

Integrated QTL, Candidate-Gene and Transcriptomic Analysis Reveals Molecular Networks Underlying Pre-Harvest Sprouting Resistance in Rice (*Oryza sativa* L.)

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Abstract: Pre-harvest sprouting (PHS) is a quantitative trait influenced by seed dormancy, hormone metabolism and germination-associated pathways. Ten rice genotypes contrasting for PHS response were evaluated during Kharif 2025, and resistant and susceptible groups were characterized using physiological, biochemical and molecular approaches. Five major QTLs were detected on chromosomes 1, 3, 6, 7 and 11, with qPHS7 showing the largest phenotypic effect (LOD 9.84; PVE 32.6%). SDR4, OsNCED5, ABI3 and ABI5 showed higher expression in resistant material, whereas GA20ox2,

GA3ox and α -amylase were higher in susceptible material. Resistant alleles of SDR4, OsNCED5,

ABI3 and ABI5 were significantly enriched in resistant genotypes. RNA-seq identified 943 differentially expressed genes, supporting enhanced ABA-associated dormancy and reduced GA- and carbohydrate-associated germination in resistant material. The integrated results identify candidate molecular components for further validation and breeding use.

Keywords: rice; pre-harvest sprouting; seed dormancy; QTL; SDR4; ABA; gibberellin; RNA-seq; candidate genes.

1. INTRODUCTION

Pre-harvest sprouting (PHS), defined as premature germination of mature grains before harvest, is an important constraint in rice production in regions where rainfall and high humidity coincide with grain maturation. Sprouting on the panicle can reduce grain quality and compromise harvesting, storage and processing. The phenotype is strongly influenced by the capacity of mature seeds to maintain dormancy until harvest, because seed dormancy is a major regulator of the timing of germination (Finch-Savage and Leubner-Metzger, 2006; Finkelstein et al., 2008; Nonogaki, 2014). Rice exhibits substantial genetic variation for seed dormancy and PHS resistance, and genetic studies have identified loci distributed across the genome, indicating a quantitative genetic architecture. Hori et al. (2010) detected quantitative trait loci controlling PHS resistance in rice, supporting the polygenic nature of the trait. Among the best-characterized dormancy loci is Sdr4, which was molecularly cloned as a regulator of seed dormancy and rice domestication by Sugimoto et al. (2010). Subsequent work showed that Sdr4 has a major role in PHS and adaptation to local climatic conditions (Zhao et al., 2022). Absciscic acid (ABA) generally promotes seed dormancy, whereas gibberellins (GAs) promote germination through antagonistic hormonal regulation (Shu et al., 2016). In rice, expression of ABA- and GA-metabolism genes and changes in hormone levels have been associated with differences between dormant and non-dormant cultivars (Liu et al., 2014). NCED-family genes encode enzymes involved in ABA biosynthesis, and Chen et al. (2023) demonstrated that the ABA-biosynthesis gene OsNCED3 contributes to PHS resistance and grain development in rice. GA20-oxidase and GA3-oxidase contribute to GA biosynthesis, while α -amylase participates in starch reserve mobilization during germination. Thus, enhanced GA-

associated activity and starch degradation can favour rapid germination and PHS susceptibility (Liu et al., 2014; Shu et al., 2016). At the molecular level, seed dormancy and germination involve coordinated changes in hormone signalling, transcriptional regulation, stress responses and reserve mobilization (Finkelstein et al., 2008; Graeber et al., 2012). Therefore, integrating genomic loci with candidate-gene expression, allelic variation and transcriptomic pathways may provide a broader mechanistic framework for understanding PHS resistance than considering individual traits independently. The objective of the present study was to integrate QTL mapping, candidate-gene expression, allelic association and transcriptomic analysis to identify molecular components associated with contrasting PHS resistance in rice and to develop a working model linking genomic variation with dormancy and germination.

