RESISTANCE TO LEAF RUST IN A SET OF DURUM WHEAT CULTIVARS AND LANDRACES IN SPAIN

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SUMMARY

Durum wheat (*Triticum turgidum* var. *durum*) is an important crop in the Mediterranean basin, and in southern Spain. One of the most important biotic constraints to durum wheat production is leaf rust. Breeder deployed resistant cultivars, but mutations in the pathogen create new virulent pathotypes that erode resistance. In this work, two collections of genotypes have been characterized for their leaf rust resistance both in field trials (in Spain and Mexico) and in the greenhouse (with an array of Spanish pathotypes). The first collection included 14 cultivars and lines, mostly from CIMMYT origin, plus six genotypes with known resistance genes. The second collection comprised 29 cultivars commercially available in Spain, plus 11 landraces from the Spanish germplasm bank. Besides, six resistant cultivars of particular importance were crossed with two cultivars with known effective genes (*Lr14a* and *Lr27+Lr31*), and a susceptible one to infer the genetic basis of their resistance. F_2 populations were analyzed. At adult plant stage, 20 genotypes including four landraces were resistant in greenhouse to all Spanish pathotypes. In field trials, 24 genotypes were resistant in Spain, and 22 genotypes in Mexico. The presence of resistant genes *Lr14a* and the complementary genes *Lr27+Lr31* has been deduced in some cultivars either by gene postulation or by genetic analysis. These results provide an opportunity for breeding programs in Spain and elsewhere to target their crossing and selection activities so they can yield lines with effective resistance in the Mediterranean region.

Key words: *Puccinia triticina*, resistance genes, crosses, *Triticum turgidum* var. *durum*.

INTRODUCTION

Durum wheat (*Triticum turgidum* var. *durum*) is an important cereal crop in the Mediterranean basin. The sustainability of its production is challenged by several biotic factors, such as leaf rust, caused by the fungus *Puccinia triticina*, that may provoke important yield losses. Susceptible cultivars may loose up to 33% of their potential yield in southern Spain (Cátedra, 2004), and up to 51% in Mexico under high disease pressure (Herrera-Foessel *et al.*, 2006). Cultivars with race-specific resistance such as Altar C84 remained resistant for nearly 20 years in Mexico, but became highly susceptible to a new pathotype designated as BBG/BN that appeared in 2001. This pathotype caused estimated yield losses of about 32 million US$ in three seasons in Mexico (Singh *et al.*, 2004). In Spain, leaf rust was reported on cultivars previously deemed resistant such as Gallareta (Altar C84 sister line), Don Pedro, and Simeto (RAEA, 1997). Six years later, with conditions favorable to natural rust epidemics (2003-2004), most cultivars sown in Spain proved susceptible to the prevailing pathotype(s) of *P. triticina*. Only a few cultivars such as Colosseo and Italo remained completely resistant.

Newer CIMMYT-derived cultivars with resistance to the Spanish pathotype(s), namely Don Jaime, Don Ricardo, Don Javier, and Don Valentín were released in southern Spain starting in 2005 (Agrovegetal, personal communication). The resistance genes in those cultivars are unknown and non-characterized.

Although there are several strategies to control wheat leaf rust, such as fungicide applications, plant resistance is the most viable approach for reducing yield losses. Hypersensitive resistance, controlled by major genes, is very effective to reduce the epidemic build-up. Many of the known resistance genes originated from common wheat and wild relatives (McIntosh *et al.*, 1995), and until recently, the information available specifically for durum wheat was much less than that available for common wheat.

In a study on eight durum wheat cultivars, Zhang and Knott (1990) reported the presence of different resistance genes from the known *Lr* genes. Gene *Lr14a*, coming from emmer, has been mapped to chromosome 7BL in durum line Somateria and cv. Llareta (Herrera-Foessel *et al.*, 2008a). Gene *Lr23* was transferred to common wheat from durum wheat cv. Gaza and mapped to the chromosome 2BS (McIntosh and Dyck, 1975). Genes *Lr3* and *LrCam* were mapped to the chromosome 6BL in durum line Storlom and cv. Camayo C2008, respectively (Herrera-Foessel *et al.*, 2007). Research conducted at CIMMYT has revealed the presence of the complementary genes *Lr27+Lr31* in

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the durum wheat cv. Jupare (Herrera-Foessel *et al.*, 2005). *Lr61* (from cv. Guayacán) is another new *R*-gene characterized at CIMMYT (Herrera-Foessel *et al.*, 2008b).

Knowledge of the identity and effectiveness of leaf rust resistance genes is essential for the incorporation of new such genes in durum wheat cultivars, and maintenance of a diversity of resistance genes in durum wheat.

In the present study, two different collections of durum wheat cultivars with confirmed or potential adaptation to southern Spain were characterized in the greenhouse for their seedling and adult plant reactions to 76 leaf rust isolates from Spain. The two collections were also tested in the field in Spain and Mexico, using either natural infection or single-isolate for artificial inoculation. Besides, six of the most important new resistant cultivars were crossed with two durum wheat cultivars with known *R*-genes, and a susceptible cultivar to determine the genetic basis of the resistance. The objective of the work was to characterize the leaf rust resistance genes in durum wheat cultivars and landraces grown in Spain and to determine the genetic basis of the resistance of these six cultivars.

MATERIALS AND METHODS

Plant material. The first collection (collection 1) comprised six genotypes with known resistance genes: Somateria (*Lr14a*), Llareta (*Lr14a*), Jupare (*Lr27+Lr31*), Storlom (*Lr3*), Guayacán INIA (*Lr61*), and Camayo (*LrCam*). Besides, 11 CIMMYT-derived cultivars/breeding lines, the Italian cv. Colosseo (*Lr14a*) and the line TDA725S (susceptible Don Patricio sister line) were also included (Table 1). The Spanish cv. Don Rafael was used as a susceptible control in greenhouse and field experiments.

