on that genotype. In pathogen infection, 26 and 34 genes were up and downregulated, respectively. Interestingly, more no. of genes were upregulated in BPT5204. In combined stress, 29 genes were upregulated and 27 genes

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Fig. 5 Number of upregulated and downregulated genes under combined and individual drought and pathogen stress. A Percentage of upregulated genes in molecular function, cellular components and biological processes under pathogen, drought and combined stress. B Percentage of downregulated genes in molecular function, cellular components and biological processes under pathogen, drought and combined stress.
| Annotation | Functional relevance studies | Reference |
|------------|-------------------------------|-----------|
| **Upregulated in BPT5204 all stresses** | Os01g0106400 Similar to Isoflavone reductase homolog IRL (EC 1.3.1.) | Oxidoreductase activity; Overexpression leads to tolerance to ROS in rice | Kim et al., 2009 |
| Os01g0162500 Leucine-rich repeat-containing N-terminal protein | Stress (Cold and drought) tolerance in rice; Required for Xa-21 transcript expression and Xa-21 mediated immunity in rice against Xoo | Liao et al.; 2016; 2. Caddell et al., 2017 |
| Os01g0844300 Peptidylprolyl isomerase | Aids in protein folding | Shaw, 2002 |
| Os02g0178800 Glossy1(GL1) homolog | Cuticular wax biosynthesis, Drought resistance in rice | Islam et al., 2009 |
| Os02g0323000 Felis catus multi-drug resistance related (Fragment) | ATPase activity, ATPase-coupled transmembrane transporter activity and ATP binding | UNIPROT |
| Os02g0820200 Serine/threonine protein kinase | Involved in abiotic stress (salt and osmotic) tolerance in Arabidopsis | Zang et al., 2016 |
| Os03g0140400 Cytochrome P450 protein CYP96B4 | Involved in growth regulation and drought stress response in rice | Tamiru et al., 2015 |
| Os04g0266900 Transketolase C-terminal-like domain containing protein | Acts as a regulatory molecule binding site | Pfam, InterPro |
| Os06g0544100 Tyrosine protein kinase domain containing protein | Abiotic stress (drought, heat, salt and submergence) tolerance | Elangovan et al., 2020 |
| Os07g0194100 OSK2 | Play important role in the early stages of endosperm development in rice seeds | Takano et al., 1998 |
| Os07g0674400 Pollen Ole e 1 allergen/extensin domain containing protein | Pollen germination and pollen tube growth | Jimenez-Lopez et al., 2012 |
| Os09g0472900 Blight-associated protein p12 precursor | p12 is responsible for systemic acquired resistance in citrus plants against citrus blight | Uniprot |
| Os12g013300 Carbohydrate transporter/ sugar porter/ transporter | Involved in long distance transport of sucrose from source to sink where sugars are used or stored | Doidy et al., 2012 |
| Os12g045800 Receptor-like serine/threonine kinase | Cytokinin signaling | Afzal et al., 2008 |
| **Upregulated in TN1 all stresses** | Os01g0858000 WD40 repeat domain containing protein | Function as molecular hubs mediating supramolecular interactions, involved in plant cell wall biosynthesis. Histone modification, transcription regulation and signal transduction | Guerriera et al., 2015; Hu et al., 2018 |
| Os02g0536300 Leucine-rich repeat 2 domain containing protein | Initiate immune response in plants by sensing PAMP | Ng and Xavier, 2011 |
| Os02g0599151 Probable protein phosphatase 2C 17 | Regulate various plant signal transduction pathways (MAPK signalling and ABA signalling) | Rodriguez et al., 1998; Endo et al., 2018 |
| Os03g0188100 transparent testa 12 protein | Encodes a multidrug secondary transporter-like protein required for flavonoid sequestration in vacuoles of the seed coat endothelium in Arabidopsis | Debeaujon et al., 2001 |
| Os03g0305800 Galactosyl transferase family protein | Biosynthesis of plant cell wall, synthesis of diverse secondary metabolites and modification of plant hormones | Cao et al., 2008 |
| Annotation | Functional relevance studies | Reference |
|------------|-----------------------------|-----------|
| Os03g0332900 Protein kinase, core domain containing protein | Central components in plant responses to environmental stresses such as drought, high salinity, cold, and pathogen attack | Wang et al., 2020 |
| Os03g0448500 Calcineurin B-like protein | Decoding Ca2+ signatures elicited by a variety of abiotic stresses | Cho et al., 2016 |
| Os03g0575200 Potassium transporter 1 (OsHAK1) | Positively regulates drought stress responses in rice | Chen et al., 2017 |
| Os03g0785900 Similar to Glutathione-S-transferase | Involved in abiotic stress response and heavy metal (Arsenic) detoxification | Kumar and Trivedi (2018) |
| Os03g0820500 Similar to WCOR719 | Involved in dynamic reorganisation of cytoskeleton during low temperature acclimatisation | Danyluk et al., 1996 |
| Os04g0127500 Serine/threonine protein kinase-related domain containing protein | Involved in abiotic stress (salt and osmotic) tolerance in Arabidopsis | Zang et al., 2016 |
| Os04g0416100 Transcription factor E2F1 (E2F-1) | Expessed throughout the cell cycle, Elevates CDK levels and activity, even under hormone-free conditions | TAIR (The Arabidopsis Information Resource) |
| Os04g0584300 Similar to Catalytic/ protein phosphatase type 2C | Regulate various plant signal transduction pathways (MAPK signalling and ABA signalling) | Rodriguez et al., 1998; Endo et al., 2018 |
| Os05g0576800 Blast and wounding induced mitogen-activated protein kinase | Activate MAPK pathway | Cheong et al., 2003 |
| Os06g0191900 Serine threonine kinase | Act as receptors for external factors like environmental conditions and mediate cellular responses | Afzal et al., 2008 |
| Os06g0229000 FtsH protease (VAR2) (Zinc dependent protease) | Involved with abiotic (light) stress response in Arabidopsis | Lopes et al., 2018 |
