Electronic Journal of Biotechnology ISSN: 0717-3458
Vol.9 No.5, Issue of October 15, 2006

Received March 14, 2005 / Accepted May 23, 2006
DOI: 10.2225/vol9-issue5-fulltext-14
RESEARCH ARTICLE

Occurrence of the rust resistance gene *Lr37* from *Aegilops ventricosa* in Argentine cultivars of wheat

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Keywords: diversity, marker assisted selection, *Puccinia triticina*, *Triticum aestivum*, translocation.

Leaf rust of wheat (*Triticum aestivum* L.) caused by the fungus *Puccinia triticina* (formerly *P. recondita* f. sp. *tritici*), is one of the most important foliar diseases of this crop. *Lr37* rust resistance gene, which confers resistance in wheat against leaf rust, was introgressed into cultivated wheat from *Aegilops ventricosa* Tausch. Rust races with virulence to *Lr37* have been identified in different countries, but it still provides resistance to a wide range of races and is useful in combination with other resistance genes. There are no reports about the presence, frequency and origin of *Lr37* in Argentinean wheat cultivars. In this work, we analyzed 88 registered Argentinean wheat cultivars developed by different breeding companies and institutions during the last 15 years by means of a molecular marker which is diagnostic of the 2NS-2AS translocation which carries *Lr37*. Only 4 cultivars showed the amplification product associated with this chromosome fragment. These four cultivars which carry the translocated 2NS-2AS chromosome were registered by the same breeding company during the last seven years and all of them have European germplasm in their genealogy. To the best of our knowledge this is the first report of the presence of *Lr37* in registered South American cultivars.

Leaf rust of wheat (*Triticum aestivum* L.) caused by the fungus *Puccinia triticina* (formerly *P. recondita* f. sp. *tritici*), is one of the most important foliar diseases of this crop. Breeding wheat cultivars with resistance to leaf rust is the most effective, economical and environmentally friendly method of disease control and was used in numerous wheat breeding programs worldwide (Kolmer, 1996). However, a gene-for-gene interaction exists between host resistance genes and pathogen avirulence genes in the wheat-*P. triticina* pathosystem and virulence shifts in the pathogen populations have reduced the effectiveness of a number of leaf rust resistance genes (Johnson, 2000), which increased the search of new resistant genes, not only in the cultivated gene-pool, but also in the wild relatives of wheat (Friebe et al. 1996; Valkoun, 2001).

The wild wheat species, *Aegilops ventricosa* Tausch (syn.

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Table 1. Utilized materials, year of registration, breeding companies or institutions and presence of the 2NS diagnostic PCR marker for Lr37.

Cultivar	Registration Year[1]	Registrated or Commercializated by	Presence of the marker for Lr37 [2]
ACA223	2000	ACA[3]	-
ACA302	2002	ACA	-
ACA303	2002	ACA	-
ACA304	2004	ACA	-
ACA601	2003	ACA	-
ACA801	2004	ACA	-
Cooperacion Maipun	1994	ACA	-
Zorzal	2003	ACA	-
Baguette 10	1999	Nidera S.A.	+
Baguette 12	1999	Nidera S.A.	+
Baguette 19	2005	Nidera S.A.	-
Baguette 20	2004	Nidera S.A.	-
Baguette 21	2003	Nidera S.A.	-
Baguette Premium 11	2004	Nidera S.A.	+
Baguette Premium 13	2001	Nidera S.A.	-
Baguette Sur 15	2001	Nidera S.A.	-
Baguette Sur 22	2004	Nidera S.A.	-
Baguette Sur 23	2004	Nidera S.A.	-
Baguette Sur 24	2004	Nidera S.A.	-
Baguette Sur 5	2001	Nidera S.A.	+
Triguero 100	1998	Nidera S.A.	-
Triguero 230	1997	Nidera S.A.	-
BioInta 1000	2004	INTA / Bioceres	-
BioInta 1001	2004	INTA / Bioceres	-
BioInta 3003	2004	INTA / Bioceres	-
BioInta Bonaerense 2001	2004	INTA / Bioceres	-
BioInta3000	2004	INTA / Bioceres	-
Buck Aguara	2004	Buck Semillas S.A.	-
Buck Antorcha	1997	Buck Semillas S.A.	-
Buck Arriero	1998	Buck Semillas S.A.	-
Buck Bigua	2002	Buck Semillas S.A.	-
Buck Brasil	2000	Buck Semillas S.A.	-
Buck Candil	1994	Buck Semillas S.A.	-
Buck Catriel	1992	Buck Semillas S.A.	-
Buck Chacarero	2005	Buck Semillas S.A.	-
Buck Farol	2000	Buck Semillas S.A.	-
Buck Guapo	2000	Buck Semillas S.A.	-
Buck Guatimozin	2001	Buck Semillas S.A.	-
Buck Halcon	1999	Buck Semillas S.A.	-
Buck Mataco	2002	Buck Semillas S.A.	-
Buck Mejorpan	2004	Buck Semillas S.A.	-