The second collection (collection 2) included 29 commercial cultivars tested in Andalusia (southern Spain) by RAEA. Moreover 10 Spanish landraces previously recorded as resistant to leaf rust, and Senatore Cappelli, an obsolete Italian cv., were requested to the Centro de Recursos Fitogenéticos (CRF) or Spanish gene bank (Table 2).

Five resistant cultivars were selected for crossing (Don Jaime, Don Javier, Don Juan, Don Ricardo, Don Valentín). These cultivars (from CIMMYT origin) are new to Spain and the genetic basis of their resistance is unknown and non characterized. Cv. Colosseo from Italy was also included. These cultivars were crossed with cultivars with the two most deployed genes (or groups of genes) in the resistance to durum leaf rust: Somateria (*Lr14a*), and Jupare (*Lr27+Lr31*). Crosses were performed between resistant cultivars to check whether they possess the same *R*-gene (allelism test). Furthermore, crosses of the resistant cultivars (cv. Don Juan was not included in this case) with the susceptible cv. Gallareta were performed to deduce the genetic basis of the resistance. F_1 plants were harvested individually to obtain F_2 progenies of about 100 plants each.

Fungal material. A set of 76 isolates collected from different Spanish locations were available, which were named according to a standard nomenclature (Singh *et al.*, 2004), with some modifications. The line Thatcher with the gene *Lr19* was replaced by a line containing *Lr14a*. The 19 resistance genes were grouped into five sets as follows: (1) *Lr1*, *Lr2a*, *Lr2c*, *Lr3*; (2) *Lr9*, *Lr16*, *Lr24*, *Lr26;* (3) *Lr3ka*, *Lr11*, *Lr17*, *Lr30;* (4) *Lr3bg*, *Lr13*, *Lr15*, *Lr18*; and (5) *Lr10*,

Table 1. Relative field disease severity (RFDS) of leaf rust observed in collection 1 from 2006 to 2011 at two locations in Southern Spain.

Genotype	Jerez (FDS)						Conil (FDS)			
	2006	2007	2008	2009	2011	Mean[3]	2006	2008	2011	Mean
Don Rafael	100S (90)[1]	100S (25)	100S (70)	100S (55)	100S (15)	100a	100S (20)	100S (35)	100S (25)	100 a
Gallareta	100S[2]	40MS	93S	82S	147S	92ab	71S	94S	75S	80 a
Don Sebastián	83S	80S	100S	73S	167S	101a	71S	100S	100S	91 a
Yavaros	89S	80S	100S	64MS	47MS	76abc	71MS	134S	75MS	94 a
Don José	83MS	40MS	79MS	73MS	53MS	66c	114S	68MS	60MS	87 a
Avispa	89S	40MS	100S	64MS	67MS	72bc	86MS	129S	90S	101 a
TDA725 S	100S	60MS	93S	55MS	80S	78abc	71S	114S	75S	87 a
Don Patricio	1MR	0R	6MR	0R	0R	1d	36MR	0R	5R	14 b
Don Jaime	0R	0R	4MR	0R	0R	1d	7R	0R	0R	2 b
Don Juan	0R	0R	3MR	0R	0R	1d	7R	0R	0R	2 b
Don Ricardo	0R	0R	6MR	0R	0R	1d	14R	0R	5R	6 b
Don Javier	0R	0R	6MR	0R	0R	1d	0R	0R	5MR	2 b
Don Valentín	0R	0R	6MR	0R	0R	1d	0R	0R	0R	0 b
Colosseo	0R	0R	3MR	0R	0R	1d	0R	0R	5R	2 b

[1] RFDS (percentage) was calculated using *cv.* Don Rafael as reference for each location and year. Actual disease severity for Don Rafael is shown between parentheses.

[2] Host infection response R= resistant; MR = moderately resistant; MS = moderately susceptible; and S = susceptible.

[3] Per column, data with the same setter are not statistically different (Duncan, $p < 0.05$).

Table 2. Virulence of 76 leaf rust isolates, field disease severity at Seville and at El Batán fields (Mexico) with two different pathotypes, and presence of a SSR marker linked to *Lr14a* gene in cultivars of collection 1 and 2.