| Os06g0275000 Zinc finger protein, Heading date | Involved in abiotic stress tolerance | Jin et al., 2018 |
| Os06g0681200 Cuprodoxin domain containing protein | Involved in abiotic stress responses | Jangam et al., 2016 |
| Os06g0702100 Methyl-CpG DNA binding domain containing protein | Involved in DNA methylation and abiotic stress responses | Parida et al., 2018 |
| Os07g0175600 Plant lipid transfer protein and hydrophobic protein, | Act as defense proteins in plant innate immunity, bind and transfer lipids and constitute one of the most clinically important classes of plant allergens | Finkina et al., 2016 |
| Os07g0521500 NB-ARC domain containing protein | Regulate the activity of R protein | Ooijen et al., 2008 |
| Os07g0614300 Von Willebrand factor type A domain containing protein, | Component of C-terminus of Lagging Growth and Development 1 (LGD1) and is responsible for the nuclear targeting and RNA binding activity | Thangasamy et al., 2012 |
| Os10g0471000 Protein of unknown function DUF810 | Involved in tolerance to drought and salt stress | Li et al, 2018 |
| Os10g0521000 TRE1 protein (Fragment) | Required for glycogen metabolism | Zhang et al., 2021 |
| Os10g0529700 glutathione transferase35 | Involved in abiotic stress response and heavy metal (Arsenic) detoxification | Kumar and Trivedi, 2018 |
| Os11g0201400 Histone deacetylase | Negatively regulates plant innate immunity by modulating histone H4 acetylation of defense-related genes in rice | Ding et al., 2012 |
| Os11g0693800 ATP-citrate synthase | Negatively regulates plant innate immunity in rice | Ruan et al., 2018 |
| Annotation | Functional relevance studies | Reference |
|------------|-------------------------------|-----------|
| Os12g0512100 Sugar/inositol transporter domain containing protein | Involved in transport of sugar from source to sink | Kong et al., 2019 |
| **Downregulated in BPT in all stresses** | | |
| Os02g0226200 HAD-superfamily subfamily IB hydrolase, hypothetical 1 protein | Involved in intracellular or extracellular organic phosphorous recycling under inorganic phosphorous stress conditions in plants | Du et al., 2021 |
| Os02g0249300 Zinc finger, RING/FYVE/PHD-type domain containing protein | Involved in abiotic stress (salt and osmotic) tolerance in Arabidopsis | Zang et al., 2016 |
| Os03g0181750 ABC transporter, transmembrane domain domain containing protein | Required for normal plant development, detoxification and pathogen defense via the transport of xenobiotics and secondary metabolites across the plants | Hwang et al., 2016 |
| Os04g0364800 Barwin-related endoglucanase domain containing protein | Aids in polysaccharide binding and Barwin is a putative plant defense protein; 2. All PR-4 proteins have Barwin domain | Todd et al., 2002; Franco et al., 2019 |
| Os04g0385600 Tetra-tripeptide-like helical domain containing protein | Involved in plant stress (osmotic stress) and hormone signaling (abscisic acid) | Schapire et al., 2006; Sharma and Pandey, 2016 |
| Os04g0618700 Protein kinase, core domain containing protein | Central components in plant responses to environmental stresses such as drought, high salinity, cold, and pathogen attack | Wang et al., 2020 |
| Os04g0686000 Zinc finger, RING/FYVE/PHD-type domain containing protein | Involved in abiotic stress (salt and osmotic) tolerance in Arabidopsis | Zang et al., 2016 |
| Os05g0170600 CLE family OsCLE503 protein | Regulate cell proliferation and differentiation in plant shoots, roots, vasculature, and other tissues | Liu et al., 2019 |
| Os07g0162700 Alpha/beta hydrolase fold-3 domain containing protein | Associated with housekeeping roles that participate in the breakdown and recycling of cellular metabolites, processing of external nutrients and detoxification of xenobiotics | Gershater and Edwards, 2007 |
| Os08g0113000 Peroxidase 47 precursor (EC 1.11.1.7) (Atperox P47) (ATP32) | Removal of H2O2, oxidation of toxic reductants, biosynthesis and degradation of lignin, suberization, auxin catabolism, response to environmental stresses such as wounding, pathogen attack and oxidative stress | UniProt (Reviewed) |
| Os10g0376400 Phosphate-induced protein 1 conserved region containing protein | Respond to abiotic stress (drought, cold, heat and salt) | Quan et al., 2018 |
| **Downregulated in TN1 in all stresses** | | |
| Os01g0311600 Sulphotransferase family protein | Respond to abiotic stress in rice; Associated with drought, salt and ABA stress in Chinese cabbage | Chen et al., 2012; Jin et al., 2019 |
| Annotation                                                                 | Functional relevance studies                                                                 | Reference                                      |
|---------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------|-----------------------------------------------|
| Os01g0823100 Alpha-expansin OsEXPA2                                       | Involved in stem elongation; important for seed germination                                    | Maro wa et al., 2016; Huang et al., 2000       |
| Os01g0871600 Peptide transporter PTR2-B                                     | Various role in rice development mainly in grain filling and germination stages, Also upregulated upon drought and salt stresses | Ouyang et al., 2010                           |
| Os01g0953400 NB-ARC domain containing protein                             | Regulate the activity of R protein                                                             | Ooi jen et al., 2008                          |
| Os02g0538400 Armadillo-like helical domain containing protein             | Role in plant development and abiotic stress signalling                                        | Sharma et al., 2014                           |
