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RESEARCH ARTICLE

Expression analysis of genetic loci linked with bakanae disease resistance in *japonica* rice of North Western Himalayas

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Abstract

Bakanae disease caused by *Fusarium fujikuroi* is emerging as a serious threat in the production of *japonica* rice under high altitudes of the western Himalayas and basmati rice in northern regions. To identify resistant sources for Bakanae disease of rice, a population of 165 doubled haploids (DHs) was screened through artificial inoculation using a uniform *F. fujikuroi* 'A30'virulent isolate under controlled conditions. Out of 165 inbred lines, 105 were identified as resistant, 51 moderately susceptible and nine susceptible to disease. A polymorphism survey was conducted with the help of 502 KASP markers on 12 chromosomes. On chromosome 6, a 6.25% polymorphism was found between parents, GS-88 and K-332. Two DHs, D5-2 and D5-3, showed 6% polymorphism among themselves, while DHs D49-3 and D49-4 revealed just 3.6%. Further, 19 defense-related genes located between markers, ALK_SNP_ff_2 (6.8Mb) and RM19817_SNP_nn_3 (9.7 Mb), were identified through *in silico*. Primers for three transcripts (*Os06g0279900, Os06g0249500* and *Os06g0267400*) were designed through Primer 3Plus. Based on the disease severity index, two extreme resistant and susceptible DH lines, along with parents, were selected for expression studies using reverse transcription polymerase chain reaction. Expression pattern between resistant and susceptible lines varied at two different stages, viz., 10 days post inoculation (dpi) and 24 dpi. Genes *Os06g0279900* and *Os06g0249500* were significantly enriched at 10 dpi and *Os06g0267400* at 24 dpi in resistant lines, but were absent in susceptible lines. This indicates that the response shown by resistant DH against the pathogen was much faster than that of susceptible ones. These responses at 10 and 24 dpi are suggestive of an active defense system induced by the pathogen in resistant cultivars, which involves pathogenesis-related gene expression and enrichment pathways to defense of rice plants.

Keywords: Rice, Bakanae, DH population, phenotyping, SNP genotyping, transcriptomics

Introduction

Rice is a staple food for most of the human population in Jammu & Kashmir. It is grown on 274 thousand hectares with an annual production of 605 thousand tonnes and productivity of 22.03 quintals per hectare (Digest of Statistics, 2022-23). Rice crop, particularly japonica types grown in high hills, is affected by a number of diseases, of which the necrotrophic fungus *Fusarium fujikuroi,* responsible for Bakanae 'foolish seedling' disease of rice, is of paramount importance. Ito and Kimura (1931) first reported this disease in Japan in 1928; widely distributed throughout the world in both temperate and tropical climates (Gupta et al. 2015).

Bakanae disease is showing an upward trend in rice cultivars, particularly Basmati in Haryana, Punjab and the western area of Uttar Pradesh, causing severe yield losses (quantitative as well as qualitative) under field conditions with loss even up to 70% (Bashyal et al. 2020; Kushwaha et al. 2023). This disease is presently emerging as a major constraint in the production of japonica rice varieties cultivated under high altitudes ranging from 2000-2200 m amsl (Ahanger et al. 2012), accounting for 15 to 20% of the rice area of Jammu & Kashmir. Disease infects plants from the seedling emergence to the maturity stage of rice, showing excessive internode elongation, stunted growth, lanky pale yellow seedlings, lower nodes tissue discoloration, foot rot at the collar portion of the root system, and withering of growing shoots resulting in dead heart. The disease predominantly acts as seed-borne as well as disseminates through the dispersal of spores found on plant components and in the soil. Traditionally, hot water treatment, chemicals and biological agents have been used to manage the disease. Nevertheless, genetic resistance is increasingly favoured for its economic viability, long-term sustainability and environment friendly (Kushwaha et al. 2023).

The discovery of QTLs or genes governing disease resistance can help in the precise transfer and quick development of bakanae disease-resistant varieties. Eight rice chromosomes (1, 3, 4, 6, 8, 9, 10 and 11) were analysed (Chen et al. 2019) and 29 QTLs for bakanae disease resistance. Many QTLs have been recently identified for resistance to bakanae disease, like *qBk1* (Hur et al. 2015), *qBk1.1, 1.2* and *1.3*

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(Fiyaz et al. 2016), *qBk1_628091* and *qBk4_31750955* (Volante et al. 2017) and *qBk1WD* (Lee et al. 2018). Most of these studies were carried out on the populations originating from sub-tropical sources, including the pathotypes used for disease screening.

The present study was undertaken using a doubled haploid (DH) population generated from K-332 x GS-88. Previously, we mapped a new QTL, qBk6, having a LOD score of 6.4 on chromosome number 6 between 6.8 Mb and 9.7 Mb markers (unpublished). The genomic region corresponding to the QTL with an *in-silico* analysis interval revealed 19 specific transcripts. The hypothesis in the present study was that the materials dealt with are specific to cold temperate high altitude regions and throw out the possibility of discovering certain novel genomic loci. This study aimed to compare the gene expression between the resistant and susceptible lines within the population.