Genotype	No. virulent isolates (seedling)	No. virulent isolates (adult plant)	FDS[1] (Spain)	FDS[1] (Mexico)	SSR linked to *Lr14a* gene
Don Rafael	73	73	90 S[2]	90 S	
Gallareta*	73	73	95 S	70 S	
Don Sebastián	73	73	65 S	70 S	
Yavaros	74	73	40 MS	45 MS	
Don José	74	73	45 MS	50 MS	
Avispa	73	73	65 S	70S	
TDA725 S	73	73	65 S	60S	X
Don Patricio	1	0	1 R	2R	X
Don Jaime*	3	0	1 R	0R	X
Don Juan*	1	1	70 S	0R	
Don Ricardo*	0	0	1 R	70MS	
Don Javier*	9	6	2 R	5R	
Don Valentín*	1	0	1 R	70MS	
Colosseo (*Lr14a*)*	0	0	0 R	0	X
Somateria (*Lr14a*)*	1	0	2 R	0	X
Llareta INIA (*Lr14a*)	19	1	1 R	0	X
Jupare (*Lr27+Lr31*)*	0	0	4 R	70MS	
Guayacán INIA (*Lr61*)	0	0	0 R	0	
Storlom (*Lr3*)	0	0	0 R	5R	
Camayo (*LrCam*)	0	0	1 R	20MR	
Ancalei[3]	1	0	1R	70S	
Calero	1	1	35MS	2R	
Canciller	73	73	65MS	55MS	
Cántico	76	76	55MS	60MS	
Carioca	74	76	55S	50MS	
Carpio	1	1	55MS	2R	
Catervo	73	73	65MS	0	
Core	73	73	60S	60MS	
Don Pedro	76	76	60S	70S	
Duratec	73	73	70S	55MS	
Duroflavus	73	73	45MS	50MS	
Estopa	73	73	55S	65S	
Grecale	2	0	5R	20MR	
Ismur	1	0	1R	0	X
Italo	0	0	0	0	X
Kanakis	1	0	5R	60MS	
Kiko Nick	72	72	60S	55MS	
Licinius	0	0	0	0	X
Miradoux	1	1	3R	5R	X
Pharaon	2	0	0	2R	X
Próspero	3	0	0	0	
Ramírez	2	0	1R	40MS	
Saragolla	0	0	0	15MR	
Sculptur	1	1	2R	0	X
Severo	71	71	75S	70S	
Simeto	74	73	70S	60S	
Vitronero	0	73	80S	80S	
Vitrosol	74	73	70S	70S	
Vivadur	69	70	55S	40MS	
Senatore Capelli	73	73	70S	65S	
BGE002881[4]	3	0	6R	--	
BGE013068	72	72	6R	--	
BGE013084	4	5	6R	--	
BGE013102	14	10	13S	--	
BGE013587	1	0	6R	--	
BGE013652	0	0	6R	--	
BGE013678	67	67	6R	--	
BGE018600	76	74	6S	--	
BGE018630	0	0	13R	--	
BGE018641	1	1	25R	--	

[1] Artificial inoculation was performed in both fields. Pathotype DGB/BN was employed at Seville site (Spain), and pathotype BBG/BP at El Batán (Mexico).

[2] S represents a susceptible reaction and R a resistant reaction (hypersensitive response). Those reactions were assessed in the greenhouse at fifth leaf stage in Spain, and the field in Mexico.

[3] The line above separates collection 1 and 2. (*) shows cultivars used as parental genotypes in the crossing experiment.

[4] FDS of Spanish landraces were taken from Martínez (2002), data not published.

Lr14a, *Lr23* and cv. Jupare with the complementary genes *Lr27+Lr31*.

Pathotype DGB/BN collected in 2003 from Cadiz was selected for artificial field inoculation at Seville (Spain). This pathotype was virulent on *Lr2c*, *Lr10*, *Lr14b*, *Lr16*, *Lr20*, *Lr23*, *Lr33*, *Lr34*, and *LrB*. In the other Spanish field trials (Jerez and Conil), natural infection of leaf rust was sufficient to provide a good source of inoculum and reliable cultivar differentiation. A pathotype from Mexico, designated as BBG/BP (Huerta-Espino *et al*., 2009), was used to inoculate the two collections (without the landraces) for studying leaf rust responses at adult plant stage in Mexico during the 2012 crop season. This pathotype was virulent on Jupare (*Lr27 + Lr31*).

Inoculation of plants derived from the crosses was performed using the Spanish pathotype DBB/CN, and the Mexican pathotype BBG/BP, which was used for field inoculations and evaluations in Mexico.

Greenhouse tests. Both collections were evaluated in the greenhouse for seedling and fifth leaf reaction against 76 Spanish isolates of *P. triticina*. Plants were grown in soil trays (60 × 40 × 10 cm). Six seeds of each cultivar were sown to obtain four plants, in one replication. Twenty cultivars were sown in a tray. Twelve days after sowing, plants were inoculated at seedling stage at growing stage DC 11 (Zadoks *et al*., 1974). Primary leaves of plants were fixed on the soil surface in a horizontal position by metallic clips, in order to perform a uniform inoculation. Each tray was inoculated with 6 mg of urediniospores mixed with talcum powder (1 : 50). The mixture was blown over the plants, which were then incubated in a dew chamber overnight at 20ºC, and 100% relative humidity. The next morning, plants were transferred to a greenhouse compartment at 20-25ºC. Twelve days after inoculation, plants were evaluated. Infection type (IT) was scored using the 0-4 scale described by Stakman *et al.* (1962). IT 3 and 4 indicated a compatible reaction between pathogen and cultivars, whereas IT 0, 1 and 2 meant an incompatible reaction. Inoculation at the adult plant stage was performed when the plants reached the 5th leaf stage (DC 15). All leaves, except the fully extended last one were excised in order to inoculate only the fifth leaf of each plant. Inoculation and incubation were as described above.

The parental lines of the crosses, the F_1 plants, and the F_2 populations, were sown in soil trays and were inoculated at the fifth leaf stage with 25 mg of spores mixed with talcum powder in the proportion described above. The mixture was distributed on the leaves with a paintbrush. Incubation and IT evaluation were performed as previously mentioned.

Field trials. Collection 1 was evaluated in Spain under field conditions and natural infection in 2006, 2007, 2008, 2009, and 2011 at Jerez, and in 2006, 2008, and 2011 at Conil, while the trials in Seville (Spain) and Mexico received artificial inoculum. Trials in Jerez and Conil consisted of six-row plots (5 × 1.2 m) with 13 cultivars and the susceptible control Don Rafael in three replications (equilibrated square lattice). Seville trials were sown in 2008, 2009 and 2010 crop seasons in four-row plots (2 × 1.2 m) in three replications (random blocks). Just before flag leaf emergence, leaf rust-infected pots from the greenhouse (inoculated with the Spanish pathotype DGB/BN) were evenly distributed on the borders of the field, and between replications and plots.

