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ORIGINAL PAPER

Lr67/Yr46 confers adult plant resistance to stem rust and powdery mildew in wheat

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Abstract

Key message **We demonstrate that *Lr67/Yr46* has pleiotropic effect on stem rust and powdery mildew resistance and is associated with leaf tip necrosis. Genes are designated as *Sr55*, *Pm46* and *Ltn3*, respectively.**

Abstract Wheat (*Triticum aestivum*) accession RL6077, known to carry the pleiotropic slow rusting leaf and yellow rust resistance genes *Lr67/Yr46* in Thatcher background, displayed significantly lower stem rust (*P. graminis tritici*; *Pgt*) and powdery mildew (*Blumeria graminis tritici*; *Bgt*) severities in Kenya and in Norway, respectively, compared to its recurrent parent Thatcher. We investigated the resistance of RL6077 to stem rust and powdery mildew using Avocet × RL6077 F_6 recombinant inbred lines (RILs) derived from two photoperiod-insensitive F_3 families segregating for *Lr67/Yr46*. Greenhouse seedling tests were conducted with Mexican *Pgt* race RTR. Field evaluations were conducted under artificially initiated stem rust epidemics with *Pgt* races RTR and TTKST (Ug99 + *Sr24*) at Ciudad Obregon (Mexico) and Njoro (Kenya) during 2010–2011; and under natural powdery mildew epiphytotic in Norway at Ås and Hamar during 2011 and 2012. In Mexico, a mean reduction of 41 % on stem rust severity was obtained for RILs carrying *Lr67/Yr46,* compared to RILs that lacked the gene, whereas in Kenya the difference was smaller (16 %) but significant. In Norway, leaf tip necrosis was associated with *Lr67/Yr46* and RILs carrying *Lr67/Yr46* showed a 20 % reduction in mean powdery mildew severity at both sites across the 2 years of evaluation. Our study demonstrates that *Lr67/Yr46* confers partial resistance to stem rust and powdery mildew and is associated with leaf tip necrosis. The corresponding pleiotropic, or tightly linked, genes, designated as *Sr55*, *Pm46,* and *Ltn3*, can be utilized to provide broad-spectrum durable disease resistance in wheat.

Introduction

Stem rust, caused by *Puccinia graminis* Pers. f. sp. *tritici* (*Pgt*), has historically been a threat to wheat (*Triticum aestivum* L.) production world-wide (Saari and Prescott 1985). In Mexico for example, 30 % of the wheat crop in the major production region of Bajio was destroyed by stem rust epidemics during 1947–1948; Chile lost 40 % of its wheat crop in 1951; and in central India, 20 % of total wheat production was lost during 1946–47 epidemic (reviewed in Dubin and Brennan 2009). Powdery mildew, caused by *Blumeria graminis* f. sp. *tritici* (*Bgt*), is an important disease in maritime or temperate climates in regions such as

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China, South and North America, and Northern Europe, causing losses of up to 34 % (Johnson et al. 1979).

The most effective way to control these two diseases is by deploying resistant cultivars. Stem rust was successfully controlled for several decades via extensive breeding efforts to incorporate resistance and the eradication of the alternate host of *Pgt* (Singh et al. 2011a). However, the disease once again became a threat to global food security when a new *Pgt* race (TTKSK or Ug99), with virulence to most of the widely deployed resistance genes, was detected in Uganda in 1998. Since then, several variants of TTKSK with virulence to additional stem rust resistance genes have been detected, and races belonging to the same lineage have spread to Kenya, Ethiopia, Zimbabwe, South Africa, Sudan, Yemen, Iran (Singh et al. 2011a), and Tanzania (Hale et al. 2013).

More than 50 and 40 resistance genes for stem rust and powdery mildew, respectively, have been designated (McIntosh et al. 2012). Most of them are classified as race-specific resistance genes associated with a hypersensitive reaction in the host, which results from recognition and incompatible host–pathogen interactions based on a gene-for-gene system (Parlevliet 1985). This type of resistance is usually short-lived due to the high evolutionary potential of the pathogen (McDonald and Linde 2002). Of the few race-specific resistance genes effective against the prevailing pathogen populations, some are not considered useful due to linkage drag problems where undesirable traits may be introduced along with the translocation or insertion (Hsam and Zeller 2002).

Partial resistance and slow rusting, or slow mildewing, are often associated with race non-specificity and a compatible host–pathogen interaction (Parlevliet 1985). This type of resistance is associated with durable resistance; it is often referred to as adult plant resistance (APR), because it is expressed in adult plants as slow disease development in the field, compared to a susceptible check, despite a susceptible infection type. The accumulation of genes for partial resistance results in additive gene effects, whereby in general, up to four or five such resistance genes can result in near-immunity and stable resistance across different environments (Singh et al. 2011b).

