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Resistance to leaf rust in cultivars and wheat lines from Paraguay

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Abstract

Leaf rust (LR) of bread wheat (*Triticum aestivum* L.), caused by the fungus *Puccinia triticina* Eriks, is one of the most important diseases in Paraguay, the Southern Cone of America and worldwide. The economic importance of the disease is clear considering that two or more fungicide applications are necessary to control the disease in susceptible cultivars. The best strategy for the management of this disease is through genetic resistance. This research was conducted in Uruguay aiming to postulate the LR resistance genes present in 116 wheat cultivars and lines from Paraguay, and to characterize their field resistance. The presence of 12 all-stage resistance genes: *Lr1, Lr2*(*b, c*), *Lr3*(*a, bg, ka*), *Lr9, Lr10, Lr11, Lr16, Lr17a, Lr23, Lr24, Lr26, Lr30* was postulated based on the reaction of the genotypes to different races of the pathogen. The adult plant resistance gene *Lr34* was postulated in 31% of the genotypes, based on the molecular marker *csLV34*. This study also allowed differentiating genotypes with field resistance conferred by all-stage resistance genes from those with resistance expressed at the adult plant stage. Knowledge of the resistance genes present in the germplasm of breeding programs is of paramount importance to establish strategies to achieve effective and long-lasting resistance.

Keywords: leaf rust, resistance genes, adult plant resistance

Resistencia a roya de la hoja en cultivares y líneas de trigo de Paraguay

Resumen

La roya de la hoja (RH) del trigo pan (*Triticum aestivum L.*), causada por el hongo *Puccinia triticina* Eriks, es una de las enfermedades más importantes en Paraguay, el Cono Sur de América y a nivel mundial. La importancia económica de la enfermedad es clara si se considera que son necesarias dos o más aplicaciones de fungicidas para su control en cultivares susceptibles. La mejor estrategia para el manejo de esta enfermedad es a través de la resistencia genética. Esta investigación fue llevada a cabo en Uruguay con el objetivo de postular los genes de resistencia a RH presentes en 116 líneas y cultivares de trigo de Paraguay y caracterizar su resistencia a campo. Se postuló la presencia de 12

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genes de resistencia en todos los estados: *Lr1, Lr2*(*b, c*), *Lr3*(*a, bg, ka*), *Lr9, Lr10, Lr11, Lr16, Lr17a, Lr23, Lr24, Lr26, Lr30*, con base en la reacción de los genotipos frente a distintas razas del patógeno. El gen de resistencia de planta adulta *Lr34* fue postulado en 31% de los genotipos en base al marcador molecular *csLV34*. Este estudio permitió también diferenciar genotipos cuya resistencia a campo es conferida por genes de resistencia en todos los estados de aquellos que poseen resistencia expresada en el estado de planta adulta. Conocer los genes de resistencia presentes en el germoplasma de los programas de mejoramiento es de suma importancia para establecer estrategias para lograr resistencia efectiva y durable.

Palabras clave: roya de la hoja, genes de resistencia, resistencia de planta adulta

Resistência à ferrugem da folha em cultivares e linhas de trigo do Paraguai

Resumo

A ferrugem da folha (FF) do trigo-pão (*Triticum aestivum* L.), causada pelo fungo *Puccinia triticina* Eriks, é uma das doenças mais importantes no Paraguai, no Cone Sul da América e no mundo. A importância econômica da doença é clara considerando que duas ou mais aplicações de fungicidas são necessárias para o controle da doença em cultivares suscetíveis. A melhor estratégia para o manejo desta doença é através da resistência genética. Esta pesquisa foi realizada no Uruguai, com o objetivo de postular os genes de resistência a FF presentes em 116 linhas e cultivares de trigo do Paraguai e caracterizar sua resistência em campo. A presença de 12 genes de resistência em todos os estados: *Lr1, Lr2*(*b, c*), *Lr3*(*a, bg, ka*), *Lr9, Lr10, Lr11, Lr16, Lr17a, Lr23, Lr24, Lr26, Lr30,* foi postulada com base na reação dos genótipos contra diferentes raças do patógeno. O gene de resistência de planta adulta *Lr34* foi postulado em 31% dos genótipos com base no marcador molecular *csLV34*. Este estudo também possibilitou diferenciar aqueles materiais cuja resistência em campo é conferida por genes de resistência em todos os estados daqueles que possuem resistência expressa no estado de planta adulta. O conhecimento dos genes de resistência presentes no germoplasma dos programas de melhoramento é de suma importância para o estabelecimento de estratégias que alcancem resistência efetiva e durável.

Palavras-chave: ferrugem da folha, genes de resistência, resistência de planta adulta

1. Introduction

Leaf rust (LR) of bread wheat (*Triticum aestivum* L.), caused by the fungus *Puccinia triticina* (*P. triticina*) Eriks, has been reported as one of the most important diseases in Paraguay[(1)], the Southern Cone of America[(2)] and worldwide[(3)]. At present, it causes severe epidemics and losses on an annual basis in Paraguay. Two or more fungicide applications are necessary to control the disease in susceptible cultivars. Yield losses up to 50% were estimated in Alto Paraná Norte and Canindeyú[(1)], where sowing is earlier and epidemics are generally more severe.

There are scarce precedents of characterization of the population of *P. triticina* in Paraguay. From 23 samples collected in Paraguay during 2011, races TDT-10,20 and MFP were most frequently isolated, and other races identified in smaller proportion were MFP-20, MDT-10,20, TDT-10, TFT-10,20, MDP, MFT-10,20, MFP-10,20, MDP-20. These races have also been identified in Uruguay[(4)], illustrating the similarity of the pathogen population present in Paraguay and Uruguay. Furthermore, the races present in the Southern Cone countries that share the same epidemiological zone east of the Andes are generally similar since there are no geographical barriers that prevent the inoculum from moving from one country to another[(5)].

LR is the main cause for the replacement of commercial cultivars in Paraguay[6] and is also considered one of the main reasons for the increase in foliar fungicide applications in the crop, which increases production costs[1]. However, the main strategy to manage this disease is through genetic resistance[7].

Genetic resistance of wheat to LR is conditioned by a high number of genes. Most of the over 80 catalogued *Lr* resistance genes[8] are major genes expressed from the seedling to the adult plant stage (all-stage resistance, ASR), that produce a hypersensitivity response[9]. Resistance based on these genes has been widely used by breeders; however, most often it has not been durable since initially resistant varieties carrying one or few ASR genes become susceptible when the pathogen develops new virulent races to these genes[10]. Some major genes expressed in adult plants, which produce a hypersensitivity response (adult plant resistance, APR-HR, *Lr12, Lr13, Lr22a, Lr22b, Lr35, Lr37*), have similar characteristics to the ASR genes. Other genes that express in adult plants (APR-PR) have a minor and additive effect, condition quantitative resistance, are race-non-specific, and have been the focus of greater interest because these are presumed to condition durable resistance[11]. In the field, minor genes determine slow disease development[12] and do not express high levels of resistance when present alone. However, the combination of four or five genes confers resistance levels close to immunity[11]. This resistance has been called partial resistance[13] (PR), adult plant resistance[14], and slow rusting[12][14]. Four genes conditioning APR-PR to LR have been widely studied: *Lr34*[15], *Lr46*[16], *Lr67*[17], and *Lr68*[18]. An outstanding feature of *Lr34*, *Lr46,* and *Lr67* is that they have pleiotropic effects on other pathogens[11][12][19][20][21]. *Lr34*, located on chromosome 7DS, was first described in the Brazilian cultivar Frontana[22].

One of the methodologies used to study the genetic resistance conditioned by ASR genes is the postulation of their presence in wheat genotypes based on the reaction to different races of the pathogen. This method is based on the gene-by-gene concept[23][24] and has been widely used[25][26][27] because it is a fast, low-cost, and convenient method for identifying ASR conferred by one or two genes, but may not be appropriate when resistance is more complex. It is not possible to use this methodology when races do not have the virulence combination that determines compatible reaction or susceptibility, nor postulate the presence of APR-PR genes, due to the absence of specific virulence to these genes.

The use of molecular markers is another alternative to postulate or confirm the presence of disease resistance genes[28]. In the case of wheat LR, there are suitable markers for several resistance genes, for example, major genes *Lr1*, *Lr10*, *Lr19, Lr21, Lr22a, Lr25, Lr29, Lr32, Lr35, Lr37, Lr39, Lr47, Lr50, Lr51* and APR-PR genes *Lr34, Lr46, Lr67, Lr68, Lr75*[29][30]. The *Lr34* molecular marker *csLV34*[31] has been used by several researchers[32][33][34][35].

It is important to characterize the resistance and know the genes present in the germplasm of a breeding program to allow identifying sources of resistance with different genes to be introduced to increase genetic variability, as well as better characterizing the leaf rust reaction of commercially used cultivars. This study aims to characterize the field resistance and postulate LR resistance genes present in wheat cultivars and lines from Paraguay.

2. Materials and methods

2.1 Seedling tests

For the postulation of ASR genes, 116 wheat varieties from the Paraguayan Wheat Research Program's Wheat Breeding Program, some of which are introductions from CIMMYT, were evaluated at the seedling stage (**Table S1**, Supplementary material). Thatcher (Tc) was included as the susceptible control and mono-

genic differential lines with *Lr1, Lr2a, Lr2b, Lr2c, Lr3a, Lr3bg, Lr3ka, Lr9, Lr10, Lr11, Lr14a, Lr14b, Lr16, Lr17a, Lr19, Lr20, Lr21, Lr23, Lr24, Lr26, Lr30* in the Tc background developed in Canada[36] and *Lr39* (TAM107*3/TA2460), *Lr42* (KS91WGRC11=CENTURY*3/T.TAUSCHII), *Lr47* (PAVON753) were used to perform the gene postulation. These lines were selected since they represent genes commonly present in improved bread wheat germplasm.