The two collections were sown and artificially inoculated also in Mexico. Each cultivar was sown in one single row and replication (1.20 m) for one season. Differences between the experimental design in Mexico and Spain were due to the land area that was available in each location. The border was planted with cv. Jupare, and inoculated with the pathotype designated as BBG/BP. Leaf rust severity and resistance responses were recorded when the susceptible check cv. Don Rafael had a field severity of at least 70-80% in mid-August in 2011 and 2012 at El Batán (Mexico), and in mid-April at Seville (Spain) during three years. Field disease severity and response rating for the adult plant field resistance was based on the modified Cobb scale (Peterson *et al*., 1948). The host infection responses was rated as R = resistant, very small uredinia with necrosis; MR = moderately resistant, small to moderate uredinia with necrosis; MS = moderately susceptible, small to moderate uredinia with chlorosis; and S = susceptible, large uredinia without necrosis or chlorosis.

Field evaluations of a second set of F_2 and F_3 populations of the same material were conducted at El Batán (Mexico) during summer 2012 to confirm the results obtained in the greenhouse. F_2 populations were sown 5-10 cm apart, on one pair of rows with 5-7 m in length. This allowed the evaluation of 91 to 227 F_2 single plants from each cross, and 86 to 133 F_3 families. The Mexican pathotype BBG/BP was used to inoculate four rows of plants of cv. Jupare, sown around the experiment to serve as urediniospore spreader. Leaf rust epidemics were initiated by artificial inoculations of susceptible spreader rows using urediospores suspended in mineral oil 'Soltrol 170'. The infection type of the parents, and F_2 populations was recorded three times in September, when the susceptible parent cv. Jupare had a disease severity of 70%, based on the modified Cobb scale (Peterson *et al*., 1948). F_3 families were classified as homozygous resistant (HR), segregating (Seg), or homozygous susceptible (HS) based on the infection type reaction of the progeny. The comparison between the observed phenotypic distributions of segregating in the F_2 and F_3 and the expected genetic ratio for a certain number of gene(s) and gene action was checked using a Chi-square (χ^2) test at the 5% probability level. The expected segregations (resistant : susceptible) for F_2 plants from R×S crosses were: 3 : 1, a dominant *R*-gene; 9 : 7, two complementary dominant *R*-genes. The expected segregations for F_2 plants from R × R were: 1:0, same *R*-gen; 15:1,

different dominant *R*-genes; 57 : 7, two complementary dominant *R*-genes plus one different dominant *R*-gene.

Marker-assisted determination of the presence of Lr14a. DNA extraction was carried out with the CTAB method of Saghai-Maroof *et al.* (1984) modified according to CIMMYTs manual of laboratory protocols. Twenty seeds per genotype were grown in the greenhouse and, after two weeks, bulks of young leaves from 10 plants per genotype were harvested for DNA extraction.

SSR markers *Xgwm344* and *Xgwm146* were used to detect the presence of the *Lr14a* gene according to Herrera-Foessel *et al.* (2008a). PCR-reactions for both markers were carried out with an Applied Biosystem 9700 PCR systems. Each 10 µl reaction mixture contained 25 ng template DNA, 150 nM of each primer, 250 µM dNTPs, 200 mM MgCl2, 1× PCR buffer and 2.5 U *Taq*-polymerase. PCR was carried out with the following standard temperature profile: 30 cycles with a 0.5 min denaturing step at 94°C, 1 min annealing temperatures at 55°C, and 1 min extension at 72°C. Amplification products were separated by electrophoresis in 2.5% agarose gels and visualized by means of ethidium bromide and UV light.

RESULTS

Resistance to leaf rust in the two durum wheat collections. The results of field trials conducted with collection 1 at two locations under natural epidemics are shown in Table 1. Seven cultivars out of 13 were consistently resistant across the two locations and years. Only cv. Don Patricio was moderately resistant during 2006 crop season in Conil while it was highly resistant in the rest of locations and years.

In Table 2 the percentage of virulent isolates on each cultivar of both collections is presented at both plant stages. Moreover, the field severity in Seville (Spain) and in El Batán (Mexico) was also displayed. The cultivars resistant in the field were also resistant to most isolates tested. The Mexican pathotype was virulent on some cultivars that displayed low IT in the experiments done in Spain, such as Don Ricardo, Don Valentín, Ancalei, Kanakis, Ramírez, and Jupare. Two SSR markers, *Xgwm344* and *Xgwm146*, linked to *Lr14a* gene were present in cvs Don Patricio, Don Jaime, Colosseo, Somateria, Llareta, and TDA 725S. Cvs Yavaros and Don Jose displayed a high IT in greenhouse to most isolates but a moderate field severity in both Spain and Mexico respect to the other susceptible cultivars.

Fourteen genotypes out of 40 from collection 2, including four landraces, were resistant in the field in Spain and in the greenhouse to all isolates. Twelve out of 30 cultivars were resistant when this collection was sown in Mexico. Cvs Ancalei, Kanakis and Ramirez were susceptible to the Mexican pathotype, but resistant to all the Spanish isolates. The SSR marker *Xgwm344* and *Xgwm146*, linked to *Lr14a*, was present in six cultivars (Ismur, Italo, Licinius, Miradoux, Pharaon, and Sculptur).

