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Deciphering Genetic Basis of Brown Planthopper Resistance in Oryza rufipogon Through GWAS and Network Analysis

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Abstract

Wild rice, particularly *Oryza rufipogon*, represents a valuable reservoir of genetic diversity for identifying novel resistance determinants. Here, a genome-wide association study (GWAS) was conducted using 214 geographically diverse *O. rufipogon* accessions to dissect the genetic architecture of resistance to the predominant brown planthopper (BPH; Nilaparvata lugens Stål) Biotype 4. Screening through the standard seedbox screening technique (SSST) revealed considerable variation, with 43 accessions exhibiting high resistance. Among these, CR100006, CR100013A and CR100441 showed the highest resistance (damage score of 1.2), followed by five accessions scoring 1.4. GWAS using 66,401 SNPs identified 19 significant marker trait associations (MTAs) across seven chromosomes (1, 2, 3, 4, 5, 6 and 10), revealing a complex genetic basis of BPH resistance. Linkage disequilibrium analysis identified 43 putative candidate genes encoding NBS-LRR proteins, kinases, hormone signalling components and cell wall-associated proteins. Network-based prioritization highlighted 32 potentially important genes, including *OsGA2OX8*, *SKP2A*, *PIP5K1* and MAPK-related kinases, implicating diverse defence and regulatory pathways. Notably, novel resistance-associated genomic regions on chromosomes 5 and 10 were identified, while associations on chromosome 4 further supported its importance in BPH resistance. Beyond genomic discovery, the highly resistant accession CR100441 has also served as a donor for mapping the novel resistance gene *bph42* and its introgression into an elite rice cultivar. The resulting introgression lines, combining BPH resistance with improved grain yield, are undergoing advanced yield evaluation. Collectively, this study provides insights into the genetic architecture of BPH resistance in *O. rufipogon*, identifies promising genomic regions and candidate genes for further validation, and highlights the potential of wild rice diversity for developing durable BPH-resistant cultivars through genomics-assisted breeding.

Introduction

Rice is essential to more than half of the global population, profoundly shaping history, diet and economies globally (Binth 2024). However, pest-related issues have emerged as a prominent obstacle to rice cultivation and productivity in recent times. Notably, rice planthoppers, particularly the brown planthopper (BPH; *Nilaparvata lugens* (Stål) are one of the most widespread insect pests threatening rice production, feeding on phloem sap and causing nutrient loss that severely hinders plant growth and development (Hu et al. 2024). Chemical measures are the most common method for controlling brown plant hopper, which is both expensive for the farmers and hazardous to the environment. Despite this, managing BPH infestation remains a challenge, as it typically strikes late in the paddy crop cycle around the grain-filling stage or approximately a week to ten days before harvest.

Recent studies acknowledge that gram-scale populations of *Nilaparvata lugens* are categorized into four distinct biotypes, with Biotype 4 identified as the most destructive and prevalent in South-East Asia and the Indian subcontinent (Yang et al. 2021). In recent years, outbreaks of BPH have been observed in several parts of India due to the breakdown of resistance genes against Indian BPH biotype 4 (Kumar et al. 2020; Pati et al. 2023). Considering the mode of action of this insect, identifying resistance genes from diverse germplasm represents an effective and eco-friendly approach for developing BPH resistant rice lines. Therefore, identifying a core subset of accessions with the potential to contribute most effectively is necessary for optimizing breeding efforts.

O. rufipogon, a rice progenitor is a compelling choice for rice breeders as a rich source of genetic diversity, given the higher percentage of fertile hybrids resulting from inter-specific crosses with *O. sativa*. It has been utilized not only to enhance qualitative and quantitative traits, but also for the introgression of novel variability (Tanksley and McCouch 1997; Brar and Khush 2018). Through crosses between *O. rufipogon* and *O. sativa*, various populations have been generated, providing crucial resources for tackling multiple biotic and abiotic stresses (Neelam et al. 2018; Guttikonda et al. 2020). Noteworthy achievements include introgression of the novel bacterial blight resistance gene *xa49*(t) from *O. rufipogon* into *O. sativa*, enhancing resistance to *Xanthomonas oryzae* pv. oryzae (Bhatia et al. 2024). Similarly, a recent study reported the mapping and transfer of a novel BPH resistance gene *bph42* from *O. rufipogon* accession CR100441 into an elite rice cultivar for resistance against BPH biotype 4 (Kaur et al. 2022). In addition, RILs derived from *O. rufipogon* have been used to identify three genomic regions associated with resistance to bacterial leaf streak (BLS), with a major QTL, *qBLS4*.1, located on chromosome 4 (Minyi et al. 2023). Kumar and Yer (2023) identified 9 rice lines from 105 *O. rufipogon* accessions showing tolerance to iron deficiency-induced chlorosis under aerobic conditions at the tillering stage. Beyond stress tolerance, numerous QTLs related to yield and grain quality have been successfully mapped using introgression lines and backcross populations derived from *O. rufipogon* (Xie et al. 2006; Cheema et al. 2008; Bhatia et al. 2018; Kaur et al. 2020), underscoring the immense potential of *O. rufipogon* as a cornerstone of rice improvement efforts.

To date, more than 44 genes and 22 quantitative trait loci (QTLs) responsible for BPH resistance have been identified from wild rice and cultivated varieties, most of which are present in clusters on chromosomes 3, 4, 6 and 12 (Du et al. 2020). Most of the known resistance genes have proven ineffective against the rapidly evolving BPH biotypes prevalent in India, highlighting the need for the identification of novel genes or breeding strategies to combat this emerging threat. Nevertheless, the potential of *O. rufipogon* as a resource for this particular stress resistance has been relatively less recognized. Most studies on *O. rufipogon* concern only a few accessions/lines in different biparental crosses as sources of stable resistance, thus limiting the allelic diversity and genetic resolution. Notably, in the realm of rice research, the utilization of Genome-Wide Association Study (GWAS), which employs a vast panel for precise association mapping to enhance the diversity of allelic variants, for identifying BPH resistant genes from wild rice relatives remains scarce.

At Punjab Agricultural University, over 400 *O. rufipogon* accessions derived from various geographic origins have been maintained and screened for diverse phenotypic attributes. In the present study, 214 accessions have been systematically evaluated for BPH resistance against Biotype 4. Subsequently, this germplasm subset has been utilised to perform GWAS to identify significantly associated loci. The LD regions identified via GWAS, and the inherent genes linked with these have been subjected to further scrutiny for their respective functional and regulatory annotations to pinpoint the most promising candidates. Overall, this study aims to predict novel BPH-resistant candidate genes and *O. rufipogon* germplasm resources, which is the first such report to the best of our knowledge.

Materials and methods

O. rufipogon diversity panel

A set of 1110 wild species accessions belonging to different genomes of rice is maintained at the School of Agricultural Biotechnology, Punjab Agricultural University, Ludhiana. These accessions were originally procured from the International Rice Research Institute, Philippines and the Central Rice Research Institute, Cuttack. In the present study, a set of 214 accessions of *O. rufipogon* was investigated for BPH resistance against Biotype 4. The diversity panel from various locations, viz., Australia, Bangladesh, Cambodia, China, India, Indonesia, Laos, Malaysia, Myanmar, Nepal, Papua, Philippines, Sri Lanka, Taiwan, Thailand, and Vietnam was screened for BPH resistance under glass house conditions during the years 2023 and 2024. Two replicates, consisting of 30 seeds of each accession along with resistant check Ptb33 and susceptible check Taichung Native 1 (TN1), were sown in plastic trays filled with well-puddled clayey soil.