2.2 Races of *Puccinia triticina*

Nineteen races of *P. triticina* isolated from samples collected in Uruguay were used in this study (**Table 1**). Two races were selected for their high frequency in the pathogen population during 2012 (MFP and TDT-10,20)[4] and the rest were selected to represent different combinations of avirulence/virulence that allow discriminating the presence of different ASR genes.

The inoculum of these races was preserved in vacuum glass tubes in a refrigerator at 5 ºC in the Rust Laboratory of the Uruguayan National Institute of Agricultural Research (INIA) La Estanzuela. To increase the inoculum of the races, 12 to 15 seeds of the susceptible genotype Little Club (LC) were sown in a 10-cm-diameter pot with a mixture of soil, vermiculite, sand, and substrate (Biofer almácigos, Riverfilco; Biofer Ltd., Montevideo) in a 1:1:1:1 ratio. When plants emerged, each pot was treated with 20 cm^3 of a maleic hydrazide solution (0.36 g/l) to stop their development and intensify spore production. Each pot of LC was inoculated with spores of a different race, suspended in Soltrol 170 mineral oil (Phillips Petroleum Co., Borger, TX). Pots were placed in a wet chamber (100% relative humidity) for about 16 hours. Subsequently, pots were moved to the greenhouse with a temperature of 20-25 ºC and six to eight hours of supplementary light (high pressure sodium Son T 400w). To prevent cross-contamination, a PVC cage was placed on each pot and connected to a hose that released a light air flow. Approximately two weeks after inoculation, the inoculum was collected and placed in glass tubes that were vacuum sealed and stored in a refrigerator at 4-6 °C[37].

Table 1. Avirulence/virulence formula of *Puccinia triticina* races used in seedling tests

Pt races[a]	Avirulence / virulence
CHT	*1,2a,2b,2c,9,10,19,20,21,24,39,42,47/3a,3bg,3ka,11,14a,14b,16,17a,26,30*
DBB-10,20	*1,2a,2b,3a,3bg,3ka,9,11,16,17a,19,21,24,26,30,39,42,47/2c,10,14a,14b,20,23*
KDG-10,20	*1,3bg,3ka,9,16,17a,19,21,26,30,39,42,47/2a,2b,2c,3a,10,11,14a,14b,20,23,24*
LPG-10	*2a,2c,3a,3ka,16,17a,19,20,21,30,39,42,47/1,9,10,11,14a,14b,23,24,26*
MCD-10,20	*2a,2b,2c,3ka,9,11,16,19,21,23,24,30,39,42,47/1,3a,3bg,10,14a,14b,17a,20,26*
MCP-10	*2a,2b,2c,9,11,16,19,20,21,23,24,39,42,47/1,3a,3bg,3ka,10,14a,14b,17a,26,30*
MCR-10	*2a,2b,2c,3bg,9,16,17a,19,20,21,24,39,42,47/1,3a,3ka,10,11,14a,14b,23,26,30*
MCT-10	*2a,2b,2c,9,16,19,20,21,23,24,39,42,47/1,3a,3bg,3ka,10,11,14a,14b,17a,26,30*
MDT	*2a,2b,2c,9,10,16,19,20,21,23,26,39,42,47/1,3a,3bg,3ka,11,14a,14b,17a,24,30*
MFP	*2a,2b,2c,9,10,11,16,19,20,21,39,42,47/1,3a,3bg,3ka,14a,14b,17a,23,24,26,30*
MFP-10,20	*2a,2b,2c,9,11,16,19,21,39,42,47/1,3a,3bg,3ka,10,14a,14b,17a,20,23,24,26,30*
MFP-20	*2a,2b,2c,9,10,11,16,19,21,39,42,47/1,3a,3bg,3ka,14a,14b,17a,20,23,24,26,30*
MFR	*2a,2b,2c,9,10,16,17a,19,20,21,39,42,47/1,3a,3bg,3ka,11,14a,14b,23,24,26,30*
MFT-10,20	*2a,2b,2c,9,16,19,21,39,42,47/1,3a,3bg,3ka,10,11,14a,14b,17a,20,23,24,26,30*
MHP-10	*2a,2b,2c,9,11,19,20,21,23,24,39,42,47/1,3a,3bg,3ka,10,14a,14b,16,17a,26,30*
MKD-10	*2a,2b,2c,3ka,9,11,19,20,21,23,30,39,42,47/1,3a,3bg,10,14a,14b,16,17a,24,26*
MMD-10,20	*2a,2b,2c,3bg,3ka,11,16,19,21,23,24,30,39,42,47/1,3a,9,10,14a,14b,17a,20,26*
SPG-10	*3a,3bg,3ka,16,17a,19,20,21,30,39,42,47/1,2a,2b,2c,9,10,11,14a,14b,23,24,26*
TDT-10,20	*9,16,19,21,26,39,42,47/1,2a,2b,2c,3a,3bg,3ka,10,11,14a,14b,17a,20,23,24,30*

[a]Long & Kolmer, 1989. The inclusion of 10 and/or 20 after the denomination of the race indicates virulence to these genes.

2.3 Postulation of all-stage resistance genes

Twenty-eight genotypes (six to eight seeds per genotype) were planted in 45.5 × 28.0 × 7.7 cm pots filled with the abovementioned substrate. The susceptible control Tc was included in each pot. The genotypes were inoculated with the 19 races individually, following the procedure described for the increase of races' inoculum. At 12 days after inoculation, the infection type (IT) was evaluated according to the scale described by Stakman and others[38], where IT 0 = immune response, without uredinia or necrosis; IT; (fleck) = necrotic lesions without sporulation; IT 1 = small uredinia surrounded by necrosis; IT 2 = small uredinia surrounded by chlorosis; IT 3 = moderate uredinia without chlorosis or necrosis; IT 4 = large uredinia without chlorosis or necrosis. The symbols + and - were used to indicate larger and smaller uredinia compared to the typical IT, respectively. IT X = a mesothetic response of flecks, small and large uredinia. IT 0-2+ and X were considered low ITs and IT 3-4 were considered high ITs.

Two replications were planted for each genotype and race. For avirulent ITs, if the ITs differed by less than one IT unit between replications, the highest IT was considered. If the ITs differed by more than one IT unit, the data was discarded (n/a). For intermediate ITs, if one replicate had low IT and the other had high IT, the information was also discarded.

To postulate which resistance genes are probably present in the genotypes, the IT patterns of the Paraguayan genotypes were compared with the IT of the lines carrying unique LR resistance genes.

2.4 Adult plant (field) resistance

Paraguayan genotypes were evaluated under field conditions and natural infection of the pathogen during the winter-spring of 2012 at INIA La Estanzuela, Colonia Department (LE: latitude 34.3° S, Longitude 57.7° W, elevation 70 masl) and at Young, Río Negro Department (Y: latitude 32.7° S, longitude 57.6° W, elevation 76 masl). Planting dates were July 12 at Young and July 24 at La Estanzuela.

The experimental design used was incomplete randomized blocks with two replications. Tc and Avocet, used as susceptible controls, were repeated six times in each replication. Plot size was two one-meter-long rows. Spreader rows (mixture of different susceptible genotypes) were planted perpendicular to the plots to homogeneously increase the natural LR inoculum.

LR severity and reaction were assessed starting at stem elongation and approximately every two weeks (four times in La Estanzuela, and three times in Young). LR severity was determined according to the modified Cobb scale[39]. Small uredinia surrounded by distinct necrosis were considered resistant (R); moderate-large sized uredinia surrounded by necrosis were considered moderately resistant (MR); moderate to large uredinia surrounded by chlorosis were considered moderately susceptible (MS); large uredinia lacking necrosis or chlorosis were considered susceptible (S); and a mixture of large and small uredinia were considered as a mixed (M) response. The coefficient of infection (CI) was calculated as severity × reaction, using a coefficient for each reaction: R = 0.2, MR = 0.4, M, MRMS or MSMR = 0.6, MS = 0.8, and S = 1.0.

The area under the disease progress curve (AUDPC)[40] was calculated based on the LR CI. The adjusted AUDPC calculation and the ANOVA were performed using a mixed linear model with the package lme4[41] in the software R[42], using the following model:

$$Y_{ijkl} = G_i + E_j + \underline{R_{k(j)}} + \underline{B_{l(jk)}} + \varepsilon_{ijkl}$$

where Y: LR AUDPC values, G: effect of genotype *i-th* (fixed), E: effect of *j-th* location (fixed), R: repetition within location (random), B: incomplete block within location and repetition (random), and ε: experimental error with iid N (0, σ^2_ε).

The genotype × location effect was disregarded and included in the experimental residuals since we were interested in the expression of resistance over locations. Based on this, the adjusted mean AUDPC across locations and the minimum significant difference (MDS, $P < 0.05$) was calculated.

The adult plant reaction of the genotypes recorded in the field was classified into different categories based on the values of the adjusted AUDPC. According to the distribution of the AUDPC of all genotypes and comparing these with the AUDPC of the susceptible checks, those genotypes with values in the range of 0-375 were considered resistant (R), 376-780 moderately resistant (MR), 781-1000 moderately resistant to moderately susceptible (MRMS), and 1001-1500 moderately susceptible (MS).

