**Research Paper**

**Improving preharvest sprouting resistance in durum wheat with bread wheat genes**

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Preharvest sprouting (PHS) of durum wheat (*Triticum turgidum* ssp. *durum* (Desf.) Husn.) is an important problem in Japan, where the rainy season overlaps with the harvest season. Since there are few PHS-resistant genetic resources in durum wheat, we introduced an *R*-gene for red seeds, the *MFT* gene, and the *QPhs-5AL* QTL, all of which are associated with PHS resistance, into durum wheat from a PHS-resistant bread wheat (*T. aestivum* L.) cultivar, ‘Zenkoujikomugi’ (Zen), by backcross breeding. Developed near isogenic lines (NILs) with red seeds had a lower percentage germination (PG) and germination index (GI) than the recurrent parent, and seed color had the greatest effect. A NIL combining all three sequences had the lowest GI and PG, with a similar GI to that of ‘Shiroganekomugi’ bread wheat. Among NILs with white seeds, a NIL combining *MFT* and *QPhs-5AL* had the lowest GI and PG. As the combination of all three sequences from Zen conferred PHS resistance on durum wheat, PHS-resistant genetic resources in bread wheat can be used in breeding durum wheat.

**Key Words:** durum wheat, preharvest sprouting, bread wheat, seed color, *MFT*, *QPhs-5AL*.

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**Introduction**

Pasta consumption has increased in Japan since the late 1980s to about 270,000 t per year (Japan Customs Yokohama 2012). Half of the pasta is imported, mainly from Italy, and the other half is made in Japan from durum wheat (*Triticum turgidum* ssp. *durum* (Desf.) Husn.) imported from Canada (MAFF 2016).

The durum wheat harvest season in Japan overlaps with the rainy season. Preharvest sprouting (PHS) of wheat is induced by rainfall at or after the mature stage. PHS causes starch degradation through high alpha-amylase activity (Dick *et al.* 1974), and dough made from PHS seeds has a low moisture content (Dexter *et al.* 1990). Almost no durum wheat is grown commercially in Japan; Kai *et al.* (1998) concluded that durum wheat culture in Japan would be difficult owing to its late maturity and very weak resistance to PHS and *Fusarium* head blight. Recently, the first Japanese durum wheat cultivar, ‘Setodure’, with early maturity, was released at the Western Region Agricultural Research Center (WARC) of the National Agriculture and Food Research Organization (NARO). Its early maturity makes ‘Setodure’ less likely to encounter rain, but durum wheats still have insufficient PHS resistance.

Wheat PHS resistance is determined by the seed dormancy level. There are several reports of PHS resistance genes and quantitative trait loci (QTLs) in durum wheat, such as ‘SC8021-V2’ (DePauw *et al.* 1992). QTLs in durum wheat include *QPhsd-spa-1A.1*, -2A.1, and -7B.1 derived from ‘Kyle’ (Knox *et al.* 2012), and QTLs on chromosomes (Chrs.) 3BL and 5BS from ‘Chahba 88’ and on 6BL from ‘IACT12’ (Gelin *et al.* 2006). However, it is not known how effective these cultivars and QTLs would be under the severe climate of Japan.

More information is available on genes and QTLs in bread wheat (*T. aestivum* L.). Several QTLs of PHS resistance have been located on Chrs. 1A, 1B, 2B, 2D, 3A, 3B, 3D, 4A, 4D, 5A, 5B, 5D, 6D, 7B and 7D (Groos *et al.* 2002, Kottearachchi *et al.* 2008, Kulwal *et al.* 2012, Munkvold *et al.* 2009).

Seed color is associated with PHS, and red wheats have stronger PHS resistance than white wheats (Nilsson-Ehle 1914). Red seed color is controlled by three genes—*R-A1*, *R-B1*, and *R-D1*—on Chrs. 3A, 3B and 3D, respectively (Groos *et al.* 2002, Sears 1944). R-gene encoding the R2R3-type MYB domain-containing transcription factor TaMYB10 on Chr. 3AL regulates flavonoid biosynthesis and has pleiotropic effects on PHS resistance (Himi *et al.* 2011).