Few genes of this type have been cataloged and designated due to their small individual effects. Those that are known, such as *Sr2/Yr30/Lr27/Pbc1*, *Lr34/Yr18/Sr57/Pm38/Sb1/Ltn1*, and *Lr46/Yr29/Sr58/Pm39/Ltn2*, also confer resistance to multiple diseases, including leaf rust and yellow rust (caused by *P. triticina* and *P. striiformis,* respectively) and are associated with certain morphological traits such as pseudo black chaff (PBC) or post-flowering leaf tip necrosis (LTN). For example, *Sr2,* which provides partial resistance to stem rust, is associated with pseudo black chaff (*Pbc1*) and the same gene, or closely linked genes, provides resistance to yellow rust, leaf rust, and powdery mildew (Singh et al. 2000; Kota et al. 2006; Mago et al. 2011). *Lr34*, the only gene of this multi-pathogen resistance class cloned to date (Krattinger et al. 2009), provides resistance to the three rust diseases (Singh et al. 2012) and powdery mildew (Lillemo et al. 2008), and has recently been shown to provide resistance to spot blotch, caused by the hemi-biotrophic fungus *Bipolaris sorokiniana* (Lillemo et al. 2013). In addition, *Lr34* was the first gene reported to be associated with LTN (*Ltn1*; Singh 1992). *Lr46* has similar phenotypic characteristics to *Lr34*; it was associated with LTN (*Ltn2;* William et al. 2003; Rosewarne et al. 2006) and was recently confirmed to provide resistance to stem rust (Singh et al. 2013). There are, therefore, multiple benefits to breeders for introducing these genes into their elite germplasm, especially if markers are available that can assist selection.

The wheat cultivar, Thatcher, is known to be slow rusting to stem rust for almost 80 years (Parlevliet, 1985). The hexaploid wheat Newthatch (Hope/Thatcher//2*Thatcher) provided the foundation for the durable resistance referred to as the '*Sr2* complex' in CIMMYT germplasm (Rajaram et al. 1988). This resistance was based on a combination of unknown APR genes from Thatcher (originating from Iumillo durum wheat; Nazareno and Roelfs 1981) and *Sr2* [originating from emmer wheat (*T. turgidum*)], and transferred to hexaploid wheat cultivars Hope and H44 (Sunderwirth and Roelfs 1980; Kolmer et al. 2011; Singh et al. 2011a).

Wheat accession RL6077 is a near-isogenic line of Thatcher that carries slow-rusting leaf and yellow rust resistance gene(s) *Lr67/Yr46* on chromosome arm 4DL (Hiebert et al. 2010; Herrera-Foessel et al. 2011). This gene was originally transferred to Thatcher background by Dyck from a Pakistani accession PI250413 (Dyck and Samborski 1979). *Lr67/Yr46* has similar phenotypic characteristics to *Lr34/Yr18*, including the 'dying back of the leaf tissue' (LTN) and was, therefore, previously thought to be the same gene (Dyck and Samborski 1979; Dyck et al. 1994). In a population developed from a cross between RL6077 and a Thatcher isogenic line carrying *Lr34*, RL6058, Dyck et al. (1994) observed that lines segregating for resistance and parents had lower stem rust severity and infection response than the isogenic line Thatcher in the field in Canada (trace infection vs. 30MS). This was also confirmed by Herrera-Foessel et al. (2011) in a field trial in Kenya with *Pgt* race TTKST (25MS for Thatcher vs. 10R-MR and 1MR for RL6077 and RL6058, respectively). RL6077 also displayed reduced powdery mildew severity compared to Thatcher in field evaluations in Norway (45 % for Thatcher vs. 19 and 23 % for RL6077 and RL6058, respectively).

In this study, we investigated the multiple disease effect of *Lr67/Yr46* by confirming the association with resistance to stem rust and powdery mildew using an F_6

Avocet × RL6077 population that was previously used to map *Lr67/Yr46* (Herrera-Foessel et al. 2011). We also provide evidence of a third locus for LTN associated with APR to multiple diseases in wheat.