Materials and methods

Plant materials and phenotyping for disease reaction

The experimental material used in the present study consisted of a DH mapping population of 165 individuals previously developed by Sakina et al. (2020). The population was derived from a cross between K-332 x GS-88. K-332, a popular short-grain japonica rice variety, is susceptible to bakanae disease and is grown in high altitude (>2000 m amsl) parts of the Kashmir valley. On the other hand, GS-88 is again a bold grain rice genotype but resistant to bakanae disease (Lone et al. 2016). Phenotyping of individuals in a mapping population was carried out twice for resistance to bakanae disease under controlled conditions at MRCFC, SKUAST-Kashmir, Khudwani and at the School of Biotechnology in SKUAST-Jammu, Chatha Campus, J&K. *Fusarium fujikuroi* isolate 'A30' was cultured in the pathology laboratory, MRCFC, Khudwani, SKUAST-K. The pure culture was further sub-cultured and used for inoculums preparation and spore concentration adjusted to 1×10^6 conidia/mL with the help of haemocytometer to obtain the standardized inoculum for evaluating DH population against the disease (Fig. 1). The standard protocol used by Zainudin et al. 2008 was followed for inoculation and the disease severity index (DSI) was calculated for each treatment within 20 and 40 days after inoculation.

Competitive allele-specific PCR (KASP) marker genotyping and in-silico search for candidate genes

The seedlings of the 165 individual plants in the DH population were grown under the irrigated field conditions. Leaf samples were collected, followed by genotyping that was outsourced (Biosearch Technologies, Hoddesdon, Herts, U.K.). Sixteen leaf discs, each from the individuals and parents, were punched on a punching pad with the help of a leaf disc puncher and placed in each well plate. Twelve selected DHs from the K-332 and GS-88 were genotyped with 502 KASP markers. *In-silico* search was done for the hypothetical and expressed genes present within qBk6 interval on chromosome 6. *Oryza sativa* reference sequence (OsNipponbareReferenceIRGSP1.0, http://rapdb.dna.affrc.go.jp/download/irgsp1.html) was used to scan the transcripts included in the selected genomic windows in order to identify candidate genes between 6.8 to 9.7 Mb.

Expression analysis of candidate genes and primer designing

The study on the expression analysis of disease resistance genes within the QTL interval carrying qBk6 was carried out. Leaf tissues at two time intervals: first on 10 days after sowing (un-inoculated/ Control), 10 days post inoculation (10dpi) and 24dpi were collected from two parents, two extreme susceptible and two extreme resistant individuals of two DHs by Trizol method followed by cDNA synthesis using Revert Aid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific, Wilmington, US).For quantification and normalization of cDNA optical density of cDNA samples was measured at 260nm and 280nm using a spectrophotometer.

The three genes *Os06g0279900*, *Os06g0249500* and *Os06g0267400* were identified through *in-silico* analysis within the chromosome 6 interval of 6.8-9.7 Mb, which

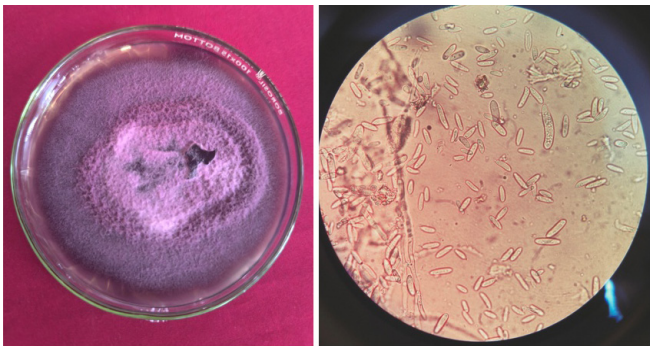

Fig. 1. *F. fujikori* culture and spore concentration

harbours QTL qBk6. Transcriptomic & genomic sequences pertaining to these genes were taken from RAP-DB and the retrieved FASTA sequences were imported into Primer3plus software (https://primer3plus.com) for primer designing (Table 1). Efficient qPCR primer designing was achieved by prioritizing amplicons shorter than 200bp, a melting temperature near 60°C, GC content (≥ 50 percent) and exon selection. To validate the annealing temperature of primers, Actin gene-specific primers were used in semi-quantitative PCR

Quantitative real-time reverse transcription PCR (qRT-PCR) and data analysis

All qRT-PCR reactions were carried out using a CFX Connect Real-Time thermal cycler machine (Bio-Rad). Each reaction mixture contained 2 µL of cDNA, 5 µL of 2× SYBR Green PCR Master Mix, 0.25 µL each of forward and reverse primers (10 pmol/µL) and nuclease-free water to a final volume of 10 µL. Each sample was run in duplicate. The *Actin gene* was used as a reference gene for data normalization. From the qPCR analysis, the threshold/quantitation cycle (CT/CQ) value was obtained. The CQ value was used for calculating the fold change in expression among the selected resistant /susceptible individuals and parents. The comparative C_T method, also known as $\Delta\Delta C_T$ method (Livak and Schmittgen 2001), was used to achieve the results for relative quantitation.

Results and discussion

The DH mapping population derived from a cross between K-332 x GS-88 was used with the aim of delineating the qBK6 interval linked with resistance to bakanae disease. The disease has been reported to cause seedling mortality and shows a quantitative inheritance pattern (Fiyaz et al. 2016). In our investigation, the high-throughput seed inoculation protocol developed (Fiyaz et al. 2016) and disease severity index (Halim et al. 2015) were scored across the doubled haploid population for resistance to bakanae disease. A virulent isolate A-30 of *Fusarium fujikuroi* was used to test the differential reaction between the two parents (K-332 and GS-88) and 165 DHs, and Pusa Basmati 1509 was used as a check. K-332 and Pusa Basmati 1509 exhibited typical

Table 1. The genes related to disease resistance were selected for real-time PCR assay