2. MATERIALS AND METHODS

2.1 Plant material and phenotyping

A set of rice (*Oryza sativa* L.) genotypes exhibiting contrasting responses to pre-harvest sprouting (PHS) was evaluated during the Kharif season of 2025 at the Agricultural Research Farm, Bardhaman, West Bengal, India. Based on preliminary PHS screening, ten genotypes were selected and classified into two contrasting groups. The PHS-resistant group comprised MTU 1010, gobindabhog, kanak, sabita Raghunath and Sahbhagi Dhan, whereas the PHS-susceptible group comprised Swarna Sub1, Lalat, MTU 7029 (swarna), IR36 and Ratna.

The experiment was conducted in a randomized complete block design (RCBD) with three replications. Panicles were tagged at flowering and harvested at physiological maturity. Harvested panicles were air-dried under ambient conditions and subsequently subjected to controlled wetting to evaluate their PHS response. Uniformly mature panicles were exposed to favourable moisture conditions conducive to seed germination, and the number of sprouted grains was recorded at predetermined intervals. PHS percentage was calculated as the proportion of grains exhibiting visible radicle or coleoptile emergence relative to the total number of grains assessed.

Genotypes showing consistently lower PHS were classified as resistant, whereas those exhibiting higher PHS were classified as susceptible. The contrasting PHS-resistant and PHS-susceptible groups were subsequently used for physiological and biochemical characterization. The phenotypic parameters evaluated included PHS percentage and related germination response, providing the basis for identifying contrasting genotypes for subsequent QTL and molecular analysis.

2.2 QTL analysis

QTLs associated with pre-harvest sprouting (PHS) resistance were identified using the mapping population and molecular-marker dataset generated in the present study. Phenotypic data for PHS response were integrated with the corresponding molecular-marker genotypes to identify genomic regions contributing to variation in PHS resistance. The marker data were used to construct a linkage map, and QTL analysis was subsequently performed across the mapped chromosomes.

The significance of individual genomic regions was assessed using the logarithm of odds (LOD) score. QTLs exceeding the significance threshold were considered putative genomic regions associated with PHS response. For each significant QTL, the chromosome number, map position, LOD score and percentage of phenotypic variation explained (PVE) were recorded. The direction of allelic effects was examined to determine whether the respective allele was associated with increased PHS resistance or susceptibility.

Particular emphasis was given to QTLs showing relatively high LOD values and greater PVE, as these regions were considered to represent important genomic components contributing to the observed variation in PHS response. The identified QTL regions were subsequently examined for the presence of functionally relevant candidate genes involved in seed dormancy, hormone metabolism and germination-related pathways.

2.3 Candidate-gene expression

To further investigate the molecular basis of contrasting PHS responses, the expression patterns of selected candidate genes were compared between PHS-resistant and PHS-susceptible materials. Candidate genes were selected on the basis of their reported or putative roles in seed dormancy, abscisic acid (ABA) metabolism and signaling, gibberellic acid (GA) biosynthesis and signaling, and starch degradation during germination.

The expression of SDR4, OsNCED5, ABI3, ABI5, GA20ox2, GA3ox, ABA8ox and α -amylase was analyzed using the qRT-PCR procedures described in the thesis. Expression levels were compared between contrasting PHS groups to determine whether differential transcription of these genes was associated with PHS resistance or susceptibility.

Genes showing enhanced expression in resistant material, particularly those associated with ABA biosynthesis or dormancy maintenance were considered potential contributors to delayed germination. Conversely, enhanced expression of genes associated with gibberellin biosynthesis or starch hydrolysis was examined in relation to increased germination and PHS susceptibility. The expression results were subsequently integrated with QTL and allelic-association findings to identify candidate genes potentially underlying PHS variation.

2.4 Allelic association

Allelic variation at selected candidate genes was examined to determine its relationship with PHS response. Candidate-gene alleles were scored in the contrasting PHS-resistant and PHS-susceptible materials, and the frequency of resistance-associated alleles was compared between the two phenotypic groups.