Raising and maintaining BPH

At PAU, the insect culture of BPH biotype 4 was maintained by rearing them on 30 days old susceptible rice cultivar Taichung Native 1 (TN1) under controlled greenhouse conditions. The environmental parameters were carefully regulated to ensure optimal insect survival and multiplication, with temperature maintained at 28 ± 2°C, relative humidity at 75% ± 5%, and a photoperiod of 14 hours light followed by 10 hours darkness (Heinrichs et al. 1985). These standardized conditions facilitated uniform growth, reproduction, and maintenance of a stable insect population. Rearing was carried out in rectangular steel cages (0.68 × 0.50 × 0.50 m) enclosed with fine nylon mesh on all sides except the top to ensure proper aeration while preventing insect escape. The cages were placed over earthen pots containing healthy, 30-day-old TN1 plants, which served as a continuous food source for the insects. Regular replacement of plants ensured sustained feeding and population buildup. For resistance screening experiments, actively feeding 2nd – 3rd instar nymphs obtained from this maintained culture were carefully collected and released onto the screening trays, as these developmental stages are most suitable for uniform infestation and reliable phenotypic evaluation (Sarao et al. 2016).

Evaluation of BPH resistance

The diversity panel, along with experimental checks, was evaluated for brown planthopper (BPH) resistance using the standard seedling box screening technique (SSST) (Heinrich et al. 1985). Seeds of each accession were sown in rows in plastic trays (45 × 35 × 10 cm) containing well-puddled soil, with 3.5 cm spacing between rows. The trays were maintained in an insect-proof greenhouse at 30 ± 2°C and 80 ± 5% relative humidity. At the 14-day stage (third-leaf stage), seedlings were infested with 2nd – 3rd instar BPH nymphs @ 8–10 per seedling. The trays were enclosed in iron cages covered with fine nylon mesh. When all seedlings of the susceptible check TN1 had died, damage was scored on a 0–9 scale following the Standard Evaluation System (SES) for rice (IRRI, 2014). Plants with scores of 0–1 showed no or very slight injury; score 3 indicated partial yellowing of the first and second leaves; score 5 reflected pronounced yellowing, stunting, and 10–25% wilting; and scores of 7 and 9 represented severe wilting and complete plant death. Accessions with scores of 0–3.49 were classified as resistant, 3.5–5.49 as moderately resistant, and 5.5–9 as susceptible.

Phenotypic data analysis

Hierarchical clustering was done using factoextra and cluster package in R. The clustering was performed using Ward's minimum variance method. The gap statistic metric was calculated to compare the total intra-cluster variation for different values of k with their expected values for a distribution with no clustering and optimal number of clusters were determined. Statistical significance of inter-cluster differences was determined using Kruskal-Wallis test and post-hoc pairwise comparisons were performed using the pairwise.wilcox.test function in R (R Core Team 2024).

Investigating population structure in association mapping panel

Admixture program was used to investigate the genetic structure in association mapping panel (Alexander et al. 2009). To explore the best fitting model, admixture was run from K1-K10 with cross-validation. The K value that minimized cross-validation error was selected as the most appropriate number of sub-populations. The admixture plot was generated using the barplot function in R (R Core Team 2024).

Genome-wide association mapping and candidate gene prediction

Genome-wide association mapping was performed in an association mapping panel comprising 214 accessions using Genomic Association and Prediction Integrated Tool version 3.0 (GAPIT 3.0) program (Lipka et al. 2012; Wang and Zhang 2019). The genotypic data comprising 66,401 SNPs was retrieved from our group's previous publication. The genomic regions associated with BPH were demarcated by carrying out association analysis employing genotypic data together with pooled phenotypic data. The fixed and Random Model Circulating Unification (FarmCPU) model was used to determine the associations between each SNP and the average damage score of BPH infestation. Principal component analysis (PCA) and admixture based prediction of genetic structure were conducted using prcomp function and admixture program in R and unix, respectively (R Core Team, 2024; Alexander et al. 2009). Principal Components alongwith FaSTLMM algorithm-based kinship computed from SNP data describing the relatedness among accessions of the panel, were iteratively added to the FarmCPU model to avoid false positives. The threshold for determining the significance level of the GWAS study was done by calculating p-value based on the minimum Bayes Factor (mBF).

Linkage disequilibrium decay and Candidate gene prediction

Linkage Disequilibrium (LD) was calculated using popLDdecay program in unix (Zhang et al. 2019). The physical distance where the r^2 value decayed to fifty percent of the highest value was designated to be the LD block. Candidate gene prediction was done by scanning the upstream and downstream genomic regions of significant SNP associations in the LD block. The putative candidate genes were predicted using MSU Rice Genome Annotation Project database (RGAP)v.7 (https://rice.plantbiology.msu.edu/).

Network analysis

Gene network analysis was conducted using KnetMiner (https://knetminer.com/) to explore functional relationships and biological relevance of candidate genes associated with brown planthopper (BPH) resistance in rice. Significant marker-trait associations identified through genome-wide association studies (GWAS) were used as input for network construction. The *O. sativa* genome was selected within KnetMiner, and candidate gene IDs derived from the LD block surrounding significant SNPs were queried in combination with trait-specific keywords “brown planthopper resistance”. Core candidate genes (hub genes) were identified based on their connectivity and functional associations with defense-related pathways. The resulting networks were exported for visualization and further analysis to identify key resistance-related subnetworks.

Results

Variation in damage score is predominantly contributed by genotype

The damage score recorded across the 214 genotypes of *O. rufipogon* displayed a continuous range of variation, indicating a quantitative pattern of response rather than discrete categories (Fig. 1, Supplementary Table 1). Analysis of variance suggested the variation observed in damage score to be attributable to variation in genotypes as evident from significant p-values (Table 1), whereas the effect of years was found to be non-significant. Thus, the mean trait value of both years was utilised for further analysis. Hierarchical clustering based on Ward's method identified a total of 4 clusters as suggested by Gap statistic parameter (Fig. 2a). The average score per cluster was calculated to be 2.028, 5.367, 8.303 and 3.527 with the number of individuals per cluster as 43, 30, 81 and 60, respectively (Fig. 2b). The inter-cluster distance was found to be statistically significant, as evident from the boxplots displayed in Fig. 2c.