| Os02g0616300 Similar to Protein argonaute MEL1                           | Development of pre-meiotic germ cells and the progression of meiosis                          | Komiy a et al., 2014; UniProt                 |
| Os03g0250200 TB2/DPI and HVA22 related protein family protein            | Inhibits gibberelin mediated PCD, involved in vesicular trafficking                           | Guo and Ho, 2008                              |
| Os04g0541100 Similar to Gt-2                                            | Probable glycosyl transferase, required for cell wall synthesis                              | Cao et al., 2008                              |
| Os05g0469800 Pyruvate decarboxylase                                       | Enzyme involved in alcohol fermentation, Also when over-produced enhance submergence tolerance in rice | Quimio et al., 2000                          |
| Os05g0477600 Alpha-expansin OsEXPA4                                       | Intermode elongation, Cell wall organisation in plants                                         | Wang et al., 2018                             |
| Os05g0488000 Peptidase C1A papain family protein                         | Role in seed development and stress tolerance                                                  | Wang et al., 2018                             |
| Os05g0552400 Zinc finger, RING/FYVE/PHD-type domain containing protein   | Involved in abiotic stress (salt and osmotic) tolerance in Arabidopsis                         | Zang et al., 2016                             |
| Os06g0142300 Early nodulin 93 ENOD93 protein family protein              | Candidate gene for brown plant hopper resistance in herbicide resistant rice; Required for somatic embryogenesis in oil palm | Wang et al., 2015; Chan et al., 2020           |
| Os06g0179000 Glycoside hydrolase family 79, N-terminal protein            | Response to biotic and abiotic stresses, defense against herbivores, activation of phytohormones, lignification, and cell wall remodelling | Opas siri et al., 2006                        |
| Os06g0549900 FAD linked oxidase, N-terminal domain containing protein     | Required for the import and folding of small cysteine-containing proteins in the mitochondrial intermembrane space | UniProt                                      |
| Os06g0610800 Peptidase A1 domain containing protein. Aspartic peptidase   | Aspartic-type endopeptidase activity. Involved in biotic and abiotic stress responses          | 1. UniProt (reviewed); 2. Figueirido et al., 2021 |
| Os07g0648000 Armadillo-like helical domain containing protein            | Role in plant development and abiotic stress signalling                                        | Sharma et al., 2014                           |
| Os08g0442400 BABY BOOM                                                    | Key regulators of plant cell totipotency. Induce somatic embryogenesis                         | Jha and Kumar, 2018; Khanday et al., 2020     |
| Os08g0473900 1,4-alpha-D-glucan glucanohydrolase                          | Important for breakdown of endosperm starch during germination                                 | UniProt; Guttikonda et al., 2020              |
| Os08g0507100 Cytochrome P450 family protein                              | Function in development regulation and drought stress response                               | Wei and Chen, 2018                           |
| Os09g0241700 Homeodomain-like containing protein                         | Negative regulators in abiotic stress responses; Play role in reproductive development and abiotic stress signaling in rice | Bhattacharjee et al., 2016; Jain et al., 2008 |
| Os09g0451400 ACC oxidase                                                  | Ethylene biosynthesis                                                                         | Lee and Yoon, 2018                           |
| Annotation | Functional relevance studies | Reference |
|------------|------------------------------|-----------|
| Os10g0109900 Major facilitator superfamily, general substrate transporter domain containing protein | Transport small compounds across biological membranes. Cadmium transporters in rice | Drew et al., 2021, Nino-Gonzalez et al., 2019; Yan et al., 2019 |
| Os10g0556100 beta-expansin EXPB4 | Internode elongation in rice | Lee et al., 2001 |
| Os11g0212900 Serine/threonine protein kinase-related domain containing protein | Involved in abiotic stress (salt and osmotic) tolerance in Arabidopsis | Zang et al., 2016 |
| Os11g0508600 Sugar transporter | TAL effector-mediated susceptibility to bacterial pathogen (Xanthomonas) | Teper and Wang, 2021 |
| Os11g0598300 NB-ARC domain containing protein | Regulate the activity of R protein | Ooijen et al., 2008 |
| Os11g0672300 Protein kinase domain containing protein | Central components in plant responses to environmental stresses such as drought, high salinity, cold, and pathogen attack | Wang et al., 2020 |
| Os11g0675200 NB-ARC domain containing protein | Regulate the activity of R protein | Ooijen et al., 2008 |
| Os11g0676200 NBS-LRR-like protein NBR2 (Fragment) | Component of R protein, Induce effector triggered immunity | DeYoung and Innes, 2006; Sagi et al., 2017 |
| Os12g0569800 Heat shock protein 70 | Involved in macromolecular translocation, carbohydrate metabolism, innate immunity, photosystem II repair and regulation of kinase activities | Wang et al., 2014 |
| Os12g0637400 Purple acid phosphatase (EC 3.1.3.2) | Inorganic Phosphorous remobilization from senescing to non-senescing leaves and organic Phosphorous utilization | Gao et al., 2017 |
were downregulated in both genotypes. The no. of genes upregulated in BPT5204 is less than TN1 genotype.