Sixteen different patterns of resistance were observed among the 60 genotypes analyzed when inoculated with eight representative pathotypes (Table 3). The most frequent pattern, *Co* from cv. Colosseo, was resistant to all pathotypes and present in 18 cultivars. Five cultivars had the same pattern as Jupare, with resistance to all Spanish pathotypes, and susceptible to the Mexican one. Three cultivars had the pattern *Lla*, from Llareta, that was similar to *Co* but with susceptibility to one pathotype (DBB/CS). A different pattern, only susceptible to DGB/BN, named *Ca*, from Carpio was found in three cultivars. The pattern *Ra* from cv. Don Rafael, susceptible to all pathotypes except one designated as PBC/PS, was observed in 17 cultivars. Three cultivars that had the pattern *Pe* (from cv. Don Pedro) displayed a susceptible reaction to all pathotypes.

Cvs Catervo, Severo and Vivadur formed patterns of their own. It is remarkable that Spanish landraces displayed also five different and unique patterns (*B*, *C*, *D*, *E*, and *F*) making a total of seven patterns for the 10 landraces.

All studied genotypes were resistant to pathotype PBC/PS, except for cvs Cantico, Carioca, Don Pedro, and four landraces. Interestingly, cv. Vitronero showed a low IT at the seedling stage when inoculated with the 73 isolates, whereas it displayed a high disease severity to these isolates in the field and a high IT at the adult plant stage in the greenhouse.

Segregation in F2 and F3 populations from resistant × resistant and resistant × susceptible crosses. The IT in the greenhouse and the field response of the parents inoculated with two pathotypes of *P. triticina* from Spain and Mexico are presented in the Table 4.

In Spain, the F_2 plants originated from crosses of cvs Don Valentín and Don Ricardo with Gallareta segregated according to a 9 : 7 ratio, indicating that a two dominant complementary genes conferred resistance to the Spanish pathotype (Table 4). However, in Mexico, all F_2 plants from crosses of cvs Don Valentín and Don Ricardo with Gallareta showed a high infection type in the field without segregation.

F_2 plants from crosses of cvs Don Jaime and Colosseo with Gallareta segregated to a 3 : 1 ratio, which is expected for a segregation of one dominant gene. In the cross with cv. Don Javier, a greater proportion of susceptible F_2 plants was recorded, but still it fitted to the proportion expected for a single dominant gene. In Mexico, F_2 plants from crosses of cvs Don Jaime, Don Javier, and Colosseo with the susceptible parent Gallareta showed segregation for a single dominant resistant gene.

The distributions of F_3 families in crosses involving cvs Don Jaime, Colosseo, or Don Javier with the susceptible parent Gallareta were in agreement with the results of F_2, with a 1 : 2 : 1 ratio. The F_3 lines from crosses involving cvs

Table 3. Reaction of 60 durum wheat cultivars tested with eight pathotypes of *P. triticina* from Spain and Mexico at adult plant stage.

Genotype	Virulence pattern/	DGB/BN	DBB/BN	DBB/DN	DBB/CS	PBC/PS	DBB/FN	DBB/CN	BBG/BP[2]
	Postulated gene	*Lr2c, 10, 14b, 16, 20, 23, 33, 34, B*	*Lr2c, 10, 14b, 20, 23, 33, 34, B*	*Lr2C, 10, 14b, 15, 20, 23, 33, 34, B*	*Lr2c, 10, 14a,14b,18, 23, 33, 34, B*	*Lr1, 2c, 3, 3bg, 10, 14a, 14b,15, 18, 20, 23, 30, 33, 34, B*	*Lr2c, 10, 14b, 15, 18, 20, 23, 33, 34, B*	*Lr2c, 10, 14b,18,23, 33, 34, B*	*Lr10, 11, 12, 14b, 20, 23, 27+31, 33*
Colosseo*	*Co/ Lr14a*[1]	R	R	R	R	R	R	R	R
Don Patricio**	*Co/ Lr14a*	R	R	R	R	R	R	R	R
Don Jaime**	*Co/ Lr14a*	R	R	R	R	R	R	R	R
Somateria*	*Co/ Lr14a*	R	R	R	R	R	R	R	R
Pharaon**	*Co/ Lr14a*	R	R	R	R	R	R	R	R
Italo**	*Co/Lr14a*	R	R	R	R	R	R	R	R
Licinius**	*Co/Lr14a*	R	R	R	R	R	R	R	R
Ismur**	*Co/Lr14a*	R	R	R	R	R	R	R	R
Storlom*	*Co/ Lr3*	R	R	R	R	R	R	R	R
Guayacán*	*Co/ Lr61*	R	R	R	R	R	R	R	R
Camayo*	*Co/ LrCam*	R	R	R	R	R	R	R	R
Saragolla	*Co*	R	R	R	R	R	R	R	R
BGE002881	*Co*	R	R	R	R	R	R	R	-
BGE013587	*Co*	R	R	R	R	R	R	R	-
BGE013652	*Co*	R	R	R	R	R	R	R	-
BGE018630	*Co*	R	R	R	R	R	R	R	-
Grecale	*Co*	R	R	R	R	R	R	R	R
Próspero	*Co*	R	R	R	R	R	R	R	R
Don Ricardo**	*Jup/ Lr27+Lr31*	R	R	R	R	R	R	R	S
Don Valentín**	*Jup/ Lr27+Lr31*	R	R	R	R	R	R	R	S
Jupare*	*Jup/ Lr27+Lr31*	R	R	R	R	R	R	R	S
Ancalei**	*Jup/ Lr27+Lr31*	R	R	R	R	R	R	R	S
Kanakis**	*Jup/ Lr27+Lr31*	R	R	R	R	R	R	R	S
Ramírez**	*Jup/ Lr27+Lr31*	R	R	R	R	R	R	R	S
Don Javier	*Jav*	R	R	R	R	R	R	S	R
Llareta*	*Lla/ Lr14a*	R	R	R	S	R	R	R	R
Miradoux**	*Lla/ Lr14a*	R	R	R	S	R	R	R	R
Sculptur**	*Lla/ Lr14a*	R	R	R	S	R	R	R	R
Don Juan**	*Ca/Lr16*	S	R	R	R	R	R	R	R
Calero	*Ca*	S	R	R	R	R	R	R	R
Carpio	*Ca*	S	R	R	R	R	R	R	R
BGE018641	*F*	R	R	R	R	S	R	R	-
BGE013084	*C*	R	R	S	R	S	R	R	-
BGE013678	*E*	R	S	R	R	S	R	S	-
BGE013102	*D*	S	R	R	S	S	S	R	-
Vivadur	*Vi*	R	S	R	S	R	S	S	S
BGE013068	*B*	S	S	S	R	R	S	S	-
Kiko Nick	*Ki*	R	S	S	S	R	S	S	S
Severo	*Se*	S	S	R	S	R	S	S	S
Catervo	*Cat*	S	S	S	S	R	S	S	R
Don Rafael	*Ra*	S	S	S	S	R	S	S	S
Gallareta*	Ra/Lr_{Altar}	S	S	S	S	R	S	S	S
D. Sebastián	*Ra*	S	S	S	S	R	S	S	S
Yavaros	*Ra*	S	S	S	S	R	S	S	S
Don José	*Ra*	S	S	S	S	R	S	S	S
Avispa	*Ra*	S	S	S	S	R	S	S	S
TDA725 S	*Ra*	S	S	S	S	R	S	S	S
Canciller	*Ra*	S	S	S	S	R	S	S	S
Core	*Ra*	S	S	S	S	R	S	S	S
Duratec	*Ra*	S	S	S	S	R	S	S	S
Duroflavus	*Ra*	S	S	S	S	R	S	S	S
Estopa	*Ra*	S	S	S	S	R	S	S	S
Simeto	*Ra*	S	S	S	S	R	S	S	S
Vitronero	*Ra*	S	S	S	S	R	S	S	S
Vitrosol	*Ra*	S	S	S	S	R	S	S	S
Sen. Cappelli	*Ra*	S	S	S	S	R	S	S	S
BGE018600	*Ra*	S	S	S	S	R	S	S	-
Cántico	*Pe*	S	S	S	S	S	S	S	S
Carioca	*Pe*	S	S	S	S	S	S	S	S
Don Pedro	*Pe*	S	S	S	S	S	S	S	S
Thatcher		S	S	S	S	S	S	S	S

