

Supplementary Information for:

Three previously characterized resistances to yellow rust are encoded by a single locus *Wtk1*

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Table S1. A list of SSR, CAPS and KASP markers used in the current study.

Name of marker	Type of marker	Primer sequence	Reference
<i>gwm413</i>	SSR	TGCTTGTCTAGATTGCTTGGG GATCGTCTCGTCCTTGGCA	Röder <i>et al.</i> (1998)
<i>wmc406</i>	SSR	TATGAGGGTTCGGATCAATACAA CGAGTTTACTGCAAACAATGG	Somers <i>et al.</i> (2004)
<i>barc8</i>	SSR	GCGGGAATCATGCATAGGAAAACAGAA GCGGGGGCGAAACATACACATAAAAACA	Song <i>et al.</i> (2005)
<i>uhw290*</i>	CAPS	ATAGCAGGATCGCAGCAAAA GAGGATAAACAATGTGATCGTTCTG	Current study
<i>uhw250</i>	EST	CTGCTCACTTTTTGCCTGTG AAAAGTTGTTGCTCTGCTTTT	Klymiuk <i>et al.</i> 2018
<i>uhw256</i>	EST	GTTACCTCCACAGCAAGGT GCGCATTACTTCCACTTCTTG	Klymiuk <i>et al.</i> 2018
<i>RAC875_c826_839</i>	KASP	ACGAAGGTTCTGTTTTCACCA ACGAAGGTTCTGTTTTCACCG TCTTCTTGCTCAAAGGTAAAGAT	Klymiuk <i>et al.</i> 2018
<i>uhw255</i>	CAPS	GATGCTCTGCACATGTGTTATG GCAGCTCCAGCTTATTCGTC	Klymiuk <i>et al.</i> 2018
<i>uhw264</i>	CAPS	GGTCTCTTGCAACATACAGTAAACA GAGTGGTAGTCTAGTAGAGGTTGGTG	Klymiuk <i>et al.</i> 2018
<i>uhw267</i>	CAPS	TGGTAATCAAGTTTCAATTTGTTCA GGAAGGACACCTTTCGGTATT	Klymiuk <i>et al.</i> 2018
<i>uhw297</i>	CAPS	CAGATGACCAACCAAAAGCA GTCATATTGGTGCCCAGTGA	Klymiuk <i>et al.</i> 2018
<i>uhw292</i>	dominant	GACTTCTTCCCTCGGGACT CTCGCACGCCTATAAAAGGA	Klymiuk <i>et al.</i> 2018
<i>uhw300</i>	dominant	CCGTGTCAGCCACCTACAAT GCACTCTACCACCGAACACA	Klymiuk <i>et al.</i> 2018
<i>uhw301</i>	dominant	GTAGTGGCTCGTTCGGTGAT TTTCGCATCCCAACCTACTG	Klymiuk <i>et al.</i> 2018
<i>uhw296</i>	CAPS	CAACCGTGCCTCCAAACA CGGGTGTGTGCCGTTGAG	Klymiuk <i>et al.</i> 2018
<i>uhw302</i>	dominant	CATCCATTCTCCGACAAAGT CACTGCAATGCAAAAATGCT	Klymiuk <i>et al.</i> 2018
<i>uhw276</i>	CAPS	TCTGTGATGCCTGTGATGGT AAAGTTTGGGATTTGGCAAT	Klymiuk <i>et al.</i> 2018
<i>uhw273</i>	CAPS	GGTGA CGCGAGTGTACG GACGCAATTGTCCGCTGT	Klymiuk <i>et al.</i> 2018
<i>uhw274</i>	CAPS	AAGCTCCGCTGCAATGAC ACCTGACATCCTCGAACCAAC	Klymiuk <i>et al.</i> 2018
<i>uhw259</i>	CAPS	CTGTATTCTAATGCAGATTAGCTGTT CACGCATAATTTGTCCACAC	Klymiuk <i>et al.</i> 2018
<i>BS00022902_51</i>	KASP	ATGTGCGGCAAGGAGAGA ATGTGCGGCAAGGAGAGG ATACTCTTCACGTCTGCTTC	Klymiuk <i>et al.</i> 2018
<i>gwm273</i>	SSR	ATTGGACGGACAGATGCTTT AGCAGTGAGGAAGGGATC	Röder <i>et al.</i> (1998)

* Temperature of elongation 59°C; Enzyme *AciI*; Fragment sizes: *T. dicoccoides* H52 = 266 bp, 148 bp; *T. durum* cv. Langdon = 414 bp.

Table S2. A list of KASP markers from the *Yr15* region developed based on SNPs from the wheat 15K SNP array.