2.5 Confirmation of the presence of *Lr34* based on the *csLV34* molecular marker

The molecular marker *csLV34*[31] was used to postulate the presence of the APR-PR *Lr34* gene. DNA extraction, PCR amplification and determination of alleles in agarose gel electrophoresis were performed according to CIMMYT protocols[43]. The wheat line Parula was used as the positive control for the expected band of the allele associated with the presence of *Lr34*.

3. Results

In seedling tests, the susceptible control Tc had high IT (3 to 4). It was not possible to postulate the ineffective (*Lr14a, Lr14b*) and effective genes (*Lr19, Lr21, Lr39, Lr42, Lr47*) to all races. The rest of the tested genes had high and low IT to different races (**Table S2**, Supplementary material). The seedling IT information of the studied genotypes is presented in three tables, according to their reaction pattern to the races: resistant to all races (**Table 2**), resistant to the most frequent races (**Table 3**), and susceptible to one or both most frequent races, MFP and TDT-10,20 (**Table 4**).

Average field infection of susceptible controls Avocet and Tc was high (final infection severity of 99% and 90%, adjusted AUDPC of 5196 and 4746, respectively). The infection of both susceptible controls was consistently high over locations and reps. The phenotypic correlation of AUDPC between locations was 0.96. The estimated AUDPC of the genotypes ranged from 0 to 1438 (**Table 2**, **Table 3**, and **Table 4**) and these values were significantly lower than the AUDPC of the susceptible controls ($MDS_{0.05}$ 1163). The percentage of field R, MR, MRMS and MS genotypes was 61, 22, 11 and 6, respectively.

Forty-five Paraguayan genotypes were resistant in the seedling stage to the 19 races (**Table 2**), which did not allow identifying the genes expressed at that stage. Their AUDPC in the field ranged from 0 (R) to 994 (MRMS).

The presence of 12 ASR genes: *Lr1, Lr2*(*b, c* alleles), *Lr3*(*a, bg, ka* alleles), *Lr9, Lr10, Lr11, Lr16, Lr17, Lr23, Lr24, Lr26,* and *Lr30* was postulated in 46 genotypes (**Table 3** and **Table 4**). Additional resistance that could not be identified was present in many of the genotypes.

Table 2. Seedling infection type to 19 *Puccinia triticina* races, AUDPC, field reaction and presence of the *csLV34* marker in Paraguayan genotypes resistant to all races

	Puccinia triticina races																					
Entry	CHT	DBB-10,20	KDG-10-20	LPG-10	MCD-10,20	MCP-10	MCR-10	MCT-10	MDT	MFP	MFP-10,20	MFP-20	MFR	MFT-10,20	MHP-10	MKD-10	MMD-10,20	SPG-10	TDT-10,20	AUDPC	Field reaction	csLV34[a]
53	0	0;	0	0	0;	0	0	0;	0;	0	0	0	0	0	0	0;	0;	0;	0	0	R	+
15	0	0;	0;1=	0;	0;	0	0	0	1-	1-;	12	1	1	1=;	0	1-;	0	0;	;1-	5	R	-
98	0;	0;	1-;	0	;1=	0;	0;	0;	0;	0;	1=;	0	12-;	1=;	;	1-;	;1=	0;	;	8	R	-
38	0	;	0	0	1-;	;1-	0;	1-;	;1-	1-;	1-;	1-;	1-;	1=	1=;	12-;	0	0;	;1=	8	R	-
76	0	0;	0;1-	n/a[b]	0	0	0	0	0	0;	;1-	0;	0;	2	0	;	0	2	0;	20	R	-
37	n/a	0	;1=	0;	0;	0	0;	0;	n/a	0	0	0;	n/a	n/a	0	0;	0;	0;	0;	22	R	-
86	0	0;	0;	0	0	0	0	0	0;	0;	2=;	0;	0;	0	0	2	0	0	0;	25	R	+
35	1-;	0;	0	0;	;1=	;1-	0;	;1-	0;	;1=	;	;1=	;1=	1=;	0;	1-;	0	0	0;	26	R	-
39	0	;1=	1=;	1-	2-	;12	0;	;1=	0;	0	;1-	0;	0;	1-;	1-	2	21	0;	;1-	27	R	-
102	0;	0	0;	0;	0;	0	0	0	0;	0;	0;	0;1=	;1-	0	0;	0	;1=	0	0;	28	R	+
88	0;	0;	1	0	;	;1=	;	1-;	1-;	1-;	;1=	;1=	1-	0	0	1-;	0;	0;	0;	30	R	-
19	0	;1=	1	0;	22+	;1-	;	;1-	0;	0	1-;	0;	0;	1=;	1-;	22+	n/a	;1-	0;1-	37	R	-
23	0	0;	1	;1=	0;	0	0;	0;	1-;	;1=	1-;	12-	1	1=;	0	1	0	0;	;1-	45	R	-
87	0	0;	0	0	0	0;	0	0	0	0	0;	0	0	0;	0	0;	2	0	0;	46	R	-
21	1-	0;	;1-	;	2=;	1-;	;1=	;1-	1-;	;1=	0;	1-;	1-;	1=;	0;1=	1-;	0;	;	;1=	57	R	-
70	n/a	;1-	1	0	0;	0;	2-;	0;	0;	2;	;1-	1	1	n/a	0;	;1-	1-;	0	n/a	68	R	-
54	0;	0;	0;1=	1-	;1=	0;12=	0;	2=;	12	2-	X-	X	1-;	n/a	n/a	2	0;	0;	;1-	77	R	-
40	0;	;1-	0	1-	0;	0	0;	0;	1-;	1-	1-;	12-	11+	n/a	0	12-	0	0;	1-	81	R	-
73	0;	0	0;	12	0;	0;	;1=	0	0	0;	;1=	1-;	1-;	0	0	0	1-;	0;	0;	84	R	-
77	0;	0;	0;	1	0;	0;	0;	0	0;	0;	n/a	0;	0;	0	0;	;	0;	1-;	0;	112	R	-
13	12-;	0	0;	1-	0;	;1=	0	;1=	0;	1-;	;1=	1-;	0;	1=;	0;	0;	0;	0	;1=	113	R	-
101	1-;	0;	n/a	1-	0;	0;1=	1-	;	0;	0;	1-;	0;1-	n/a	1=	0;	;1=	0;	0	0;	153	R	+
36	1-;	0;	;1=	0;	1=	;1-	0;	;1=	n/a	;1=	n/a	1-	1-;	0	1=;	1-	0;	0;	0;	157	R	-
114	0	;1-	0;	0	0;	;1=	2-	;1=	0;	0;	0;	0;1-	1-;	1=;	0;	;	0;	0	0;	165	R	-
49	0;	0;	0	0;	0;	0;	0;	2=;	n/a	0;	0;	0;	n/a	2	0;	22+	0	0	0;1=	187	R	-
105	;1=	;	1-	n/a	0;1-	0;	n/a	0;	0;	2-;	1=;	0;1-	n/a	12	0;	;	0;	0;	;1-	195	R	-
71	0	0	0	1=	1-;	0;1=	2	2	n/a	0	0;	0;1-	n/a	n/a	0	1-;	0;	1	2;	207	R	-
68	;1-	0;	0;	0	0;	0;	0;	;1=	0;	;1=	0;	;1=	0;	0	0	;1=	0;	0	0;	249	R	-
44	0	0	0;	0	0;	0;	0;	;1=	0;	0	0;	0;	0;	1=;	;	;1=	0;	0;	;1=	252	R	H
74	;	0;	0;	1	;	0;	;1=	0	0	0;	;	1-;	1-;	0	0	0;	2=;	0;1=	0;	257	R	+
80	0;	0;	0;	0	12-	0;	0	0;	0;	0;	;1=	1-;	0	0;	;1=	2-;	2=;	0	0;	314	R	-
106	0;	1-	1-;	1-	12;	12-;	0;	0;	1-;	1-	1=;	n/a	1;	0	0	n/a	12;	0;	12-;	318	R	H
85	0;	1-	1-	1	1=;	0;	1-	0	0;	1-	;1=	1;	1-	1=;	n/a	;	0;	;1=	21	418	MR	+
4	0	0;1=	0	1=	;1=	;	;	n/a	0;	1-	1-;	1-	1	2=	0	;1-	1-;	1-;	2	439	MR	-
92	0;	0;	0;	1+	;1=	0;1=	;	0;	;1-	2-	0;	0;1-	2-1	n/a	1=;	;	0;	0	12-	454	MR	+
111	0;	0;	0;	1=	0;	;	;1=	;1=	0;	;1-	;1=	1-;	n/a	0	0;	;	;1=	0;	;1=	531	MR	-
14	X	2=	0;1=	0	1-;	1=;(X=)	;1=	2-;	;1=	1-;	1-;	1-;	23 (X)	1-	2	2	;1-	0;	0;1=	539	MR	-
112	0	;1-	0	1-	;1=	;	0;	;	1-;	1-	;1=	1	1-;	1-	0;	1-;	0	0;	2-;	602	MR	H
91	0;	1-;	0;	0;	;1=	0;	0;	0;	0;	1-;	;1=	0;1-	1=	0;1=	0	;1=	0;1=	;1=	;1=	660	MR	+
75	n/a	0;	1-	1	n/a	0;	n/a	0	;1=	1-;	;	n/a	n/a	n/a	n/a	1-;	n/a	n/a	1-	785	MRMS	+
78	0	0;	0	0	2	;1=	;1=	2-;	0	0	2-;	0;	0;	0	0;	1-2-	0;	0	2-;	824	MRMS	H
95	0;	1-	1-	1=	1-;	0;1=	1-;	;	0;	1-	0;	11-	1	1	;1=	;1=	1-;	1=	1-	909	MRMS	-
84	n/a	;1=	0;1-	1	n/a	n/a	0;	0	n/a	1=;	;	n/a	n/a	n/a	1=;	0;	n/a	n/a	0;	960	MRMS	H
113	1-;	1-;	0;	1-	;1=	;	;1=	;	;	1-;	0;	0;1-	1-;	1=;	0;	;1-	0	0;1=	;1=	982	MRMS	-
3	1-;	;1=	0;1=	1-;	0;	;	1-;	0;	0;	1-;	;1=	1-;	1-;	n/a**	0	0;	0;	;1=	1	994	MRMS	-
Avocet																				5196	S	
Thatcher																				4746	S	