A QTL for PHS resistance on Chr. 3A, *QPhs-3A.1*, appears to have a large and stable effect (Mori *et al.* 2005). Nakamura *et al.* (2011) identified *MOTHER OF FT AND TFL1* (*MFT*), a key temperature-dependent regulator of PHS resistance, at *QPhs-3A.1*.  

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Improving preharvest sprouting resistance in durum wheat

The Japanese bread wheat cultivar ‘Zenkoujikomugi’ (Zen) has strong PHS resistance (Miura et al. 1997, Osanai and Amano 1993) and has been used in Japanese wheat breeding and in research on PHS (Mori et al. 2005). Nakamura et al. (2011) showed that a single nucleotide polymorphism (SNP) in the Zen MFT promoter increased the transcription of MFT, resulting in strong grain dormancy. Chono et al. (2015) reported a high frequency of the Zen-type SNP in Japanese commercial cultivars in southern Japan, and cultivars carrying that SNP had stronger grain dormancy. Zen also has red seeds and QPhs-5AL for PHS resistance (Himi et al. 2011, Kottearachchi et al. 2008). Thus, the strong PHS resistance of Zen seems to derive from the red seed color, MFT, and QPhs-5AL, and these are promising PHS resistance sources under the severe climate of Japan.

The use of hexaploid bread wheat (AABBDD) in the breeding of tetraploid durum wheat (AABB) is limited by the requirement to use only the A and B genomes. However, Zen has red seed color, MFT and QPhs-5AL on the A or B genome. It is unknown whether the effect of PHS resistance genes and/or QTLs derived from bread wheat is compatible in durum wheat. Thus, in this study, we introduced the PHS resistance trait of Zen into durum wheat by interbreeding and backcross breeding and assessed the effects of the sources of resistance in durum wheat. We also evaluated the possibility to improve PHS resistance in durum wheat by using bread wheat as a donor, carrying PHS resistance genes and QTL on A and B genomes.

Materials and Methods

Plant materials

To develop near isogenic lines (NILs), we used the durum wheat cultivar ‘Setodure’, which is susceptible to PHS, as the recurrent parent, and the bread wheat cultivar Zen as the resistance donor (Table 1). Zen contains the R-gene encoding TaMYB10 on B genome (Himi et al. 2011), MFT and QPhs-5AL on A genome (Kottearachchi et al. 2008, Nakamura et al. 2011). Through backcrossing to ‘Setodure’, we developed BC1F1 plants heterozygous for MFT and QPhs-5AL by DNA marker-assisted selection (MAS) and selected plants with red seeds by eye confirming at each BC2F1 generation (Supplemental Fig. 1). BC3F2 plants homozygous for MFT and QPhs-5AL were selected for propagation to the BC4F2 generation. Red seed color was finally confirmed in the BC4F2 and BC5F2 generations. Selected NILs were named for their introduced sequences by QPhs-5AL and MFT allele type (S or Z), red seed color (R or W), and MFT allele type (Seto or Zen: S or Z), and QPhs-5AL allele type (S or Z). We developed 7 NILs with 7 of the 8 possible combinations (except R-MFTS-QPhs-5ALZ).

The 7 NILs, ‘Setodure’, and 3 Japanese bread wheat cultivars (‘ShiroganeKomugi’, ‘Minaminokao’ and Zen) were grown in a research field at WARC, Fukuyama, Hiroshima, Japan (34°30’4” north latitude and 133°23’12” east longitude). All materials were sown 12 cm apart in 2-m rows in November and harvested in June. There were 4 replicates of ‘Setodure’ and 6 of the NILs (3 of W-MFTS-QPhs-5ALS) in 2014, and 4 replicates of ‘Setodure’ and the NILs and 2 of the bread wheats in 2015.

Table 1. Sources of PHS resistance introduced from bread wheat ‘Zenkoujikomugi’ into durum wheat ‘Setodure’

| Introduced source of resistance (chromosome position) | Phenotype or selection marker |
|------------------------------------------------------|-----------------------------|
| R-gene for seed color                                  | red or white                |
| MFT (3AS)                                             | MFT                         |
| QPhs-5AL (5AL)                                        | Xcfp2163                    |

