Genetic Dissection of Seed Storability and Validation of Candidate Gene Associated with Antioxidant Capability in Rice (*Oryza sativa* L.)

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Supplementary Figures and Tables

![Heatmap of Pearson's correlation coefficients among seed storability parameters](image)

**Figure S1.** Correlation among germination behaviors and antioxidant capability. Heatmap of Pearson’s correlation coefficients among seed storability parameters (means in triangle) in the BRILs. G1, G2, G4, and G6 represents germination rates after 1-, 2-, 4-, 6-month storage, respectively. P50 is the time required for a reduction of viability to 50% under natural storage. GH indicates germination rate under hydrogen peroxide. Asterisks indicate significant correlations using a two-tailed t-test (*p* < 0.01).
Figure S2. Effect of the peak bin harboring qSS1. (a) Genotypes of five selected BRILs and the parents (NIP and 9311). Black rectangle represents the homozygous NIP genotype, and white rectangle shows the homozygous 9311. (b) G4 ± SE, means of germination rate at G4 with three replicates. Double asterisks in the left panel indicate significant difference compared with 9311 at $p < 0.01$. (c) Three annotation genes in the peak bin for qSS1.
Figure S3. QTL analysis for germination rate in the BRILs under hydrogen peroxide and the effect of the peak bin harboring \( q_{SS3.1} \). (a) Frequency distribution of germination rate of the G1 seeds under hydrogen peroxide. Arrows indicate the means of parental lines NIP and 9311. (b) Manhattan plot of QTL analysis for the germination rate under hydrogen peroxide. (c) Graphical genotypes of five BRILs and the parents. Black rectangle represents the homozygous NIP, and white rectangle shows the homozygous 9311. (d) Means of seed germination rate of five BRILs and parents at G4 with three replicates. Double asterisks indicate significant difference compared with 9311 at \( p < 0.01 \). (e) Candidate genes for \( q_{SS3.1} \) in the peak bin in Table 1.
Figure S4. Comparison of sequence variation and expression of OsFAH2 between NIP and 9311. (a) Expression level in different tissues of NIP. 1, Young leaf; 2, Shoot; 3, Pre-emergence inflorescence; 4, Postemergence inflorescence; 5, pistil; 6, anther; 7, Seed at 5 days after pollination (DAP); 8, Seed at 10 DAP; 9, Embryo at 25 DAP, 10, Endosperm at 25 DAP. (b) Relative expression of OsFAH2 in NIL (9) and NIL(N). Error bar represents mean ± SE with three replicates. Double asterisks indicate significant difference at $p < 0.01$. (c) Schematic gene model showing a nonsynonymous mutation occurred in the coding region of OsFAH2 in NIP and 9311.

Table S1. Distribution of Bins in chromosomes in the BRILs.

| Chromosome | Bin Number | Average Distance between Adjacent Bin (cM) | Bin Size (kb) |
|------------|------------|-------------------------------------------|--------------|
|            |            | Mean | Min | Max |
| 1          | 423        | 0.48 | 102.04 | 3.79 | 2,556.59 |
| 2          | 363        | 0.46 | 98.16 | 1.71 | 2,498.17 |
| 3          | 289        | 0.61 | 125.43 | 6.53 | 3,881.13 |
| 4          | 284        | 0.49 | 124.88 | 5.50 | 5,862.09 |
| 5          | 277        | 0.47 | 106.91 | 4.31 | 3,070.50 |
| 6          | 125        | 0.72 | 249.68 | 11.95 | 5,098.52 |
| 7          | 210        | 0.54 | 140.45 | 3.89 | 1,973.62 |
| 8          | 154        | 0.60 | 183.89 | 2.59 | 7,340.89 |
| 9          | 182        | 0.50 | 125.27 | 4.22 | 2,123.23 |
| 10         | 149        | 0.45 | 155.68 | 7.33 | 4,079.71 |
| 11         | 228        | 0.53 | 126.71 | 5.22 | 2,902.89 |
| 12         | 180        | 0.51 | 152.69 | 6.41 | 2,204.44 |
| Total      | 2,864      | 0.53 | 129.67 | 1.71 | 7,340.89 |
Table S2. Candidate genes in the peak bin harboring the QTL of interest.

| QTL   | Locus Name       | Annotation Genes                                                                 |
|-------|------------------|--------------------------------------------------------------------------------|
| qSS1  | LOC_Os01g55730   | AGAP003732-PA                                                                    |
|       | LOC_Os01g55740   | OsRhmbd4 - Putative Rhomboid homologue                                           |
|       | LOC_Os01g55750   | TCP family transcription factor                                                  |
| qSS3.1| LOC_Os03g01750   | Dual specificity protein phosphatase                                             |
|       | LOC_Os03g01760   | Exostosin family domain containing protein                                      |
|       | LOC_Os03g01770   | Rhodanese                                                                        |
|       | LOC_Os03g01780   | Transposon protein                                                               |
|       | LOC_Os03g01790   | Zinc finger, C3HC4 type domain containing protein                                |
|       | LOC_Os03g01800   | Glycosyl hydrolases family 16                                                   |
|       | LOC_Os03g01810   | SNF7 domain containing protein                                                  |
|       | LOC_Os03g01820   | Fatty acid hydroxylase                                                          |

Table S3. Primers used in this study.

| Primer       | Sequence (5'-3')                                  | Purpose      |
|--------------|---------------------------------------------------|--------------|
| OsFAH2-pc1301s| Forward: atgatgatgataaaaggtaccatgcctccgtacgcgacggcggcggg  |
|              | Reverse: ctagaggatcggctgaccttgtttccctgtgacgaatcc    | OX vector    |
| Hn           | Forward: acggttgtgtctcatcagctggttgc                | Positive test|
|              | Reverse: ttcggaagttgtgacatggggag                 |
| Ubq          | Forward: aaccagctgaggcccaaga                       | qRT PCR      |
|              | Reverse: acgattgatatttaaccagtccatga              |
| OsFAH2       | Forward: catctacgggactgacagaggc                   | qRT PCR      |
|              | Reverse: ctgtgtcccttgtgacgaatcc                  |
| ID0115       | Forward: tgttgttctcctggtatctg                     | Mapping      |
|              | Reverse: acaaaaacag1tatgcac                 |
| ID0117       | Forward: gtctatggaaattgtgaacaa                    |              |
|              | Reverse: acaaaatgggtcacaagg                     |
| ID0118       | Forward: tggagtgaagcctttgctc                     |              |
|              | Reverse: ttcacaatacgtcagttc                      |
| ID     | Forward              | Reverse              |
|--------|----------------------|----------------------|
| ID01C101 | tttgtgcgaaaactttttat | gaccacatgaatatgcttt |
| ID01C104 | ggagagcaagacgagtagtt | acgaggtactacgacttgatg |
| RM5914  | tgtgataaggatgcaattgagc | caagatetccacatctgacag |
| RM11698 | atcacgctcgaagctagaacc | aaccgtatattgagggagcaagc |
| RM11716 | ccctcatcteggcccaacagc | gaggaacctccctgatcg |
| RM11743 | aaggtcaaggaacagggacctgg | agccacgaatccactttcagc |