Materials and methods

Plant material

Avocet-*YrA*, a selection from the Australian wheat cultivar Avocet, is susceptible to leaf rust and yellow rust but is known to carry the 6AL translocation from *T. elongatum* with stem rust resistance gene *Sr26*. For simplicity, Avocet-*YrA* will be referred to as Avocet throughout this paper. RL6077 (Thatcher*6/PI 250413) is a Thatcher backcross line with slow rusting gene(s) *Lr67/Yr46* mapped to chromosome arm 4DL (Dyck and Samborski 1979; Hiebert et al. 2010; Herrera-Foessel et al. 2011). RL6077 is photoperiod sensitive and, therefore, late-maturing compared with photoperiod-insensitive Avocet. To avoid a confounding effect of phenology in the rust assessment in Mexico, the Avocet × RL6077 F_6 RILs previously described by Herrera-Foessel et al. (2011) were derived from two photoperiod-insensitive (based on visual selection of normal-maturity phenotype in Ciudad Obregon, which is a good location to identify the photoperiod sensitive and insensitive wheat lines) F_3 families segregating for *Lr67/Yr46*. A total of 148 F_6 RILs were developed; 74 lines derived from each F_3 family.

Molecular analyses

The original parents, Avocet and RL6077, and the 148 F_6 RILs were evaluated with marker *Sr26#43* associated with *Sr26* (Mago et al. 2005). Leaf tissue of the lines was harvested and DNA was extracted according to a CTAB method (CIMMYT 2005). The PCR program was run with the most favorable annealing temperature according to the established protocols and the PCR product was loaded on 12 % acrylamide (29:1) gels. Bands were detected using silver staining (CIMMYT 2005).

Stem rust evaluation in the greenhouse

The 148 Avocet/RL6077 RILs, plus Avocet and RL6077, were evaluated for stem rust resistance in greenhouse seedling tests with Mexican *Pgt* race RTR. One replication was used for the subset of 74 RILs carrying *Sr26* (based on the linked marker) originating from the first F_3 family, whereas two replicates were used for Avocet, RL6077, and the other 74 RILs that lacked *Sr26* which originated from the second F_3 family. Two sets of stem rust differentials were also included in the greenhouse test; one set with 20 differential lines from the Cereal Rust Disease Laboratory, Minnesota, and another set with 50 differential lines available at CIMMYT. The avirulence/virulence formula of RTR is: *Sr7a, 9e,10,12,13,14,22,23,24,25,26,27,29,30,31,32,33,35,Dp2, H/5,6,7b,8a,8b,9a,9b,9d,9f,9g,11,15,17,21,28,34,36* (Singh 1991). The avirulence/virulence formula of TTKST is: *Sr3 6,Tmp/5,6,7b,8a,9a,9b,9d,9e,9g,10,11,17,24,30,31,38,McN* (Rouse et al. 2011).

Approximately, eight kernels per line were sown as clumps in trays and inoculated at the two-leaf stage (10 days after sowing), using an atomizer with urediniospores suspended in Soltrol 170 (Chempoint.com). Plants were placed in a dew chamber overnight, with 3-h light exposure period during drying after the dew period, and then transferred to a greenhouse with minimum, maximum, and average temperatures of 15.3, 28.4, and 21.8 °C, respectively. Infection types were recorded 14 days after inoculation using the 0–4 scale as described in Roelfs et al. (1992). Infection types below ‘3’ were considered resistant, whereas higher infection types were considered susceptible. Symbols ‘+’ and ‘−’ describe somewhat larger and smaller uredinia, respectively, than normal for the infection type classification. More than one designation represents a range of infection types.

Stem rust evaluation in the field

The 74 Avocet/RL6077 RILs lacking *Sr26* (based on marker and seedling data) were evaluated together with Avocet and RL6077 at two field sites, Ciudad Obregon (Mexico) and Njoro (Kenya), during the 2010–2011 season under artificially initiated stem rust epidemics with *Pgt* races RTR (Cd. Obregon) and TTKST (Njoro). One of the RILs possessing *Sr26* (but lacking *Lr67/Yr46*) was included as check in the field trial. Two replicates for each RIL were used at the Cd. Obregon field site, whereas a non-replicated trial was established in Njoro. Each plot of about 80 (Cd. Obregon) and 120 (Njoro) kernels comprised a 1 m double row, 20 cm apart, on 80 cm wide raised beds in Mexico and on flat beds in Kenya where one row was left unplanted between plots. Spreaders surrounding the experimental field consisted of a mix of seven susceptible lines at Cd. Obregon and seven different lines at Njoro. The same mix in each respective site was also planted as hills in the middle of a 0.5 m pathway on one side of each plot to assure a uniform disease epidemic. At Cd. Obregon, the spreaders and hills were inoculated by spraying urediniospores of *Pgt* race RTR suspended in Soltrol 170 about 8 weeks after sowing. At Njoro, spreaders and hills were inoculated with race TTKST by spraying with a mixture of urediniospores and Tween 20 suspensions and needle inoculations using urediniospores suspended in distilled water.