Table 1
Analysis of variance (ANOVA) for determining the effect of *O. rufipogon* genotypes on average damage score after BPH infestation

Genotype	Df	Sum Sq	Mean Sq	F value	Pr(> F)
	213	**2775.49**	**13.0305**	**52.6835**	**< 2e-16*** **
Year	1	0.2	0.1978	0.7996	0.3722
Residuals	213	52.68	0.2473		
Significance codes: 0‘***’0.001‘**’0.01‘*’0.05‘.’0.1‘’1					

Genetic structure in association mapping panel revealed four sub-populations

The distribution of SNPs across the genotypic dataset is illustrated in Fig. 3a. Notably, Chromosomes 1 and 4 exhibited the highest SNP density among all chromosomes. Investigation of population structure in association mapping panel led to the identification of four sub-populations consisting of 151 accessions. With the membership criterion of 50 percent, the remaining 63 accessions were deemed admixed (Fig. 3b-3c). Genetic differentiation among four sub-populations was studied by calculating F_{st}. The highest level of genetic differentiation was observed between first and third sub-population. The pairwise F_{st} estimates indicated a moderate level of genetic differentiation among most sub-population pairs, with values ranging from 0.23 to 0.34, whereas a comparatively lower differentiation was observed between sub-populations 2 and 3 (Table 2).

Table 2
Pairwise F_{st} among different sub-populations

Pop1	Pop1	Pop2	Pop3
Pop2	0.287		
Pop3	0.346	0.138	
Pop4	0.23	0.278	0.341

Genome wide association mapping for the identification of BPH resistance loci

To control the confounding effects that may lead to the generation of false positives, kinship and 4 principal components (PCs) were added as covariates in the GWAS model. P-value based on the minimum Bayes Factor was calculated to be 4.545e-4, which was used as a threshold for determining the significance level. The analysis led to the identification of 19 SNPs significantly associated with BPH infestation (Fig. 4), distributed on chromosomes 1, 2, 3, 4, 5, 6 and 10 (Table 3). The most significant marker-trait associations (MTAs), S1_852606 and S4_33682882 were found on chromosomes 1 and 4. The effect of significant associations varied in the range of 0.78–2.04. The MTAs showing maximum effect on BPH resistance were found on chromosomes 3, 4 and 2. Survey of the upstream and downstream LD region adjacent to significant MTAs led to the identification of 43 candidate loci, of which 6 genes belonged to R-gene categories, encoding proteins with kinase domains, F-box, MADS box, NB-ARC/LRR (Table 3). Majority of the remaining loci belonged to plant hormone biosynthesis/metabolism/signalling, cell wall organisation, or developmental pathways, including floral development, suggesting a tentative role in biotic stress resistance.

Table 3
Candidate genes predicted in the proximity of 19 significant MTAs and their GO annotations

S.No.	SNP_Position	Chr	p-value	MAF	Effect	Loci in LD region		GO Annotation	
						MSU-IDs	RAP-IDs	Biological Process	Functional annotation
1	S1_852606	1	5.94E-05	0.44	0.89	LOC_Os01g02550	OS01G0115500	disease resistance	protein kinase domain containing protein, expressed
						LOC_Os01g02560	OS01G0115600	disease resistance	Ser/Thr receptor-like kinase, putative, expressed
						LOC_Os01g02590	Os01g0115800		receptor-like kinase, putative, expressed
2	S1_855912	1	6.07E-05	0.39	0.95	-			-
3	S1_5568286	1	4.48E-04	0.08	1.38	LOC_Os01g10504	OS01G0201700	flower development, auxin metabolic process	OsMADS3 - MADS-box family gene with MIKCc type-box, expressed
4	S1_14759940	1	3.89E-04	0.14	1.06	LOC_Os01g26010	-		expressed protein
						LOC_Os01g65920	OS01G0881900	auxin activated signalling pathway, hydrotropism, root development, auxin transport	F-box/LRR-repeat protein 2, putative, expressed
						LOC_Os01g65950	Os01g0882000 Os01g0882100		Thioesterase family protein, putative, expressed
						LOC_Os01g65970	OS01G0882200	auxin biosynthetic pathway, disease resistance, flavonoid content, amylose content, anthocyanin content, potassium content, heat tolerance	transcription factor HBP-1b, putative, expressed
5	S1_38284718	1	3.59E-04	0.22	1.00	-			-
6	S1_38739278	1	2.87E-04	0.23	0.97	LOC_Os01g66680	OS01G0890600		S-domain receptor-like protein kinase, putative, expressed
						LOC_Os01g66690	OS01G0890900	abcisic acid activated signaling pathway, gravitotropism, transpiration	ZIP4/SPO22, putative, expressed
						LOC_Os01g66700	OS01G0891000		beta-hexosaminidase precursor, putative, expressed
						LOC_Os01g66710	OS01G0891100	cell wall organization, pollen germination	polygalacturonase, putative, expressed
7	S2_19488010	2	3.61E-04	0.12	1.23	LOC_Os02g32814	OS02G0530100	cell wall organization, abcissic acid activated signalling pathway	heavy metal-associated domain containing protein, expressed

S.No.	SNP_Position	Chr	p-value	MAF	Effect	Loci in LD region		GO Annotation	
						MSU-IDs	RAP-IDs	Biological Process	Functional annotation
						LOC_Os02g32840	OS02G0530300		zinc finger A20 and AN1 domain-containing stress-associated protein, putative, expressed
8	S2_27833965	2	1.23E-04	0.22	1.14	-			NB-ARC/LRR disease resistance protein, putative, expressed
9	S3_8130501	3	3.70E-04	0.07	1.57	LOC_Os03g14900	OS03G0254000	plant type hypersensitive response, plant organ morphogenesis	phosphatidylinositol-4-phosphate 5-kinase, putative, expressed
10	S3_8568591	3	1.09E-04	0.05	1.91	LOC_Os03g15550	OS03G0262000	auxin activated signalling pathway, gravitotropism, flower development, auxin transport, disease resistance	wibg, putative, expressed
						LOC_Os03g15560	OS03G0262100	root morphology	STE_MEKK_ste11_MAP3K.12 - STE kinases include homologs to sterile 7, sterile 11 and sterile 20 from yeast, expressed
						LOC_Os03g15570	OS03G0262200	abcisic acid activated signaling pathway	nodulation-signaling pathway 2 protein, putative, expressed
11	S3_8662621	3	1.93E-04	0.20	0.98	LOC_Os03g15680	OS03G0263300	nodulation, della protein	phosphate carrier protein, mitochondrial precursor, putative, expressed
						LOC_Os03g15690	Os03g0263400		splicing factor 3A subunit 2, putative, expressed
						LOC_Os03g15700	OS03G0263500		strictosidine synthase, putative, expressed
						LOC_Os03g15710	OS03G0263600		ZOS4-11 - C2H2 zinc finger protein, expressed
12	S4_28413838	4	2.10E-04	0.06	2.05	LOC_Os04g47860	OS04G0566400	root development	autophagy-related protein, putative, expressed
13	S4_31728064	4	1.63E-04	0.10	1.39	LOC_Os04g53240	OS04G0624000		polyphenol oxidase, putative, expressed
						LOC_Os04g53260	Os04g0624400		polyphenol oxidase protein, putative, expressed
						LOC_Os04g53250	Os04g0624100		pelota, putative, expressed
14	S4_33682882	4	5.22E-05	0.13	1.25	LOC_Os04g56480	OS04G0659900	root development	anthranilate phosphoribosyltransferase, putative, expressed
15	S5_17823427	5	3.01E-04	0.10	1.45	LOC_Os05g30750	OS05G0370600	Auxin biosynthetic process, flower development	esterase, putative, expressed
						LOC_Os05g30760	OS05G0370700		OsFBT10 - F-box and tubby domain containing protein, expressed
16	S5_27903500	5	2.52E-04	0.45	0.98	LOC_Os05g48670	OS05G0560400		transcription factor like protein, putative, expressed
						LOC_Os05g48690	Os05g0560600		gibberellin 2-beta-dioxygenase, putative, expressed