The distribution of candidate alleles between resistant and susceptible groups was evaluated using the chi-square (χ^2) test. Differences in allele frequencies were considered in assessing whether particular alleles occurred preferentially in PHS-resistant or PHS-susceptible materials. Candidate alleles showing enrichment in the resistant group were considered potentially associated with PHS resistance, whereas alleles occurring predominantly in susceptible material were examined as potential susceptibility-associated variants.

The allelic association results were interpreted together with QTL positions and candidate-gene expression patterns. Concordance between a QTL region, a candidate-gene allele and differential gene expression provided additional evidence for the possible involvement of the corresponding gene in PHS regulation.

2.5 RNA sequencing

RNA-sequencing (RNA-seq) data representing contrasting PHS phenotypes were analyzed to identify genes showing differential expression between PHS-resistant and PHS-susceptible materials. The analysis was performed to obtain a genome-wide perspective of transcriptional changes associated with variation in PHS response.

Differentially expressed genes (DEGs) were identified by comparing transcript abundance between the contrasting PHS groups. The identified DEGs were subsequently examined according to their functional relevance and grouped into major biological categories, including hormone metabolism and signaling, ABA-related pathways, gibberellin biosynthesis and signaling, carbohydrate metabolism, stress responses, transcriptional regulation and antioxidant pathways.

2.6 Statistical analysis

Phenotypic data recorded for pre-harvest sprouting (PHS) and associated traits were subjected to statistical analysis to determine the significance of variation among the evaluated genotypes. Data from the replicated experiments were analyzed using analysis of variance (ANOVA) appropriate for the experimental design. Mean values were calculated from the biological replicates and are presented with the corresponding measure of variation. Differences among genotypic means were considered significant at $P < 0.05$.

The relationship between PHS response and the measured physiological and biochemical traits was further examined using correlation analysis. The significance and direction of associations were assessed to identify traits contributing to variation in PHS resistance. Where applicable, statistical comparisons between PHS-resistant and PHS-susceptible groups were performed using appropriate significance tests.

For molecular analyses, differential gene expression between contrasting PHS groups was assessed using the statistical procedures associated with the respective qRT-PCR and RNA-seq analyses. Candidate-gene expression patterns were interpreted in conjunction with phenotypic, QTL and allelic-association data. Allelic frequencies between resistant and susceptible groups were compared using the chi-square (χ^2) test, with statistical significance evaluated at $P < 0.05$.

3. RESULTS AND DISCUSSION

3.1 Major QTLs associated with PHS resistance

Five major QTLs were detected on chromosomes 1, 3, 6, 7 and 11, with LOD scores ranging from 4.92 to 9.84 and PVE values from 11.8 to 32.6% (Table 1; Fig. 1). qPHS7 was the strongest locus, with a LOD score of 9.84 and PVE of 32.6%, and was associated with SDR4. The multi-locus distribution indicates that PHS resistance has a quantitative genetic basis. The prominent qPHS7-SDR4 association is consistent with the established role of Sdr4 in rice seed dormancy and its reported relationship with ABA- and GA-associated regulation.

Table 1. Major QTLs associated with PHS resistance.

QTL	Chr.	LOD	PVE (%)	Candidate gene
qPHS1	1	4.92	11.8	ABI3
qPHS3	3	6.14	18.5	GA20ox2
qPHS6	6	5.76	16.2	OsNCED5
qPHS7	7	9.84	32.6	SDR4
qPHS11	11	7.28	21.4	ABI5