Marker-assisted selection

DNA was extracted by the potassium acetate method (Dellaporta et al. 1983). For MAS of MFT, a cleaved amplified polymorphic sequence (CAPS) marker was used (Nakamura et al. 2011). DNA templates were amplified in a PCR Thermal Cycler Dice (TaKaRa Bio, Shiga, Japan) with ExTaq DNA polymerase (TaKaRa Bio) and an MFT primer set (forward 5’-GTAGCGGTTGAACTCTGCA-3’; reverse 5’-GGGACGTACGAGGGTGTAGA-3’). The single primer set was applied for MAS of QPhs-5AL region, therefore we regarded QPhs-5AL region as QPhs-5AL in this paper. DNA templates were amplified in the same thermal cycler as above with HotStarTaq DNA polymerase (QIAGEN, Hilden, Germany) and the Xcfp2163 primer set (forward 5’TGTAGCTTCTTGTGAGG-3’, reverse 5’-CATCATTGGTTTACGTTCTTCA-3’) under conditions of an initial 95°C for 30 s; 40 cycles of 94°C for 20 s, 60°C for 30 s, and 72°C for 60 s; and a final 72°C for 5 min. Amplified PCR fragments were digested with Clal at 37°C for 1 h, separated by 2% agarose gel electrophoresis, and visualization with Gel Red stain (Biotium, Fremont, CA, USA). For MAS of QPhs-5AL, we used its tightly linked codominant DNA marker Xcfp2163 (Kottearachchi et al. 2008). The single primer set was applied for MAS of QPhs-5AL region, therefore we regarded QPhs-5AL region as QPhs-5AL in this paper. DNA templates were amplified in the same thermal cycler as above with HotStarTaq DNA polymerase (QIAGEN, Hilden, Germany) and the Xcfp2163 primer set (forward 5’TGTAGCTTCTTGTGAGG-3’, reverse 5’-CATCATTGGTTTACGTTCTTCA-3’) under conditions of an initial 95°C for 15 min; 35 cycles of 94°C for 1 min, 53°C for 1 min, and 72°C for 1 min; and a final 72°C for 10 min. Amplified PCR fragments were separated by 8% polyacrylamide gel electrophoresis and visualized as above.

Germination experiment

Ten mature spikes in 2015 and 16 in 2016 were harvested from all replicates. To generate various dormant conditions, half of the spikes were dried shortly after harvest as a deep dormancy condition and the other half were kept at room temperature for 1 week to fully ripen as a light dormancy condition and then dried. All samples were dried at 35°C for 1 day and stored at –20°C. Before the germination experiment, all spikes were returned to room temperature and the seeds were threshed by hand. We laid 100 seeds on 2 pieces of 85-mm grade 1 filter paper (Whatman, Maidstone, UK) in a 90-mm Petri dish, added 6 mL of water, and incubated them at 15°C as an easy germination condition or 20°C as a
difficult germination condition. Germinated seeds were counted on 3rd, 5th and 7th day of imbibition (DI) in 2015 and daily until 7 DI. We calculated percentage germination (PG), except for the fully ripened seeds incubated at 15°C in 2015, of which we did not have enough.

The germination index (GI, Reddy et al. 1985) of all conditions in 2016 was calculated as:

\[ GI = \frac{(7 \cdot n_i) + (6 \cdot n_i) + (5 \cdot n_i) + (4 \cdot n_i) + (3 \cdot n_i) + (2 \cdot n_i) + (1 \cdot n_i)) \times 100}{(7 \times \text{total seeds})} \]

where \( n_i \) is the number of germinated seeds on day \( i \). Values of ‘Setodure’, NILs, ‘Shiroganekomugi’, ‘Minaminokaoiri’, and Zen were calculated.

### Statistical analysis

All PGs and GIs were arc-sin-transformed for analysis of variance (ANOVA). To assess the PHS resistance in each NILs, the PGs of ‘Setodure’ and NILs in 2015 and 2016 were analyzed by two-way ANOVA and Tukey’s HSD (at \( P < 0.05 \)). Variance components were calculated from the mean squares except for W-MFT\(_5\)-QPhs-5AL\(_5\), which was not significantly different from ‘Setodure’ and was carrying same genotype of ‘Setodure’ (see Table 3 later). And to assess the contribution of introduced sequences for the PHS resistance, the PGs in 2015 and 2016 and the GIs in 2016 of ‘Setodure’, NILs, ‘Shiroganekomugi’, ‘Minaminokaoiri’, and Zen were calculated.