Locus ID	Primer name	Putative function
Os06g0279900	ABS-6	Similar to the NB-ARC domain-containing protein
Os06g0249500	ABS-7	Pentatricopeptide Repeat domain containing protein
Os06g0267400	ABS-8	Cytochrome P450

bakanae disease symptoms: yellowing, excessive growth and withering, while GS-88 showed resistance. The seedlings after inoculation recorded a DSI of 0 for GS-88 in contrast to K-332, which recorded a DSI of 3.22. On the other hand, the check variety Pusa Basmati 1509 recorded DSI of 3.45. Out of 165, 15 DH lines were found immune (DSI: 0–0.29), 90 as resistant (DSI: 0.30-1.99), 51 with DSI 2-2.99 as moderately susceptible and 9 lines with DSI: 3-4 showed susceptible reaction. Nearly uniform results were found across the population over two replications of screening. Similar approaches have been followed by Martic et al. (2016), where they found Selenio as a resistant cultivar and Dorella as a susceptible cultivar, after screening 12 rice genotypes against bakanae disease.

Previously, we identified a novel single major QTL for seedling mortality explaining 65.2% of phenotypic variation on chromosome 6 between molecular markers ALK_SNP_ff_1 (6.8 Mb) and RM527_SNP1 (9.7 Mb) at a LOD score of 6.4 (unpublished). Therefore, our present investigation on the DH population was used for SNP genotyping. A polymorphism survey was conducted with the help of 502 KASP markers distributed on 12 chromosomes. On chromosome 6, 21 out of 83 markers were identified as polymorphic between the parents (25%). At qBk6 (6.8 to 9.7 Mb) 10 markers (ALK_SNP_ff_2, gs_id1002_fn, RM8_11_SNP_nn_1, gs_id1003_ff, HD1_SNP_nn_1, HD1_SNP_nn_2, HD1_SNP_nf_1, HD1 SNP nn 3, gs_id1004_ff and gs_id1005_fn) showed polymorphism. Identification of a sufficient number of markers revealing polymorphism among the parental lines is a prerequisite for the identification of disease resistance genes. The two DHs, D5-2 (susceptible) and D5-3 (moderately resistant), were polymorphic for 6% of which four (HD1_SNP_nn_1, HD1_SNP_nn_2, gs_id1004_ff and gs_id1005_fn were found within a 6.8 to 9.7Mb distance. Further, between families D49_3 and D49_4, 3.6% markers were found to be polymorphic (Table 2). The existence of narrow genetic variations between the parents is because they belong to the same ecotype and are adapted to the same rice ecosystem. This has also been reported in earlier findings (Gomez et al. 2010; Yadav et al. 2015).

In-silico analysis of genes and transcripts

Quantitative trait locus is a multi-locus cluster carrying several gene sequences with minor or major effect, or the genes that could be possible candidates governing

Table 2. SNP genotyping of parents and families using polymorphic markers at chromosome 6

S. No.	Marker	locus (Mb)	K-332	GS-88
1	Wx_SNP_nn_1	1.6	T:T	G:G
2	Amy_RM1910_func_1	1.6	A:A	C:C
3	SNP_4_SNP_ff_1	2.1	G:G	A:A
4	RM587_SNP_fn_1	2.1	T:T	A:A
5	gs_id980_nn	3.7	G:A	G:G
6	ALK_SNP_ff_2	6.8	T:C	C:C
7	gs_id1002_fn	8.8	T:T	C:C
8	RM8_11_SNP_nn_1	9.0	T:T	C:C
9	gs_id1003_ff	9.0	C:C	T:T
10	HD1_SNP_nn_1	9.2	T:G	T:T
11	HD1_SNP_nn_2	9.2	G:C	C:C
12	HD1_SNP_nf_1	9.2	A:G	A:A
13	HD1_SNP_nn_3	9.2	G:A	G:G
14	gs_id1004_ff	9.2	T:C	C:C
15	gs_id1005_fn	9.5	T:A	T:T
16	RM19817_SNP_nn_3	9.9	T:T	G:G
17	gs_id1009_ff	10.6	A:A	G:G
18	gs_id1057_fn	19.6	C:C	A:A
19	Sub1A_SNP_nn_3	23.1	T:C	T:T
20	RM6811_SNP_nn_2	30.6	C:C	T:T
21	RM6811_SNP_nn_4	30.6	T:T	C:C
			5_2	5_3
1	gs_id980_nn	3.7	G:A	A:A
2	HD1_SNP_nn_1	9.2	T:G	G:G
3	HD1_SNP_nn_2	9.2	G:C	G:G
4	gs_id1004_ff	9.2	T:C	T:T
5	gs_id1005_fn	9.5	T:A	A:A
			49_3	49_4
1	gs_id1004_ff	9.2	T:C	C:C
2	gs_id1005_fn	9.5	T:A	T:T
3	RM6811_SNP_nn_4	30.6	T:C	C:C

resistance to disease (Ishikawa et al. 2017). *In-silico* search was done between the marker Wx_SNP_nn_1 (1.6 Mb) on the proximal end to RM6811_SNP_nn_4 (30.6 Mb) at the distal end of chromosome 6. Marker intervals were scanned to identify candidate genes in RAPDB (OsNipponbareReferenceIRGSP1.0,http://rapdb.dna.affrc.go.jp/download/irgsp1html). A total of19genes were identified within the marker interval ALK_SNP_ff_2 (6.8Mb) and RM19817_SNP_nn_3 (9.7 Mb) flanking the qBK6 interval. The genes carrying defence-related functions are listed in Table 3. A detailed analysis of these candidate genes helps in understanding the molecular mechanisms governing resistance to bakanae disease. The defence-related transcripts included cytochrome P450 monooxygenases (P450s) implicated in the oxidation of many substrates with defence-related metabolites by the activation of molecular oxygen. Transcripts encoding PR proteins like glucoamylase, thaumatin-like, peptidase A1, protease serine type peptidase, xyloglucan endoglucoslase/hydrolase, endo beta 1,4 glucanase, threonine endopeptidase were also located within the region. These transcripts have antifungal activity against many phytopathogenic fungi, such as *Phytophthora infestans, Phytophthora parasitica, Uromyces fabae, Erysiphe graminis* and also *Fusarium fujikuroi* (Hugot et al. 2004). In a similar study, Matic et al. (2016) revealed transcripts encoding Avr9/Cf-9 elicited protein, two zincfinger RING/FYVE/PHD-type genes (*Os03g0240600* and *Os01g0755700*), cytochrome P450 *(Os12g0582700*; family 94), thaumatin-like genes and β-1, 3-glucanases to be highly upregulated in the resistant genotype.