Name of marker	Primer A	Primer B	Primer Common
<i>RFL_Contig2160_617</i>	cgtgtgaatgtgactctacca	cgtgtgaatgtgactctaccg	ggaccagaatgccagtg
<i>IACX502</i>	cacgcctaacaaaacatatccatt	cacgcctaacaaaacatatcatc	tgggagttcttatttaattcttcgg
<i>Ra_c16879_977</i>	cagagcaaaagccatcaatctta	cagagcaaaagccatcaatcttc	tctgagaagatgccagaacg
<i>BS00087784_51</i>	gagctgacagatgggggt	gagctgacagatgggggc	ccatgacatcagaaaagttgatgat
<i>Excalibur_c17202_1833</i>	gttagagcccaatttcaagctta	gttagagcccaatttcaagctg	aggagcttatattgctatgtacagt
<i>w SNP_Ku_c4911_8795151</i>	gttcatcaactgttgagctggt	gttcatcaactgttgagctgtc	gctgctgtagtcttctgattgtg
<i>w SNP_Ex_c2111_3963161</i>	agcagcatacgattaactcagtt	agcagcatacgattaactcagtc	ctgttggcgagaagctg
<i>RAC875_c79370_378</i>	tggaaactgatgtgtccagt	tggaaactgatgtgtccagc	gcggcatcaactccccg

Table S3. Molecular characterization of the *yr15*[#], *yrG303* and *yrH52* EMS mutants

Domain	Base substitution*	Effect on amino acid†	Gene	Mutant No.	Name of mutant
KinI	G 160 A	G 54 S	<i>yr15</i>	EMS1	Sunce+ <i>yr15</i> -L18
KinI	G 161 A	G 54 D	<i>yrH52</i>	EMS14	M52-4
KinI	G 340 A	E 114 K	<i>yr15</i>	EMS2	Sunce+ <i>yr15</i> -L89
KinI	G 445 A	A 149 T	<i>yrG303</i>	EMS11	M2298-410-16
KinI	G 445 A	A 149 T	<i>yrH52</i>	EMS15	M52-9
KinI	G 448 A	E 150 K	<i>yr15</i>	EMS3	B9+ <i>yr15</i> -L1351
KinI	C 482 T	P 161 L	<i>yr15</i>	EMS4	Avocet+ <i>yr15</i> -1
KinI	C 509 T	A 170 V	<i>yrH52</i>	EMS16	M52-14
KinI	C 602 T	T 201 I	<i>yrH52</i>	EMS17	M52-18
KinI	C 632 T	P 211 L	<i>yr15</i>	EMS5	Avocet+ <i>yr15</i> -L90
KinI	G 686 A	G 229 E	<i>yrH52</i>	EMS18	M52-2
KinI	G 705 A	M 235 I	<i>yrG303</i>	EMS12	A95-126
KinII	G 2922 A	A 460 T	<i>yr15</i>	EMS6	Avocet+ <i>yr15</i> -13
KinII	G 2922 A	A 460 T	<i>yrH52</i>	EMS19	M52-8
KinII	G 3114 A	D 524 N	<i>yr15</i>	EMS7	Excalibur+ <i>yr15</i> -6L306
KinII	C 3229 T	T 562 I	<i>yr15</i>	EMS8	Avocet+ <i>yr15</i> -L72
KinII	G 3281 A	W 581 *	<i>yrG303</i>	EMS13	M2298-767-16
KinII	C 3315 T	P 591 S	<i>yr15</i>	EMS9	B9+ <i>yr15</i> -LF
KinII	G 3469 A	V 614 M	<i>yr15</i>	EMS10	Excalibur+ <i>yr15</i> -L137

[#]Full description of *yr15* mutants is provided in Klymiuk *et al.* (2018).

*The first letter indicates the wild-type nucleotide, the number indicates its position relative to the ATG start codon, and the last letter shows the mutant nucleotide. The complete WTK1 coding regions of the above 19 mutants were sequenced; no additional mutations were detected.

†The first letter indicates the wild-type amino acid, the number indicates its position relative to the start methionine, and the last letter shows the mutant amino acid.