[a]+ presence, - absence of the allele associated with *Lr34*. H: heterozygous

[b]n/a: not available information

Table 3. Postulated genes, seedling infection types to 19 *Puccinia triticina* races, AUDPC, field reaction and presence of the *csLV34* marker in Paraguayan genotypes resistant to prevalent races (MFP and TDT-10,20)

		Puccinia triticina races																					
Entry	Postulated genes	CHT	DBB-10,20	KDG-10-20	LPG-10	MCD-10,20	MCP-10	MCR-10	MCT-10	MDT	**MFP**	MFP-10,20	MFP-20	MFR	MFT-10,20	MHP-10	MKD-10	MMD-10,20	SPG-10	**TDT-10,20**	AUDPC	Field reaction	*csLV34*[a]
103	*Lr1,3bg,9,10,11,23,26*	0	0	0	3	0	0	0	0	0	**0**	0	0	0	0	0	0;	2-;	n/a[c]	**0;**	13	R	+
24	*Lr10,11,23,24,26,+*[b]	0	0;	12	3	0;	0;	0	0	0;	**0;**	0	0;	0;	1-	0	2;	0	3+	**0;1=**	34	R	-
46	*Lr10,11,23,24,+*	0;	0	3	12	0;	0;	0	0	0;	**0;**	0;	0;	1-;	0	0	0;	0;	3	**0;**	61	R	+
33	*Lr1,2c,10,11,23,24,26*	0;	0	0;	2=	0;	0;	;1=	0	0	**0;**	0;	0;	0;1-	0;	0	0;	0;	3+	**0;**	68	R	+
12	*Lr11,23,26,+*	;1=	0;	0;	0	0;	0;	1-;	0;	0;	**0;**	;	0;1=	1-	1=;	0;	0;	0;	3	**0;**	72	R	+
51	*Lr3a,10,11,23,+*	;1=	0;	3+	0	0;	0;	0;	0;	0;	**0;**	0;	;	n/a	12	n/a	;1=	2-	0	**0;**	75	R	+
66	*Lr1,11,23,24,26,+*	0;	0	0	3	0;	0;	0;	0;	0	**0;**	0;	;1=	1-	0	0	;	0;	3+	**0;**	80	R	+
72	*Lr10,11,23,24,+*	0	0	3+	0	0	0	0	0	0;	**0**	;1=	;	0;	n/a	0	;2	0	3+	**;2=**	86	R	-
48	*Lr3(a or ka),10,11,23,26,30,+*	0	0	12	0	;1=	0;	n/a	0;	0;	**0;**	1-;	0;1=	0;	3	n/a	0;	0;	0	**2**	91	R	-
96	*Lr23,26,+*	0;	0	1	3	;1=	0;	n/a	0;	;1=	**2-;**	1-;	12	4	n/a	0;	0;	0;	3+	**12-;**	95	R	-
25	*Lr10,24,+*	0	0	3	3+	0;	0	0;	0	0;	**0;**	n/a	0;1=	0;	3+	0	32;	0	3+	**0;1=**	115	R	-
89	*Lr3a,23,+*	0;	0;	3	0;	;1=	0;	0	0	0;	**0;**	4	4	0;	0;1=	0;	0;	0;	0;	**;1-**	137	R	-
32	*Lr10,11,23,24,+*	0;	0;	3+4	12	0;	0;	1-;	0;	0;	**0**	0;	0;	1-;	0;	0	0;	0;	3+	**0**	162	R	+
82	*Lr3(a or bg),10,17a,26,+*	0	0;	0;1-	0	3+	;12-	;1=	;1-	0	**0;**	X	0;	0;	3+	2+	3+	2=;	0;	**2**	170	R	+
83	+	X-	2-;	0;	0	33+	2;	;1=	2	12	**2-;**	2	22+(X)	4	n/a	3+	3+	2=;	0	**1**	175	R	-
67	*Lr10,11,23,24,26,+*	0;	0	1	3	0;	0;	0;	0;	0;	**0;**	0	0;	1-;	0	0;	0;	0	3	**0;**	193	R	+
52	*Lr1,3bg,23,26,+*	0;	0;	0	3	;1=	n/a	0;	0;	0;	**0;**	0;	2+3+	12=	2=	n/a	;1=	;1=	0;	**21;**	210	R	H
26	*Lr3(a,bg or ka),17a,23,26,30,+*	0;	0	;1=	0	;1=	0;	0	0;	0;	**0;**	4	3+4	0;	0;	0;	;	0	0;	**0;1=**	239	R	+
108	+	n/a	;1=	;1=	0;	3	3	2	2-	;1-	**2**	2	n/a	23	n/a	3+	2-;(X-)	0;	0;	**2=;**	283	R	+
107	*Lr30,+*	n/a	2=;	;1=	n/a	n/a	32	2	2-;	12	**0;**	n/a	2	n/a	n/a	3+	2-	0;	0;	**2=**	348	R	-
8	+	12	3+	12	3+	2=	;1=	4	2=;	2	**2=;**	2	2	4	3+	0	;1=	3+	3	**2**	367	R	+
79	*Lr1,10,26,+*	0	0	0	2+3+	32	23	1-	23	0	**0;**	n/a	0;	0;	n/a	n/a	3+	0;	21	**2**	392	MR	H
97	*Lr23,+*	0;	2=;	1=	3+	0;	;	n/a	0;	1-;	**2=;**	12-;	12	3+4	2+3+	0;	;	0	3+	**12-;**	401	MR	-
57	+	n/a	2-;	0	3	1-	n/a	n/a	;1=	n/a	**2-**	n/a	n/a	23	3+	3+	0;	3+	n/a	**2-;**	414	MR	H
47	*Lr3a,10,+*	0	0	3	0	2-	12-;	0	2=;	0;	**0**	2	0;	0	n/a	3+	2	0;	0	**1**	424	MR	-
90	*Lr23,+*	0;	2-;	1-	n/a	;	0;	4	0	;1-	**2;**	2+	n/a	n/a	0	0	;	0;	3	**2;**	438	MR	+
43	*Lr3(a or bg),10,16,26*	n/a	0;	1-	0	n/a	12-	0;	;1=	1-;	**0;**	;1-	n/a	1-;	1-	n/a	3+	0;	0;	**;1=**	479	MR	+
109	+	;1-	;1-	;1=	0;	3+	23	2	2	2=;	**2**	2	n/a	23	3+	3+	2;(X-)	2=;	0;	**2-**	598	MR	+
55	*Lr3bg,+*	n/a	;1=	0	n/a	3-	3	n/a	3	n/a	**0**	1+2	2	21	n/a	3	3	22+	n/a	**2**	716	MR	-
64	*Lr3bg,26,+*	3	0	;1-	3+4	n/a	n/a	n/a	3	;	**2-**	1-;	22+	4	3+	3+	n/a	n/a	n/a	**2**	723	MR	+
81	+	n/a	;1=	0;1=	0	2	23	2	23-	n/a	**2-;**	2	X-	23	2+	2+	22+	2	0;	**1+2**	795	MRMS	+
116	+	23	12-	12	1	12	21	0;	2	1-;	**1-**	1-;	12	1-1	1=	0	3+	;1=	0;	**1=**	869	MRMS	-
45	*Lr10,+*	;1=	;1-	3+	3+	2	;1-	0;	12-	1-	**1-**	1-;	12	1-;	1;	1=;	3+	21	0;	**1-;**	1238	MS	-
110	*Lr10,+*	0	;1-	n/a	1	3+	n/a	n/a	2-	;1=	**2=;**	;1-	0;1-	n/a	1=	0	n/a	n/a	;1=	**12-;**	1367	MS	+
50	*Lr3(a or bg),17a,26,+*	2	0	0	0	2+3	2=;	0	2=;	0	**2-;**	2	X	0;	3+	n/a	2	2	0;	**2-**	1438	MS	+
Avocet																					5196	S	
Thatcher																					4746	S	

[a]+ presence, - absence of the allele associated with *Lr34*. H: heterozygous

[b]+: presence of additional unidentified resistance gene(s)

[c]n/a: not available information

Table 4. Postulated genes, seedling infection types to 19 *Puccinia triticina* races, AUDPC, field reaction and presence of the *csLV34* marker in Paraguayan genotypes susceptible to one or both prevalent races (MFP and TDT-10,20)