Expression analysis of disease resistance genes within the QTL interval carrying qBk6

In the present investigation among identified candidate genes, three genes viz., Os06g0267400 (8.8 Mb), *Os06g0249500* (7.7 Mb) and *Os06g0279900* (9.7) were selected for studying their role in defence response against *Fusarium fujikuroi* in GS-88 and K-332 (parental lines), DHs 5-3 (5-3-11 & 5-3-19) and 23-2 (23-2-7 & 23-2-15) at two different time periods i.e. 10 and 24 dpi. The said protocol was followed from Matic et al. (2016), who suggested that after 1week post germination (wpg) and 3 wpg transcriptional changes are desirable to study the resistance mechanism.

RNA gel assay

RNA samples isolated from untreated checks and at 10dpi and 24dpi were tested for quality and intactness on a 1.5% agarose gel. The gel electrophoresis resolved distinct bands for 28S and 18S ribosomal RNA (Fig. S1). The confirmation of cDNA was carried out through semi-quantitative PCR. The samples amplified a 496 bp fragment at the GAPDH locus after 35 cycles. First, all three primers (Os06g0279900, Os06g0249500 and Os06g0267400) were validated on cDNA of GS-88 and K-332 for amplification. qRT-PCR analysis of all three genes was carried out on the selected resistant and susceptible individuals and parents of the doubled haploid population at 10 and 24 dpi against untreated controls under controlled conditions. The differential gene expression among the different individuals during the seedling stage was worked out for all the individuals with actin (housekeeping gene) for normalization (Fig. 2).

Os06g0267400 (Cytochrome P450)

In the present study, gene *Os06g0267400* exhibited increased expression in the resistant lines in contrast to susceptible lines and showed maximum fold change of 12.99x and 3.78x at 24dpi in GS-88 and 5-3-11, respectively. The resistant line

Table 3. Location of different transcripts identified within the qBK6 interval

S. No	Physical location (Mb)	Transcript
1.	6.8	Protein kinase
2.	6.9	Similar to DEGP9 (Protease: serine type peptidase)
3.	7.1	Similar to xyloglucan endoglucoslase/hydrolase
4.	7.6	Similar to Avr9/Cf 9 rapidly elicited
5.	7.6	Similar to endo beta- 1,4 glucanase precursor
6	7.7	Pentatricopeptide Repeate domain containing protein
7	8.1	Similar to endo beta- 1,4 glucanase precursor
8	8.3	Similar to TMV response related
9	8.7	Threonine endo peptidase
10	8.7	Endo/exonuclease/ phosphatise
11	8.8	Similar to Gibberellin-stimulated protein
12	8.8	Cross-talk between GA & BR signalling
13	8.8	Cytochrome P450
14	8.9	Peptidase A1
15	9.1	Zinc Finger Nuclease
16	9.2	Chitin-binding lectin 1 precursor
17	9.5	Glucoamylase
18	9.7	Similar to the NB-ARC domain-containing protein
19	9.8	Thaumatin like

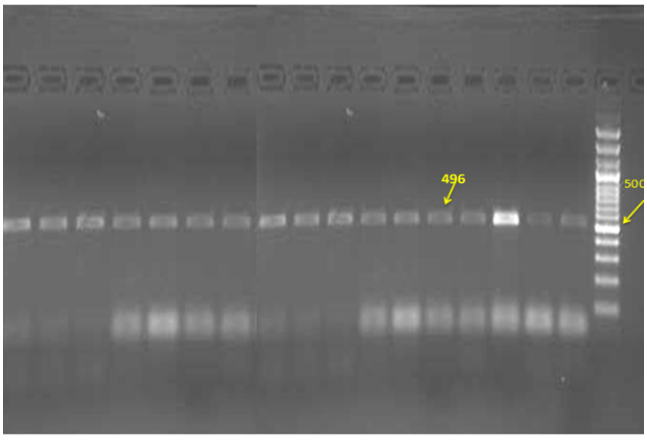

Fig. 2. Gel image of isolated cDNA of parents GS-88, K-332 and DH progenies; 5-3-11, 5-3-17, 23-2-7, 23-2-5) at control, 10dpi and 24dpi using GAPDH