Stem rust severity was assessed on adult plants using the modified Cobb Scale (Peterson et al. 1948). Description of the host responses to infection (infection response) followed Roelfs et al. (1992), where 'R' indicated resistant, or miniature uredinia surrounded by necrosis or chlorosis; 'MR' indicated moderately resistant, or small uredinia surrounded by chlorosis or necrosis; 'MS' indicated moderately susceptible, or moderate sized uredinia without chlorosis or necrosis; and 'S' indicated susceptible, or large uredinia without chlorosis or necrosis. At least, two scorings at different intervals were made per site, but the final statistical analysis was conducted using the terminal reading for each site (19 March and 29 March 2011 for Cd. Obregon and Njoro, respectively).

Powdery mildew evaluations

The 148 Avocet/RL6077 RILs and parents Avocet and RL6077 were evaluated for powdery mildew resistance under natural epidemics during the 2011 and 2012 growing seasons at two sites in southeastern Norway: Vollebekk research farm in Ås and Staur research farm close to Hamar. Thatcher, RL6058, and Saar were also included in the evaluations. Saar is a CIMMYT line with high levels of stable partial resistance to powdery mildew (Lillemo et al. 2008, 2010). These two sites are characterized by having severe epidemics but vary in virulence composition of *Bgt* (Skinnes 2002). The *Bgt* race composition at Ås is more similar to the composition in other countries of Northern Europe (UK, Germany, and Poland) possibly due to spore migration (Clarkson 2000; Skinnes 2002), whereas the Hamar area which experiences a more stable snow cover during winter time is characterized by a more diversified *Bgt* population often leading to a faster breakdown of resistance in local cultivars (Skinnes 2002). An augmented alpha-lattice design was used where about half of the lines were sown with two reps at each location. Each line was planted as hill plot with 50 cm between plots. The percentage leaf tissue covered with powdery mildew was recorded on a whole canopy basis according to the modified Cobb Scale (Peterson et al. 1948). Post-flowering LTN was scored in the RILs as absent (0) or present (1) at the two sites in 2011. The absence/presence of LTN recorded in Norway was compared with the absence/presence of *Lr67*, based on previous phenotypic assessments conducted at Cd. Obregon for leaf rust resistance (Herrera-Foessel et al. 2011).

Statistical analyses

The SAS/STAT® program (SAS Institute Inc 1999) was used for statistical analyses. For analysis of variance, we used the MIXED procedure, by considering replications (reps) and blocks within rep as random factors, whereas for the across sites (combined) analysis: site, rep(site), block(rep site) and site*entry were considered as random factors. Least square means were compared with paired *t* tests using the PDIFF statement. Phenotypic associations among stem rust, powdery mildew and previously recorded leaf rust and yellow rust severities (Herrera-Foessel et al. 2011) were assessed using Pearsons' correlation coefficient (*r*).

Results

Marker for *Sr26* and greenhouse response to *Pgt* race RTR

Avocet and the 74 F_6 lines derived from the first photoperiod-insensitive F_3 family were positive for the presence of the *Sr26#43* marker (Mago et al. 2005) associated with *Sr26*, and the presence of *Sr26* was also confirmed by greenhouse seedling tests. With the Mexican *Pgt* race RTR, Avocet and all the RILs originating from the first F_3 family displayed infection type '2−' to '2' (Table 1). RL6077 and the 74 RILs developed from the second F_3 family lacked the *Sr26* associated marker and, therefore, in greenhouse seedling tests with the *Pgt* race RTR, the infection type of RL6077 was high ('33+') (Table 1). The 74 RILs generated from the second F_3 family displayed susceptible infection types that varied from '3' to '4', with the exception of one RIL that was mixed with plants displaying two types of infection (3+ and 2).