#RIL	barc8	IACX502	Ra_c16879_977	uhw290	Excadibur_c17202_1833	w SNP_Ka_c4911_8795151	w SNP_Ex_c2111_3963161	RAC875_c826_839	uhw255	uhw264	RAC875_c79370_378	uhw297	uhw292	uhw300	YrG303 seedling	YrG303 adult	uhw301	uhw296	uhw302	uhw276	uhw273	uhw274	uhw259	RS00022902_51	gwm273
2.6.38.3	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	A
1.1.82.1	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	A
1.1.84.5	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	A
2.2.92.10	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	A
2.6.50.1	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	A
2.3.51.3	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	A	A	A	A	A	A	A
2.4.25.3	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	A	A	A	A	A	A	A	A
1.3.19.4	B	B	B	B	B	B	B	B	B	B	B	A	A	A	A	A	A	A	A	A	A	A	A	A	A
2.1.12.10	B	B	B	B	B	B	B	B	B	B	B	A	A	A	A	A	A	A	A	A	A	A	A	A	A
1.2.66.10	B	B	B	B	B	B	B	B	B	B	B	A	A	A	A	A	A	A	A	A	A	A	A	A	A
2.4.23.5	B	B	B	B	B	B	B	B	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
2.1.54.2	B	B	B	B	B	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
2.3.32.4	B	B	B	B	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
1.2.70.1	B	B	B	B	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
3.2.74.4	B	B	B	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
2.1.64.1	B	B	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
2.4.88.4	B	A	A	A	A	A	A	A	A	A	A	A	A	A	B	A	A	A	A	A	A	A	A	A	A
3.2.64.2	B	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
2.2.60.2	B	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
1.1.66.4	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	A	A	A	A	A	A	A	A	A	B
2.7.4.3	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B
3.3.48.3	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B
2.1.58.1	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	A	A	A	A	A	A	A	A	B	B
2.1.59.2	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B
1.1.79.1	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B
2.5.74.8	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B
2.5.80.4	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	B	B	B
1.3.58.9	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B
2.4.45.3	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B
3.2.5.3	A	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B
2.3.30.2	A	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B
3.3.10.2	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B
3.2.26.2	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B
3.2.86.2	A	A	A	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B
3.2.18.3	A	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B
2.2.7.4	A	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B

Fig. S1. Graphical genotype of selected recombinant lines (RILs) from *YrG303* tetraploid mapping population. Allele A – resistant allele from *T. dicoccoides* acc. G303; allele B – susceptible allele from *T. durum* acc. D447. *YrG303* seedling and *YrG303* adult columns correspond to the phenotypes observed at seedling and adult screens with *Pst* (14 and 21 dpi, respectively). RILs marked in red showed different seedling and adult resistance response.

#RIL	barc8	RFL_Contig2160_617	BS00087784_51	uhw267	uhw290	Excalibur_c17202_1833	w SNP_Ku_c4911_8795151	RAC875_c826_839	w SNP_Ex_c2111_3963161	uhw255	uhw264	RAC875_c79370_378	uhw297	uhw292	uhw300	YrH52	uhw301	uhw296	uhw259	BS00022902_51	gwm273	
V1_31_1	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	A
V1_60_6	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	A
GA19_37_12_1	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	A	A
GA_10_61_14_2	B	B	B	B	B	B	B	B	B	B	B	B	A	A	A	A	A	A	A	A	A	A
D11_86_6_4	B	B	B	B	B	B	B	B	B	B	A	A	A	A	A	A	A	A	A	A	A	A
GA_22_37_10_3	B	B	B	B	B	B	B	B	B	A	A	A	A	A	A	A	A	A	A	A	A	A
D16_5_11_2	B	B	B	B	B	B	B	B	B	A	A	A	A	A	A	A	A	A	A	A	A	A
V4_5_3	B	B	B	B	B	B	B	B	B	A	A	A	A	A	A	A	A	A	A	A	A	A
V2_90_4	B	B	B	B	B	B	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
D130_4_14_4	B	B	B	B	B	B	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
402_9_20_1_2	B	B	B	B	B	B	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
D128_47_7_4	B	B	B	B	B	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
V3_101_2	B	B	B	B	B	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
GA_26_27_6_2	B	B	B	B	B	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
d131_46_9_1	B	B	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
V4_1_2	B	B	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
GA26_73_4_1	B	B	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
d133_42_16_1	B	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
d127_9_21_1	B	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
V4_14_2	B	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
V1_46_3	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B
d16_76_5_1	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B
402-18-8-3_2	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B
371_8_11_4_2	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B
D14_54_1_2	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B
GA_18_23_15_2	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B
V3_70_3	A	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B
D13_39_5_2	A	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B
GA_17_40_11_2	A	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B
d12_149_3_1	A	A	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B
d16_57_13_1	A	A	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B
d12_74_6_1	A	A	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B
GA19_118_5_1	A	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B

Fig. S2. Graphical genotype of selected RILs from *YrH52* mapping population. Allele A – resistant allele from *T. dicoccoides* acc. H52; allele B – susceptible allele from *T. durum* cv. LDN. *YrH52* column corresponds to the phenotypes observed at seedling screen with *Pst* at 14 dpi.

Reference list:

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