23-2-7 showed maximum fold change of 7.26x at 10dpi that declined to 3.052x at 24dpi (Fig. 3). A group of redox proteins like cytochrome P450 monooxygenases which catalyse different oxidative reactions, facilitating plant defence, including the metabolism and synthesis of various chemical compounds which mediate plant defence against various pathogenic micro organisms and insect pests (Isin and Guengerich 2007). The expressed resistant lines, along with the parent GS-88, showed enhanced resistance to *F. fujikuroi*, implicating that this gene underlying the QTL qBk6 region can significantly contribute to plant disease resistance. CYP74A plays a significant role in wound-induced defence mechanism against different biotic stresses, by catalyzing of dehydration of hydroperoxide to an unstable allene oxide in the biosynthesis of jasmonic acid (Park et al. 2002). Yan et al. (2016) also observed that a family gene of Soybean (Soybean Cytochrome P450) was involved in the ethylene and jasmonic acid pathway, which enhanced resistance of plants to biotic and abiotic stress. However, Jasmonic acid mediates defence against necrotrophic or hemibiotrophic pathogens was observed by Pieterse et al. 2009 and 2012). Therefore, this may be the reason for showing increased expression of Os06g0267400 in 5-3-11, 23-2-7 and GS-88. In earlier studies, Matic et al. (2016) found a putative cytochrome P450 (Os12g0582700; family 94) as the most upregulated gene in Selenio (resistant rice genotype) at 3 weeks post germination with a log2FC 6.60, and showed cytochrome P450 as a positive regulator of bakanae disease resistance. (Lee et al. 2019) investigated that cytochrome P450 monooxygenase gene expression was significantly higher in the resistant variety compared to the susceptible one in all three stages of infection (6, 9 and 12 dpi).

Os06g0249500 (pentatricopeptide repeat domain)

PPR (Pentatricopeptide repeat domain) proteins function as an important gene expression regulators of organelles at post-transcriptional levels, besides their role in RNA splicing, editing, cleavage, translation and RNA stability (Wu et al. 2016). In the present finding, Os06g0249500 revealed upregulated signal transduction in the resistant lines as compared to susceptible lines. It showed maximum fold change of 1.505x and 10.33x at 10dpi in 5-3-7 and 23-2-7, respectively. Still, in GS-88 maximum fold change of 12.99x was found at 24dpi (Fig. 3). PPRs usually control the expression of genes that are associated with different processes like growth, development and defence against diseases via post-transcription modification of RNA (Ichinose and Sugita 2017). Researchers have revealed that biotic and abiotic stresses usually cause severe damage to the function or structure of plant mitochondria and the PPR protein has the potential to regulate mRNA processing by splicing and editing mitochondrial RNA, therefore playing a vital role in plant stress management (Baxter et al. 2007). In earlier studies, Cui et al. (2022) found that the hub gene (arahy. EJT9JS) encoding PPR was upregulated in the resistant genotype as compared to the susceptible genotype and showed resistance against *Aspergillus flavus*.

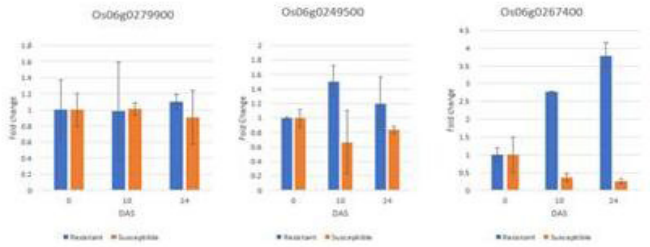

Fig.3a.Fold change diagram (2^-ΔΔCt) of Os06g0279900, Os06g0249500 and Os06g0267400 in 5-3-11 (resistant) and 5-3-17 (susceptible) at different points of time

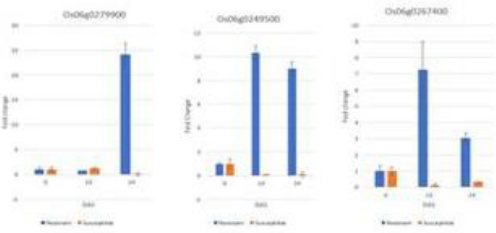

Fig.3b Fold change diagram (2^-ΔΔCt) Os06g0279900, Os06g0249500 and Os06g0267400 in 23-2-7 (resistant) and 23-2-15 (susceptible) at different points of time

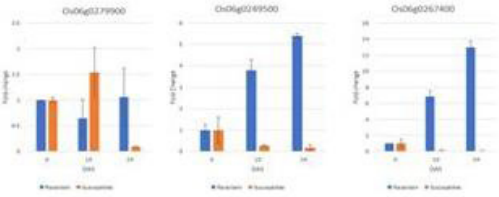

Fig.3c Fold change diagram (2^-ΔΔCt) of Os06g0279900, Os06g0249500 and Os06g0267400 in parents GS-88 (resistant) and K-332 (susceptible) at different points of time

Fig. 3. Expression studies of DHs (5-3), DHs (23-2) and K-332 and GS-88

Os06g0279900 (Similar to NB-ARC)

Nucleotide-binding adaptor shared by Apoptotic protease activating factor-1 (APAF-1), R proteins and CED-4 (NB-ARC) proteins are critical signalling pathway regulators and play significant roles in signal transduction and effector recognition in disease-free plant growth and development (Chen et al. 2018). The expression patterns of pathogen-inoculated susceptible and resistant parents were examined for *Os06g0279900* at the transcript level. In the present study, *Os06g0279900* showed maximum fold change of 24.04x, 1.1x and 1.05x at 24dpi in 23-2-7, 5-3-11 and GS-88, respectively, but at 10 dpi, susceptible lines 23-2-15, 5-3-17 and K-332 also recorded expression of fold change of 1.29x,1.01 and 1.54x. However, at 24 dpi, low fold change was observed in susceptible lines only (Fig. 3). (Cheng et al. 2020) Also reported that PRRs like kinases were upregulated in resistant and downregulated in susceptible cultivars at 7 dpi of *F. fujikuroi*. *PRRs* like kinases encoding LRR receptors were also reported to participate in participation of regulating cell cycling and brassinosteroid signalling in rice (Jiang et al.2012). The current findings are supported by earlier research (Li et al. 2013) on the upregulation of the NBS-LRR gene PnAG3 in the peanut fruit tissues that show resistance to *Aspergillus flavus* infection. It has been observed that the regulation of the *Arabidopsis thaliana* gene RPP8 was induced in response to challenge with *Hyaloperonospora arabidopsidis* and salicylic acid. The RPP8 promoter contains three W box cis elements that are the only known cis elements overrepresented in *Arabidopsis* nucleotide-binding leucine-rich repeat promoters (Mohr et al. 2010). The NB and ARC domains together work as a single platform, with the result that downstream signal transduction processes occur Lukasik and Takken, 2009. In the avoidance of pathogens, they remain inactive with the ADP-bound state via intramolecular interactions in their various domains or extramolecular interactions with other host proteins (Noman et al. 2019). (Baker et al. 2021) identified that the NB-ARC protein was expressed differentially in responsive to *E. mallotivora*.

