Registration of wheat lines carrying the partial stripe rust resistance gene *Yr36* without the *Gpc-B1* high grain protein content allele

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Abstract

While the high-temperature adult plant resistance gene *Yr36* represents a promising source of quantitative and potentially race non-specific resistance to wheat stripe rust (causal organism *Puccinia striiformis* Westend. f. sp. *tritici*), its tight linkage (0.3 cM) with the high-grain protein content gene *Gpc-B1* may hinder its introgression in certain cases, such as in soft wheat varieties requiring low grain protein content or in lines where the *Gpc-B1* allele may be associated with a yield penalty. The development and registration of two donor lines, one tetraploid (*Triticum turgidum* L. ssp. *durum*; PI 656793) and one hexaploid (*T. aestivum* L. ssp. *aestivum*; PI 664549), each carrying the resistant wild emmer (*T. turgidum* ssp. *dicoccoides*) allele for *Yr36* linked with the non-functional *Gpc-B1* allele, are intended to overcome this potential limitation. Meiotic recombination events breaking the linkage between these two genes were discovered during the systematic screening of a population of 4,500 F₂ durum plants (cv. Langdon background) used to fine map *Yr36*. One of the critical recombination events was selected for fixation by self-pollination and transferred to a California adapted spring hexaploid background (breeding line UC1110₅₊₁₀) through five generations of backcrossing. Genotypic and phenotypic data confirm the presence of *Yr36* and the non-functional *Gpc-B1* allele in both registered lines.

Wild relatives of crop species are invaluable sources of genetic variation and thus are instrumental to the continued improvement of disease resistance, quality, and other traits important to the success of modern cultivars. Effective utilization of genes of interest from such wild genetic stocks, however, requires not only their identification and characterization but also their isolation from undesired neighboring genes, common in wild genetic backgrounds. The widespread adoption and deployment of exotic genes, therefore, is greatly facilitated by the development and availability of donor germplasm carrying precise introgression events. Such germplasm provides an enhanced range of options to breeders, allowing them to target objectives specific to their respective programs.

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A 30 cM region from wild emmer [*Triticum turgidum* L. ssp. *dicoccoides* (Korn. Ex Asch. and Graebn.)], introgressed into the short arm of chromosome 6B in the durum [*T. turgidum* ssp. *durum* (Desf.) Husn.] cultivar Langdon, was found to carry novel alleles of two tightly-linked (0.3 cM) genes of agronomic interest and potential utility to wheat improvement programs: 1) A grain protein-enhancing allele for *Gpc-B1*, a gene which affects grain protein and micronutrient content via modulation of the terminal senescence process (Uauy et al., 2006a); and 2) A resistant allele for *Yr36*, a gene conferring high-temperature adult plant (HTAP) resistance to stripe rust (causal organism *Puccinia striiformis* Westend. f. sp. *tritici*) (Fu et al., 2009). Both genes have been positionally cloned and extensively characterized (Fu et al., 2009; Uauy et al., 2006b).

While increased grain protein content (and, in some cases, micronutrient content) is a common goal of pasta and bread wheat breeding programs, it has been shown that introgression of the wild emmer allele of *Gpc-B1* into some advanced, optimally-adapted lines can result in slight yield penalties, particularly in tetraploid wheat (Brevis and Dubcovsky, 2010). Such penalties may also be observed in growing environments amenable to extended periods of grain fill due to the fact that the wild emmer allele of *Gpc-B1* accelerates the terminal senescence process. The linkage of the high-grain protein *Gpc-B1* allele may also limit the introgression of *Yr36* into soft wheat breeding lines, generally bred for low levels of grain protein content for use in cookies and cakes (Finney et al., 1987; Huebner et al., 1999). To facilitate the use of *Yr36* by a wide range of interested wheat improvement programs, we here register one tetraploid (*T. turgidum* ssp. *durum*) and one hexaploid (*T. aestivum* L. ssp. *aestivum*) wheat line in which the tight linkage (0.3 cM) between the wild emmer alleles of *Yr36* and *Gpc-B1* has been broken, such that the *Yr36* resistant allele is now associated with the non-functional *Gpc-B1* allele.