S.No.	SNP_Position	Chr	p-value	MAF	Effect	Loci in LD region		GO Annotation	
						MSU-IDs	RAP-IDs	Biological Process	Functional annotation
						LOC_Os05g48700	OS05G0560900	auxin biosynthetic, giberelin biosynthetic and catabolic process, abcisic acid activated signaling pathway	histone-like transcription factor and archaeal histone, putative, expressed
17	S5_28584052	5	3.73E-04	0.41	0.92	LOC_Os05g49780	OS05G0573500	flower development,	DUF538 domain containing protein, putative, expressed
						LOC_Os05g49790	Os05g0573600		ketol-acid reductoisomerase, chloroplast precursor, putative, expressed
						LOC_Os05g49800	OS05G0573700		armadillo/beta-catenin-like repeat containing protein, expressed
						LOC_Os05g49810	Os05g0573800	developmental gene	chloroplast unusual positioning protein, putative, expressed
						LOC_Os05g49820	OS05G0573900		acanthoscurrin-1 precursor, putative, expressed
18	S6_29873079	6	3.34E-04	0.50	0.78	LOC_Os06g49290	Os06g0706700		glycosyltransferase protein, putative, expressed
						LOC_Os06g49300	OS06G0707000	Plant-type Cell Wall Organization, Plant Epidermis Development, Root Morphogenesis	MATE efflux family protein, putative, expressed
						LOC_Os06g49310	Os06g0707100		possible lysine decarboxylase domain containing protein, expressed
19	S10_18005430	10	3.77E-04	0.20	1.02	LOC_Os10g33900	OS10G0479500	root development, nodulation	mitochondrial import inner membrane translocase subunit Tim16, putative, expressed
						LOC_Os10g33910	OS10G0479600	auxin-activated signalling pathway	transporter-related, putative, expressed
						LOC_Os10g33920	OS10G0479700	disease resistance	Functional annotation

Analysis of candidate genes for regulatory network and biochemical pathways

The genes associated with significant MTAs were further analysed for their regulatory and biochemical networks to pinpoint the candidate genes with putative involvement in BPH resistance (Fig. 5). This analysis yielded the genes displaying strong association with various biological pathways related to BPH resistance, including signal transduction, cell wall biosynthesis, and plant hormone metabolism. Of the 43 genes listed, 32 putative candidates were further used to find literature evidence for their involvement in various mechanisms of BPH resistance. These genes predominantly included those involved in plant hormone signalling (*OsGA2OX8*, *SLAH2*, *PAN*, *PIP5K1*, etc.), cell wall biosynthesis (*XYX1*, *HEXO3*, etc.), various classes of kinases (*MEKK1b* and *RLCK10*/*11*), and other NLRs such as *SLAH1*, *SKP21*, *LML1*, *LOG1*, etc. (Table 4). As many as 22 genes could be categorized as stress-responsive, whereas 9 genes remained uncharacterized. Of these, the strongest associations were displayed by 7 genes involved in hormone signalling, viz. *OsGA2OX8*, *SKP2A*, *PAN*, *PIP5K1*, *SLAH2*, *RAG*, and *ZIP4* based on the relevance score assigned by knetminer. In addition, *LOG1* and *IDD4* also showed evidence for involvement in BPH resistance, even though these were not characterized as conventional R-genes. Further, none of the MAP-kinases displayed a relevance score higher than 5.7. However, an uncategorized gene *NSP2*, involved in nodulation signalling, also displayed substantial relevance in context to BPH resistance.

Table 4

Categorization of 32 candidate genes according to resistance pathways, along with their positions and knetminer scores

Biochemical/Physiological/ Regulatory response to BPH	Gene Name	Gene ID	Chromosome	Location	LD region	knetminer score	Reference
Plant Hormone Signalling							
SA/JA/GA	*OsGA2OX8*	LOC_Os05g48700*	Chr05	27910754–27912924	S5_27903500	**17.70**	Wang et al. 2024
ABA	*SKP2A*	LOC_Os01g65920	Chr01	38275062–38279030	S1_14759940	**50.70**	
	PAN	LOC_Os01g65970	Chr01	38291672–38292452	S1_14759940	**43.57**	
	PIP5K1	LOC_Os03g15550	Chr04	8558993–8562806	S4_28413838	**15.21**	
	SLAH2	LOC_Os02g32814	Chr01	19472233–19486248	S1_5568286	7.95	
Calcium	*ZIP4*	LOC_Os01g66690	Chr01	38736349–38739981	S1_38739278	**14.53**	
	FTIP7	LOC_Os05g30750	Chr05	17812076–17816392	S5_17823427	14.31	
MAPK Cascade							
MAPK	*MEKK1B*	LOC_Os03g15570	Chr03	8573083–8577492	S3_8568591	5.61	Ren et al. 2024
Ser/Thr kinases	*SRK6*	LOC_Os01g66680	Chr01	38732782–38734779	S1_38739278	1.22	Naithani et al. 2021
RLKs	*RLCK10*	LOC_Os01g02550	Chr01	839809–840828	S1_852606	3.67	Vij et al. 2008
	RLCK11	LOC_Os01g02560	Chr01	843530–847399	S1_852606	2.90	Vij et al. 2008
Cell wall biosynthesis and lignocellulose deposition							
	XYXT1	LOC_Os06g49300	Chr06	29872566–29874424	S6_29873079	8.55	
	TXR1	LOC_Os10g33910	Chr10	18006974–18009702	S10_18005430	4.79	Scheible et al. 2003
	HEXO3	LOC_Os01g66700	Chr01	38740059–38745917	S1_38739278	5.74	
Other biotic/abiotic stress response mechanisms							
	LOG1	LOC_Os10g33900	Chr10	18000856–18005796	S10_18005430	**48.30**	Chen et al. 2022
	IDD4	LOC_Os04g47860	Chr04	28408769–28416001	S4_28413838	**21.64**	Sun et al. 2020
	HAP3C	LOC_Os05g49780	Chr05	28572426–28575154	S5_28584052	8.38	
	RGA3	LOC_Os03g14900	Chr03	8122290–8126074	S3_8130501	7.63	Thakur et al. 2024
	LML1	LOC_Os04g56480	Chr04	33665023–33672967	S4_33682882	7.57	Qin et al. 2018
	SAP5	LOC_Os02g32840	Chr02	19488188–19488930	S2_19488010	5.83	Chen et al. 2021
	TLP7	LOC_Os05g48670	Chr05	27892619–27895727	S5_27903500	2.10	Mejia et al. 2024
Uncategorized							