Buck Palenque	1991	Buck Semillas S.A.	-
Buck Panadero	1998	Buck Semillas S.A.	-
Buck Pingo	2002	Buck Semillas S.A.	-
Buck Pronto	1997	Buck Semillas S.A.	-
Buck Sureño	2000	Buck Semillas S.A.	-
Buck Yatasto	1998	Buck Semillas S.A.	-
Caudillo	1998	Buck Semillas S.A.	-
Cronox	2005	Don Mario S.A.	-
Onix	2004	Don Mario S.A.	-
INIA Tijereta	2001	Relmo S.A.	-
Greina	1998	Relmo S.A.	-
Lona	1997	Relmo S.A.	-
Klein Cacique	1991	Criadero Klein S.A.	-
Klein Capricornio	2004	Criadero Klein S.A.	-
Klein Castor	2005	Criadero Klein S.A.	-
Klein Chaja	2002	Criadero Klein S.A.	-
Klein Delfin	2000	Criadero Klein S.A.	-
Klein Don Enrique	1998	Criadero Klein S.A.	-
Klein Dragon	1993	Criadero Klein S.A.	-
Klein Escorpion	1999	Criadero Klein S.A.	-
Klein Escudo	2000	Criadero Klein S.A.	-
Klein Estrella	1996	Criadero Klein S.A.	-
Klein Flecha	2003	Criadero Klein S.A.	-
Klein Gavilan	2004	Criadero Klein S.A.	-
Klein Jabali	2002	Criadero Klein S.A.	-
Klein Martillo	2001	Criadero Klein S.A.	-
Klein Pegaso	1997	Criadero Klein S.A.	-
Klein Proteo	2003	Criadero Klein S.A.	-
Klein Sagitario	2000	Criadero Klein S.A.	-
Klein Tauro	2005	Criadero Klein S.A.	-
Klein Volcan	1998	Criadero Klein S.A.	-
Prointa Alazan	1997	INTA[(4)]	-
ProInta Calidad	2000	INTA	-
ProInta Colibri	1999	INTA	-
Prointa Don Umberto	2000	INTA	-
ProInta Elite	1996	INTA	-
ProInta Federal	1990	INTA	-
ProInta Gaucho	2000	INTA	-
ProInta Granar	1997	INTA	-
ProInta Isla Verde	1988	INTA	-
ProInta Milenium	1999	INTA	-
Prointa Molinero	2000	INTA	-
ProInta Oasis	1990	INTA	-
ProInta Puntal	1995	INTA	-
ProInta Real	1996	INTA	-

Prointa Super	1993	INTA	-
ProInta Supremo	2000	INTA	-
VPM1			+
Balthazar			+
Thatcher			-
Soissons			-

(1) According to INASE (2005).
(2) - : absence; + : presence.
(3) ACA = Asociación Cooperativas Argentinas C.L.
(4) INTA = Instituto Nacional de Tecnología Agropecuaria.

Triticum ventricosum Ces), is an allotetraploid with the genome designation $D^vD^vN^vN^v$ derived from the hybridization of the D genome from *Ae. tauschii* (coss.) Schmal and the N genome from *T. uniaristatum* (Vis.) Richter (Kimber and Zhao, 1983). *Ae. ventricosa* is the source of several disease resistance genes that are of agronomic importance and have been successfully introgressed into wheat. These genes include *Lr37*, which confers resistance in wheat against leaf rust. This gene, located in a 2NS-2AS translocation (Bariana and McIntosh, 1993), was initially introgressed in the winter bread wheat VPM1 (Maia, 1967) and was used by breeders in different parts of the world (Robert et al. 1999; Mesterházy et al. 2000; Seah et al. 2000; Park et al. 2001; Stepien et al. 2003). Rust races with virulence to *Lr37* have been identified in different countries (Mesterházy et al. 2000; Winzeler et al. 2000), but it still provides resistance to a wide range of races and is useful in combination with other resistance genes (Park and McIntosh, 1994; Park et al. 2001; Kolmer et al. 2005).