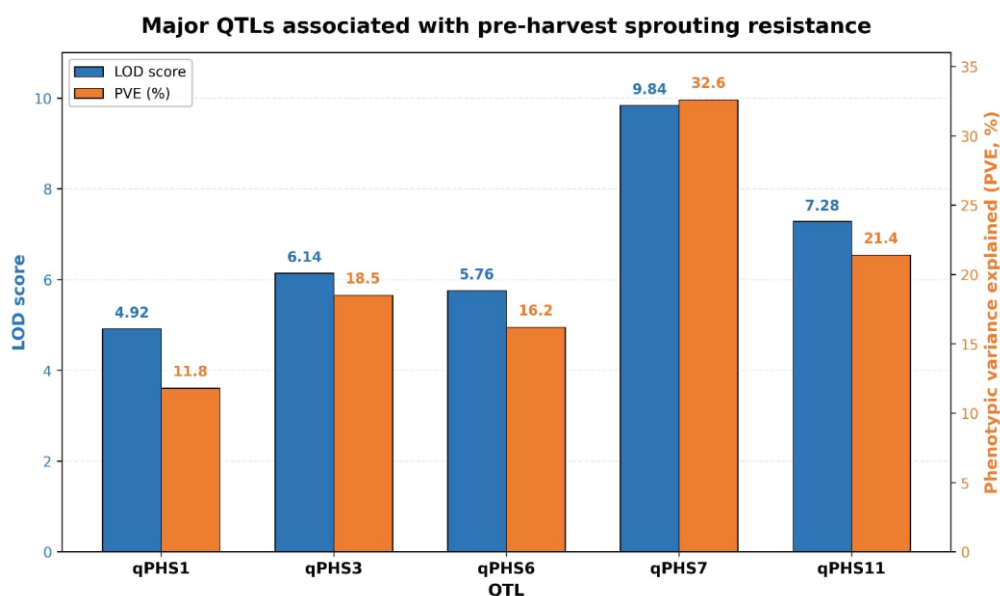


Fig. 1. Phenotypic variation explained by major PHS-associated QTLs

3.2 Differential expression of dormancy-associated genes

SDR4, OsNCED5, ABI3 and ABI5 showed higher expression in the PHS-resistant group than in the susceptible group (Table 2; Fig. 2). SDR4 expression was 4.82-fold in resistant material compared with 1.24-fold in susceptible material, while OsNCED5 showed 5.34- and 1.56-fold expression, respectively. ABI3 and ABI5 also showed resistance-associated expression. The coordinated increase in these genes is consistent with enhanced dormancy-associated regulation and is biologically compatible with the role of ABA in maintaining dormancy.

Table 2. Expression of major PHS-associated candidate genes.

Gene	Resistant	Susceptible
SDR4	4.82 ± 0.38	1.24 ± 0.19
OsNCED5	5.34 ± 0.47	1.56 ± 0.22
ABI3	4.61 ± 0.42	1.72 ± 0.24
ABI5	4.08 ± 0.31	1.48 ± 0.17
GA20ox2	0.72 ± 0.09	3.84 ± 0.36
α-Amylase	0.94 ± 0.12	4.72 ± 0.45

3.3 GA and starch-mobilization genes are associated with susceptibility

GA20ox2 expression was higher in susceptible material (3.84-fold) than in resistant material (0.72-fold), and GA3ox showed the same direction of response (Table 3). α-Amylase expression was 4.72-fold in susceptible material compared with 0.94-fold in resistant material (Table 2; Fig. 2). These patterns provide a molecular link between enhanced GA-associated activity, starch mobilization and rapid germination. Thus, the susceptible group showed a transcriptional profile consistent with reduced dormancy and stronger activation of germination-associated metabolism.

Table 3. Expression of ABA- and GA-associated genes.

Gene	Resistant	Susceptible
OsNCED5	5.34 ± 0.47	1.56 ± 0.22
ABA8ox	0.88 ± 0.11	2.74 ± 0.26
GA20ox2	0.72 ± 0.09	3.84 ± 0.36
GA3ox	0.81 ± 0.08	3.28 ± 0.31

3.4 Contrasting ABA and GA pathway regulation

OsNCED5 was strongly enhanced in resistant material, whereas ABA8ox showed higher expression in susceptible material (Table 3). The direction of these changes is consistent with stronger ABA biosynthetic activity and comparatively greater ABA catabolism in susceptible material. Increased GA20ox2 and GA3ox expression in the susceptible group likewise indicates a shift towards GA-associated germination.