Boldface = 10 highest knet-miner scores

Biochemical/Physiological/ Regulatory response to BPH	Gene Name	Gene ID	Chromosome	Location	LD region	knetminer score	Reference
Plant Hormone Signalling							
	NSP2	LOC_Os03g15680	Chr03	8651497–8653224	S3_8662621	**16.32**	
	RAG	LOC_Os01g10504	Chr01	5559532–5568924	S1_5568286	**14.51**	
	OSPGL7	LOC_Os01g66710	Chr01	38750313–38750768	S1_38739278	12.00	
	LAP3	LOC_Os03g15710	Chr03	28579255–28582615	S3_8662621	7.56	
	OS05G0573700	LOC_Os05g49800	Chr05	8664268–8666146	S5_28584052	7.32	
	OSATG8B	LOC_Os04g53240	Chr04	31716399–31719152	S4_31728064	4.71	
	OS10G0479700	LOC_Os10g33920	Chr10	18011909–18018928	S10_18005430	4.33	
	PYM	LOC_Os03g15560	Chr03	8568445–8571825	S3_8568591	2.40	
	OS05G0573900	LOC_Os05g49820	Chr05	28589103–28592602	S5_28584052	1.91	
	MES10	LOC_Os05g30760	Chr05	17821376–17827429	S5_17823427	0.54	
	OS03G0263500	LOC_Os03g15700	Chr03	8660232–8663021	S3_8662621	0.51	
Boldface = 10 highest knet-miner scores							

Discussion

BPH has emerged as a serious challenge for rice production across the globe. For sustainable rice yields, the identification, mapping, and characterization of BPH resistance genes is necessary to achieve durable resistance against the predominant biotypes. The large-scale screening was undertaken to identify potential genomic regions underlying disease response, as BPH Biotype 4 poses a significant threat to rice cultivation in the region due to its rapid adaptation and widespread distribution. To unravel the complex molecular mechanisms underlying disease resistance in plants, the presence of adequate phenotypic variation within the germplasm under study is essential (Zhou et al. 2024). When such a substantially diverse population is investigated using genome-wide molecular markers, the likelihood of identifying causal genomic regions increases multifold. *Oryza rufipogon*, the wild progenitor of cultivated rice, represents a rich reservoir of beneficial alleles for numerous agronomically important traits, including resistance to biotic and abiotic stresses.

Population Structure and Disease Response in the Association Panel

In the present study, GWAS was carried out using a moderately sized association mapping (AM) panel comprising 214 *O. rufipogon* accessions. For a successful association mapping study, the variation in the panel should capture the entire range of phenotypic variation for the trait under study. This ensures the detection of genomic regions associated with the trait of interest. The AM panel revealed pronounced phenotypic variation in disease response and consisted of 43 highly resistant, 60 moderately resistant, 30 moderately susceptible, and 81 highly susceptible accessions. Hierarchical clustering analysis corresponded well with the continuum of disease responses, from extreme susceptibility to high resistance. Unlike our study and Shi et al. 2021, which relied solely on disease response phenotypes, Zhou et al. (2024) adopted an approach integrating multiple physiological and behavioural indicators to evaluate resistance more comprehensively.

The presence of moderate genetic differentiation among four sub-populations suggested inclusion of PCs as covariate in GWAS model to avoid false positives. Different number of sub-populations have been determined in various panels of *O. rufipogon* varying from three (Huang et al. 2012), four (Zhou et al. 2003), five (Prathepha et al. 2012), and six (Kim et al. 2016) suggestive of differences in the number and representatives in the panel employed. The LD decayed at 10Kb as has been reported by Huang et al. 2012 owing to numerous recombination events over evolutionary time. This rapid LD decay facilitates finer resolution in association mapping compared to domesticated rice populations.

Chromosome 1: A hub for multi-insect-pest resistance genes

The major associated SNPs identified in this study were found on chromosome 1 and 4, indicating a potential genomic region for resistance against BPH. This observation aligns with earlier reports where other BPH resistance genes have been mapped to chromosome 1, including *Bph33(t)*, which confers stable resistance through antixenosis and antibiosis (Naik et al. 2018), *Bph37* linked to improved seedling survival and tolerance (Yang et al. 2019) and

Bph38(t) linked to *LOC_Os01g37260*, which encodes an FBXL-type F-box protein with an LRR domain involved in biotic stress resistance (Balachiranjeevi et al. 2019). Moreover, chromosome 1 is known to host genes and QTLs conferring resistance not only to BPH but also to other significant pests such as the whitebacked planthopper (WBPH) and the rice root-knot nematode (*Meloidogyne graminicola*). Rawat et al. (2018) identified three WBPH resistance QTLs—*qWBPH1*, *qWBPH1-1*, and *qWBPH1-2*—on this chromosome, each with LOD scores between 3.3 and 4.0 and explaining up to 30% of the phenotypic variation. Similarly, Hada et al. (2020) reported several SNPs significantly associated with key phenotypic traits related to *M. graminicola* infestation, such as gall number, egg masses, and multiplication factor per plant, co-localizing with a QTL region on chromosome 1. The accumulation of multiple insect resistance loci on chromosome 1 suggests a possible clustering of resistance gene families or conserved genomic regions that can be exploited in breeding. The co-localization of genes conferring resistance to different insect pests provides opportunities to develop multi-pest resistant cultivars through marker-assisted selection (MAS). Together, these findings not only validate the results of our GWAS but also highlight the broader functional significance of chromosome 1 in mediating resistance to a range of insect pests in rice.

Unexplored Genomic Regions for BPH Resistance

In earlier studies, chromosome 4 has been recognized as a hotspot for BPH resistance genes; however, only a limited number of genes have been found to confer resistance against the dominant BPH biotype 4. Several earlier reported genes located on chromosome 4 such as *bph4* (Babawee), *Bph6* (Swarnalata), *Bph20[t]* (IR71033-121-15), and *Bph27* (Balamwee) have proven ineffective against this biotype, underscoring the need for new resistant sources (Kaur et al. 2024). Thus, the significant SNPs identified on chromosome 4 in this study offer valuable opportunities to uncover novel resistance and guide the development of rice varieties resistant to BPH biotype 4 through targeted breeding efforts. Further, chromosomes 5 and 10 have not been prominently associated with any major QTLs linked to BPH resistance. The absence of previously reported resistance loci on these chromosomes suggests they may represent unexplored regions of the rice genome. In our analysis, 2 LD regions harbouring 8 genes on chromosome 5, and 1 region harbouring 3 genes on chromosome 10 were identified as strong MTAs. Therefore, the associations detected in the present study could provide novel insights and expand the current understanding of the genetic basis of BPH resistance, offering new targets for future breeding programs.

Network-based functional insights into candidate genes

To explore the potential regulatory or physiological relationships among the identified candidate genes, gene interaction networks encompassing these candidates were studied. This network-based approach enabled the prediction of functional linkages, co-expression patterns, and possible shared pathways, providing deeper insights into their coordinated roles in conferring BPH resistance. Based on the previously established BPH-responsive effectors, the defence pathways may be broadly classified as biochemical response, which includes signal transduction leading to activation of hormone signalling and development of secondary metabolites; physiological response, which includes cell wall thickening and callose depositions to prevent pest penetration; and regulatory response, which involves transcription factors and miRNAs to control the expression of crucial resistance genes (Yan et al. 2023). Network analysis illustrated the potential involvement of 33 candidate genes in various defence-related pathways, which was supported by their annotated ontology and/or previously reported functional characterization.