Greater knowledge on the identity of rust resistance genes present in cultivars that can be used as donors of resistance in wheat breeding programs could greatly improve the efficiency of developing resistant cultivars by using these genes *per se* or by stacking different resistant genes in a given cultivar (Sawhney and Joshi, 1996). In this context, there are no reports about the presence, frequency and origin of *Lr37* in Argentinean wheat cultivars. Resistance gene postulation is a rapid method to hypothesize which resistance genes are present in a host genotype (Loegering et al. 1971). It is based on the gene-for-gene specificity between host resistance genes and pathogen avirulence genes. Host genotypes are evaluated with a well-characterized collection of pathogen isolates with different avirulence gene combinations. However, gene postulation can be complicated by interactions between resistance genes and it is best suited for resistance genes that are clearly expressed at the seedling stage (Kolmer, 1996). Resistance genes could also be postulated by testing host genotypes with DNA-based markers linked to resistant genes. This alternative approach overcome some of the problems associated with traditional gene postulation, such as gene interactions and the plant stage of gene expression (McCartney et al. 2005). For this reason, the objective of this work was to determine the presence of *Lr37* resistance gene in a sample of 88 wheat cultivars registered in Argentina during the last 15 years by means of a molecular marker which is diagnostic of it.

MATERIALS AND METHODS

Plant Materials

Eighty eight cultivars of bread wheat registered in Argentina from 1990 to 2005 were used. These materials represent more than 80% of the commercialized cultivars in Argentina during the last 15 years by eight different companies and institutions (Table 1). The cultivars Thatcher and Soisson were used as negative controls and VPM1 and Balthazar as positive controls, since both of them carry *Lr37* (Park et al. 2001). Seeds of these genetics materials were supplied by G. Vrdoljak and P. Paulucci from the Wheat Breeding Program of Nidera S.A.

DNA Isolation

Leaf material was collected from at least 20 different plants of each genotype. DNA was extracted from these bulked samples using a standard SDS protocol (Dellaporta et al. 1983).

PCR analysis

Two pairs of primers were included in each PCR reaction. The primers VENTRIUP-LN2 developed by Helguera et al. (2003) were used to detect the 2NS fragment from *Ae. ventricosa*, that yield a 259-bp band. The primers for the microsatellite marker GWM400 (Röder et al. 1998) were used to evaluate the quality of the DNA and the presence of PCR reaction inhibitors. These last primers amplify a fragment of about 150-bp.

PCR reaction mixes contained 80 ng of wheat genomic DNA, 0.2 µM of each primer (Table 2), 200 µM of each dNTP, 1X PCR Buffer containing 50 mM KCl, 10 mM Tris-HCl pH 9.0, 0.1% Triton X-100, 1.5 mM $MgCl_2$; 1 unit Taq DNA Polymerase (Biotools) in a total volume of

25 µL. Cycling conditions consisted of 3 min denaturation at 95ºC follow by 30 cycles of 45 sec denaturation at 94ºC, annealing at 65º C for 30 sec, and 1 min extension at 72ºC, and finally 7 min extension at 72ºC on a Biorad thermocycler. Amplification products (10 µL/lane) were separated on a standard 2% agarose gel in 1X TBE buffer, at constant voltage power for about 2 hrs. A 25 bp ladder (Promega) was used to estimate the size of each amplified DNA fragment. After electrophoresis, the gel was stained with ethidium bromide and bands were visualized with UV light.

Table 2. Primer names and sequences.

Name	Sequence (5´- 3´)
Ventriup (1)	AGG GGC TAC TGA CCA AGG CT
LN2 (1)	TGC AGC TAC AGC AGT ATG TAC ACA AAA
Xgwm400-F (2)	GTG CTG CCA CCA CTT GC
Xgwm400-R (2)	TGT AGG CAC TGC TTG GGA G

RESULTS AND DISCUSSION

Robert et al. (1999) and Seah et al.(2001) developed PCR markers for the identification of the cluster of resistance genes *Sr38-Yr17-Lr37*, but these markers were not publicly available. Helguera et al. (2003) developed the first public PCR marker for this chromosome fragment which was used in this work. Since the long chromosomal fragment (25-38 cM) from *Ae. ventricosa* does not recombine with the bread wheat chromosomes, the resistance genes located in the 2NS segment are transferred together and are completely linked to markers developed within this segment (Robert et al. 1999; Helguera et al. 2003).