[1] Cultivars with known genes, either from the literature(*) or postulated by the present work(**).

[2] Mexican pathotype, after Huerta-Espino *et al.* (2009).

Don Valentín and Don Ricardo with Gallareta were not evaluated in Mexico.

The results of the crosses between resistant cultivars performed in Spain and Mexico are displayed in Table 5. In Spain, all F_2 plants from the crosses Jupare × Don Valentín and Jupare × Don Ricardo displayed a low IT. The proportions of F_2 plants from crosses involving cvs Don Jaime, Colosseo, Don Javier, and Don Juan with Jupare

Table 4. IT of the parents, the F_1, and frequencies of F_2 (resistant, R, and susceptible, S), and F_3 plants (homozygous resistant, HR, segregating, Seg, and homozygous susceptible, HS) from crosses of five resistant cultivars with the susceptible cultivar Gallareta (♀).

Resistant parent	Parents Spain	F_1 Spain	Number of F_2 plants, Spain				Number of F_2 plants, Mexico				Number of F_3 lines, Mexico				
	IT[1]	IT	R	S	Exp. ratio	P[2] χ^2	R	S	Exp. ratio	P χ^2	HR[3]	Seg[3]	HS[3]	Exp. ratio	P χ^2
D. Jaime	X	X	135	44	3:1	0.90	154	47	3:1	0.60	33	63	26	1:2:1	0.63
D. Valentín	2	0;1	99	74	9:7	0.80	0	227	-	-	-	-	-	-	-
D. Javier	2	1	149	63	3:1	0.11	152	58	3:1	0.38	25	46	30	1:2:1	0.52
D. Ricardo	1	;	101	68	9:7	0.36	0	192	-	-	-	-	-	-	-
Colosseo	X	X	112	36	3:1	0.85	77	19	3:1	0.24	24	49	23	1:2:1	0.97

[1] Infection type response follow a 0-4 scale (Stakman *et al.*, 1962). Tests were performed in Spain with pathotype DBB/CN (greenhouse) and in Mexico with pathotype BBG/BP (field).

[2] P value of χ^2 test. Values above 0.05 indicate that the observed proportions fit the expected ratio.

Table 5. IT of F_1, and frequencies of F_2 (resistant, R, and susceptible, S), and F_3 plants (homozygous resistant, HR, segregating, Seg, and homozygous susceptible, HS) from crosses between resistant cultivars.