Candidate genes noted in this study were found to be predominantly involved in biochemical defence pathways, i.e., hormone signalling and MAPK signal transduction. Several genes, including *SKP2A, PAN, PIP5K1*, and *SLAH2*, were putatively involved in auxin biosynthesis or signalling, whereas genes involved in GA *(OsGA2OX8)* (Wang et al. 2024) and Calcium signalling were also marked. Since plant hormone pathways constitute a major line of plant defence against pests, investigating the inherent genetic variation in the *O. rufipogon* orthologs of these genes may further clarify the role of these genes in BPH response. Further, signal transduction mediated via different classes of kinases is an indispensable component of pest resistance. In accordance, 4 crucial genes, including MAP-kinase *MEKK1B*, Ser/Thr-kinase *SRK6*, and receptor-like kinases *RLCK10* and *RLCK11* were unearthed in BPH-associated regions. Besides these known genes, 6 other genes annotated as protein kinases appeared in our analyses. Analyzing these genes for their location, identity, function, and sequence variation in *O. rufipogon* might lead to the identification of hitherto unknown sources of resistance. Besides signal transduction cellulose biosynthesis and cell wall-related structural proteins are also crucial players in preventing damage caused by BPH attacks. Hence, candidate genes potentially associated with these traits were also listed, which included *XYXT1*, responsible for xylan-lignin crosslinking (Zhong et al. 2018), and *TXR1*, involved in cellulose biosynthesis. Finally, in addition to these classified candidate genes, about 8 genes implicated in various biotic and biotic stress response pathways were discovered, of which *RESISTANCE GENE ANALOG3* (*RGA3*) has been reported in *Fusarium* (Thakur et al. 2024) and blast (Xu et al. 2014) resistance mechanisms, while a close family member of *IDD4*, *IDD3* is involved in sheath blight resistance (Sun et al. 2020). Furthermore, genes such as *HAP3C*, *SLAH1*, and *OsGA2OX8* have been implicated in various abiotic stresses, such as cold and high salinity. Overall, the list of putative BPH-responsive genes predicted in the candidate regions identified from GWAS include several crucial genes, with a strong association with biotic stress response pathways, which can serve as a solid foundation for future mapping and cloning ventures.

Conclusion

This study provides the first comprehensive GWAS-based identification of genomic regions associated with BPH resistance in a large *O. rufipogon* panel against BPH biotype 4. The discovery of 19 significant MTAs across seven chromosomes, including novel loci on chromosomes 5 and 10, expands the known genetic landscape of BPH resistance. The candidate genes identified within these regions were predominantly involved in hormone signaling, cell wall biosynthesis, and kinase-mediated signal transduction—core pathways in biotic stress responses. Notably, genes such as *OsGA2OX8, SKP2A, RLCK10/11*, and *TXR1* were highlighted through network analysis as potential regulators of BPH resistance. The phenotypic variability and rich allelic diversity captured in the *O. rufipogon* accessions underscore the value of this wild species in resistance breeding. Our findings establish a foundational framework for targeted breeding strategies to combat the evolving threat of BPH in rice cultivation.

Abbreviations

BPH
Brown Planthopper
O. sativa
Oryza sativa
O. rufipogon
Oryza rufipogon
IRRI
International Rice Research Institute
PAU
Punjab Agricultural University
CRRI
Central Rice Research Institute
GWAS
Genome-Wide Association Study
SNP
Single Nucleotide Polymorphism
MTA
Marker-Trait Association
LD
Linkage Disequilibrium
QTL
Quantitative Trait Locus/Loci
R-gene
Resistance Gene
NB-LRR
Nucleotide-Binding Site – Leucine-Rich Repeat
NB-ARC
Nucleotide-Binding Apoptosis and Resistance-Associated domain
MAPK
Mitogen-Activated Protein Kinase
RLK
Receptor-Like Kinase
RLCK
Receptor-Like Cytoplasmic Kinase
MEKK
Mitogen-Activated Protein Kinase Kinase Kinase
MADS-box
MCM1/AGAMOUS/DEFICIENS/SRF transcription factor family
SST
Standard Seedling Box Technique
RIL
Recombinant Inbred Line
ANOVA
Analysis of Variance
PCA
Principal Component Analysis
FarmCPU
Fixed and Random Model Circulating Probability Unification
FaST-LMM
Factored Spectrally Transformed Linear Mixed Model
mBF
Minimum Bayes Factor
GO
Gene Ontology
GAPIT
Genomic Association and Prediction Integrated Tool
RGAP
Rice Genome Annotation Project
MSU

Michigan State University Rice Genome Database
KnetMiner
Knowledge Network Miner for Genes
popLDdecay
Population Linkage Disequilibrium Decay program
ABA
Abscisic Acid
GA
Gibberellin
JA
Jasmonic Acid
SA
Salicylic Acid
TN1
Taichung Native 1 (susceptible rice check variety)
Ptb33
Resistant rice check variety

Declarations

Ethics approval and Consent to participate

This article does not contain any studies with human participants or animals performed by any of the authors. Informed consent was obtained from all individual participants included in the study.

Consent for publication

Informed consent was obtained from all individual participants included in this study.

Competing Interest

The authors declared no conflict of interest.

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Author Contribution

KN- Conceived the idea; PK- Screening of the germplasm and phenotypic data analysis; PM- Performed GWAS, PK, TS- analysed the results; KN, YV, KS- Handling and maintenanceof the germplasm; PS- provided BPH biotype 4 insect culture, PK, PM, TS- Writing and proofreading of the manuscript. All the authors read the manuscript carefully and approved it.

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Data Availability

All the data generated and analysed in this study are available in this article and as supplementary material .