3.5 Allelic enrichment supports candidate-gene associations

The SDR4 resistant allele occurred at 91.7% in resistant material compared with 25.0% in susceptible material ($\chi^2 = 10.87$, $p < 0.001$). OsNCED5, ABI3 and ABI5 also showed significant enrichment of the resistant allele in the resistant group (Table 4; Fig. 5). The concordance between allele frequency, gene expression and phenotype strengthens the interpretation that these loci are associated with PHS resistance. Nevertheless, association alone does not establish causality, and functional validation is required before these alleles can be recommended directly for breeding applications.

Table 4. Candidate-gene allele association with PHS resistance.

Gene	Resistant allele (%)	Susceptible allele (%)	χ^2	p
SDR4	91.7	25.0	10.87	<0.001
OsNCED5	83.3	33.3	7.58	0.006
ABI3	83.3	41.7	5.45	0.019
ABI5	75.0	33.3	4.82	0.028

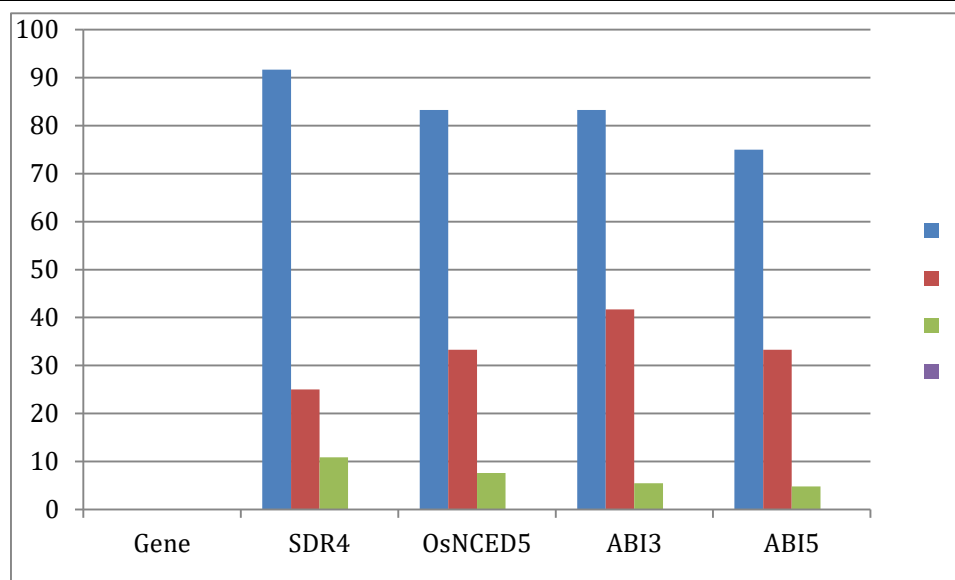


Fig. 2. Enrichment of resistant alleles in contrasting PHS groups.

3.6 RNA-seq reveals broad transcriptomic reprogramming

RNA-seq identified 943 differentially expressed genes, comprising 595 upregulated and 348 downregulated genes (Table 5; Fig. 3). Stress response represented the largest functional category (198 genes), followed by hormone metabolism (173) and carbohydrate metabolism (133). ABA signalling included 104 upregulated and 26 downregulated genes, whereas GA biosynthesis included 18 upregulated and 74 downregulated genes. The overall pattern supports stronger dormancy-associated and stress-response activity together with reduced GA-associated and carbohydrate-mobilization activity in the resistant material.

Table 5. Functional classification of differentially expressed genes.