Methods

The tetraploid critical recombinant line PI 656793 was developed from a cross between tetraploid wheat cv. Langdon (LDN) [CI 13165, (Heyne, 1959), pedigree Mindum / Carleton // Khapli /// Heiti / Stewart // Mindum / Carleton /4/ Stewart /5/ Carleton] and RSL65, a near-isogenic line of LDN carrying a 30-cM segment of chromosome arm 6BS from *T. turgidum* ssp. *dicoccoides* (accession FA15-3, hereafter designated DIC). A total of 4,500 F₂ plants from the cross LDN / DIC were screened for recombination between CAPS marker *Xucw110* and BAC end sequence marker *Xucw125* (Fu et al., 2009); among these, only twelve recombinant lines (0.3%) were found. A selected plant from one of those lines was self-pollinated and a recombinant substitution line (RSL) homozygous for the recombinant chromosome was obtained. Based on genotype, this line (PI 656793) carries the non-functional, low grain protein content allele (LDN) of *Gpc-B1* and the resistant allele (DIC) of *Yr36* (see Table 1). In terms of agronomic traits, the recombinant line otherwise resembles the recurrent parent cv. Langdon (Heyne 1959).

The hexaploid critical recombinant line PI 664549 was developed from a cross between the tetraploid RSL described above (PI 656793) and an elite hard white spring (HWS) breeding line from the UC Davis wheat breeding program [UC1110 = GSTR 13501 (Lowe et al., 2011), pedigree Chukar /// Yding // Bluebird / Chanate] into which the high-molecular

weight glutenin subunits 5+10 at the *Glu-D1* locus had been introgressed from donor line UC1041 (Uauy et al., 2009) via six generations of marker-assisted backcrossing. Hereafter, this line will be designated UC1110₅₊₁₀. The pentaploid F₁ was backcrossed five times to the recurrent hexaploid parent UC1110₅₊₁₀ and plants heterozygous for the *Yr36-Gpc-B1* recombinant chromosome were selected with molecular marker *WKS1* (see Figure 1). The heterozygous BC₅ plants were self-pollinated and a plant homozygous for the recombinant chromosome was obtained. Based on genotype, this line (PI 664549) carries the non-functional allele [LDN] of *Gpc-B1* associated with the resistant allele [DIC] of *Yr36* (see Table 1). In terms of agronomic traits, the recombinant line otherwise resembles the elite HWS breeding line UC1110 (see common wheat entry #1110, UCD 95-111W; Jackson et al., 1998).

Characteristics

Functional allele of *Yr36*

The presence of the *Yr36* resistant allele in the recombinant lines can be demonstrated with certainty via genotyping due to the fact that a diagnostic molecular marker (*WKS1*) has been developed for this gene (see Figure 1). Nevertheless, for the purpose of this registration, we also performed a phenotypic confirmation of the presence of *Yr36* in PI 656793 and PI 664549 by subjecting these recombinant lines, alongside their near isogenic controls (Langdon and UC1110₅₊₁₀, respectively), to intense stripe rust pressure in the field during the 2010–2011 growing season in Davis, CA (see Figure 2). Included in this replicated trial were near-isogenic materials in four other hexaploid backgrounds to demonstrate the effect of *Yr36* across a broader selection of germplasm, information which may be of value to breeding programs interested in utilizing this gene.

It is clear from Figure 2 that, in addition to *Yr36*, both PI 664549 and PI 656793 carry other sources of quantitative adult plant resistance to stripe rust. In the case of PI 664549, those sources of resistance are known to include a major QTL on chromosome 3BS (*QYr.ucw-3BS*) and a minor resistance QTL on chromosome arm 2BS (*QYr.ucw-2BS*) (Lowe et al., 2011). For PI 656793, the genes responsible for the observed basal level of adult plant resistance are as yet unknown, since the sources of stripe rust resistance in the recurrent parent LDN have not been characterized. Regarding the other hexaploid lines in the trial, Anza is known to carry *Yr18/Lr34* (Krattinger et al. 2009) and UC1041 carries the major resistance gene *Yr1* (X. Chen, unpublished data); the sources of resistance in UC1037 are unknown. While Yecora Rojo is known to carry one dominant and one recessive stripe rust resistance gene (Zwer 1986), it exhibits a highly susceptible phenotype in this trial because both genes were completely defeated by post-2000 races of stripe rust (e.g., see Jackson et al. 2003). Except for Yecora Rojo, then, the effects of *Yr36* presented in Figure 2 should not be interpreted as its performance in otherwise susceptible backgrounds but rather as the range of effects exhibited by this source of HTAP resistance in combination with other effective sources of stripe rust resistance.