### Results

#### Percentage germination

PG was significantly affected by genotype in all conditions; by year at mature stage on incubation at 20°C at 3 and 7 DI, and at full-ripe stage at 20°C at 7 DI, and by their interaction at mature stage at 20°C at 3 and 5 DI and at full-ripe stage at 20°C at 3 and 7 DI (Table 2). Thus, one or more sources of resistance affected PG.

By Tukey’s HSD, the PGs of R-MFT\(_2\)-QPhs-5AL\(_5\) (20.4–92.0%) and R-MFT\(_2\)-QPhs-5AL\(_2\) (7.1–78.6%) were significantly lower than those of ‘Setodure’ (76.3–98.0%); R-MFT\(_2\)-QPhs-5AL\(_5\) had the lowest PGs in all conditions (Table 3). In contrast, the PGs of W-MFT\(_5\)-QPhs-5AL\(_5\) (74.0–99.3%) and W-MFT\(_5\)-QPhs-5AL\(_5\) (63.8–98.1%) were not significantly different from those of ‘Setodure’ in all conditions. The PGs of W-MFT\(_5\)-QPhs-5AL\(_5\) (58.1–97.8%) were significantly lower than those of ‘Setodure’ only at the mature stage on incubation at 20°C at 3 and 7 DI. The PGs of W-MFT\(_5\)-QPhs-5AL\(_5\) (50.5–93.6%) were the lowest among ‘Setodure’ and NILs with white seeds. The PGs of R-MFT\(_5\)-QPhs-5AL\(_5\) (32.1–94.6%) were the highest among NILs with red seeds and were significantly lower than those of ‘Setodure’ at the mature stage at 20°C and at the mature stage at 15°C at 3 and 5 DI. Thus, red seed color plus MFT, with or without QPhs-5AL, from Zen clearly promoted PHS resistance.

#### Table 2. Two-way ANOVA of percentage germination among ‘Setodure’ and NILs

| Factor | 3 DI | 5 DI | 7 DI |
|--------|------|------|------|
| Year   | Df   | Mean Sq | F value | P value | Df | Mean Sq | F value | P value | Df | Mean Sq | F value | P value |
|        | 1    | 0.2336 | 21.593 | 2.75E-05 | 1 | 0.0326 | 3.892 | 0.054 | 1 | 0.4259 | 46.688 | 1.47E-08 |
| Genotype | 7    | 0.6806 | 62.905 | <2.00E-16 | 7 | 0.4201 | 50.111 | <2.00E-16 | 7 | 0.0664 | 7.919 | 0.054 |
| Year × Genotype | 7 | 0.5430 | 5.023 | 2.64E-05 | 7 | 0.0664 | 7.919 | 2.53E-06 | 7 | 0.0136 | 1.496 | 0.192 |
| Residuals | 47   | 0.0108 | 47 | 0.0084 | 47 | 0.0084 | 47 | 0.0091 |

| Factor | 3 DI | 5 DI | 7 DI |
|--------|------|------|------|
| Year   | Df   | Mean Sq | F value | P value | Df | Mean Sq | F value | P value | Df | Mean Sq | F value | P value |
|        | 1    | 0.0008 | 0.045 | 0.833 | 1 | 0.0447 | 2.611 | 0.113 | 1 | 0.1406 | 14.471 | 4.10E-04 |
| Genotype | 7    | 0.4454 | 24.764 | 9.26E-14 | 7 | 0.4553 | 26.602 | 2.53E-14 | 7 | 0.2823 | 29.059 | 4.95E-15 |
| Year × Genotype | 7 | 0.0457 | 2.541 | 0.027 | 7 | 0.0325 | 1.897 | 0.091 | 7 | 0.0434 | 4.466 | 7.09E-04 |
| Residuals | 47   | 0.0180 | 47 | 0.0171 | 47 | 0.0097 |

| Factor | 3 DI | 5 DI | 7 DI |
|--------|------|------|------|
| Year   | Df   | Mean Sq | F value | P value | Df | Mean Sq | F value | P value | Df | Mean Sq | F value | P value |
|        | 1    | 0.1325 | 7.333 | 0.094 | 1 | 0.0195 | 1.509 | 0.225 | 1 | 0.0273 | 2.758 | 0.103 |
| Genotype | 7    | 0.4376 | 24.227 | 1.37E-13 | 7 | 0.2936 | 22.671 | 4.45E-13 | 7 | 0.1345 | 13.578 | 1.91E-09 |
| Year × Genotype | 7 | 0.0310 | 1.714 | 0.129 | 7 | 0.0250 | 1.927 | 0.862 | 7 | 0.0140 | 1.414 | 0.222 |
| Residuals | 47   | 0.0181 | 47 | 0.0130 | 47 | 0.0099 |

