



# Evolution of wheat blast resistance gene Rmg8 accompanied by differentiation of variants recognizing the powdery mildew fungus

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Supplementary Table 1. Primers used in this study

| Objectives                                      | Primer name       | Sequence( 5' -> 3')                 | Description                                                                                                                                                       |
|-------------------------------------------------|-------------------|-------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Mapping of <i>Rmg8</i>                          | KM9-F             | TGATGGTCTGACGTCCGTGC                | CAPS marker ( <i>Spe</i> I)                                                                                                                                       |
|                                                 | KM9-R             | CAATCACTACCAGTAACGTTACACGGTG        |                                                                                                                                                                   |
|                                                 | KM12-F            | TAGAGCTCTCAAGCACTTCCCTTGAG          |                                                                                                                                                                   |
|                                                 | KM12-R            | GAAGTGGAAAGGTTAGACACTGAGGAAG        | CAPS marker ( <i>Hinf</i> I)                                                                                                                                      |
|                                                 | KM13-F            | GTGCCACCGAGGCGACTTGTTT              |                                                                                                                                                                   |
|                                                 | KM13-R            | AGTCTCCCCCAAAGTTCCAGCGG             |                                                                                                                                                                   |
|                                                 | KM25-F            | AGGTAGATGCTGCTATGTGACTTGTG          | Presence/absence marker (presence in Sch)                                                                                                                         |
|                                                 | KM25-R            | AGACGGAGATTGTGATGAGGAG              |                                                                                                                                                                   |
|                                                 | KM27-F            | AGGCACGAAGGCAATTTCAC                |                                                                                                                                                                   |
|                                                 | KM27-R            | TGCTCGCTGATAGCATCAACAAG             | Presence/absence marker (presence in S-615)                                                                                                                       |
|                                                 | KM30-F            | AACCGTGAGATTTCCTACTGC               |                                                                                                                                                                   |
|                                                 | KM30-R            | TATCTCCGTAAAGAGCTTCCAAGA            |                                                                                                                                                                   |
|                                                 | KM65-F            | ACGATGCATCCTTGATACATCAAC            | CAPS marker ( <i>Hae</i> III)                                                                                                                                     |
|                                                 | KM65-R            | TCCGTGTCACAGTTCAGAAATAGATAG         |                                                                                                                                                                   |
|                                                 | KM109-F           | TGGATTGTCTTTCATCGCTTC               |                                                                                                                                                                   |
|                                                 | KM109-R           | TGTTGGAACGTAGGGTTCACC               | Presence/absence marker (presence in S-615)                                                                                                                       |
|                                                 | KM138-F           | ATAACGCTGAGTCAAAGTCTCCAC            |                                                                                                                                                                   |
|                                                 | KM138-R           | TAGATACGGTTGAAATGCTTCTTCTC          |                                                                                                                                                                   |
|                                                 | KM140-F           | AGCATCATCCAAAAAGTCCC                | Presence/absence marker (presence in S-615)                                                                                                                       |
|                                                 | KM140-R           | TGTATTGAGAAATCGTTACCTGG             |                                                                                                                                                                   |
|                                                 | KM155-F           | AGATGGGGATTGACAGCTTG                |                                                                                                                                                                   |
|                                                 | KM155-R           | AGAACATGTGCCACTACAAGGC              | Presence/absence marker (presence in Sch)                                                                                                                         |
|                                                 |                   |                                     |                                                                                                                                                                   |
|                                                 |                   |                                     |                                                                                                                                                                   |
| Mapping of <i>Rmg8</i>                          | KM171-F           | TGACGCTAGAGACCAACAGATG              | Presence/absence marker (presence in S-615)                                                                                                                       |