To validate a few RP encoding genes using qRT-PCR, tissues were collected from 4 and 6 dpi. The expression of RPL28, L25, L27, L46, L5e/L18, L5, L23, L10, RPS8, S18, S17, S14, S12 and S4 genes were assessed in the RNA seq from both genotypes showing differential expression profile (Fig. 6A). The expression analysis study of RP encoding genes in drought, at 4 d showed upregulation in BPT5204. RPL25, RPL27, RPL5, RPL46, RPS12 and RPS14 were upregulated > 2 fold at 4 days (Fig. 6B) and at 6 days varied expression levels observed. However, RPL27 and RPL5 has maintained > 2 fold expression in BPT5204 (Fig. 6C). In pathogen infected condition at 4 dpi, all the RP encoding genes were upregulated in BPT5204 except RPL25. Expression of RPS8, RPS17 and RPS12 was upregulated > 2 fold (Fig. 6D). At 6 dpi, RPL5 and RPS17 transcript levels were >2 fold in BPT5204 compared to TN1 (Fig. 6E). In combined stress of drought and pathogen, the levels of RP encoding genes were significantly upregulated than individual stresses in resistant BPT5204 genotype. In combined stress, at 4 dpi transcripts of RPL25, RPL27, RPL5, RPS8, and RPS12 were > 4 fold upregulated in BPT5204 compared to TN1. Other genes were also upregulated in resistant BPT5204 genotype (Fig. 6F). At 6 dpi transcripts of RPL27 and RPL5 were maintained > 8 fold in BPT5204 compared to TN1. The transcripts of other genes were maintained at higher levels in BPT5204 than TN1 genotype (Fig. 6G).