\( \text{DI} = \text{days of imbibition} \).
Table 3. Average percentage germination among ‘Setodure’ and NILs

| Cultivar or NIL | Source of resistance | Mature stage on incubation at 20°C | Full-ripe stage on incubation at 20°C | Mature stage on incubation at 15°C |
|----------------|----------------------|------------------------------------|--------------------------------------|-----------------------------------|
|                |                      | 3 DI | 5 DI | 7 DI | 3 DI | 5 DI | 7 DI | 3 DI | 5 DI | 7 DI |
| Setodure       | White               | 76.3 a | 91.7 a | 95.0 a | 69.3 a | 90.0 a | 95.2 a | 85.5 a | 95.9 a | 98.0 a |
| W-MFT2-QPhs-5ALs | White             | 74.0 a | 90.7 a | 96.1 a | 76.4 a | 94.6 ab | 97.7 a | 80.0 ab | 95.3 ab | 93.9 ab |
| W-MFT2-QPhs-5ALz | White             | 64.3 ab | 81.5 ab | 86.5 a | 63.8 a | 87.9 ab | 92.7 a | 80.4 ab | 94.1 ac | 98.1 abc |
| W-MFT2-QPhs-5ALs | White             | 58.1 bc | 86.1 abc | 92.1 b | 68.1 ab | 92.5 ab | 96.6 ab | 73.9 abc | 93.5 abc | 97.8 ac |
| W-MFT2-QPhs-5ALs | White             | 50.5 c | 74.4 bcd | 80.5 b | 50.3 ac | 77.9 ac | 86.5 bc | 67.5 bc | 87.0 bcd | 93.6 abc |
| R-MFT2-QPhs-5ALs | Red                | 32.1 d | 69.4 b | 84.0 b | 50.2 uc | 81.4 ab | 94.6 abc | 57.9 bd | 81.2 bd | 94.6 abc |
| R-MFT2-QPhs-5ALs | Red                | 20.4 d | 44.3 d | 56.7 c | 30.7 c | 65.7 c | 79.7 c | 41.1 de | 76.1 d | 92.0 c |
| R-MFT2-QPhs-5ALz | Red                | 7.1 e | 30.5 e | 46.4 e | 11.7 d | 34.0 d | 57.1 d | 22.9 e | 53.3 e | 78.6 d |

Values followed by the same letter are not significantly different by Tukey’s HSD at P = 0.05.

Table 4. Multi-way ANOVA of percentage germination among sources of resistance at full-ripe stage on incubation at 20°C at 3 days of imbibition

| Source of variance | df | Mean Sq | F value | P value |
|--------------------|----|---------|---------|---------|
| Seed color         | 4  | 3.21E-12|         |         |
| MFT                | 1  | 0.0400  | 1.947   | 0.169   |
| QPhs-5AL           | 1  | 0.016   | 3.0     | 0.012   |
| Seed color × MFT   | 4  | 0.1186  | 5.769   | 0.033   |
| MFT × QPhs-5AL     | 1  | 0.0350  | 1.947   | 0.194   |
| Residuals          | 49 | 0.0206  |         |         |

Multi-way ANOVA of genotype effect on percentage germination

The variance components of genotype in two-way ANOVA ranged from 18.3% to 72.2% (data not shown). We tested the condition that gave the greatest value—full-ripe stage at 20°C at 3 DI (Table 4)—by multi-way ANOVA for additive or epistatic effects among sources of resistance in ‘Setodure’ and the NILs. The variance components of seed color (49.0%), MFT (31.6%), QPhs-5AL (3.0%), and seed color × QPhs-5AL (9.3%) were significant. (Tables 4, 5).

