

A century of wheat breeding in Brazil: the origin and inheritance of the *Lr34* locus in wheat varieties released from 1922 to 2016

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Abstract: *Wheat breeding in Brazil began in 1919. A cross between the varieties Fronteira and Mentana gave rise to Frontana, the most popular ever released, being recommended for more than 40 years. Frontana played an important role as a source of resistance to leaf and stripe rust as well as resistance to pre-harvest sprouting. Possessing an early maturity cycle, it contributed to change the predominant varietal pattern at that time. The resistance gene *Lr34* was first described in Frontana. The gene confers durable adult plant resistance to leaf rust and other biotrophic fungi. Because of its durability, stability across environments, haplotype diversity and wide geographical distribution, *Lr34* is probably the most important single resistance gene in wheat. We evaluated 453 wheat varieties that have been released or recommended for cultivation in Brazil from 1922 to 2016 using KASPar markers to detect those varieties carrying the diagnostic mutations corresponding to the presence of the *Lr34* resistance allele.*

Keywords: *Adult plant resistance, leaf rust, diagnostic molecular markers, KASPar.*

INTRODUCTION

Wheat breeding in Brazil, which began in 1919, is completing a century in 2019 (Del Duca 1999, Sousa and Caierão 2014). The first lines were selected from among primitive wheats introduced by European immigrants in the 16th century that displayed little adaptability to local growing conditions (Carmo 1911). In 1924, the first Brazilian wheat varieties - Polyssú and PG 1 (a Polyssú selection) - were released, and soon after were used intensively in crosses, giving rise to many descendants among cultivars in Brazil (Sousa 2003). In the 1920s, the Italian sib cultivars Mentana and Ardito, released in Italy in the early 1900s (Borghi 2001), were introduced and subsequently widely cultivated in Brazil; on account of their outstanding adaptability and disease resistance, they were used as parents in many local breeding programs (Sousa and Caierão 2014). In 1940 a cross between Fronteira and Mentana gave rise to Frontana (Del Duca 1999), the most popular variety ever released, now sowed across the country for more than 40 years (from 1940 to 1987) (Sousa and Caierão 2014). Frontana is the genotype with the greatest number of offspring among the commercial cultivars in Brazil, with 175 descendants in total. Frontana played an important role as a source of resistance to leaf and stripe rust, resistance to pre-harvest sprouting and as source of the early maturity cycle, inherited

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from Mentana, contributing to change the predominant varietal pattern at that time (Sousa and Caierão 2014). Frontana has been traced as the source of the *Lr34* quantitative trait locus in a significant proportion of International Maize and Wheat Improvement Center (CIMMYT) cultivars as well as in Hard Red Spring Wheats from North America (Kolmer et al. 2008).

At present, *Lr34* is the best-known and studied adult plant resistance (APR) gene. It provides durable adult plant resistance to leaf rust (*Puccinia triticina*) as well as to other biotrophic fungi causing stripe rust (*P. striiformis*), stem rust (*P. graminis tritici*) and powdery mildew (*Blumeria graminis*) (Dyck 1977, German and Kolmer 1992, Bossolini et al. 2006, Spielmeyer et al. 2008). Co-segregation of *Lr34* with other traits such as leaf tip necrosis (*Ltn*), tolerance to barley yellow dwarf virus (*Bdv1*) and spot blotch (*Bipolaris sorokiniana*) have been documented (Singh 1992a, b, McIntosh 1992, Singh 1993, Joshi et al. 2004, Spielmeyer et al. 2005). Because of its durability (Johnson 1984), stability across environments (Lillemo et al. 2011), pleiotropy (Krattinger et al. 2009), haplotype diversity and wide geographical distribution (Dakouri et al. 2010, Dakouri et al. 2014), *Lr34* is probably the most important single resistance gene in wheat.