**Discussion**

Rice is affected by plethora of stresses like drought, bacterial blight caused by Xoo, that are major constraints causing substantial crop loss. During drought stress in rice plants, reduction in fresh and dry biomass, plant height, tiller number, panicle number lead to crop loss. Many QTLs for drought stress tolerance are introgressed into elite varieties to improve crop yields. Similarly, many QTLs against Xoo has been identified to improve resistance to bacterial leaf blight (BB) disease. Efforts has been made to introgress BB resistant Xa genes with drought QTLs. Rice genotypes having Xa4/qDTY2.2 + qDTY4.1 showed improved resistance for combined drought stress and Xoo infection. Many genes have been identified and characterized for individual stresses, however, tolerance traits for abiotic and biotic stresses are multigenic in nature. From this context, developing durable climate resilient crops are in demand. To develop durable resistant crops, the candidate genes are prerequisite for improving combined stress tolerance.

Studying the simultaneous stress occurrence / combined stress experiment on plants in laboratory conditions are challenging due to the lack of stress imposition.

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**Table 2** Differentially expressed genes from pathogen, drought and combined stress from RNA sequencing data generated from this study and microarray data from public domain

|                | Upregulated genes | Downregulated genes |
|----------------|-------------------|---------------------|
|                | Unique | Common | Microarray | Unique | Common | Microarray |
| Drought        |         |         |           |         |         |           |
| BPT 5204       | 230    | 79      | 6120      | 297    | 54      | 6046      |
| TN1            | 264    | 75      | 6124      | 460    | 94      | 6006      |
| Common         | 14     | 5       |           | 22     | 6       |           |
| Pathogen       |         |         |           |         |         |           |
| BPT 5204       | 278    | 51      | 5041      | 432    | 72      | 3496      |
| TN1            | 194    | 70      | 5022      | 374    | 39      | 3530      |
| Common         | 19     | 11      |           | 36     | 5       |           |
| Drought + Pathogen |     |         |           |         |         |           |
| BPT 5204       | 394    | 53      | 2593      | 423    | 33      | 1930      |
| TN1            | 487    | 108     | 2538      | 585    | 45      | 1918      |
| Common         | 84     | 26      |           | 0      | 9       |           |

* The list of these genes were given in additional file 5

**Table 3** Differential expression of ribosomal protein encoding genes from RNA sequencing data

|                | Upregulated | Downregulated |
|----------------|-------------|---------------|
|                | Unique | Common | Unique | Common |
| Drought        |         |         |           |         |
| BPT5204        | 12     | 19     | 7       | 19      |
| TN1            | 37     | 11     |         |         |
| Pathogen       |         |         |           |         |
| BPT5204        | 22     | 26     | 35      | 34      |
| TN1            | 7      | 27     |         |         |
| Drought + Pathogen |     |         |           |         |
| BPT5204        | 16     | 29     | 27      | 27      |
| TN1            | 22     | 26     |         |         |
Fig. 6 Response of ribosomal protein encoding genes in combined and individual pathogen and drought stress. A Multiple gene expression viewer showing differential response of ribosomal protein encoding genes from RNA sequencing data under pathogen, drought and combined stress in BPT5204 and TN1 genotypes. Average FPKM values from each stress was plotted using graphpad. (B and C) Expression of ribosomal protein encoding genes at 4 and 6 days after drought stress, (D and E) Xoo pathogen stress, and (F and G) combined stress. The leaf samples were collected after 4 and 6 dpi and total RNA was isolated. The cDNA was prepared and used for qRT-PCR analysis. Values are means ± SE with three biological replicates. Significant differences were determined at p < 0.0001 (estimated by one-way ANOVA using Tukey’s HSD analysis).
methods. In rice, transcriptome data was developed in combined drought and pathogen infection when plants reached 20% FC [18]. At 20% FC in drought condition, pathogens do not infect rice to cause disease, because of higher ROS that is cytotoxic. However, during severe drought stress, tissue water status reduces which inhibit bacterial growth in intracellular spaces [21]. To overcome this, we infected the plants with Xoo at mild drought stress (80% FC). At mild drought stress condition, water status of leaf reduces moderately and when pathogens are challenged, they can cause severe infection as observed in our study. When Xoo was infected at 80% FC, pathogen multiplied at higher rate causing more lesion length as evident in combined stress in both genotypes. At 80% FC, tissue water status does not reduce drastically which is favouring pathogen to infect the rice plants efficiently. The infection of Xoo on rice plants by leaf clipping method does not add additional water supply as in Arabidopsis and do not change relative water content of leaf. In this condition, pathogen infection is increased in combined stress. In resistant genotype BPT5204, higher ROS at 60% FC decreased the bacterial growth.

BPT5204 was introgressed with Xa5, Xa13 and Xa21 to improve resistance against Xoo [22]. To identify common and unique genes which can be involved in resistance under combined stress, comparative transcriptome data from resistant genotype with sensitive genotype and public data sets may provide relevant genes [23, 24]. A meta-analysis can integrate multiple transcriptomic data from different set of experiments, which provide an option to identify overlapping genes between drought and BB infection, to improve multi-stress tolerant plants using relevant candidate genes.