Non-functional allele of *Gpc-B1*

The presence of the non-functional (LDN) *Gpc-B1* allele carrying the frame shift mutation reported by Uauy et al. (2006b) was confirmed in both tetraploid and hexaploid homozygous recombinant lines by sequencing (see Figure 3). The protein content of the field-grown isogenic lines described in the previous section were not compared due to the fact that differential levels of stripe rust resistance (e.g. +/- *Yr36*) are known to have a large effect on grain protein content.

Discussion

As an HTAP stripe rust resistance gene conferring partial, or quantitative, resistance to a broad spectrum of races of wheat stripe rust under field conditions, *Yr36* should be of interest to breeders who are pursuing a disease management strategy based on combining multiple minor genes of complementary effect. The positional cloning of *Yr36* demonstrated that the gene encodes a novel Kinase-START protein, heretofore unknown in the grasses (Fu et al., 2009). The *Yr36* resistance gene has not been detected in any of the commercial tetraploid or hexaploid wheat varieties screened so far (Fu et al., 2009), suggesting that it may be a useful source of resistance in many wheat breeding programs. In controlled environments (i.e. growth chambers), a comparison of *Yr36* isogenic lines in various genetic backgrounds revealed that adult plants with the gene are partially resistant to stripe rust at relatively high temperatures (25° – 35°C). This suggests that *Yr36* may complement well the resistance afforded by other stripe rust resistance genes, many of which are effective at the seedling stage and at lower temperatures (Qayoum and Line, 1985).

The wild emmer (DIC) allele of *Gpc-B1* encodes a NAC transcription factor that increases grain protein, zinc, and iron content via regulation of the terminal senescence process (Uauy et al., 2006b). While the size of the effect on grain protein content (GPC) depends on both environment and genetic background, a study using near isogenic lines showed that the DIC allele confers an average increase in grain protein concentration of approximately 10% in tetraploid wheats and 6% in hexaploid wheats (Brevis and Dubcovsky, 2010). The increase in total protein content (GPC × grain yield) was approximately 5% in both species (Brevis and Dubcovsky, 2010). Given the importance of high grain protein content to pasta and bread making quality, and in light of the current global epidemics of stripe rust, it is likely that many pasta and bread wheat breeding programs would prefer to introgress the original DIC segment carrying both genes.

That being said, the linkage of these two genes is not necessarily beneficial in all market classes and environments. The most evident benefit of breaking this linkage is for soft wheat breeding programs working to develop varieties with relatively low levels of grain protein content. Generally speaking, such programs may be hesitant to use the *Yr36* stripe rust resistance because of the possible negative effect of the linked *Gpc-B1* gene on cookie and cake quality (Finney et al., 1987; Huebner et al., 1999). Durum breeding programs may also find the separation of the *Yr36* gene from the functional *Gpc-B1* allele useful for introgression of HTAP stripe rust resistance into varieties that already possess high grain protein content. In such varieties, further increase in grain protein content may not provide adequate compensation for the potential associated yield penalty. There may also be value in

breaking the linkage between *Gpc-B1* and *Yr36* in hexaploid wheat varieties grown in environments amenable to longer grain filling periods, since the presence of the functional *Gpc-B1* is always associated with a shorter grain filling period. As an example, in California, we found that the hard red spring isogenic lines we tested were able to compensate for the reduced grain filling period (i.e. smaller grains) with other yield components (Brevis and Dubcovsky, 2010), but such may not be the case for other genotype×environment combinations.

The diagnostic marker *WKS1* (see Figure 1), the development of which was made possible by the positional cloning and extensive characterization of *Yr36* (Fu et al., 2009), is a useful resource for molecular-assisted breeding programs interested in introgressing *Yr36* into their materials. It is important to note that, due to the separation of *Yr36* from *Gpc-B1* in these recombinant lines, previously reported markers like *Xuhw89* (0.1 cM distal of *Yr36*; see <http://maswheat.ucdavis.edu/protocols/HGPC/index.htm>) should not be used in marker-assisted selection protocols using PI 656793 or PI 664549 as donors. Also, due to the dominant nature of the *WKS1* marker reported here, progeny tests will be required to distinguish heterozygous and homozygous *Yr36* genotypes in crossing programs.