		Puccinia triticina races																				
Entry	Postulated genes	CHT	DBB-10,20	KDG-10-20	LPG-10	MCD-10,20	MCP-10	MCR-10	MCT-10	MDT	**MFP**	MFP-10,20	MFP-20	MFR	MFT-10,20	MHP-10	MKD-10	MMD-10,20	SPG-10	**TDT-10,20**	AUDPC	Field reaction	*csLV34*[a]
7	+[b]	2-;	;	3+4	3+	4	X	4	n/a[c]	3+	**3+**	4	4	4	4	4	4	n/a	X-	**3**	64	R	-
17	+	1-;	;2	3+4	3	3+	X+	4	2	3	**3+**	3+	4	3+4	3+	3+	33+	n/a	X-	**3+**	70	R	-
104	+	0;	X	3	X	2-;	;	3+	n/a	2	**3+**	2	4	4	3+	1=;	;1=	;1=	12	**2+3+**	78	R	-
27	*Lr3(a,bg or ka),23,30,+*	0;	0;	1-	0	;1=	0;	1-	;	1-;	**2**	;1=	11+	3+	2+3+	0;	;1-	12-	0;	**3+2**	91	R	-
34	*Lr23,+*	0;	1-;	12	2+3	;1=	;	4	0	1-;	**3+**	X	2+3+	4	3	0	;1-	0;	X-	**X+**	108	R	-
20	*Lr23,30,+*	;1=	;1-	12	n/a	;1=	0;	4	0;	1-	**3+**	2	n/a	3+4	3+	0	0;1=	0;	X-	**3+4**	114	R	-
58	*Lr3(a,bg or ka),17a,23,30,+*	;2	0;	n/a	0	1-;	;21=	n/a	n/a	0;	**0;**	X	n/a	0;	3+	0;	;1=	0	0;	**3+**	144	R	-
59	+	;1=	;1-	3+	3+	3+	0;2=	2-;	n/a	3+	**2**	4	4	n/a	3+	n/a	3+4	3+	3	**3+**	160	R	+
6	*Lr3a,10,+*	0;	0;	3	0	3+	;	3+	n/a	0;	**0;**	X	;	0;	3+	3+	33+	;2	0;	**23**	174	R	-
42	+	23	;	3	4	23	X-	n/a	2-;	3+	**3+**	4	3+	4	4	3+	4	n/a	;1-	**3+4**	223	R	-
5	*Lr26,+*	3+	n/a	1	1-	3+	2+3+	3	;1=	12	**3**	2	2+3+	4	4	4	4	0;	1-;	**21**	243	R	-
94	*Lr23,30,+*	0	2=;	;1=	n/a	;1=	0;	0;	;1=	1-;	**3**	1-;	X	X	2+3+	0	;	;2=	X-	**n/a**	259	R	+
61	+	n/a	23;	3+	3	4	X	n/a	2-;	3+	**3+**	n/a	4	n/a	3+	3+	4	4	n/a	**4**	262	R	-
93	+	0;	2	3	3+	n/a	0;	3+	0;	2	**3+**	X	X	4	n/a	n/a	;1-	1-;	3	**3+**	302	R	+
22	+	;1-	1-;	1	3+	3+	2-;(X)	1-;	;1=	4	**3+**	3+	33+	23(X)	3+	2+	33+	X-	X	**2-**	319	R	-
10	*Lr23,+*	0;	n/a	12	3+	n/a	1-;	1-;	;	2	**3**	2	33+	23(X)	3+	0;	;1-	;2	32	**4**	327	R	-
56	*Lr1,26,+*	n/a	0;	0	n/a	3+	3+	0	n/a	n/a	**3**	2+3+	3+	23	3+	3+	3+	3+	23	**3**	328	R	+
16	*Lr23,+*	1-;	;1=	2	3	2-	1-;	4	;1=	2	**3+**	2+	4	4	3+	0;	0;	0;	0;	**3+**	366	R	+
9	+	12	n/a	3+	3+	3+	23-	1-;	2=;	3+	**3**	3	33+	32(X)	3+	n/a	33+	2+	12-	**3+**	450	MR	-
41	*Lr23,+*	;1=	n/a	2+	4	2-;	0;	4	0;	0;	**3+**	2(X)	n/a	n/a	2+3+	0	1-;	2=;	n/a	**3+**	498	MR	+
28	+	3+	12-;	1	3+	n/a	3+	4	2	3+	**3+**	3+	4	4	3+	3+	3+	3+	X	**3+**	528	MR	-
99	+	0;	2	n/a	0	2=;	0;	0	0;	2	**3+**	;1-	n/a	4	n/a	0;	0;	0;	0	**3+**	560	MR	-
18	*Lr3a,10,+*	0	0;	3	n/a	2+3+	23	0;	2	;	**0;**	22+	0;1=	0;	3+	3+	3	3	0;	**3+**	575	MR	-
62	+	0;	2-;	2	4	4	X	n/a	2-;	n/a	**3+**	n/a	2+3+	n/a	3+	3+	4	n/a	n/a	**4**	586	MR	-
63	*Lr10,+*	;1=	;	3	3+	n/a	n/a	3+	;1=	n/a	**;1-**	X	n/a	n/a	n/a	2+3+	0;	;1-	n/a	**3+**	609	MR	H
2	*Lr23,+*	0	3	0	0	0;	0;	0;	0;	12	**3+**	2	2	4	3+	n/a	0;	0;	0	**3+4**	646	MR	-
30	+	;1=	23;	0	1-	3	23	2+3+	;1=	3+	**3+**	4	4	3+	3+	n/a	3+	;1=	0;	**3+**	667	MR	-
1	*Lr23,26,+*	12	n/a**	1	3+	n/a	n/a	3+	;1=	1-;	**23**	2	1	4	3+	1-;	;1=	2-;	3+	**n/a**	783	MRMS	-
60	+	2	;1=	3+	1	4	X-	4	2=;	3+	**3+**	4	4	4	3+	n/a	4	;1-	2-;	**4**	801	MRMS	+
69	+	;1=	12	3	0	2+3	0;	;12-	1-;	2	**3**	2	4	n/a	n/a	0	12-;	n/a	2-	**3+**	813	MRMS	+
100	*Lr3a,+*	n/a	0	3	1=;	3	X	2+3+	3+	n/a	**3**	n/a	n/a	4	3+	3+	3+	0;	0;	**n/a**	819	MRMS	-
29	+	0	3	3	12	3+	3+4	4	3	3+	**3+**	4	4	4	3+	n/a	4	X-	X	**3+**	835	MRMS	-
65	*Lr2b,10,11,23,24,+*	0	0	4	3	0	0;	0	;	0	**0**	0	0;	0;	0	n/a	0;	n/a	X-	**4**	1156	MS	-
11	+	0;	n/a	3+	3+	3+	X	X	;1=	3+	**3**	3+	33+	3+	3+	3+	33+	33+	32	**3+**	1181	MS	-
31	+	0;	23;	3+	3	4	23	2-;	2=;	3+	**3+**	3+	4	3+(X)	3+	n/a	3	3+	n/a	**4**	1259	MS	-
115	*Lr3a,10,+*	0;	0;	3+	0	3+	4	n/a	3+	;1=	**1-;**	3+	0	0;	3+	3+	3+	0;	0	**3+**	1321	MS	H
Avocet																					5196	S	
Thatcher																					4746	S	

[a]+ presence, - absence of the allele associated with *Lr34*. H: heterozygous
[b]+: presence of additional unidentified resistance gene(s)
[c]n/a: not available information

The number of genes postulated in each genotype ranged from 1 to 7. One gene was postulated in 14 genotypes, seven with *Lr23*, three with *Lr10* and one with *Lr3a, Lr3bg, Lr26* or *Lr30*; all these genotypes had additional resistance conferred by ASR genes which was not possible to identify (+). Two genes were postulated in 12 genotypes, three genes were postulated in four genotypes, four genes were postulated in eight genotypes, five genes were postulated in five genotypes, six genes were postulated in one genotype, and seven genes were postulated in two genotypes. Other 25 genotypes only possessed seedling resistance that could not be identified with the races used in the study.

Most genotypes have different genes or gene combinations, although some might carry the same or similar resistance base. Genotypes 32, 46, and 72 probably have *Lr10, Lr11, Lr23,* and *Lr24*, while genotypes 24 and 67 could carry *Lr26* in addition to these genes; genotypes 1 and 96 probably have *Lr23* and *Lr26*; genotypes 20 and 94 probably have *Lr23* and *Lr30*; genotypes 50 and 82 might share *Lr3(a* or *bg), Lr17a,* and *Lr26*; gen-

otypes 26 and 58 could share *Lr3(a, bg* or *ka), Lr17a, Lr23,* and *Lr30*; genotypes 50 and 82 probably share *Lr3(a* or *bg), Lr17a* and *Lr26*; genotypes 26 and 58 might share *Lr3(a, bg* or *ka), Lr17a, Lr23,* and *Lr30*, and genotypes 6, 18, 47 and 115 were postulated to have *Lr3a* and *Lr10*.

Thirty-five genotypes were resistant to the races prevalent during 2012 (MFP and TDT-10,20)[4] and were susceptible to at least one of the other races tested (**Table 3**). These genotypes had a range of AUDPC from 13 (R) to 1438 (MS). The 36 seedling S genotypes to MFP and/or TDT-10,20 had a range of AUDPC from 64 (R) to 1321 (MS) (**Table 4**).