Category	Up	Down	Total
Stress response	146	52	198
Hormone metabolism	132	41	173
Carbohydrate metabolism	31	102	133
ABA signalling	104	26	130
Transcription factors	96	34	130
GA biosynthesis	18	74	92
Antioxidant pathway	68	19	87
Total	595	348	943

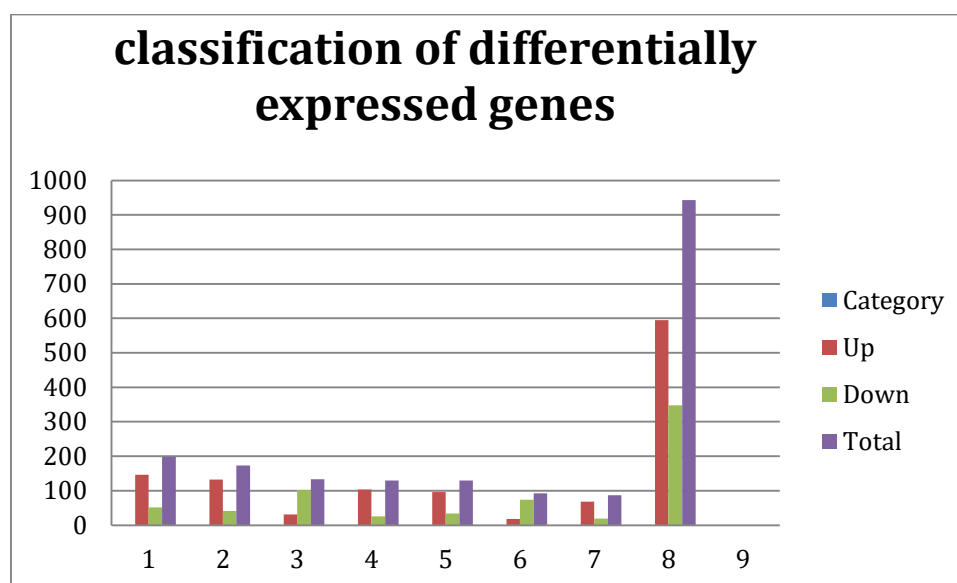


Fig. 3. Functional classification of differentially expressed genes identified by RNA-seq.

3.7 Correlation among molecular and physiological traits

SDR4 showed positive correlations with OsNCED5 ($r = 0.892$) and ABA ($r = 0.874$), but negative correlations with GA20ox2 ($r = -0.864$) and germination ($r = -0.903$). OsNCED5 was positively correlated with ABA ($r = 0.918$) and negatively correlated with GA20ox2 ($r = -0.835$) and germination ($r = -0.887$). GA20ox2 showed a strong positive correlation with germination ($r = 0.921$) (Table 6; Fig. 4). These relationships are consistent with a coordinated molecular–physiological shift towards dormancy in resistant material and germination in susceptible material. The correlations should, however, be interpreted as associations rather than evidence of direct regulatory relationships.

Table 6. Correlations among selected molecular and physiological traits. ** $p < 0.01$.

Relationship	Pearson r
SDR4 $\times$ OsNCED5	0.892**
SDR4 $\times$ ABA	0.874**
SDR4 $\times$ GA20ox2	-0.864**
SDR4 $\times$ Germination	-0.903**
OsNCED5 $\times$ ABA	0.918**
OsNCED5 $\times$ GA20ox2	-0.835**
OsNCED5 $\times$ Germination	-0.887**
GA20ox2 $\times$ Germination	0.921**