Field evaluations to *Pgt* races RTR and TTKST

Stem rust infection developed well in the absence of additional diseases at Cd. Obregon (apart from leaf rust that

Table 1 Seedling greenhouse infection types of Avocet, RL6077 and 148 Avocet/RL6077 RILs tested with Mexican *Pgt* race RTR

Parents/RILs	Infection type[a]	RILs (No.)	
		Rep I	Rep II
Avocet	2	–	–
RL6077	33+	–	–
RILs-1st family	2−	6	0
	2	68	0
RILs-2nd family	3	2	0
	3+	56	41
	4	15	32
	2, 3+	1	1

[a] Infection types follow 0–4 Scale as described in Roelfs et al. (1992). Symbols '+' and '−' describe somewhat larger and smaller uredinia, respectively, than normal for the infection type classification. More than one designation represents a range of infection types

Table 2 Stem rust mean severity and range of severities for 74 F_6 lines of the Avocet/RL6077 population derived from the second F_3 family, based on evaluations with two replications at Ciudad Obregon, Mexico, in 2010–2011 with *Pgt* race RTR and with one replication at Njoro, Kenya, in 2010–2011 with *Pgt* race TTKST

Parent/RIL	Ciudad Obregon (*Pgt* race RTR)			Njoro (*Pgt* race TTKST)		
	Severity[a] (%)	Infection response[b]	Severity range (%)	Severity (%)	Infection response	Severity range (%)
Avocet	25	R-MR		10	R-MR	
RL6077	1	MR-MS to MS		1	R-MR	
−*Lr67* RILs[c]	57 A[d]	MS-S	20–85	37 A	MS-S	10–70
+*Lr67* RILs	16 B	MS-S	1–40	21 B	MS-S	1–50
SEG RILs	31 B	MS-S	30–70	22 B	MS-S	5–70

[a] Stem rust severity (%) assessed on adult plants using the modified Cobb Scale (Peterson et al. 1948)

[b] Host response to infection as described in Roelfs et al. (1992)

[c] Homozygous susceptible (−*Lr67*), homozygous resistant (+*Lr67*) and segregating (SEG) for *Lr67/Yr46* based on previous phenotypic assessments (Herrera-Foessel et al. 2011)

[d] Means with different letters in the same column within each location are significantly different at $P < 0.001$ based on paired *t* test comparisons

Table 3 Phenotypic correlations (Pearsons') among powdery mildew, leaf rust, stem rust and yellow rust for Avocet/RL6077 population

	PMÅs[a]	PMHamar[b]	LRObregon[c]	YRToluca[d]	SRObregon[e]
PMHamar	0.89**				
LRObregon	0.73**	0.72**			
YRToluca	0.53**	0.50**	0.52**		
SRObregon	0.66**	0.62**	0.80**	0.42**	
SRNjoro[f]	0.44**	0.42**	0.43**	0.23*	0.67**

* $P < 0.05$, ** $P < 0.01$

[a] *PMÅs* Mean final powdery mildew severity across two crop cycles 2011/2012 for Ås, Norway

[b] *PMHamar* Final powdery mildew severity recorded at Hamar, across two crop cycles 2011/2012

[c] *LRObregon* Final leaf rust severity recorded at Cd. Obregon, Mexico in 2009–2010 crop cycle and previously reported in Herrera-Foessel et al. (2011)

[d] *YRToluca* Final yellow rust severity recorded at Toluca, Mexico in 2010 crop cycle and previously reported in Herrera-Foessel et al. (2011)

[e] *SRObregon* Final stem rust severity recorded at Ciudad Obregon in 2010–2011 crop cycle

[f] *SRNjoro* Final stem rust severity recorded at Njoro, Kenya at 2010–2011 crop cycle

appeared very late); therefore, this site was identified as useful for conducting stem rust evaluations. At Njoro, in addition to stem rust, a high incidence of yellow rust (that occurs naturally in the region) was observed on Avocet and some Avocet/RL6077 RILs. Avocet (carrying *Sr26*) displayed a terminal mean stem rust severity and infection response of 25 R-MR at Cd. Obregon and 10 R-MR at Njoro, with *Pgt* races TTKST and RTR, respectively (Table 2). The photoperiod-sensitive RL6077 developed late at both sites and displayed 1 MR-MS to 1 MS severity and infection response. The *Sr26* check lacking *Lr67/Yr46* from the Avocet/RL6077 RIL population was scored as 40 R-MR and 5 R-MR at Cd. Obregon and Njoro, respectively. Adult plants of all seedling-susceptible (lacking *Sr26*) Avocet/RL6077 RILs displayed MS to S infection response to stem rust in the field (Fig. S1). Terminal mean severities were significantly correlated between Cd. Obregon and Njoro (Table 3). At Cd. Obregon, a mean reduction of 41 % in stem rust severity was obtained for the RILs carrying *Lr67/Yr46*, compared to RILs that lacked it (Table 2). The classification of presence or absence of *Lr67/Yr46* in the RIL population was based on previous phenotypic assessments (Herrera-Foessel et al. 2011).