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Figures

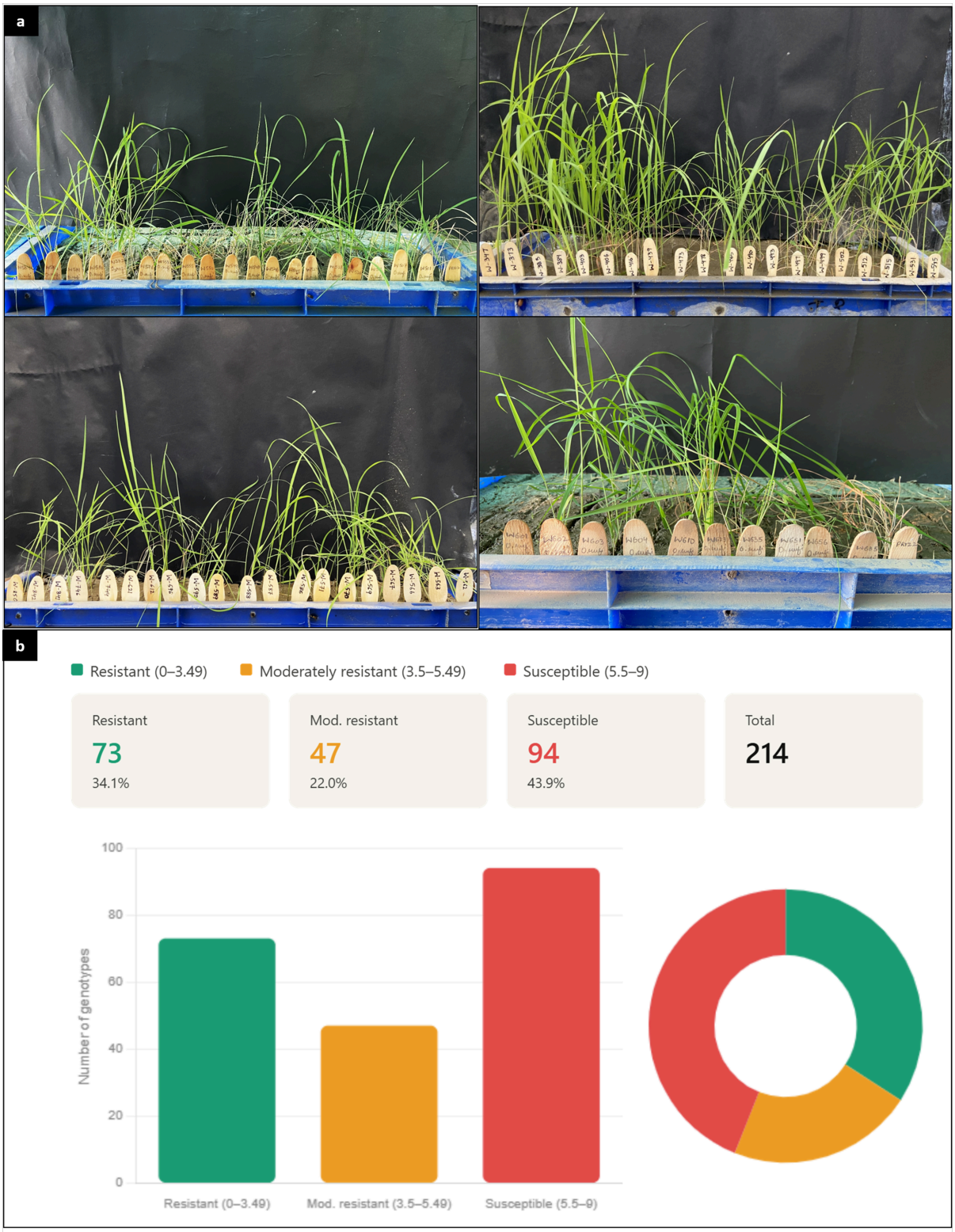

Figure 1

a) Screening of *O. rufipogon* accessions against BPH biotype 4. Red arrows indicate susceptible cultivar PR122, b) Percentage distribution of BPH damage score among *O. rufipogon* accessions.

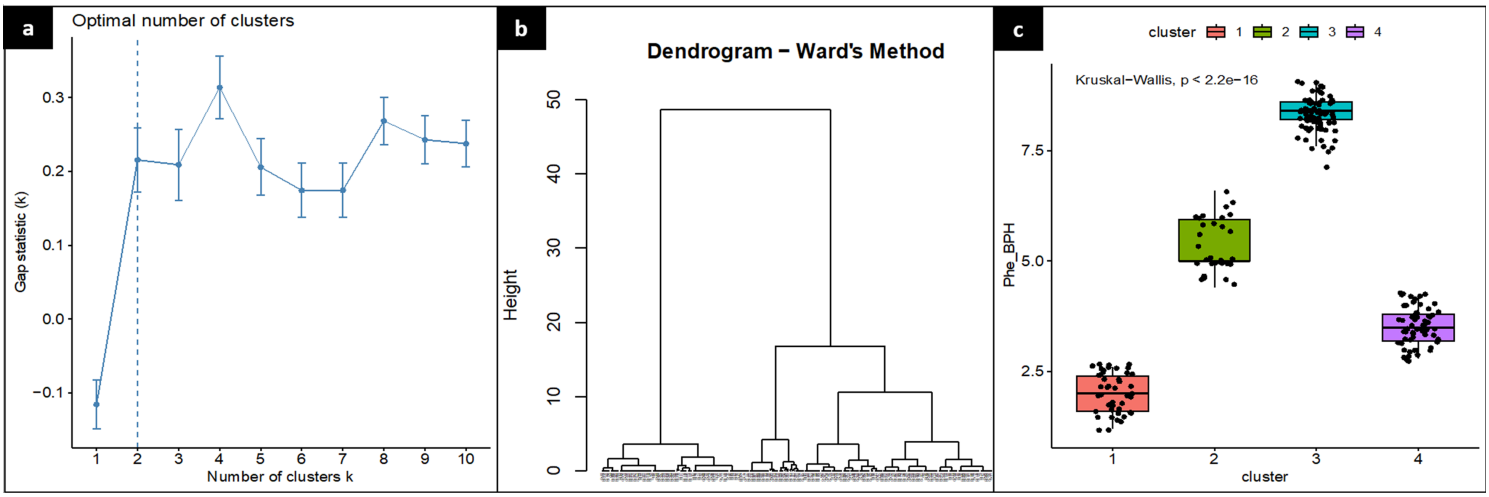

Figure 2

a) Gap statistic metric versus number of clusters for determining optimal number of clusters, b) Hierarchical clustering of genotypes based on Ward's method, c) Box plots representing phenotypic variation among four clusters.

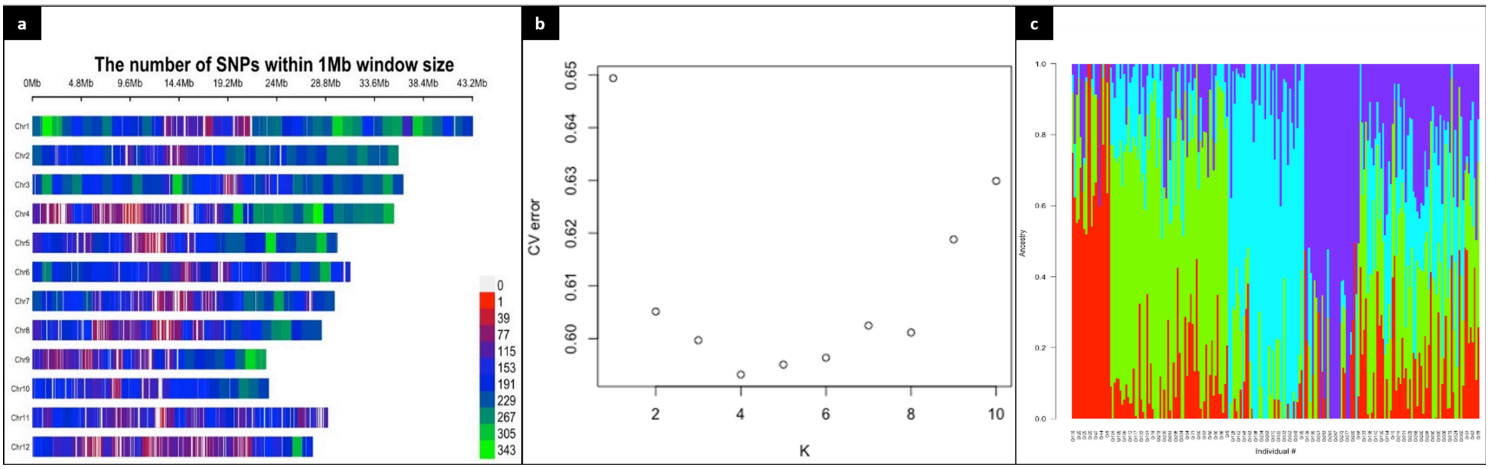

Figure 3

a) Marker density plot showing distribution of SNP markers on all the 12 chromosomes of *O. rufipogon* employed in the current study, b) Admixture-based determining optimal number of clusters, c) Admixture-based bar-plot of accessions in the association mapping panel.