From the 12 genes postulated in Paraguayan genotypes, *Lr23* was the most frequent, postulated in 28 genotypes. Other genes frequently present in the genotypes were *Lr10, Lr26* and *Lr3* (jointly considering its three alleles). *Lr11, Lr24, Lr30*, *Lr1*, *Lr17a, Lr2* (*b* or *c*) were postulated in progressively fewer genotypes, while *Lr9* and *Lr16* were postulated in only one genotype (**Figure 1**).

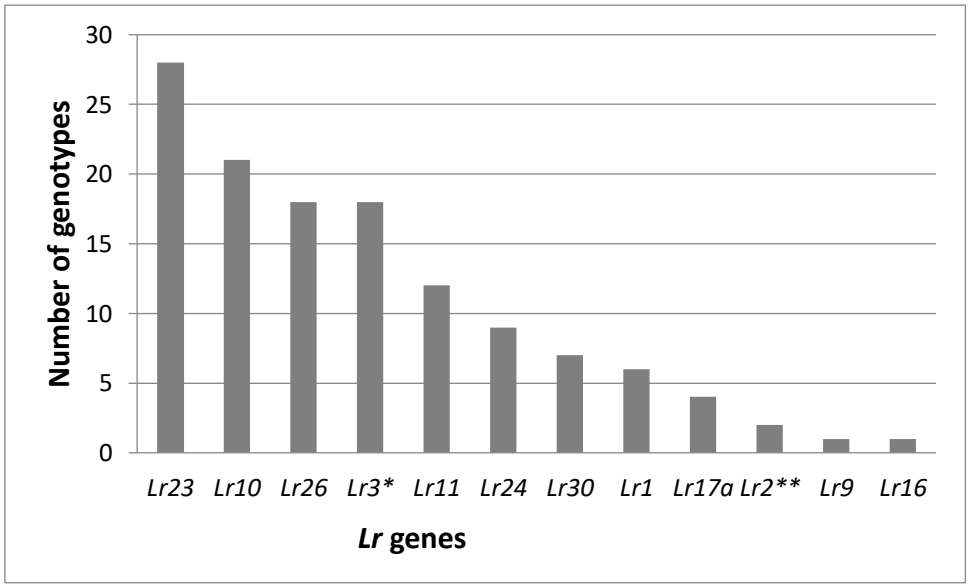

Figure 1. Number of genotypes with the postulated genes

**Lr3 (a, bg and ka), **Lr2 (b and c)*

Based on the *csLV34* molecular marker *Lr34* was postulated in 31%, absent in 60% and heterozygous in 9% of the genotypes (**Table 5**). *Lr34* was postulated in 20% of all-race-resistant genotypes in the seedling stage, 54% of prevalent race-resistant genotypes, and 22% of seedling susceptible genotypes to one or both prevalent races. *Lr34* was postulated in 31% of field R genotypes, 32% of MR genotypes, 31% of MRMS genotypes, and 29% of the MS genotypes.

Table 5. Percentage of genotypes with different seedling and field reaction with presence, absence or heterozygous for the *csLV34* marker, and total number of genotypes

	% of genotypes			
Category	+[a]	-[b]	H[c]	N° of genotypes
Seedling				
R to all races	20.0	68.9	11.1	45
R to prevalent races	54.3	37.1	8.6	35
S to the two prevalent races	22.2	72.2	5.6	36
Field				
R	31.0	64.8	4.2	71
MR	32.0	52.0	16.0	25
MRMS	30.8	53.8	15.4	13
MS	28.6	57.1	14.3	7
Total	31.0	60.3	8.6	116

[a]presence, [b]absence, [c]heterozygosity of *csLV34*

4. Discussion

The postulated ASR genes *Lr1, Lr2*(*a, b* alleles), *Lr3*(*a, bg, ka* alleles), *Lr9, Lr10, Lr11, Lr16, Lr17, Lr23, Lr24, Lr26,* and *Lr30* were detected alone or in combinations of up to seven genes per genotype. From the three reported *Lr2* alleles (*Lr2a, Lr2b, Lr2c*)[(44)], two (*Lr2b* and *Lr2c)* were postulated in only one genotype each. All *Lr3* alleles (*Lr3a, Lr3bg, Lr3ka*)[(45)] were postulated in some genotypes, although these could not be differentiated in some cases. The methodology used in this study is not very precise for genotypes with complex resistances[(27)]. Other studies have postulated up to five rust resistance genes in wheat genotypes.

As expected, genotypes derived from the same cross or closely related generally had similar basis of resistance: genotypes 24, 25 and 72 (E-92225/FCEP30) share *Lr10* and *Lr24*, while 24 and 72 also have *Lr11* and *Lr23*; genotypes 96 and 97 (ITAPUA40/3/ITP35/PF84432//CORD4) have *Lr23*, and 96 also has *Lr26*; genotypes 12 and 66 (ITAPUA40/CARCOVE//JUP*5/AMIGO) share *Lr11, Lr23* and *Lr26*, while 66 also has *Lr1* and *Lr24*; genotypes 32, 33, 46 and 67 (ITAPUA45/CORDILLERA4) share *Lr10, Lr11, Lr23, Lr24*, while genotypes 33 and 67 also have *Lr26*; genotypes 48 and 58 (MILAN/KAUZ//PASTOR/3/PASTOR) share *Lr3*(*a or ka*), *Lr23* and *Lr30*; genotypes 41 and 90 (PARULA/IAN10) had *Lr23*. All genotypes derived from ITAPUA 40 had either *Lr23* or *Lr26*, or both genes.

Most of the studied genotypes were introduced from CIMMYT or derived from local crosses with lines of this origin or from Brazil. Both origins have traditional regional cultivars in their foundation germplasm. Therefore, the resistance genes identified in the studied genotypes most probably come from CIMMYT germplasm and/or regional breeding programs. Eight of the postulated genes in the studied genotypes were previously reported in CIMMYT germplasm (*Lr1, Lr3a* and *Lr3bg* alleles, *Lr10, Lr16, Lr17a, Lr23, Lr24, Lr26*)[(9)(46)(47)(48)]. *Lr1, Lr3a*, *Lr10, Lr16, Lr17a, Lr24,* and *Lr26* were reported in cultivars and lines from Argentina, Brazil and Uruguay[(2)(25)(33)(49)]. *Lr3bg* and *Lr23* are present in genotypes from Brazil and Uruguay[(2)(25)(49)]. *Lr3ka* was first reported in the Argentinean cultivar Klein Aniversario[(45)] and has also been postulated in genotypes from Argentina and Brazil[(33)(49)]. *Lr9*, postulated in a single genotype, was transferred from *Aegilops umbellulata* to wheat[(50)]. This gene is not widely distributed in breeding germplasm but it was reported in some Brazilian and Argentinean cultivars[(33)(49)]. *Lr11* was described in the Argentinean cultivar El Gaucho[(51)] and was reported in some Brazilian cultivars[(49)]. *Lr30* was initially described in the Brazilian cultivar Terenzio[(52)]. The origin of *Lr2b* and *Lr2c* could not be traced back to known sources[(53)].

The seedling resistance that could not be identified probably corresponds to uncatalogued genes or known genes for which no genetic stocks were available. It is possible that the complementary genes *Lr27+31*, which have a low IT of X = to X+[53], are present in the tested germplasm, since some genotypes showed this characteristic IT and these genes are present in CIMMYT germplasm[9][47][48]. Another reason why the genes could not be identified probably relies on the genetics of the pathogen, since the races used might not possess the adequate virulence combination for the postulation.

It was not possible to postulate genes in 45 genotypes that were resistant to all races (**Table 2Table 3**), since by using this methodology only the presence of genes or combinations of genes that are ineffective to one or more races can be postulated. Some genotypes probably possess the resistance genes present in some of their parents. Genotypes 21, 35, 36, 37, and 38 probably possess *Lr42*, as they are derived from one parent with this gene. *Lr42* was transferred from *Triticum tauschii* to wheat[54]; it is found in synthetic wheat and is present in modern CIMMYT lines. Genotype 37 is derived from a cross of parents with *Lr42* and *Lr47* (BABAX/LR42//BABAX*2/3/PAVON 7S3+*LR47)* and probably has *Lr47*, since it expressed very low IT to all races similar to its respective single gene line, or both genes. *Lr47* was transferred from *Triticum speltoides*[55] and was effective to all races. Resistance in other genotypes in this group could be conferred by combinations of catalogued or uncatalogued ASR genes or by single *Lr* genes effective to the pathogen population of the region, and, therefore, valuable for LR resistance breeding. To identify or confirm the resistance genes present in these genotypes, it is necessary to use other methodologies as available molecular markers for ASR genes[29], confirm the presence of the genes through allelism tests or perform genetic analysis to map the resistance.

The high, uniform, and consistent LR infection achieved in the experimental fields at both locations, indicated by the high phenotypic correlation between locations, allowed a reliable characterization of the resistance of Paraguayan genotypes in Uruguay in 2012. All genotypes evaluated in the field had LR infection significantly lower than the susceptible controls, expressing a degree of resistance from R to MS. Most genotypes of all categories (**Table 2**, **Table 3** and **Table 4**) were R or MR (83%) and a low proportion was MRMS and MS (17%), which indicates that an effective selection for LR resistance was accomplished. Based on the phenotype, it was not possible to infer the presence of resistance expressed in adult plants in lines with seedling resistance to all races (**Table 2**), or resistant to the most frequent races (**Table 3**), since effective ASR masks the presence of APR-PR[27].