The RNA Sequencing data from BPT5204 and TN1 showed upregulation of many peroxidases, cinnamoyl-CoA genes, starch and sugar metabolism genes that are involved in phenylpropanoid biosynthesis pathway in both the genotypes. In combined stress, genes encoding thiolase-like protein, WRKY70, fatty acid elongase 1, Calcium dependent kinases were upregulated. Many kinases were upregulated that in turn triggered many hormone signalling genes. Many candidate genes were identified for combined stress tolerance from these genotype. Meta-analysis identified 110 genes in combined stress, which were upregulated from different studies (Table 2). Interestingly, many of these genes were characterized for individual stresses (Table 1). In combined stress, kinases like serine kinase (Os05g0466900, Os12g0454800) were upregulated, similarly leucine-rich repeat genes (Os01g0162300, Os01g0162500), ras-related protein (Os01g0750000), MTN3 (Os01g0606000), phosphofructokinase (Os06g0326400), cyclin-dependent kinase inhibitor (Os09g0459900) genes were upregulated. Overexpression of serine/threonine kinases showed improved abiotic stress tolerance in Arabidopsis [25]. The role of Os12g0454800 in cytokinin signalling has been deciphered [26]. A leucine-rich protein Os02g0536300 involved in sensing PAMP responses to trigger the plant immunity [27]. Like that many serine threonine protein kinases have been characterized which are involved in improving the stress conditions to mediate cellular responses. In individual and common drought stress, 12 transcription factors like Zinc finger RING-type domain, Hox29, heat shock transcription factor 31, bZIP (Os02g0578500), transcription activator for tolerance to drought, high-salt and cold stresses (Os09g0522200), elongation factor, NAC, NAM, Class-B HSF (Os08g0546800), HSF29, Hox22, Homeodomain-leucine zipper (HD-Zip) genes were upregulated. AP2 domain containing protein RAP2.6 was downregulated. Transcription factors like NAC, WRKY, bHLH, bZIP were induced upon drought stress and bacterial blight infection. TFs regulate many downstream target genes [28–32]. WRKY45 showed broad spectrum resistance and acts as a negative regulator for pathogen, salt, cold and drought stress is upregulated in combined stress [33, 34]. WRKY11 acts as a positive regulator of defence response against Xoo and drought tolerance is upregulated in combined stress [35]. Genes encoding domains of unknown function (DUF) 250, 868, 761 were upregulated in combined stress. DUF 810 improved drought and salt stress [36]. In combined stress, ACC oxidase (Os09g0451400) was upregulated and showed to involve in ethylene pathway indicating hormonal biosynthesis, play key role in combined stress tolerance [37].

Few peptidases A1, aspartic proteinase nepenthesin-1 (Os06g0610800), serine carboxypeptidase 1 (Os04g0176400), peptidase S8 (Os10g0524600), and M50 family genes (Os03g0729000) were upregulated in combined stress. Peptidase A1 encoding aspartic type endopeptidase activity showed improved biotic and abiotic stress [38]. Similarly peptidase C1A showed to play a role in seed development and improved stress tolerance [39]. Many UDP-glucuronosyl and UDP-glucosyltransferase were upregulated in combined stress [40]. Few chaperons like Cpn60, copper chaperone homolog CCH, HSPs, dehydrins were upregulated in combined stress and involved in improving biotic and abiotic stress tolerance in many plants [41]. The DEGs identified in combined stress have potential to improve multiple stress tolerance in rice.

Translation associated genes are differentially expressed in combined stress. Ribosomal proteins are a class of highly conserved proteins across the living system involved in translation mechanisms. Among them, many are considered to have an important role during growth, development and stress condition in plants [42].
Many omics reports, represent genes associated with translation mechanism that are differentially regulated in individual as well as combined stress [7]. Recent studies, have shown extra-ribosomal function of ribosome encoding genes. There are 29 ribosomal protein encoding genes that were upregulated in both the genotypes in combined stress. Subsequent, validation of these genes confirmed upregulation in BPT5204 in combined stress, however, response of these genes varied from individual drought and pathogen infection. RPL10 was upregulated at early time points in resistant genotypes, however, in TN1 upregulation was at 6 dpi indicating mechanism of early sensing of stress in tolerant variety compared to sensitive genotype. Mutation/silencing of RPL10 in Arabidopsis, Nicotiana benthamiana showed susceptible phenotype and weak ABA response [43, 44].

Genome wide expression analysis of rice in drought and Xoo showed upregulation of RPL12, L28, L36, L44 and L51. In combined stress, expression of RPL28, L25, L27, L5, L46, L18 was upregulated, similarly RPS14, S12, S9, S4 were upregulated in resistant genotypes, RPS6, RPS9 and RPS10 were responsive to biotic stress [45]. RPL10 play a vital role during both viral and bacterial infection acting as a positive and negative regulator [46]. Virus-induced gene silencing of RPS12 and RPS19 in N. benthamiana showed compromised non-host disease resistance [47]. Mutated rpl23 plants showed to have impaired growth and developmental abnormalities [48]. rpl27 mutant plants showed impaired shoot development and seed setting [49]. Our study shows elevated levels of RPL23A and RPL27 during drought and combined stress. RPS14 showed higher transcript levels upon hormonal treatment [50] and pathogen infection [51]. RPS14 also showed a similar pattern of upregulation during all stress conditions. These evidences clearly show their extra-ribosomal functions in regulating stress adaptation. These ribosomal proteins play critical role in both transcriptional and translational mechanisms and differential expression of these genes indicate their potential role in improving multi-stress tolerance. More detailed studies are required to unravel these genes potential in stress adaptation.

**Conclusion**

Developing climate resilient crops are in demand to supply the food for growing population. The relevant genes to improve multi-stress tolerance can be identified from plants which are simultaneously exposed to different combination of stresses. We have optimized combination of drought and bacterial infection process in rice and developed transcriptome information from contrasting genotypes. We demonstrate that, the role of many candidate genes which showed to improved stress tolerance for both drought and pathogen infection. Many of the genes were functionally validated by different research groups. These genes could be used to develop durable multi-stress tolerant crops in changing climatic conditions. Many candidate genes can be used for introgression in elite genotype background and also can be targeted for genetic manipulation using gene editing approaches.