| & Detection of <i>Rmg8</i> variants             | KM171-R           | ACCATTGGAAGGATGAGCTG                |                                                                                                                                                                   |
| Mapping of <i>Rmg8</i> and <i>Rmg7</i>          | KM200-F           | TTCGACGGCATCTGCAAGTGAAGAACCC        | Presence/absence marker (presence in S-615)                                                                                                                       |
| & Detection of <i>Rmg8</i> variants             | KM200-R           | ACTGCGCGCGCTCCCCCTGCAT              |                                                                                                                                                                   |
| Mapping of <i>Rmg7</i>                          | KM201-F           | AAAAATAGAGGTGCGTGGGTAG              | CAPS marker ( <i>Mbo</i> I)                                                                                                                                       |
|                                                 | KM201-R           | AAAAGAGGGGGATTGAAGGAG               |                                                                                                                                                                   |
|                                                 | KM202-F           | AAGGGTCAGGCGTTAATGG                 | CAPS marker ( <i>Xho</i> I)                                                                                                                                       |
|                                                 | KM202-R           | ATCCAGCATCCTGCACATTT                |                                                                                                                                                                   |
| Transgene detection*                            | HPT-F             | GTGTCACGTTGCAAGACCTG                | for detection of transgene                                                                                                                                        |
|                                                 | HPT-R             | GATGTTGGCGACCTCGTATT                |                                                                                                                                                                   |
| Protoplast assay<br>& transformation of Fielder | InF-Rmg8-V1-F     | TGTGTGTGCAGATCGGGTTCTACTGACTGCAAATG | for In-Fusion cloning of <i>Rmg8-V1</i> and <i>Rmg7-V1</i>                                                                                                        |
|                                                 | InF-Rmg8-V1-R     | GGAAATTCGAGCTCGAACCACATTTACAAGAGAG  |                                                                                                                                                                   |
|                                                 | InF-Rmg8-V2-F     | TGTGTGTGCAGATCGGGTTCTACTGACTGCAAATG | for In-Fusion cloning of <i>Rmg8-V2</i> and <i>Rmg7-V2</i>                                                                                                        |
|                                                 | InF-Rmg8-V2-R     | GGAAATTCGAGCTCGCGACGTCATCAATACTTCAT |                                                                                                                                                                   |
|                                                 | InF-Rmg8-genome-F | TGTGTGTGCAGATCGGGTTCTACTGACTGCAAATG | for In-Fusion cloning of <i>Rmg8</i> -genome                                                                                                                      |
|                                                 | InF-Rmg8-genome-R | GGAAATTCGAGCTCGCGACGTCATCAATACTTCAT |                                                                                                                                                                   |
| Protoplast assay                                | InF-PWT3dSP-F     | TGTGTGTGCAGATCGATGAGTGACTTTTGAAAGTA | for In-Fusion cloning of <i>PWT3</i> without its signal peptide                                                                                                   |
|                                                 | InF-PWT3dSP-R     | GGAAATTCGAGCTCGTTACGGCGATGCAACACAGC |                                                                                                                                                                   |
|                                                 | InF-AVR-Rmg8dSP-F | TGTGTGTGCAGATCGATGCTGCCTGCGCCGCAG   | for In-Fusion cloning of <i>AVR-Rmg8</i> without its signal peptide                                                                                               |
|                                                 | InF-AVR-Rmg8dSP-R | GGAAATTCGAGCTCGCTACTGCCTTCTAGTACCG  |                                                                                                                                                                   |
| <i>Rmg8</i> variant analysis                    | Rmg8-E5-F         | GGTTCTACTGACTGCAAATGGA              | for amplification of Exon 1-5 of <i>Rmg8</i> variants in <i>Triticum</i> spp.                                                                                     |
|                                                 | Rmg8-E5-R         | TGTAGCAACCCAATTAAAGGAAG             |                                                                                                                                                                   |
|                                                 | Rmg8-V1V2-F       | GAAACGTGCTACCAGACAGAATC             | for amplification of Exon 6 (V1) and Exon 7 (V2) of <i>Rmg8</i> variants                                                                                          |
|                                                 | Rmg8-V1V2-R       | CAACATGAAGAAATTCATCGTCA             |                                                                                                                                                                   |
|                                                 | Rmg8U-V1-1R       | ATTTCACAAGAGAGCTAGCGG               | for amplification of <i>AeuRmg8</i> V1 variant from cDNA of <i>Ae. umbellulata</i> accessions in combination with Rmg8-E5-F                                       |
|                                                 |                   |                                     |                                                                                                                                                                   |
|                                                 | Rmg8U-V2-1R       | AAATTCATCATCACAGGAGCAC              | for amplification of <i>AeuRmg8</i> V2 variant from cDNA of <i>Ae. umbellulata</i> accessions (KU-4026, KU-4103, KU-5934, KU-12198) in combination with Rmg8-E5-F |
|                                                 |                   |                                     |                                                                                                                                                                   |
|                                                 | Rmg8U-V2-2R       | AAGTAATTGCAACATGAAGAAATTC           | for amplification of <i>AeuRmg8</i> V2 variant from cDNA of <i>Ae. umbellulata</i> accessions (KU-8-5, KU-4035, KU-4043, KU-5954) in combination with Rmg8-E5-F   |