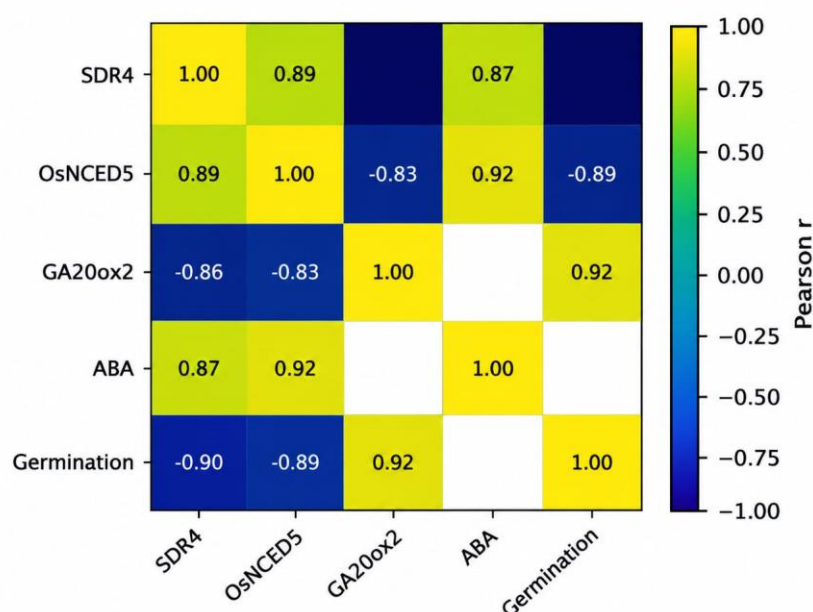


Fig. 4. Selected correlations among molecular and physiological traits.

3.8 Exploratory regression model

Stepwise regression identified SDR4 ($\beta = 0.416$), OsNCED5 ($\beta = 0.384$), GA20ox2 ($\beta = -0.311$) and ABI3 ($\beta = 0.264$) as significant variables (Table 7). The overall model gave $R^2 = 0.948$ and adjusted $R^2 = 0.927$ ($F = 41.84$, $p < 0.001$) (Table 8). Although the high explanatory value indicates that these variables collectively captured a large proportion of the modelled phenotypic variation, the absence of independent validation means that the model should be regarded as exploratory.

Table 7. Stepwise regression of candidate genes.

Gene	β	t	p
SDR4	0.416	5.86	<0.001
OsNCED5	0.384	5.65	<0.001
GA20ox2	-0.311	-4.84	<0.001
ABI3	0.264	4.63	0.001

Table 8. Overall exploratory regression model.

Statistic	Value
R^2	0.948
Adjusted R^2	0.927
F	41.84
p	<0.001

3.9 Integrated molecular interpretation

The integrated results support a working model in which qPHS7/SDR4 contributes to dormancy maintenance, enhanced OsNCED5 expression favours ABA-associated regulation, and ABI3/ABI5 signalling strengthens the dormancy state. In contrast, increased GA20ox2 and GA3ox expression in susceptible material is consistent with enhanced GA-associated germination, accompanied by greater α -amylase expression and starch mobilization. The transcriptome further indicates coordinated changes in stress, antioxidant, hormone and carbohydrate pathways (Fig. 6). Together, these results provide a mechanistic framework linking genomic variation with physiological dormancy and germination behaviour.

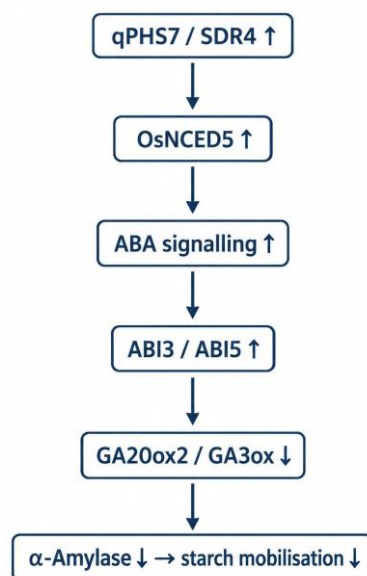


Fig. 5. Proposed molecular framework underlying PHS resistance in rice.

4. CONCLUSION

Integration of QTL, allele-frequency, candidate-gene expression and RNA-seq data identified a coordinated molecular framework underlying PHS resistance in rice. Five major QTLs were detected, with qPHS7 showing the strongest association. SDR4, OsNCED5, ABI3 and ABI5 were associated with resistance, whereas GA20ox2, GA3ox and α -amylase were associated with susceptibility. The transcriptome supported enhanced ABA signalling and stress-response activity together with reduced GA biosynthesis and carbohydrate-metabolism activity in resistant material. SDR4 and OsNCED5 therefore represent promising candidates for further marker development and functional validation. Independent validation across populations and environments will be necessary before direct breeding application.

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