**Material and methods**

**Plant materials and growth conditions**

Rice seeds of BPT5204 and TN1 genotypes collected from National Seed Project (NSP), University of Agricultural Sciences, GKVK, Bengaluru were used for this study. BPT5204 rice genotype is resistant to bacterial blight and is highly cultivated and TN1 genotype is susceptible for drought and bacterial blight. Four different sets i.e. control, drought, pathogen and combined stress were imposed. Seeds were soaked in water for O/N followed by germination on wet filter paper in Petri-plate. The germinated seedlings were transferred to individual pots, kept in green house condition (28 °C, 60% relative humidity and 16 h light / 8 h dark condition) and maintained. 45—day-old plants were used for imposing drought, pathogen and combined stress.

**Xanthomonas oryzae pv. oryzae (Xoo) inoculation and leaf sampling**

Xoo culture was grown in nutrient broth (NB) medium (1% polypeptone, 0.5% yeast extract, 1% sucrose, pH 6.8) at 28°C for 48 h. The Xoo inoculum was prepared by suspending the bacterial cells in 10 mM MES buffer. Leaves of 45-day-old plants were infected with 0.5 × 10⁶ CFU/mL of Xoo inoculum by leaf clipping method [52]. Bacterial disease symptoms were observed at 4 days of post infection (dpi), 6 dpi, 8 dpi, 10 dpi, 12 dpi and 14 dpi and bacterial growth was measured from the infected leaves.

**Drought and combined stress imposition**

For drought stress, 45-days-old rice plants were exposed to gradual reduction in soil moisture content till 60% field capacity (FC) and further maintained along with the respective control. The samples were collected after plants reaching 60% FC for RNA sequencing. For combined stress imposition, 45-days-old rice plants were exposed to moisture stress by gradual reduction in watering till they reach 80% FC, then plants were infected with Xoo (5*10⁶ CFU/mL) and further maintained till 60% FC. Bacterial disease symptoms and CFU was measured at 6,
8, 10 and 12 dpi. After 51 days, tissues from minimum 5 plants were pooled in each sample, three biological replicates were collected from each pathogen, drought and combined stress exposed plants along with their respective controls.

**Determination of hydrogen peroxide (H₂O₂) by Diaminobenzidine (DAB) staining**

Detached rice leaves were immersed in 1 mg/mL DAB (SRL- Sisco Research Laboratories, New Delhi, Cat no. 17076) solution at 3.8 pH. Leaves were infiltrated and kept in box for 5–6 h until brown precipitation was observed. Chlorophyll, was removed from the leaves with ethanol washing. Stained leaves were fixed in ethanol: acetic acid: glycerol (3:1:1) and photographs were taken. For quantification, stained leaves were ground and accumulation of formazan (reddish brown colour) was quantified by measuring the absorbance at 450 nm.

**Determination of superoxide anion radicals by using Nitroblue tetrazolium chloride (NBT)**

Superoxide ion (O₂⁻) react with NBT to form blue colour. Leaves were excised and kept in 0.1% (w/v) Nitro Blue Tetrazolium (NBT), 10 mM sodium azide and 50 mM potassium phosphate solution (pH 6.4). Leaves were vacuum infiltrated for 2–3 times until leaves were completely infiltrated. Further, leaves were kept in 10 mL of 0.1% NBT for 15 min. Chlorophyll was removed from leaves by washing with ethanol. Photographs were taken and quantification was done by measuring the absorbance at 560 nm.

**Cell membrane damage by Evan's blue staining assay**

Evans blue (Sigma-Aldrich, Cat no. E2129) solution was prepared in 0.1 M CaCl₂ solution at pH 5.6. Tissues were dipped in Evans blue solution for overnight and excess unbound dye washed with water. Images were taken under microscope. To quantify Evans blue dye was extracted in 1% SDS from the stained tissues and centrifuged for 5 min at room temperature to remove debris and elute dye into the supernatant. Optical density was measured at 600 nm and 1% SDS was used as blank. Concentration of Evans blue dye was estimated using standard curve method [53].

**RNA extraction and quantitative real-time PCR analysis**

For RNA isolation from drought, pathogen and combined stress, plant samples were collected at 4 dpi and 6 dpi from both genotypes along with respective control. Samples were frozen in liquid nitrogen, crushed to powder and RNA was isolated using TRIzol reagent (Sigma-Aldrich, Cat no. T9424). Total 5 µg of RNA was then converted to cDNA using MMLV reverse transcriptase (ThermoFisher Scientific, Cat no. EP0451) with oligo dT primers. Specific primers for quantitative real-time PCR (qRT-PCR) were designed (Additional file 7). The qRT-PCR was performed using diluted cDNA and SYBR green (Takara Bio, Cat no. RR820A) on a Quant studio 6 Real Time PCR system (ABI-Quant studio 6 Real Time PCR system, ThermoFisher Scientific, Singapore). The expression data was collected and further processed to calculate 2^−ΔΔCT method [54]. Rice actin was used as internal control for normalization and three biological replicates were used for each gene.