Availability

Seeds of the two recombinant lines are available from the National Small Grains Collection, part of the USDA-ARS National Germplasm System (www.ars-grin.gov/npgs). The deposited seeds for both PI 656793 and PI 664549 are BC₅F₆, homozygous for the recombination event between *Gpc-B1* and *Yr36*. Recipients of seed are asked to make appropriate recognition of the source of these genetic stocks when using them for the development of new cultivars, germplasm, parental lines, or hybrids.

Conclusion

With the registration of PI 656793 and PI 664549, and the development of the diagnostic molecular marker *WKS1* reported here, both durum and common wheat improvement programs now have the option to introgress the HTAP stripe rust resistance gene *Yr36* into their materials without also introgressing the high-grain protein allele of *Gpc-B1*, which may be unwanted in some circumstances due to its impact on either protein or yield. Specifically, in those instances of unacceptable yield penalty or when enhanced stripe rust resistance is needed in varieties with otherwise suitable quality profiles, the recombinant lines registered here provide the opportunity to deploy *Yr36* without the high grain protein content allele. Ultimately, individual breeding programs will need to investigate the effect of *Gpc-B1* on yield in their own materials in order to assess the suitability of these donor lines to their breeding objectives.

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Abbreviations

BAC	bacterial artificial chromosome
CAPS	cleavage amplification polymorphic sequence
cM	centiMorgan
DIC	<i>Triticum turgidum</i> L. ssp. <i>dicoccoides</i> (accession FA15-3)
GPC	grain protein content
HTAP	high-temperature adult plant
LDN	<i>Triticum turgidum</i> L. ssp. <i>durum</i> cultivar Langdon
RSL	recombinant substitution line

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Figure 1.

The primer pair *WKS1-F1/WKS1-R1* amplifies a 501-bp segment from the final intron and exon of HTAP stripe rust resistance gene *Yr36* (also known as *WKS1*). Due to the fact that *Yr36* is unique to DIC and was likely lost during the domestication process, these primers provide a dominant marker for the presence of the gene, as the sample gel image indicates. Primer sequences and recommended PCR conditions are shown

Figure 2.

Average flag leaf disease severities at anthesis of near-isogenic lines in multiple backgrounds, both hexaploid and tetraploid, illustrate the effect of the *Yr36* resistance during the 2010–2011 field season in Davis, CA. The complementary effect of *Yr36* with *Yr18/Lr34* can be seen in both the Yecora Rojo and Anza backgrounds (note: Anza carries *Yr18/Lr34*). In the Yecora Rojo, UC1037, Anza, and UC1041 NILs, *Yr36* is linked to the wild emmer high protein (DIC) allele of *Gpc-B1*. In the UC1110₅₊₁₀ and Langdon NILs (PI 664549 and PI 656793), *Yr36* is linked to the Langdon low-protein (LDN) allele of *Gpc-B1*. Near isogenicity was achieved in all backgrounds through a program of five backcrosses. Averages are of three replications; error bars are ± 1 standard error

		M	G	S	S	D	S	S	S
	DIC	ATG	GGC	AGC	T C C	GAC	TCA	TCT	TCC
	LDN	ATG	GGC	AGC	T T C	CGA	CTC	ATC	TTC
PI 664549		ATG	GGC	AGC	T T C	CGA	CTC	ATC	TTC
PI 656793		ATG	GGC	AGC	T T C	CGA	CTC	ATC	TTC
		M	G	S	F	R	L	I	F

Figure 3.

Sequence data of the first 24 nucleotides of the coding region of *Gpc-B1* show that both the tetraploid critical recombinant PI 656793 and the hexaploid critical recombinant PI 664549 carry the single-nucleotide frame shift mutation shown by Uauy et al. (2006b) to render the gene non-functional. DIC = sequence from DIC (i.e. high protein *Gpc-B1* allele). LDN = sequence from LDN (i.e. non-functional *Gpc-B1* allele, carrying a 1-bp insertion after the tenth coding nucleotide)

Table 1

Genotypes of the tetraploid critical recombinant PI 656793 and the hexaploid critical recombinant PI 664549.

		← to 6BS telomere					
	Gpc-B1	Xucw110	Xucw70	Xucw112	Xucw128	Xucw125	Yr36
PI 656793	L	L	D	D	D	D	D
PI 664549	L	L	D	D	D	D	D

L = allele from LDN (i.e. non-functional *Gpc-B1* allele and susceptible *Yr36* allele). D = allele from DIC (i.e. high protein *Gpc-B1* allele and resistant *Yr36* allele).