Because the expression of the *Lr34* multi-pathogen resistance locus is frequently masked by major R genes, the development of molecular markers has been a priority in many breeding programs (Bossolini et al. 2006, Lagudah et al. 2006, Kolmer et al. 2008, McCallum et al. 2008, Lagudah et al. 2009). The *Lr34* gene has been isolated, sequenced and predicted to encode a pleiotropic drug resistance (PDR-like) ATP-binding cassette (ABC) transporter of 1,401 amino acids (Krattinger et al. 2009). This allowed the development of a group of perfect diagnostic markers (Lagudah et al. 2009), including the gene-specific marker caIND11 (that detects the TCC indel in exon 11) the best diagnostic marker for marker-assisted selection of *Lr34* due to its co-dominancy and robustness (Dakouri et al. 2010).

In a century of wheat breeding in Brazil, the joint efforts of many public and private research institutes resulted in the release of more than 540 varieties to farmers. Traits such as disease resistance, aluminum tolerance, cycle, plant height and architecture, as well as yield and technological quality were the focus of these breeding programs (Sousa and Caierão 2014). In this paper, we evaluated 453 wheat varieties released or recommended for cultivation in Brazil from 1922 to 2016 using KASPar markers to detect those varieties carrying the diagnostic mutation corresponding to the *Lr34* resistance allele. Based on the results of both DNA and pedigree analysis, we discuss the likely inheritance of this locus through generations of varieties released in Brazil, from the very beginning of breeding programs to the present day. We also present the reactions to leaf rust in adult plant stages of a group of varieties possessing the *Lr34* resistance locus and discuss the stability of the rust reaction conferred across environments.

MATERIAL AND METHODS

Plant material

The germplasm collection comprised 453 hexaploid wheat genotypes that have been under cultivation in Brazil from 1922 to 2016 (Table S1). The majority (431) are improved varieties produced by local breeding programs. A few varieties were introductions from Italy (Ardito and Mentana), Uruguay (Centenário), Australia (Florence), Argentina (Buck Manantial), Mexico (Ciano 67, Inia 66, Jupateco 73, Pitic 62, Sonora 64, Tanori 71 and Anahuac 75), and Paraguay (Paraguay 214, Paraguay 281 and Itapua 5). Some landraces (Branco, Veadeiros, Sales, Camacrânia and Colonista) and varieties of unknown origin (Bandeirantes, General Vargas and Peladinho) that have been cultivated in Brazil were also analyzed. Seeds were provided by the Embrapa Wheat germplasm bank located in Passo Fundo, in the state of Rio Grande do Sul.

DNA extraction and marker analysis

Seeds from each accession were sown in commercial substrate and grown in a chamber under controlled temperature (22/17 °C, day/night) at 14 h daylength for 15 days. When the seedlings were at the two-leaf stage, leaf segments from one seedling per accession were cut and placed in 2 mL microtubes. Immediately after, the leaf samples were lyophilized for 48 h. The dried tissue was macerated using TissueLyser II (Qiagen) and the DNA was extracted according to the hexadecyltrimethylammonium bromide (CTAB) buffer protocol (Lefort and Douglas 1999). DNA concentrations were measured using a spectrophotometer (Thermo Scientific, USA) and normalized to about 100 ng μL^{-1} . The DNA samples were placed in 96-well plates (PCR-96-FLT-C, Axygen) and sent to CenGen (Pty) Ltd., Worcester, South Africa, to be

analyzed using the Kompetitive Allele Specific PCR (KASPar) marker. Trait-based KASP assays for the presence of *Lr34/Yr18/Sr57/Pm38* alleles (delTCC/delTCC) or (insTCC/insTCC) were run as previously described (Dreisigacker et al. 2016). The wheat variety Kariega was used as positive control and a no template control (NTC) was used as a negative control.