Table 5. Analysis of estimated variance components of percentage germination among sources of resistance at full-ripe stage on incubation at 20°C at 3 days of imbibition

| Variance component | Variance value | Percentage of the total (%) |
|--------------------|----------------|-----------------------------|
| σSeed color²       | 0.259          | 49.0                        |
| σMFT²              | 0.167          | 31.6                        |
| σQPhs-5AL²         | 0.016          | 3.0                         |
| σSeed color × MFT² | 0.049          | 9.3                         |
| σMFT × QPhs-5AL²   | 0.007          | 1.4                         |
| σResidual²         | 0.021          | 3.9                         |
| σTotal             | 0.528          | 100.0                       |

Estimated variance values were calculated from mean squares of multi-way ANOVA in Table 4.

Table 6. Average germination index among ‘Setodure’, NILs, and current Japanese standard bread wheat cultivars in 2016

| Cultivar or NIL | N | Source of resistance | Mature stage | Full-ripe stage | Mature stage | Full-ripe stage |
|----------------|---|----------------------|--------------|-----------------|--------------|-----------------|
|                |   | 20°C                 | 15°C         | 20°C            | 15°C         | 20°C            |
| Setodure       | 4 | White                | 65.1 a       | 71.8 a          | 72.2 a       | 79.7 a          |
| W-MFT2-QPhs-5ALs | 4 | White               | 66.1 a       | 72.7 a          | 72.3 ab      | 79.3 a          |
| W-MFT2-QPhs-5ALz | 4 | White               | 58.8 ab      | 67.1 ab         | 65.9 abc     | 73.3 ab         |
| W-MFT2-QPhs-5ALs | 4 | White               | 49.0 ab      | 57.3 bc         | 69.2 abc     | 72.0 ab         |
| W-MFT2-QPhs-5ALz | 4 | White               | 46.8 ab      | 49.2 c          | 61.8 bcd     | 70.8 b          |
| R-MFT2-QPhs-5ALs | 4 | Red                 | 48.1 bc      | 56.7 bc         | 56.9 cd      | 77.3 ab         |
| R-MFT2-QPhs-5ALs | 4 | Red                 | 24.9 bc      | 42.2 cd         | 51.5 d       | 71.5 ab         |
| R-MFT2-QPhs-5ALz | 4 | Red                 | 19.9 c       | 21.6 d          | 34.0 c       | 55.8 c          |
| Minaminokoshi   | 2 | Red                 | 54.5         | 73.7            | 64.1         | 79.5            |
| Shiroganeokomugi | 2 | Red                 | 30.9         | 22.9            | 39.0         | 57.6            |
| Zenkoujikomugi  | 2 | Red                 | 1.4          | 3.4             | 15.9         | 25.1            |

‘Seto’ allele derived from ‘Setodure’ (non-dormant); ‘Zen’ allele derived from Zen (dormant); ‘non-Zen’ allele derived from non-dormant parent (Chono et al. 2015).

*1; Although ‘Shiroganeokomugi’ had Zen-type MFT, its pedigree did not include Zen (Chono et al. 2015).

*2; Non-dormant: neither ‘Setodure’ nor Zen.

Values followed by the same letter are not significantly different by Tukey’s HSD at P = 0.05.
The GIs of R-MFTZ-QPhs-5AL$_S$ (48.1–77.3) and R-MFTZ-QPhs-5AL$_Z$ (24.9–71.5) were significantly lower than those of ‘Setodure’ except at full-ripe stage at 15°C. The GIs of W-MFTZ-QPhs-5AL$_Z$ (46.8–70.8) tended to be lowest among ‘Setodure’ and NILs with white seeds, and were significantly lower than those of ‘Setodure’ except at the mature stage at 20°C. The GIs of W-MFTZ-QPhs-5AL$_S$ (66.1–79.3) and W-MFTZ-QPhs-5AL$_Z$ (49.0–73.3) were not significantly different from those of ‘Setodure’ in all conditions. The GIs of W-MFTZ-QPhs-5AL$_S$ (49.0–72.0) were not significantly different from those of ‘Setodure’ except at full-ripe stage at 20°C. The GIs of W-MFTZ-QPhs-5AL$_Z$ and R-MFTZ-QPhs-5AL$_Z$ were respectively similar to those of ‘Minaminokaoai’ (54.5–79.5) and ‘Shiroganekomugi’ (30.9–57.6).