In *Os06g0279900* and *Os06g0267400* defence genes, expression mostly peaked during 24 dpi stage of infection. On the other hand, at the 10dpi of infection, the *Os06g0249500* gene expressed itself strongly and then declined during the later stages (24 dpi). This could be explained by noting that resistant plants respond quickly to infection, as evidenced by the instantaneous up-regulation of several resistant genes, in contrast to susceptible plants, which either experience down-regulation or mild up-regulation. In later stages of infection in resistant plants, expression of *Os06g0249500* genes was either steady or lowered. This means that resource allocations in the resistant line of defensive response might be stopped during the early stage of infection to avoid adding to the stress on plant machinery. The upregulated genes *Os06g0267400*, *Os06g0249500* and *Os06g0279900* during 10 dpi and 24 dpi in resistant plants contribute to positive defence response.

The genes within the marker interval ALK_SNP_ff_2(6.8)-RM19817_SNP_nn_3 (9.9 Mb) associated with bakanae resistance were analysed for the possible candidate genes and in the process, three loci Os06g0267400, *Os06g0249500* and Os06g0267400 were identified. All three loci showed elevated gene expression in resistant DH lines as compared

to susceptible ones. The derived lines shall serve as a useful resource to study gene structure and function and also for the improvement of japonica rice cultivars grown in the high hills of the North-Western Himalayas.

Supplementary materials

The Supplementary Table S1 and Supplementary Fig. 1 are provided, which can be accessed at www.isgpb.org

Authors' contribution

Conceptualization of research (NRS, ABS); Contribution of experimental materials (ABS, NRS, KZM, SMZ, AS); Execution of field/lab experiments and data collection (MI, NN, RSK, HA, NUN, MAM); Analysis of data and interpretation (NRS, ABS, MAM, MI, NN); Preparation of the manuscript (NRS, ABS, MI, SHW).

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References

Ahangar M. A., Najeeb S., RatherA. G. Bhat Z. A., Parray G. A., Sanghara G. S., Kashap S. C., Ahangar, F. A. and Ahmad, H.2012. Evaluation of fungicides and rice genotypes for the management of bakanae. Oryza, **49**: 121-126.

Bakar N. S. A., Saidi, N. B., Rozano, L., Abdullah, M. P. and Supian, S. 2021. In-silico characterization and expression analysis of NB-ARC genes in response to Erwinia mallotivora in Carica papaya. Sains Malaysian, **50**(9): 2591-2602.

Bashyal B. M., Aggarwal R., Rawat, K., Sharma S., Gupta A. K, Choudhary R., Bhat J., Krishnan S. G. and Singh A. K. 2020. Genetic diversity and population structure of *Fusarium fujikuroi* causing Bakanae, an emerging disease of rice in India. Indian J.Exp. Biol., **88**: 45-52.

Baxter C. J., Redestig H., Schauer N., Repsilber D., Patil K. R. and Nielsen J. 2007. The metabolic response of heterotrophic *Arabidopsis* cells to oxidative stress. Plant Physiol., **143**: 312–325.

Chen S. Y., Lai M. H., Tung C. W., Wu D. H., Chang F. Y., Lin T. C. and Chung C. L. 2019. Genomewide association mapping of gene loci affecting disease resistance in the rice-*Fusarium fujikuroi*. Pathosystem Rice, **12**: 85.

Chen X., Chen H., Yuan J. S., Köllner T. G., Chen Y., Guo Y., Zhuang X., Chen X., Zhang Y. J., Fu J., Nebenführ A. and Guo Z. F. C. 2018. The rice terpene synthase gene *OsTPS19* functions as an (S)-limonene synthase in planta and its overexpression leads to enhanced resistance to the blast fungus *Magnaporthe oryzae*. Plant Biotechnol., **16**: 1778–1787.

Cheng A. N., Chen S. Y., Lai M. H., Wu D. H., Lin S. S., Chen C. Y. and Chung C. L. 2020. Transcriptome analysis of early defenses in rice against *Fusarium fujikuroi*. Rice., **13**: 65-73.

Cui M., Han S., Wang D., Haider M. S., Guo J., Zhao Q., Du P., Sun Z., Qi F., Zheng Z., Huang B., Dong W., Li P. and Zhang X. 2022. Gene co-expression network analysis of the comparative transcriptome identifies hub genes associated with resistance to *Aspergillus flavus* L. in cultivated peanut (*Arachis hypogaea* L.). Front. Plant Sci., **13**: 89-97.