At Njoro, the reduction was significant but smaller (16 %) possibly due to interference with natural yellow rust infections. A range of stem rust severities of 1–40 and 1–50 % were observed for the RILs with *Lr67/Yr46* at Cd. Obregon and Njoro, respectively, whereas for RILs lacking *Lr67/Yr46*, the range was 20–85 and 10–70 % at Cd. Obregon and Njoro, respectively (Table 2). A significant correlation was found between stem rust, powdery mildew, and previously assessed leaf rust and yellow rust severities (Herrera-Foessel et al. 2011), confirming the pleiotropic effect of *Lr67* (Table 3). The correlations for stem rust severity for Cd. Obregon with other diseases were in general higher (0.42–0.66) than for correlations with stem rust severity from Njoro (0.23–0.44). A continuous distribution for stem rust severity was observed for both non-*Lr67/Yr46* and *Lr67/Yr46* RILs at both sites, indicating that other genes of minor effect also segregated in the population and contributed to enhanced stem rust resistance (Fig. 1).

Powdery mildew and LTN in Norway

The severity of powdery mildew on Thatcher ranged between 61 and 57 % at the two sites (across 2011 and 2012) in Norway (Table 4). The presence of *Lr67/Yr46* and *Lr34/Yr18/Sr57/Pm38* in the same background (RL6077 and RL6058, respectively) reduced the severity by 32–37 and 32–35 %, respectively. Avocet displayed an intermediate (40–41 %) response. Avocet/RL6077 RILs lacking *Lr67* displayed mean powdery mildew severities of 43 and 36 % at Ås and Hamar, respectively, whereas the RILs with *Lr67* had significantly lower severities (23 and 16 %, respectively). *Lr67,* therefore, reduced powdery mildew severity by approximately half. At Ås and Hamar, powdery mildew severity ranged from 5–42 and 0–33 %, respectively, for RILs carrying *Lr67/Yr46*; whereas RILs without *Lr67/Yr46* ranged from 16–76 and 11–75 % at the same two sites (Table 4). A significant correlation (0.89) for powdery mildew severity was obtained between the two sites (Table 3). The correlations between powdery mildew recorded at Ås and Hamar with the three rust diseases were similar (0.44–0.73 vs. 0.42–0.72), and a remarkably high correlation between powdery mildew and leaf rust was obtained (Table 3). These high correlations support the finding that the same gene provides protection to multiple diseases. However, a range of severity responses (Table 4) as well as a continuous severity response for RILs with and without *Lr67* (Fig. 2) indicate that additional minor genes also segregated for resistance to powdery mildew in this population.

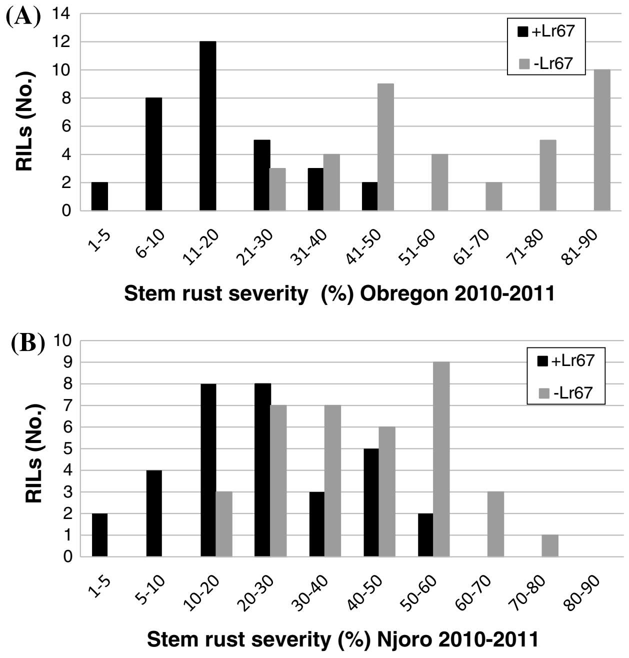

Fig. 1 Distribution of *Lr67/Yr46* homozygous susceptible (−*Lr67*) and homozygous resistant (+*Lr67*) Avocet/RL6077 RILs, based on the final disease evaluation at Ciudad Obregon, Mexico, in 2010–2011 with *Pgt* race RTR (**a**) and in Njoro, Kenya, with race TTKST in 2010–2011 (**b**)

Table 4 Mean terminal powdery mildew severity and range of severity for parents, checks, and 148 F_6 lines of the Avocet/RL6077 population when evaluated in two Norwegian sites, Ås and Hamar, across 2011 and 2012