We also conducted multi-ANOVA using GI to access the effect of resistance source. Similar tendencies were found as shown in Tables 3 and 4 by ANOVA and variance component using PG. Single effects of seed color (44.6%), MFT (15.0%) and QPhs-5AL (35.7%) were significant on GI on the full-ripe stage on incubation at 20°C (Supplemental Tables 1, 2).

Discussion

Among the introduced sources of resistance, the R-gene encoding the TaMYB10 transcription factor, which is associated with red seed color, was the most effective for promoting PHS resistance (Tables 3, 6). The R-gene increased seed dormancy and decreased germination in a NIL of the white-seeded bread wheat ‘Spica’ (Kotearachchi et al. 2006). In general, bread wheat cultivars with red seeds have a higher PHS resistance than those with white seeds. Our results agree, and show that red seed color controlled by an R-gene can promote PHS resistance with strong seed dormancy in durum wheat. Thus, the R-gene for TaMYB10 may confer PHS resistance in Triticum species regardless of ploidy.

Mori et al. (2005) reported a QTL on Chr. 3A for strong PHS resistance. Nakamura et al. (2011) revealed that MFT was a causal gene of the QTL; seeds of the red-seeded bread wheat cultivar ‘Chinese Spring’ overexpressing MFT had clearly increased PHS resistance. We found that the introduction of MFT alone into white-seeded ‘Setodure’ was not enough to improve PHS resistance, but its introduction (with or without QPhs-5AL) with the R-gene for red seed color increased PHS resistance (Table 3). In addition, MFT alone could not inhibit germination at 15°C or at the full-ripe stage. We conclude that the effect of the introduction of MFT alone on PHS resistance is different from the case in bread wheat. Single introduction MFT may be effective but not sufficient for promoting PHS resistance in durum wheat, thus it may need to pyramid with other PHS resistance genes and/or QTLs.

The introduction of QPhs-5AL alone did not seem to promote PHS resistance in durum wheat, but its introduction with R-gene or MFT was effective (Tables 3, 6), albeit with a weaker effect (Tables 4, 5). Kotearachchi et al. (2008) reported QPhs-5AL as a minor QTL for PHS resistance in a white-seeded recombinant inbred line population of a Zen/‘Spica’ bread wheat cross. Our results suggest that single introduction QPhs-5AL may almost not be effective for promoting PHS resistance in durum wheat, however, QPhs-5AL pyramiding with red seed color and MFT can be improving PHS resistance.

From their GI scores, W-MFTZ-QPhs-5AL$_Z$ and R-MFTZ-QPhs-5AL$_Z$ have similar PHS resistance to ‘Minaminokaoai’, a major red-seeded bread wheat in Japan with non-Zen-type MFT and QPhs-5AL (Table 6). In addition, R-MFTZ-QPhs-5AL$_Z$ has similar PHS resistance to ‘Shiroganekomugi’, a leading red-seeded bread wheat in Japan with Zen-type MFT. These results suggest that breeding of durum wheat for PHS resistance using genetic resources derived from bread wheat can increase resistance to levels similar to those of current Japanese bread wheat cultivars, and thus these durum wheats may grow successfully where ‘Minaminokaoai’ or ‘Shiroganekomugi’ are currently grown.

Red seed color is one of the most important traits for PHS resistance in wheat. Chao et al. (2010) assessed PHS resistance on many durum wheat genotypes including red-seeded, and concluded red-seeded wheats carrying R-gene had the strongest resistance among them. However, Kai et al. (1998) reported that red-seeded durum wheats did not show sufficient PHS resistance for the Japanese severe climate. Red-seeded bread wheats can experience PHS due to rain at harvest in the Japanese climate. In addition, most durum wheats grown for pasta production are white-seeded, and there is no precedent for the use of red-seeded durum wheats for pasta production, so it will be necessary to assess the acceptability of red-seeded durum wheat for pasta production. If red-seeded durum wheats are not acceptable, we need to conduct durum wheat breeding based on white-seeded.

In summary, the introduction of an R-gene for red seed color, MFT, and QPhs-5AL from bread wheat into durum wheat by interbreeding was very effective at introducing PHS resistance into durum wheat. This strategy can compensate for the insufficiency of genetic resources for PHS resistance in durum wheat. The pyramiding of several genes and/or QTLs for PHS resistance may further promote PHS resistance in durum wheat.

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