**RNA sequencing and data analysis**

For RNA sequencing analysis, infected leaf samples were collected at 4 dpi and frozen in liquid nitrogen. Since the disease progression was slow at 4 dpi and at 6 dpi the bacterial load is very high, plants trigger many transcriptional reprogramming and to capture the differences we have collected the tissue at 4 dpi. Samples were collected in three biological replicates from both BPT5204 and TN1 genotypes. RNA sequencing was performed using Illumina HiSeq2500 platform from cDNA library by Thracus Innovations Pvt. Ltd., Bengaluru, India. The raw data was trimmed and low-quality reads were removed by the sickle trimming tool. The transcriptome analysis was performed using CLC Genomics Workbench v.12. The default parameters and analysis procedure followed as per CLC Genomics Workbench manual instructions. The control versus stress comparison [drought, pathogen and combined stresses (drought+pathogen)] was done in both genotypes and the IRGSP1.0 rice genome was considered as a reference for the analysis. The False Discovery Rate (FDR) ≤ 0.05, and log₂FC ≥ 1.5 (for up-regulation), ≤ -1.5 (for down-regulation) rigorous filtering parameters were applied for the mining of differentially expressed genes (DEGs). The functional descriptions of the DEGs were retrieved from the Rice Annotation Project (RAPDB) database. The downstream analysis like pathway mapping, Gene ontology were carried out for DEGs using web-based tools KEGG mapper using mapping parameters mismatch cost = 2, insertion cost = 2, deletion cost = 3, length fraction = 0.8 and similarity fraction = 0.8. Transcripts Per Million (TPM) was used for the expression calculation. The agrigo (v2) (https://agriGO. rw/) analysis was performed using default settings.

**Meta-analysis**

Meta-analysis was performed using RNA sequencing data of BPT5204 resistant and TN1 susceptible genotypes and microarray data curated from individual drought and pathogen infection from public domains (RiceMetasysA http://14.139.229.201/RiceMetaSys/ and RiceMetasysB http://14.139.229.201/RiceMetaSysB/).
Abbreviations
Xoo: Xanthomonas oryzae Pv. Oryzae; TN1: Taichung native 1; BPT5204: Samba Mahsuri; QTL: Quantitative trait loci; Xo: Xanthomonas oryzae pv. oryzae resistant allele; DEGs: Differentially expressed genes; FC: Field capacity; Dpi: Days post infection; RDS: Reactive oxygen species; NBT: Nitroblue tetrazolium chloride; DAB: Diaminobenzidine; BDF: BPT5204 exposed to drought and Xoo-Combined stress; BD: BPT5204 exposed to drought; BP: BPT5204 exposed to pathogen; TDP: TN1 genotype exposed to drought and Xoo-Combined stress; TD: TN1 exposed to drought; TP: TN1 exposed to pathogen; RNA seq: RNA sequencing; RP: Ribosomal proteins; qRT-PCR: Quantitative real time polymerase chain reaction; BB: Bacterial leaf blight BB; qDTY2.2: QTL for drought tolerance and yield; CFU: Colony forming unit.

Supplementary Information
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Additional file 1. Differentially expressed genes from contrasting genotypes identified in RNA sequencing data.
Additional file 2. Gene ontology of DEGs from BPT5204 and TN1.
Additional file 3. List of upregulated and downregulated genes having biological processes, molecular function and cellular components.
Additional file 4. List of DEGs from microarray data set.
Additional file 5. List of upregulated and downregulated genes in combined stress.
Additional file 6. DEGs of ribosomal protein encoding genes.
Additional file 7. List of primers used in this study.

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Authors’ contributions
RSV conceived the project, analysed data and wrote manuscript. GR, RB, SP and SA performed the experiments. SD, VN and AD performed the experiments. RSV, GP, MKP wrote and edited the manuscript. The author(s) read and approved the final manuscript.

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Availability of data and materials
All the data generated and analysed during this study are included in additional files. RNA sequencing datasets associated with this manuscript have been deposited on Gene Expression Omnibus (GSE197133) and can be accessed using the following link https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE197133. Go analysis—agrIGO (v2) (https://agrigo.rw/); KEGG Mapper- https://www.genome.jp/kegg/mapper/; RiceMetaSysA http://14.139.229.201/RiceMetaSys/ and RiceMetaSysB http://14.139.229.201/RiceMetaSysB/; Rice Annotation Project (RAPDB) database- https://rapdb.dna.afrc.go.jp

Declarations
Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interest.

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