Resistant parents (♀×♂)	F_1 response in Spain	Number of F_2 in Spain				Number of F_2 plants in Mexico				Number of F_3 families in Mexico				
	IT[1]	R	S	Exp. ratio	P[2] χ^2	R	S	Exp. ratio	P χ^2	HR	Seg	HS	Exp. ratio	P χ^2
S×D.Jaime[3]	X	157	0	1:0	1	186	0	1:0	1	133	0	0		1
S×DValentín	0;	123	21	57:7	0.16	140	45	3:1	0.83	22	44	20	1:2:1	0.93
S×D. Javier	;X	141	7	15:1	0.45	176	17	15:1	0.14	49	55	5	7:8:1	0.77
S×D.Ricardo	0;	101	11	57:7	0.71	106	35	3:1	0.96	25	46	22	1:2:1	0.90
S×D. Juan	;	146	13	15:1	0.32	204	18	15:1	0.25	60	59	7	7:8:1	0.68
S×Colosseo	X	144	0	1:0	1	163	0	1:0	1	101	0	0	1:0:0	1
J×D. Jaime	0	117	16	57:7	0.69	117	39	3:1	1	20	51	26	1:2:1	0.61
J×DValentín	1	137	0	1:0	1	0	221	1:0	1	0	0	98	1:0:0	1
J×D. Javier	1	97	11	57:7	0.80	96	37	3:1	0.45	26	66	33	1:2:1	0.56
J×D. Ricardo	;1	151	0	1:0	1	0	142	1:0	1	96	0	0	1:0:0	1
J×D. Juan	0;	98	13	57:7	0.79	107	40	3:1	0.54	29	52	27	1:2:1	0.90
J×Colosseo	0	86	14	57:7	0.33	69	22	3:1	0.86	22	45	24	1:2:1	0.95

[1] Infection type response follow a 0-4 scale (Stakman *et al.*, 1962). Tests were performed in Spain with pathotype DBB/CN (greenhouse) and in Mexico with pathotype BBG/BP (field).

[2] P value of χ^2 test. Values above 0.05 indicate that the observed proportions fit the expected ratio.

[3] S = Somateria; J = Jupare.

fitted a ratio of 57:7, indicating the presence of three genes, one dominant and other two dominant complementary genes (from cv. Jupare). Similarly, the segregation of F_2 plants from the cross of Somateria with cvs Don Valentín or Don Ricardo fitted the same ratio. The segregation in F_2 populations from crosses of cvs Don Jaime or Colosseo with Somateria did not yield any susceptible plants. However, the F_2 populations from crosses involving cvs Don Javier and Don Juan with Somateria, segregated according to a 15:1 ratio, which is the ratio expected for two dominant resistance genes.

In Mexico, all the individuals from the F_2 and F_3 populations of the crosses involving cv. Jupare with Don Ricardo or Don Valentín displayed a high disease response, since the Mexican pathotype was virulent on *Lr27+Lr31*. The F_2 populations from crosses involving cv. Jupare with Don Jaime, Don Javier, Don Juan, or Colosseo segregated according to a 3:1 ratio. All the plants from the F_2 and F_3 populations from crosses involving Somateria with cvs Don Jaime or Colosseo showed a high resistance response. The frequencies of the resistant and the susceptible plants in the crosses involving Somateria with either cvs Don Valentín or Don Ricardo fitted a 3:1 ratio. F_2 populations from crosses involving Somateria with either cvs Don Javier or Don Juan segregated according to a 15:1 ratio as obtained in Spain. Distribution of F_3 lines in each cross was in agreement with the F_2 results.

Two SSR markers linked to *Lr14a*, *Xgwm146* and *Xgwm344*, were segregating in the F_2 populations of cvs Gallareta and Jupare with Colosseo. The frequencies of resistant and susceptible plants in these two crosses were in accordance with a 3:1 ratio (data not shown).

DISCUSSION

Until recently, leaf rust has not been considered a yield-limiting disease on durum wheat (Martínez *et al.*, 2007). But since the appearance of the new virulent pathotype in 2001 in Mexico (Singh *et al.*, 2004) works to characterize resistance genes to *P. triticina* in durum wheat are being carried out in Italy (Maccaferri *et al.*, 2010), France (Goyeau *et al.*, 2012), and Mexico (Herrera-Foessel *et al.*, 2005; Huerta-Espino *et al.*, 2010). There is no a standardized differential set of durum wheat cultivars, to be used for characterization of pathotypes and resistance gene postulation. The situation is different in common wheat where a set of differential of near-isogenic lines with different *Lr* genes in the same background (cv. Thatcher) are available and used worldwide.

In this work we have been provided with a set of durum wheat cultivars with known resistance genes from CIMMYT, and the discussion is largely focused on the presence of those genes in our material.

Cultivars Llareta and Somateria are both reported to possess *Lr14a* gene (Herrera-Foessel *et al.*, 2008a). This gene, located on the long arm of chromosome 7B, originated from emmer wheat Yaroslav (McFadden, 1930). *Lr14a* is a gene present in many current durum wheat cultivars worldwide (Herrera-Foessel *et al.*, 2008a; Goyeau *et al.*, 2010).

In this work, the presence of this gene has been postulated in the cvs Don Patricio, Don Jaime, Colosseo, Ismur, Italo, Licinius, Miradoux, Pharaon, and Sculptur, based on their virulence pattern. Moreover, the two SSR markers *Xgwm344* and *Xgwm146* were identified in these cultivars. Besides, genetic analysis of the F_2 and F_3 populations confirmed that cvs Don Jaime and Colosseo carried the *Lr14a* gene. However, some cultivars carrying *Lr14a* (Llareta, Miradoux, and Sculptur) showed a high infection type to one pathotype (DBB/CS). It seems that these cultivars only possess *Lr14a*, while the other cultivars may have additional resistant genes with additive effect. Cv. Colosseo showed the marker linked to *Lr14a*, although cv. Creso (present in Colosseo ancestry) has been reported to possess a different allele at the *Lr14* locus, named tentatively *Lr14c* (Marone *et al.*, 2009), but this finding has not been proven. The long lasting resistance of cv. Creso seems to be based on a combination of different mechanisms, i.e., hypersensitive plus partial resistance (Maccaferri *et al.*, 2008). Virulence on cv. Creso, and other genotypes with the resistance gene *Lr14a*, was recently found in France (Goyeau *et al.*, 2012).