Parents and checks	Severity (%) Ås		Severity (%) Hamar	
	Mean[a]	Range	Mean	Range
Avocet	40	–	41	–
Thatcher	61	–	57	–
RL6077	29	–	20	–
RL6058	26	–	25	–
Saar	8	–	3	–
+*Lr67*RILs[b]	23A[c]	5–42	16A	0–33
−*Lr67* RILs	43B	16–76	36B	11–75
SEG RILs	34C	15–67	27C	11–46

[a] Powdery mildew severity based on the modified Cobb Scale (Peterson et al. 1948)

[b] Homozygous susceptible (−*Lr67*), homozygous resistant (+*Lr67*) and segregating (SEG) for *Lr67/Yr46* based on previous phenotypic assessments (Herrera-Foessel et al. 2011)

[c] Means with different letters in the same column within each location are significantly different based on paired *t* test ($P < 0.05$)

At both Ås and Hamar, the presence and absence of LTN concurred with that of *Lr67* (Herrera-Foessel et al. 2011). Just one exception was found at each site for non-*Lr67* RILs (1/70 and 1/74 RILs at Ås and Hamar, respectively) where LTN was scored as being present instead of absent. Similarly, only one and three RILs out of 62 and 66 *Lr67*-carrying RILs, at Ås and Hamar, respectively, were scored as not having LTN; the remaining were scored as having LTN.

Discussion

The Thatcher-derived wheat line RL6077, which possesses *Lr67/Yr46*, was shown in earlier studies to have reduced stem rust severity compared with Thatcher in Canada and Kenya (Dyck et al. 1994; Herrera-Foessel et al. 2011). Our study confirmed the effect of *Lr67/Yr46* on stem

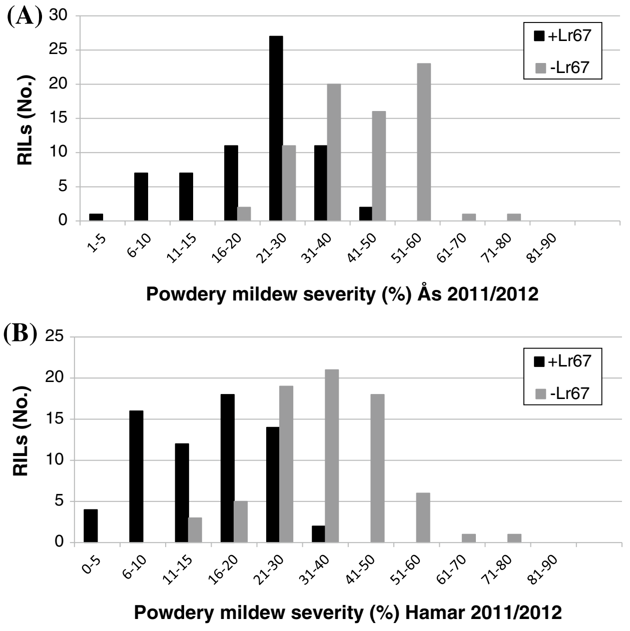

Fig. 2 Distribution of *Lr67/Yr46* homozygous susceptible (−*Lr67*) and homozygous resistant (+*Lr67*) Avocet/RL6077 RILs based on the final evaluation with *Bgt* across two crop cycles (2011/2012) at two Norwegian sites; Ås (**a**) and Hamar (**b**)

rust and powdery mildew resistance using RILs from the Avocet/RL6077 population previously used to map *Lr67/Yr46* to 4DL. *Lr67/Yr46* reduced mean stem rust severity by 41 % in Mexico with *Pgt* race RTR, whereas a lower but significant stem rust reduction of 16 % was obtained in Kenya with race TTKST. An average reduction in powdery mildew severity of 20 % was obtained for lines carrying *Lr67/Yr46* at the Norwegian sites Ås and Hamar. This study also provides evidence of a third locus for post-flowering LTN associated with APR. We hereby demonstrate that *Lr67/Yr46* contributes to stem rust and powdery mildew resistance and is associated with LTN. The corresponding genes were, therefore, designated as *Sr55*, *Pm46,* and *Ltn3*, respectively.

In Kenya, the early presence of yellow rust affected stem rust development; nevertheless, a significant correlation for stem rust resistance was obtained between the Mexican and Kenyan sites. Even though the Mexican *Pgt* races are avirulent to many more seedling resistance genes than TTKST, Cd. Obregon was shown to be a useful site for stem rust evaluations, in particular for slow rusting resistance, since yellow rust is normally not present and leaf rust, if not inoculated in spreaders, comes late in early sown material.