Fiyaz R. A., Krishnan S. G., Rajashekara H., Yadav A. K., Bashyal B. M., Bhowmick P. K., Singh N. K., Prabhu K. V. and Singh A. K. 2014. Development of high throughput screening protocol and identification of novel sources of resistance against bakanae disease in rice (*Oryza sativa L.*). Indian J. Genet. And Plant Breed., **74**: 414-22.

Fiyaz R. A., Yadav A. K., Krishnan S. G., Ellur R. K., Bashyal B. M., Grover N., Bhowmick P. K., Nagarajan M., Vinod K. K., Singh N. K., Prabhu K. V. and Singh A. K. 2016. Mapping quantitative trait loci responsible for resistance to bakanae disease in rice. Rice, **9**: 3–10.

Gomez S. M., Manikanda B. N., Kumar S., Ramasubramanian T., Zhu C., Jeyaprakash P., Senthil A. and Chandra Babu R. 2010. Molecular mapping and location of QTLs for drought-resistance traits in indica rice (*Oryza sativa* L.) lines adapted to target environments. Acta Physiologiae Plantarum, **32**: 355-364.

Government of Jammu and Kashmir 2022-23. Digest of Statistics. Directorate of Economics and Statistics, Government of Jammu and Kashmir.

Gupta A. K., Solankil. S., Bashyal B. M., Singh Y. and Srivastava K. 2015. bakanae of rice-an emerging disease in Asia. J. Animal Plant Sci., **25**: 1499–1514.

Halim W., Naw A., Razak A. A., Zainudin. and Naim Z. 2015. Susceptibility of Malaysian rice varieties to *Fusarium figuro* and in vitro activity of *Trichoderma harzianum* as biocontrol agent. Malaysian J. Microbiol., **11**: 20-26.

Hugot K., Riviere M. P., Moreilhon C., Dayem M. A., Cozzitorto J. and Arbiol G. 2004. Coordinated regulation of genes for secretion in tobacco at late developmental stages: association with resistance against oomycetes. Plant Physiol., **134**: 858-70.

Hur Y. J., Lee S. B., Kim T. H., Kwon T., Lee J. H., Shin D. J., Park S. K., Hwang U. H., Cho J. H., Yoon Y. N., Yeo U. S., Song Y. C., Kwak D. Y., Nam M. H. and Park D. S. 2015. Mapping of qBK1, a major QTL for bakanae disease resistance in rice. Mol. Breed., **35**: 78-89.

Ichinose M. and Sugita M. 2017. RNA editing and its molecular mechanism in plant organelles. Genes, **8**: 5–19.

Isin E. M. and Guengerich F. P. 2007. Complex reactions catalyzed by cytochrome P450 enzymes. Biochemistry and Biophysics Acta., **1770**(3): 314–329.

ItoS. and Kimura J. 1931. Studies on the 'bakanae' disease of the rice plant. In: Report of Hokkaido National Agriculture Experimental Station., **27**: 1–95.

Ishikawa R., Iwata M., Taniko K., Monden G., Miyazaki N., Orn C., et al. 2017. Detection of quantitative trait loci controlling grain zinc concentration using Australian wild rice, *Oryza meridionalis*, a potential genetic resource for biofortification of rice. PLOS ONE., **12**(10): 187-224.

Jiang Y., Bao L., Jeong S. Y., Kim S. K., Xu C., Li X. and Zhang Q. 2012. XIAO is involved in the control of organ size by contributing to the regulation of signalling and homeostasis of brassinosteroids and cell cycling in rice. Plant J., **70**: 398–408.

Kushwaha A. K., Ellur R. K., Maurya S. K., Krishnan S. G., Bashya B. M., Bhowmick P. K., Vinod, K. K., Bollinedi H., Singh N. K. and Singh A. K. 2023. Fine mapping of qBK1.2, a major QTL

governing resistance to bakanae disease in rice. Front. Plant Sci., **14**: 126-176.

Lee S. B., Hur Y. J., Cho J. H., Kim T. H. Cho C. M., Song Y. C., Song O. C., Seo Y. S., Lee J. K., Kim T. S., Park Y. J., Oh M. L. and Park D. S. 2018. Molecular mapping of qBKI^{WD}, a major QTL for bakanae disease resistance in rice. Rice, **11**: 3-9.

Lee S. B., Kim N., Hur Y. J., Cho S. M., Kim T. H., Lee J. Y., Cho J. H., Lee J. H., Song Y. C., Seo Y. S., Ko J. M. and Park D. S. 2019. Fine mapping of qBK1, a major QTL for bakanae disease resistance in rice. Rice, **12**: 36 45.

Li W., Shao M., Yang J., Zhong W., Okada K., Yamane H., Qian G. and Liu F. 2013. Oscyp71Z2 involves diterpenoid phytoalexin biosynthesis that contributes to bacterial blight resistance in rice. Plant Sci., **207**: 98–107.

Lone Z. A., Bhat Z. A., Najeeb S., Ahanger M. A., Shikari A. B., Parray G. A. and Hussain S. 2016. Screening of rice genotypes against bakanae disease caused by *Fusarium fujikuroi* Nirenberg. Oryza, **53**: 91-97.

Lukasik E. and Takken F. L. 2009. Standing strong, resistance proteins instigators of plant defence. Curr. Opinion Plant Biol., **12**: 427–436.

Matić S., Bagnaresi P., Biselli C., Carneiro G. A., Siciliano I., Valé G., Gullino M. L. and Spadaro D. 2016. Comparative transcriptome profiling of resistant and susceptible rice genotypes in response to the seedborne pathogen *Fusarium fujikuroi*. BMC Genomics, **17**: 608.