However, using the molecular marker *csLV34* APR-PR, the presence of the *Lr34* gene was postulated in 31% of all genotypes. The *csLV34* marker used to postulate the presence of *Lr34* has also been used by other researchers[32][33][34][35]. Although it is not a perfect marker, it is very close to *Lr34* (0.4 cM)[31] and it is considered to have a high quality diagnostic power[34]. *Lr34* was first described by Dyck and others[22] in the Brazilian cultivar Frontana[22] and it is present in the wheat germplasm from CIMMYT[11][12][56] and the region[2][5][25][33]. The presence of *Lr34* in the regional germplasm is a relevant contribution to the control of wheat diseases since it has conferred durable resistance to LR and has a pleiotropic effect for resistance to stripe rust (*Yr18*)[21], stem rust (*Sr57*)[57], powdery mildew (*Pm38*)[19][20] and spot botch (*Sb1*)[12][58].

Lr34 was postulated in 20% of the genotypes resistant to all races (**Table 2**) and in 54% of the genotypes resistant to the two prevalent races (**Table 3**). The significance of the presence of *Lr34* in the germplasm with ASR relies on the enhanced resistance it confers when combined with major genes[59]. Additionally, the presence of *Lr34* could help mitigate future losses if new virulent races that affect genotypes with ASR to all races emerge, or if low frequency virulent races to genotypes resistant to the most prevalent races increase in frequency.

Genotypes that in the seedling stage were susceptible to the most frequent races (**Table 4**) most probably possess resistance expressed in advanced stages of development. MFP and TDT-10,20, the most frequent races identified in the 2012 survey[4], were also the predominant races at La Estanzuela Experimental Station (17% and 32% of 65 analyzed samples, respectively) and at Young experimental field (20% and 34% of 79 samples, respectively). These two races are virulent to *Lr12, Lr13* and *Lr37* APR-HR genes, frequent in the regional germplasm[5]. Therefore, these genotypes' field resistance is most probably related to the presence of APR-PR genes that confer PR. APR-PR genes do not express high levels of resistance on their own, however, the combination of four or five APR-PR genes confers resistance levels close to immunity[11]. Lines under field conditions with MS to R response would have a progressively greater number of APR-PR genes. Silva and others reported the presence of *Lr34* and *Lr68* APR-PR genes in line 59 (SUZ6/OPATA)[60], which is consistent with the presence of the *Lr34* molecular marker and R field reaction of this genotype.

Lr34 was postulated in 22% of the genotypes with APR-PR to LR (**Table 5**). According to the levels of resistance expressed in the field, other APR-PR genes would also be present in addition to *Lr34*, since this gene expresses moderate levels of resistance when present alone. *Lr34* is probably present alone in entries with *csLV34* that expressed MRMS field reaction (**Table 4**); therefore, higher levels of field resistance would be explained by the presence of additional APR-PR genes. Furthermore, there are a considerable number of genotypes with R to MS field reaction which lack *csLVLr34*, indicating the presence of APR-PR genes other than *Lr34*. Diversity in this characteristic is desirable to achieve high and stable PR levels. It would be desirable to confirm the presence and/or introgress other genes that confer this type of resistance[12], such as *Lr46, Lr67, Lr68,* and other QTLs associated with PR that have not yet been characterized[61]. The introgression of *Lr68* in Paraguayan germplasm would be especially interesting since this gene has a greater effect than *Lr34* in some South American countries[32][62]. Molecular markers could be used to accelerate the introgression of PR in the Paraguayan germplasm.

When using major resistance genes, it is essential to know which genes are present in the germplasm to combine them with different effective resistance, increasing the diversity and possible duration of the resistance. *Lr42*, possibly present in entries 21, 35, 36, 37, and 38, and *Lr47*, possibly present in entry 39, are effective to the leaf rust population in the region. These genes are not present in the traditional Paraguayan germplasm, and could be used to develop new resistant cultivars. While the presence of race-specific ASR genes is common in cultivars used by farmers and in the germplasm of wheat breeding programs, the use of APR-PR race-non-specific gene combinations that confer PR is the best alternative to achieve high levels of durable resistance. This decreases the need for LR chemical control, being environmentally and economically friendly, and it could also allow for better integrated disease management.

Before designing the resistance improvement strategy in the Paraguayan Wheat Improvement Program, the field resistance of the genotypes should be confirmed locally, since environmental conditions can affect the expression of PR[62] and the resistance conferred by major genes[63]. The pathogen's diversity can affect the effectiveness of the resistance of genotypes with major genes. While there is evidence of similarity in the population of *P. triticina* in the epidemiological zone comprising Uruguay and Paraguay, the pathogen's population is highly variable both spatially and temporally, including many races during each growing season[5], which also indicates the need to further test the genotypes locally and under the current pathogen population.

In conclusion, this study demonstrates that there is a significant number of genotypes with APR-PR to LR as a foundation to expand diversity and accumulate additional genes, to achieve stability of cultivars' field resistance and the pathogen population. Alternatively, genotypes with resistance conferred by ASR genes effective to all races of the pathogen may be used in combination with PR genes, to avoid high losses in case new virulent races of the pathogen to the ASR emerge. If only genotypes with effective ASR genes are used, it is

advisable to increase the genetic diversity introducing other effective genes, and their strategic deployment should be associated with ongoing pathogen monitoring, as an essential tool to take early measures such as alerts for disease control and replacement of affected varieties if new virulent races are detected.

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Transparency of data

Available data: The entire data set that supports the results of this study was published in the article itself.

Author contribution statement

RS: Data curation; Formal analysis; Investigation; Writing - original draft; Writing - Review & editing

SP: Writing - Review & editing

PS: Data curation; Formal analysis; Writing - Review & editing

SG: Conceptualization; Data curation; Formal analysis; Writing - original draft; Writing - Review & editing

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Supplementary material

Table S1. Wheat genotypes used in the study

Entry	Genotype	Cross	Origin
1	ITAPUA40	BOW´S´/VEE´S´	CIMMYT
2	ITAPUA 70	RAYON//VEE#6/TRAP1	CIMMYT
3	ITAPUA 75	VEE"S"/RL6010/JUP73/3/PRINIA	CIMMYT
4	CANINDE 1	MILAN/MUNIA	CIMMYT
5	CANINDE 11	WEEBILL2	CIMMYT
6	Y-06068	WBLL4//BABAX.1B.1B*2/PRL/3/PASTOR	CIMMYT
7	Y-06070	WBLL1*2/TUKURU	CIMMYT
8	E-06247	BABAX/PASTOR/3/KAUZ*2/YACO//KAUZ	CIMMYT
9	E-07056	ITAPUA40/KURUKU	PIT
10	E-08057	ITAPUA40/KURUKU	PIT
11	E-08071	ITAPUA40/KURUKU	PIT
12	E-07094	ITAPUA40/CARCOVE//JUP*5/AMIGO	PIT
13	E-08158	ITAPUA40/IAN 10	PIT
14	E-08356	ITAPUA50/ITAPUA40	PIT
15	Y-08008	PFAU/WEAVER*2//TRANSFER#12,P88.272.2	CIMMYT
16	Y-06069	WBLL4/KASO2//PASTOR	CIMMYT
17	Y-06074	WBLL1*2/TUKURU	CIMMYT
18	Y-07096	CS/TH.SC//3*PVN/3/MIRLO/BUC/4/MILAN/5/TILHI	CIMMYT
19	Y-07090	VEE/MJI//2*TUI/3/PASTOR/4/BERKUT	CIMMYT
20	2525	ND643/2*WBLL1	CIMMYT
21	2526	BABAX/LR42//BABAX*2/3/KUKUNA	CIMMYT
22	2527	PRL/SARA//TSI/VEE#5/3/FINSI	CIMMYT
23	Y-09035	PFAU/WEAVER*2//TRANSFER#12,P88.272.2	CIMMYT
24	E-09246	E-92225/FCEP30	PIT
25	E-09250	E-92225/FCEP30	PIT
26	E-09318	PRL/VER#6//CLMS/3/ORL99393	PIT
27	Y-08027	PRINIA/STAR//PISUPERI0R/CRDN	CIMMYT
28	Y-09006	EMB16/CBRD//CBRD	PIT
29	Y-09003	GUS/3/PRL/SARA//TSI/VEE#5/4/FRET2	CIMMYT
30	CD150	CD150	PIT
31	E-08069	ITAPUA40/KURUKU	PIT
32	E-08031	ITAPUA45/CORDILLERA4	PIT
33	E-08032	ITAPUA45/CORDILLERA4	PIT
34	Y-08202	ND643/2*WBLL1	CIMMYT
35	Y-08006	BABAX/LR42//BABAX*2/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KAUZ	CIMMYT
36	Y-09096	BABAX/LR42//BABAX*2/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KAUZ	CIMMYT
37	Y-09038	BABAX/LR42//BABAX*2/3/PAVON 7S3,+LR47	CIMMYT
38	Y-09023	BABAX/LR42//BABAX*2/3/VIVITSI	CIMMYT
39	Y-07088	VEE/MJI//2*TUI/3/PASTOR/4/BERKUT	CIMMYT
40	Y-09036	PFAU/WEAVER*2//TRANSFER#12,P88.272.2	CIMMYT
41	Y-08086	PARULA/IAN10	PIT
42	Y-09033	WBLL1*2/BRAMBLING	CIMMYT
43	Y-09003	GUS/3/PRL/SARA//TSI/VEE#5/4/FRET2	CIMMYT
44	Y-09004	T.DICOCCON PI94625/AE.SQUARROSA (372)//3*PASTOR	CIMMYT
45	Y-10005	PRINIA/STAR//MILAN/MUNIA	CIMMYT
46	E-08032	ITAPUA45/CORDILLERA4	PIT
47	Y-10132	IAN10/CANINDE3	PIT
48	Y-10385	MILAN/KAUZ//PASTOR/3/PASTOR	CIMMYT
49	Y-10139	IAN10/CANINDE3	PIT
50	Y-10145	IAN10/ITAPUA70	PIT
51	Y-10228	CEP99173/PEWIT1	PIT
52	Y-10236	PRL/VEE#6//CLMS/3/ITAPUA 55	PIT
53	Y-10239	PRL/VEE#6//CLMS/3/ITAPUA 45	PIT
54	Y-10376	KAUZ/MILAN/3/CROC_1/AE.SQUARROSA (205)//KAUZ	CIMMYT
55	Y-10378	SITE/FINSI	CIMMYT
56	Y-10379	KAUZ*2//K134(60)/VEE/3/ATTILA/4/MILAN/KAUZ	CIMMYT
57	Y-10381	KAUZ*2//K134(60)/VEE/3/ATTILA/4/MILAN/KAUZ	CIMMYT
58	Y-10383	MILAN/KAUZ//PASTOR/3/PASTOR	CIMMYT
59	Y-10388	SUZ6/OPATA	CIMMYT
60	Y-10587	TNMU/6/CEP80111/CEP8165/5M/7/IAPAR 85	PIT
61	Y-10482	KAMBARA2//MILAN/AMSEL	CIMMYT
62	Y-10416	WBLL2//MILAN/AMSEL	CIMMYT
63	Y-10456	WBLL4/5/BABAX.1B.1B*2/PRL/3/PASTOR/4/OC 946/PF906	PIT
64	Y-10417	WBLL2/BR18	PIT
65	Y-10209	PARAGUA-CIAT//MILAN/MUNIA	PIT
66	E-07095	ITAPUA40/CARCOVE//JUP*5/AMIGO	PIT