|                                                 |                   |                                     |                                                                                                                                                                   |
|                                                 | Rmg8M-V1-1R       | GTCAGGTCAGCAGGTGG                   | for amplification of <i>AecRmg8</i> V1 variant from cDNA of <i>Ae. comosa</i> (KU-17-2) with Rmg8-E5-F                                                            |
|                                                 |                   |                                     |                                                                                                                                                                   |
|                                                 | Rmg8M-V2-1R       | AAATTCATCGTCACAGGAGC                | for amplification of <i>AecRmg8</i> V2 variant from cDNA of <i>Ae. comosa</i> (KU-17-2) with Rmg8-E5-F                                                            |
|                                                 |                   |                                     |                                                                                                                                                                   |
|                                                 | M13_fw            | GTA AAAACGACGGCCAGT                 | for sanger sequencing                                                                                                                                             |
|                                                 | T3_promoter       | ATTAACCCCTCACTAAAGGGAA              | for sanger sequencing                                                                                                                                             |
|                                                 | Rmg8-seq-F1       | GAAACCCGCCAATATACTGCTC              | for sanger sequencing                                                                                                                                             |
|                                                 | Rmg8-seq-F2       | ATGCTATGAGACAGCTTTAGAGTGC           | for sanger sequencing                                                                                                                                             |
|                                                 | Rmg8-seq-F3       | GAAACGTGCTACCAGACAGAATC             | for sanger sequencing                                                                                                                                             |
|                                                 | Rmg8-seq-F4       | AAGTGGTTCAGCCTGTTGGTG               | for sanger sequencing                                                                                                                                             |
|                                                 | Rmg8-seq-F5       | ATGACGCTAGAGACCAACAGATG             | for sanger sequencing                                                                                                                                             |
|                                                 | Rmg8-seq-F6       | AGTATGGCAAACCGCAGCTC                | for sanger sequencing                                                                                                                                             |
|                                                 | Rmg8-seq-F7       | ATCCTTCCAATGGTGTTACTGTG             | for sanger sequencing                                                                                                                                             |
|                                                 | Rmg8-seq-R1       | TCCATAAGAGCATTGGACATCC              | for sanger sequencing                                                                                                                                             |
|                                                 | Rmg8-seq-R2       | TTTGTCGGTTCAGTATTTCTAGG             | for sanger sequencing                                                                                                                                             |
|                                                 | Rmg8-seq-R3       | CACGACCTTGAGGTACATGAGTT             | for sanger sequencing                                                                                                                                             |
|                                                 | Rmg8-seq-R4       | TCCGTCTCAAGGCTCAGATG                | for sanger sequencing                                                                                                                                             |
|                                                 | Rmg8-seq-R5       | GGACATGAAGCGGTAGAAAGTTG             | for sanger sequencing                                                                                                                                             |
|                                                 | Rmg8-seq-R6       | TGTTGTCCATCTTGTGGAGC                | for sanger sequencing                                                                                                                                             |
|                                                 | Rmg8-seq-R7       | CAACATGAAGAAATTCATCGTCA             | for sanger sequencing                                                                                                                                             |
|                                                 | Rmg8U_V1_seq_F1   | ACCTTCATGATAATCATATTATT             | for sanger sequencing                                                                                                                                             |
|                                                 | Rmg8U_V1_seq_R1   | TTGGCGGGTTTTAGATCCAAGTG             | for sanger sequencing                                                                                                                                             |
|                                                 | Rmg8U_V2_seq_F1   | GCCGTGAAAAGGATGTCCAATGC             | for sanger sequencing                                                                                                                                             |
|                                                 | Rmg8U_V2_seq_F2   | AGCGCTGACTTGTATAGTCTTGG             | for sanger sequencing                                                                                                                                             |
|                                                 | Rmg8U_V2_seq_R1   | AGCGGCGGGTCGGCCTGCTCCAG             | for sanger sequencing                                                                                                                                             |
|                                                 | Rmg8U_V2_seq_R2   | CGAGCTCGGCGTGTGACAGCGTC             | for sanger sequencing                                                                                                                                             |

\* Refer to Abe, F. et al. Genome-edited triple recessive mutation alters seed dormancy in wheat. *Cell Rep* . 28, 1362-1369 (2019).