Cloning of *Lr34* confirmed that the same gene (and not tightly linked genes) controlled *Ltn1* (Krattinger et al. 2009). This was further supported by induction of an equivalent response in transgenic *Lr34* wheat (Risk et al. 2012). It is suggested that *Lr34* may be involved in the export of a senescence-related compound that stimulates senescence like-processes in the flag leaf tips and edges (Krattinger et al. 2009; Risk et al. 2012). The few lines found in this study showing differences between *Ltn3* assessed in Norway and the presence of *Lr67*, based on previous assessments in Mexico (Herrera-Foessel et al. 2011), are most likely due to environmental effects and the difficulty in assessing this trait. A phenotypic error during the evaluation (such as seed mix) cannot be excluded. Cloning this gene will ultimately confirm whether the same gene is responsible for both traits. Deletion mutants can also be highly valuable for assessing this association. LTN is useful as a morphological marker to select for partial resistance to multiple pathogens in breeding programs.

Additional genes of minor effect for resistance to both stem rust and powdery mildew were segregating in the Avocet/RL6077 RIL population. In previous studies, Avocet has been shown to contribute powdery mildew resistance at a locus on 4BL that also has a minor effect on leaf rust and yellow rust resistance (Lillemo et al. 2008). The same QTL was also reported for stem rust resistance in a recent study (Njau et al. 2013). Another minor QTL from Avocet on chromosome 5A was also recently reported to enhance stem rust resistance (Njau et al. 2013), as well as leaf rust resistance (Rosewarne et al. 2012). Additional loci contributing to stem rust resistance could have partly originated from Thatcher. At least two slow rusting stem rust resistance genes have been shown to contribute to durable resistance in Thatcher (Nazareno and Roelfs 1981; Kolmer et al. 1991), and since the durum wheat Iumillo is the origin of slow rusting stem rust resistance genes in Thatcher (Nazareno and Roelfs 1981), genes are expected to be located in the A or B genome. Brennan (1975) identified chromosomes 6A and 2B to be associated with a reduction in stem rust pustules in Thatcher. Gavin Vanegas et al. (2007) and Kolmer et al. (2011) identified a QTL on chromosome arm 2BL in Thatcher that was enhanced by the presence of *Lr34/Yr18/Pm38*. Significant interactions were found between a marker in the same region on 2BL, and at the *Lr34/Yr18/Yr38* locus, in an association mapping study using advanced breeding lines from CIMMYT (Yu et al. 2011).

Continuous evolution of the stem rust pathogen to new variants of Ug99 shows the vulnerability of deploying race-specific resistance genes. The pleiotropic mechanism of slow rusting genes such as *Lr67/Yr46/Sr55/Pm46/Ltn3,* or the previously described *Sr2/Yr30/Lr27/Pbc1*, *Lr34/Yr18/Pm38/Ltn1*, and *Lr46/Yr29/Sr58/Pm39/Ltn2,* makes them very useful not only in enhancing durable resistance but also in reducing vulnerability to more than one disease. Even if the effect of these slow rusting genes is minor, they do play an important role in gene combinations and interactions with additional genes with additive effects resulting in high levels of durable resistance. The similarities between the four above-mentioned slow rusting resistance genes suggest that they could share common defense mechanisms at the molecular level. Cloning *Lr34/Yr18* showed that it differed from other classes of plant resistance genes (Krattinger et al. 2009; Lagudah 2011). Unlike *Lr34,* where its occurrence in wheat germplasm has been validated using tightly linked or gene-based markers (Kolmer et al. 2008; Lagudah et al. 2009), very little is known about the presence of *Lr67* in wheat cultivars. Some of CIMMYT's germplasm exhibits strong LTN, no effective leaf rust seedling resistance, but APR for leaf rust. These lines do not possess *Lr34* (Lagudah et al. 2009) and may, therefore, be carriers of *Lr67.* Some of these genotypes include the old, tall Indian cultivars NP876 and Sujata, where a significant association was found between *Lr67/Yr46* linked markers and leaf rust and yellow rust resistance (Lan et al. 2013). Ultimately, the cloning of *Lr67* will be decisive in ascertaining its prevalence, or lack thereof, in bread wheat cultivars. Cloning these multi-pathogen resistance genes and their molecular characterization may provide insights into whether they share common mechanisms, as well as lay the foundations for developing diagnostic markers that can assist breeding.

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Conflict of interest The authors declare that they have no conflict of interest.

Ethical standard The experiments comply with the current laws of the country in which they were performed.

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