The two SSR markers used in this work were present in the sister line of cv. Don Patricio (TDA 725S), that displayed a high infection type to 73 isolates. Both SSR markers, *Xgwm344* and *Xgwm146*, were at a distance of 1 cM and 7.5 cM respectively from the resistance gene in cv. Llareta (Herrera-Foessel *et al.*, 2008a). The presence of these two markers in TDA725S is probably due to a recombination between the markers and resistance gene *Lr14a*.

The complementary genes *Lr27+Lr31* are present in cv. Jupare, which was the first deployed by INIFAP-Mexico from the CIMMYT germplasm as a response to the virulent pathotype BBG/BN (Singh *et al.*, 2004). Genes *Lr27* and *Lr31* are located on 3BS and 4BL respectively (Singh and McIntosh, 1984). But in 2007/08 crop season cv. Jupare became susceptible to another Mexican pathotype (BBG/BP), six years after its releasing (Huerta-Espino *et al.*, 2009). This cultivar has still proved to be consistently resistant to all Spanish tested isolates. The presence of these complementary genes has also been postulated in cvs Don Ricardo, Don Valentín, Ancalei, Kanakis, and Ramírez, according to their response to both Mexican and Spanish pathotypes. The presence of genes *Lr27+Lr31* in Don Valentín and Don Ricardo has been confirmed in the allelism tests. F_2 plants from crosses of cv. Jupare with Don Valentín and Don Ricardo showed no segregation in Spain, but a high disease response in Mexico.

Other sources of resistance identified at CIMMYT were *Lr3* (from Storlom) and *Lr61* (from Guayacán, a line released in Chile from a CIMMYT cross) (Herrera-Foessel *et al.*, 2007, 2008b). Virulence to cultivars carrying these genes has been found in Mexico but these pathotypes are still avirulent to the Altar (cv. Gallareta) gene, Lr_{Altar} (J. Huerta Espino, personal communicationc.).

Some of the *Lr* genes described in common wheat and located on genomes A and B might be present in the cultivars used in this study, but the postulation is complicated. For example, *Lr3ka*, *Lr11*, *Lr13*, *Lr17* genes are effective to all tested isolates (data not shown), hence the presence of these genes in some cultivars cannot be discarded. Martinez *et al.* (2007) did not identify any known *Lr* gene in a collection of durum wheat cultivars available in Spain. But Goyeau *et al.* (2012) postulated the presence of *Lr23* genes in some durum cultivars registered in France, and Zhang and Knott (1990) did not rule out the occurrence of *Lr16* and *Lr17* genes. According to the segregation results from the crosses, cvs Don Javier and Don Juan carry one dominant resistance gene, but both showed a different resistant pattern to the Spanish pathotypes (data not shown). Based on the high IT to pathotype DGB/BN and low to DBB/CN it seems that cv. Don Juan has the *Lr16* gene.

Although a relatively low number of landraces were included, it appears that this native gene pool may be rich in different resistance factors/gene combinations. Resistance genes to wheat leaf rust in landraces have been frequently reported (Beharav *et al.*, 1997; Huerta Espino *et al.*, 2011).

Many cultivars displayed a moderate field severity (Yavaros, Don José, Duroflavus, and Calero). These cultivars may possess genes for slow rusting or partial resistance. The old Mexican cv. Yavaros has been reported to carry slow rusting resistance to leaf rust (Singh *et al.*, 1993). Cv. Don José has Yavaros in its pedigree, from which it may have inherited the genes for slow rusting.

Cv. Vitronero has shown susceptibility in both field and greenhouse (at adult plant stage), but it showed an

immune IT at the seedling stage. This is an unusual case, because resistance at the seedling stage generally remains effective throughout the whole life of the plant. In fact, what is more common is the opposite, genes that are not expressed in seedlings but they are at the adult plant stage (e.g. *Lr13*, *Lr35*, *Lr37*). Anyway, Huerta-Espino *et al.* (2002) found some common wheat cultivars with *Lr16* gene that were resistant at the seedling but susceptible at the adult plant stages. Qi *et al.* (1998) found a QTL for partial resistance (*Rphq1*), located on chromosome 7H, effective to barley leaf rust only at the seedling stage. Cv. Vitronero may have either an only-seedling-resistance gene or maybe a suppressor gene acting at the adult plant stage. Genes suppressing rust resistance have been reported previously in the wheat rust system, but only in the D genome (Bai and Knott, 1992).

The presence of *Lr14a* and *Lr27+Lr31* was confirmed in several Spanish durum wheat cultivars. These genes still confer a good level of resistance to Spanish durum wheat pathotypes. In 2013 crop season, cultivars and lines with the resistance gene *Lr14a* such as Don Jaime displayed pustules of high infection type at Conil (Cadiz, Spain). The resistance gene *Lr14a* may have been overcome by a new pathotype that emerged at that location. More information about this new pathotype is required to determine whether it has diverged from a Spanish pathotype or it has come from France (Goyeau *et al*., 2010).

Our results indicate that currently durum wheat cultivars show substantial variability of resistance to Spanish leaf rust pathotypes as demonstrated by the numerous resistance patterns. However, much of the resistance is based on *Lr14a* and *Lr27+Lr31*, which have become ineffective in France and Mexico, respectively. Therefore, development of durum wheat germplasm with different sources of resistance is essential to secure and prolong durability to leaf rust. In southern Spain, as well as in other countries, the situation is somehow worse, for much of the durum wheat acreage is still sown with susceptible cultivars. Therefore a replacement of cultivars with new sources of resistance is required.

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