Mohr T. J., Mammarella N. D., Hoff T., Woffenden B. J., Jelesko J. G. and McDowell J. M. 2010. The Arabidopsis downy mildew resistance gene RPP8 is induced by pathogens and salicylic acid and is regulated by W box cis elements. Mol. Plant Microbe Inter., **23**: 1303–1315.

Noman A., Aqeel M. and Lou Y. 2019. PRRs and NB-LRRs: From signal perception to activation of plant innate immunity. Int. J. Mol. Sci., **20**(8): 1882.

Park J. H., Halitschke R., Kim H. B., Baldwin I. T., FeldmannK. A. and Feyereisen R. 2002. A knock-out mutation in allene oxide synthase results in male sterility and defective wound signal transduction in Arabidopsis due to a block in jasmonic acid biosynthesis. The Plant J., **31**: 1–12.

Pieterse C. M., Leon-Reyes A., Van der Ent S. and Van Wees S. C. 2009. Networking by small-molecule hormones in plant immunity. Nature Chem. Biol., **5**(5): 308–16.

Pieterse C. M., Van der Does D., Zamioudis C., Leon-Reyes A. and Van Wees S. C. 2012. Hormonal modulation of plant immunity. Annual Review Cell Development Biology., **28**: 489–521.

Sakina A., Mir S., Najeeb S., Zargar S. M., Nehvi F. A., Rather Z. A., Salgotra R. K. and Shikari A. B. 2020. Improved protocol for efficacious in vitro androgenesis and development of doubled haploids in temperate *japonica* rice. PLOS ONE., **15**(11): 241-292.

Volante A., Tondelli A., Aragona M., Valente M. T., Biselli C., Desiderio F., Bagnaresi P., Matic S., Gullino M. L. and Infantino A. 2017. Identififfication of bakanae disease resistance loci in japonica rice through genome wide association study. Rice, **10**: 29-40.

Wu Y., Wang Y., Mi X. F., Shan J. X., Li X. M., Xu J. L. and Lin H. X. 2016. The QTL *GNP1* encodes GA20ox1, which increases grain number and yield by increasing cytokinin activity in rice panicle meristems. PLoS Genet., **12**(10): 63-76.

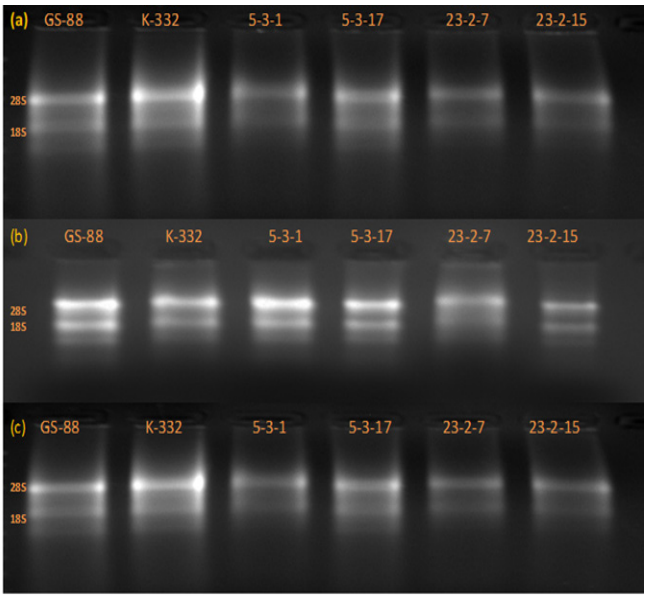
Yadav S., Anuradha G., Kumar R. R., Vemireddy L. R., Sudhakar R., Donempudi K., Venkata D., Jabeen F., Narasimhan Y. K., Marathi B. and Siddiq E. A. 2015. Identification of QTLs and possible candidate genes conferring sheath blight resistance in rice (*Oryza sativa L.*). Springer Plus, **4**: 175.

Yan Q., Cui X., Lin S., Gan S., Xing H. and Dou D. 2016. GmCYP82A3, a soybean cytochrome P450 family gene involved in the jasmonic acid and ethylene signaling pathway, enhances plant resistance to biotic and abiotic stresses. PloS One., **11**(9): 253-65.

Zainudin N. A. I. M., Razak A. A. and Salleh B. 2008. Bakanae disease of rice in Malaysia and Indonesia: Etiology of the causal agent based on morphological, physiological and pathogenicity characteristics. J. Plant Prot. Res., **48**: 475-485.

Supplementary Table S1. Concentration and normalization of cDNA

Sample Name	Time period	Concentration (ng/µL)	cDNA (µL)	NFW (µL)
5-3-11	Control	720.10	5.55	14.45
23-2-7		619.80	6.45	13.55
5-3-19		704.60	5.67	14.33
23-2-15		754.60	5.30	14.70
GS-88		928.20	4.30	15.70
K-332		486.30	8.22	11.78
5-3-11	10dpi	850.00	4.70	15.33
23-2-7		770.00	5.19	14.81
5-3-19		434.10	9.20	10.80
23-2-15		634.50	6.30	13.70
GS-88		861.30	4.64	15.46
K-332		386.70	10.34	9.66
5-3-11	24dpi	301.70	13.25	6.75
23-2-7		329.80	12.12	7.88
5-3-19		323.10	12.38	7.62
23-2-15		308.50	12.96	7.04
GS-88		335.40	11.90	8.10
K-332		283.00	14.13	15.87



Supplementary Fig. 1. Gel image of isolated RNA of parents GS-88, K-332and DH progenies (5-3-11, 5-3-17, 23-2-7, 23-2-15) at control, 10dpi and 24dpi