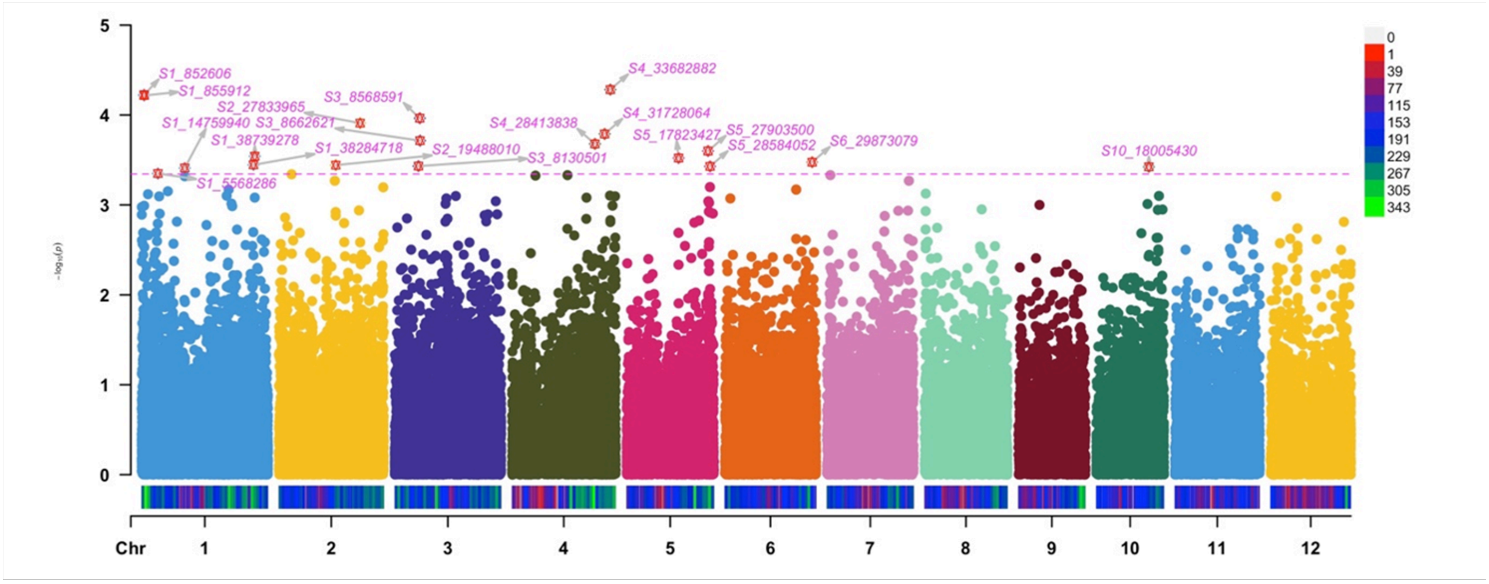

Figure 4

Manhattan plot displaying significant Marker-trait associations (MTAs) for BPH on different chromosomes in *O. rufipogon* panel.

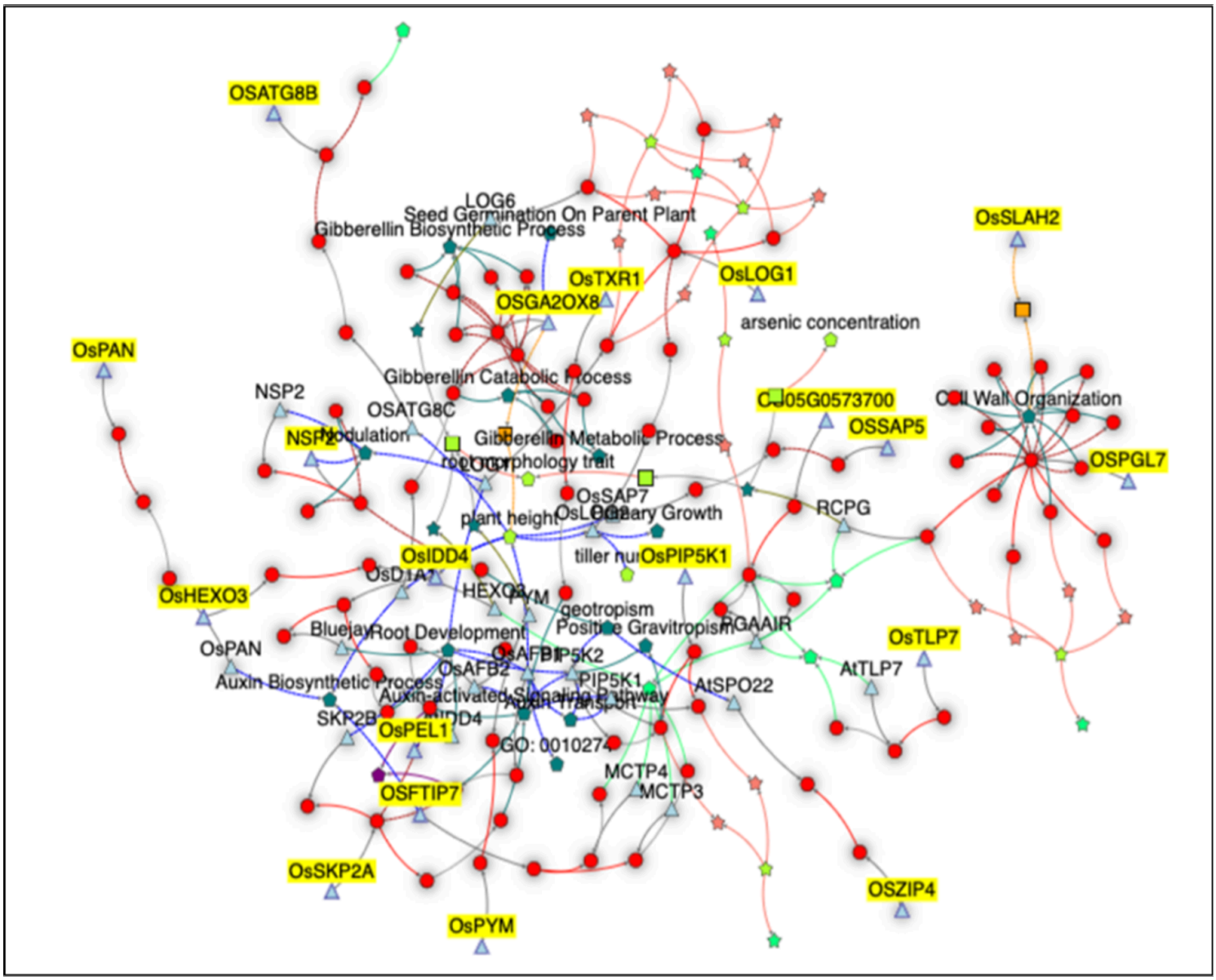

Figure 5

Network analysis representing predicted candidate genes for BPH resistance, and potential pathways and gene regulatory network.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Table11.docx
- Table2.docx
- Table3.docx
- Table4.docx
- SupplementaryTable1.xlsx