Entry	Genotype	Cross	Origin
67	E-08028	ITAPUA45/CORDILLERA4	PIT
68	E-08143	IAN7/LIRA"S"//ITAPUA 65	PIT
69	E-08078	ITAPUA40/KURUKU	PIT
70	LAJ-5018	PGO//CROC1/AE SQ(224)/3/2*BORL95/4/BAV92/5/PASTOR	CIMMYT
71	E-09091	ITAPUA40/CEP36	PIT
72	E-09244	E-92225/FCEP30	PIT
73	E-09256	E-97034/ORL99220	PIT
74	E-09259	E-97034/ORL99220	PIT
75	E-09333	EMB16/CBRD//CBRD	PIT
76	E-09245	E-92225/FCEP30	PIT
77	E-09252	E-97034/ORL980204	PIT
78	E-09200	IAN10/ORL99393	PIT
79	E-09108	ITAPUA40/E-96001	PIT
80	E-09070	ITAPUA40/CORDILLERA4	PIT
81	E-09022	ITAPUA45/ITAPUA40	PIT
82	E-09032	ITAPUA45/ITAPUA40	PIT
83	E-09317	TUI/RL4137//ITAPUA60	PIT
84	E-09367	YANGMAI 5*2/4/MOR/VEE#5//DUCULA/3/DUCULA	CIMMYT
85	E-08447	TNMU/CBRD//MILAN/SHA7	CIMMYT
86	E-09246	E-92225/FCEP30	PIT
87	E-09064	ITAPUA40/PROINTAGRANAR	PIT
88	E-09404	BABAX/LR42//BABAX*2/3/TUKURU	CIMMYT
89	E-09318	PRL/VER#6//CLMS/3/ORL99393	PIT
90	E-09393	PARULA/IAN 10	PIT
91	E-09407	ITAPUA50/4/TNMU/3/LND/BAU//BA	PIT
92	E-09415	ITAPUA60/CANINDE1	PIT
93	E-09382	ITAPUA40/PEWIT1	PIT
94	E-09389	CD112/ITAPUA50	PIT
95	E-09395	BR18/ITAPUA40	PIT
96	E-09399	ITAPUA40/3/ITP35/PF84432//CORDILLERA4	PIT
97	E-09401	ITAPUA40/3/ITP35/PF84432//CORDILLERA4	PIT
98	E-09408	ITAPUA55//PISUPERIOR/CRDN	PIT
99	E-09408	MILVUS1/ITAPUA60	PIT
100	E-09429	IAN8/KASOSRO3//CD104	PIT
101	E-09653	ITAPUA45//TUI/RL4137	PIT
102	E-09893	E-97034/ORL99220	PIT
103	E-09933	C-91181/ORL980204	PIT
104	E-10042	ND643/2*WBLL1	CIMMYT
105	E-10098	ITAPUA75/WEEBILL2	PIT
106	E-10101	ITAPUA75//PF953048/IAPAR18	PIT
107	E-10104	PARULA/CD104	PIT
108	E-10105	PARULA/CD104	PIT
109	E-10106	PARULA/CD104	PIT
110	E-10121	TAPUA75/CORDILLERA3	PIT
111	E-10126	ITAPUA75/3/ITP35/PF84432//CORDILLERA4	PIT
112	E-10132	E- 2044/CORDILLERA3	PIT
113	E-09760	ITAPUA55//HUW234+LR34*2/PASTOR	PIT
114	E-09676	ITAPUA45//TUI/RL4137	PIT
115	E-09841	ITAPUA60/PROINTAGRANAR	PIT
116	E-09628	ITAPUA40/CEP36	PIT
Susceptible control		Avocet	
Susceptible control		Thatcher	

Table S2. Infection types of differential lines with *Lr* genes to *Puccinia triticina* races used in seedling tests

Lr gene	CHT	DBB-10,20	KDG-10-20	LPG-10	MCD-10,20	MCP-10	MCR-10	MCT-10	MDT	MFP	MFP-10,20	MFP-20	MFR	MFT-10,20	MHP-10	MKD-10	MMD-10,20	SPG-10	TDT-10,20
Lr1	0;	0;	0;	4	3+	3+	3+4	3+	3+	3+	3+	3+	3+	3+	3+	3+	4	3+	3+
Lr2a	;	;1-	3+	0	0	0;	0;	;	0;	0;	0;	0;	0	0;	0;	0;	0;	3+	3+
Lr2b	0;	;	3+	n/a[a]	0	0;	0;	;	0;	0;	0;	0	0	0;	0;	0;	0	3+	3+4
Lr2c	1-	3	3+	2=	0;1-	;1=	;1=	;1=	1-;	1=;	;1=	;1=	;	;1=	;1-	1-;	;	3+	3+
Lr3a	3+	0;	3+	1=;	3+	3+	3+4	3+	3+	3+	3+	3+	3+	4	3+	3+	4	;1=	3+
Lr3bg	3+	;1=	1-;	n/a	3+	3+	12-	2+3+	3+	3+	3+	3+	23	3+	3+	3+	22+	;1=	3+
Lr3ka	3+	;	2=	2=;	2	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	2=	2=;	2=	3+
Lr9	0	0	0	3+	0	;	0	0	0;	0;	0;	0;	0	0	0;	0;	3+	3+	0
Lr10	1=;	3+	3+	4	3+	4	3+	3+	2=;	2=;	3+	2=;	;1=	4	3+	3+	4	3+	3+
Lr11	3+	0;	3+	3+	2	2-	3+	3+	3+	2=;	2=	2=;	3	3+	2=;	2=	2=;	3+	3+
Lr14a	3+	3+	3+	4	4	3+	3+	3+	3	3+	3+	3+	3+	4	3+	3+	4	3+	3+
Lr14b	3+	3+	3+	4	4	3+	3+	3+	3	3+	3+	3+	3+	4	3+	3+	3+	3+	3+
Lr16	3	1-	1-	1-;	21	22+	1-	12	1-;	1-	1-	1-	;1=	1-;	2+3+	3+	2-	1-;	1-
Lr17a	3+	;	1-;	;1=	4	3+	;1=	3+	3	3+	3+	3+	;	4	3+	3+	3+	;1=	3+
Lr19	0	0	0	0;	0	0;	0	0;	0	0	0	0	0	0;	0	0	0	0	0
Lr20	;	3+	3+	;	4	;	;	;	;	;	3+	3+	;	3+	1-;	;1=	4	;1=	3+
Lr21	2	2=	2=;	2	22+	2	12-;	2-	2	2=;	1=;	2=;	1-;	2	2	;1=	2=;	1-;	2=
Lr23	n/a	3+	3+	3+	2	1-;	3+	2-	2-	3+	3+	3	3	4	2-	2	2	3+	3+
Lr24	0;	0	3+	3+	0	0;	;1=	;	3	3+	3+	3+	3	4	;	3+	1-;	3+	3+
Lr26	3+	0	2-	3+	3+	3+	3+	3+	2	3+	3	3+	3	3+	3+	3+	3+	3+	2+
Lr30	3+	2=	2=;	2-;	2-2	3+	3+	3+	3+	3+	3+	3+	3+	4	3+	2-	2=;	2=	3+
Lr39	0	2	0	0	0;	0;	0	0	0	0	0	0	0	0	0	0	12-	0;	0
Lr42	0;	0;	2	1=;	0;	0;	0	0	n/a	12-	12	12-	1-	X	;	2	0;1=	1-;	1=
Lr47	0	0	0	0;	0;	;	0	0	0;	0	0	0	0	0;	;	0;	0;	0;	0;

[a]n/a not available information