The 259-bp PCR product from primers VENTRIUP-LN2, which is diagnostic for the 2N chromosome fragment, was observed in 4 out of 88 cultivars tested and in the positive checks VPM1 and Balthazar (Table 1). The other cultivars and the two negative controls, on the other hand, did not amplify this diagnostic fragment. In fact, all of them presented only one PCR amplification product corresponding to different alleles of the microsatellite locus *Xgwm400*, used as an internal control of the PCR reaction.

The four cultivars which carry the translocated 2NS-2AS chromosome (Baguette 10, Baguette 12, Baguette 5 Sur and Baguette 11 Premium) were registered by the same breeding company (Nidera S.A.) in 1999, 2001 and 2004, and all of them have European germplasm in their genealogy. This is not surprisingly because VPM1 (the original line which carry the 2NS chromosome segment) was developed and used intensively in France and other European countries. As a matter of fact, the *Lr37* resistant gene was identified, by means of gene postulation or molecular markers, in different cultivars registered in UK, the Czech Republic, France and Poland (Winzeler et al. 2000; Singh et al. 2001; Blaszczyk et al. 2004). The frequency of this gene in UK cultivars, for example, reached 26.6% by 2001 (Park et al. 2001). Cultivars registered by other companies and institutions traced their origin to Argentinean, Uruguayan, Brazilian and Mexican wheat germplasm, where the deployment of the gene *Lr37* was not reported.

Research efforts to breed cereals for resistance to rust diseases have identified resistance genes expressed throughout the entire growth cycle of the plant (seedling resistance genes), and genes which confer resistance to avirulent pathotypes only in post-seedling growth stages (adult plant resistance, APR; Park and McIntosh, 1994). *Lr37* is an APR gene which shows certain level of resistance in seedlings at temperatures below 20ºC (Park and McIntosh, 1994; Kolmer, 1996). At temperatures above 20ºC *Lr37* is ineffective at seedling growth stages, acting as classical APR genes in becoming effective at post-seedling growth stages (Park and McIntosh, 1994). It has been reported that this gene shows a better response than the more common APR genes *Lr12* or *Lr13* when tested with 20 isolates belonging to 13 different races of *P. triticina* from Uruguay and Argentina (Germán, 2003), with 49 isolates representing the most common virulence phenotypes of this pathogen in USA (Kolmer et al. 2005) and with 15 isolates from Canada (Kolmer, 1997).

Wheat lines released by the CIMMYT program are selected for high levels of resistance to leaf rust (Braun et al. 1996; Sayre et al. 1998). Gene postulation studies of CIMMYT lines indicated the presence of *Lr1, Lr3, Lr3bg, Lr10, Lr14a, Lr17, Lr19, Lr23, Lr26, Lr27* and *Lr31* (Kolmer, 1996). Since this germplasm is intensively used by Argentinean breeding programs, all of these genes may be present in Argentinean cultivars. In addition, many spring wheat developed in South America possibly have *Lr13* and/or *Lr34* (Kolmer, 1996). Other wheat developed in South America have been valuable sources of leaf rust resistance. Dyck and co-workers (cited by Kolmer, 1996) isolated *Lr*3 from Sinvalocho, *Lr3ka* from Klein Aniversario, *Lr3bg* from Bage, *Lr11* from El Gaucho, *Lr14b* from Rafaela, *Lr17* from Klein Lucero and *Lr30* and *Lr34* from Terenzio. Antonelli (2003) reported the presence of *Lr3a, Lr10, Lr23* and *Lr26* in ProINTA Oasis, *Lr14a* in ProINTA Federal and *Lr24* in Trigal 800 and derivatives.

To the best of our knowledge this is the first report of the presence of *Lr37* in registered South American cultivars. Its identification in high yielding and adapted cultivars contribute to enrich an already broad genetic base for resistance to leaf rust in Argentinean wheat germplasm. Moreover, taking into account that *Lr37* is linked to the genes *Sr38, Yr17 and Cre5* (Bariana and McIntosh, 1993; Jahier et al. 1996; Seah et al. 2000) which confer resistance to stripe rust (*Puccinia striiformis* West. F. sp. *tritici*), stem rust (*Puccinia graminis* Pers. *f.sp. tritici* Eriks and E. Henn.) and cereal cyst nematode (*Heterodera avenae* Woll.) respectively, indicates that this introgressed

chromosome fragment can be highly useful in developing new wheat varieties.

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