Disease scoring

The reaction to leaf rust of some wheat varieties was evaluated under field conditions, from 2006 to 2010 and in 2013. Because of the limited amount of seeds available at the Embrapa Wheat germplasm bank, only varieties with adequate number of seeds required to establish field plots were included. Genetic stocks comprising near-isogenic lines and varieties from diverse origins known to possess the *Lr34* gene were also evaluated for rust reaction. Highly susceptible Brazilian varieties were used as controls. Each genotype was sowed in approximately 1-m rows, 20 cm apart, and 40 to 50 seeds were planted per plot. Spreader rows of the susceptible variety CD 104 were sowed perpendicular to plots and inoculated in early June with a mixture of races of *P. triticina* covering a wide range of the virulence occurring in Brazil. Races chosen were as follows: B25 (LCJRS), B27 (MBRMQ), B34 (MCJCN), B35 (MCHCN), B39 (CGTCQ), B43 (TDDQR), B48 (MCJMT), B49 (TFTCS), B50 (SPJRS), B51 (MFTCT), B52 (MFJMN), B54 (TFTCT), B55 (MFTMT), B56 (MFPCT), B57 (TDTMR), B58 (MDPMR) and B59 (MFTMT+). The mixture of isolates was suspended in Soltrol 170 oil and sprayed on the spreader rows in early October using a backpack sprayer. The severity and response rating for adult plant field resistance was based on the modified Cobb scale (Peterson et al. 1948). The host infection type 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 (Roelfs et al. 1992). Leaf rust severity and resistance responses were recorded in mid-November when the susceptible control CD 104 had leaf rust severity of at least 80%.

RESULTS AND DISCUSSION

In a century of wheat breeding in Brazil, more than 540 varieties were released to farmers. Because of the highly conducive environments, one of the major concerns addressed by these programs was the enhancement of levels of resistance to the common wheat diseases, including leaf rust. From the 453 accessions analyzed in this study, 117 (25.86%) were found to carry the mutation (delTCC/delTCC) corresponding to the *Lr34* leaf rust resistance allele (Table S1). A few lines presented a deletion in a single allele (delTCC/insTCC), and therefore the *Lr34* locus was not confirmed to be present in these lines as a functional allele. The origin of the *Lr34* resistance allele in Brazilian wheat varieties and its inheritance across decades over one century of wheat breeding is discussed below.

The 1920s and 1930s

Both Mentana and Ardito were found to carry the *Lr34* gene using the diagnostic KASP markers, which is in accordance with previous results using other molecular markers (Kolmer et al. 2008, Dakouri et al. 2013). Mentana is the older recognized source of the *Lr34* locus in South America (Kolmer et al. 2008); it was traced to be the most likely donor of the *Lr34* gene to the majority of varieties detected to carry this gene, either directly or indirectly via Frontana (Figure 1). Branco and Veadeiros are the oldest Brazilian cultivars found to carry the *Lr34* resistance gene (Table S1). Both are landraces and their pedigrees are unknown, and therefore the origin of the *Lr34* gene in these varieties remains unclear. Minuano and Surpresa are also among the old cultivars possessing the *Lr34* gene. Both were developed by Iwar Beckman from a cross between Polyssú and Alfredo Chaves 6-21. The cultivar Alfredo Chaves 6-21 is a selection from the landrace Turco (Sousa 1995), Polyssú is a landrace selected from undetermined Italian varieties in the early 1900s (Sousa 2003). Polyssú is the most likely donor of the *Lr34* gene to Minuano and Surpresa because it carries the *Ltn* gene (causing leaf tip necrosis) which is a phenotypic marker associated with the gene *Lr34* (Sousa and Barcellos 2000).

The 1940s

The first descendants from Mentana were released in the 1940s, with Frontana being the most important variety among them. Frontana is the most popular variety ever released in Brazil, being sowed across the country for 47 years (from 1940 to 1987). Frontana is the variety where the gene *Lr34* was first described (Dick 1977). Subsequently, it was included in the group of genetic stocks used for the development of the diagnostic molecular markers (Lagudah et al.

2009) used in our study. Here, we found that Frontana is the most likely source of the *Lr34* gene of more than 50 wheat varieties released for cultivation in Brazil (Figure 1). In addition to Frontana, other varieties released in the 1940's were found to possess the *Lr34* gene (Table S1), namely, Cincana, Floreana, Sales, and Lageadinho. Pitana descends from Mentana via Floreana; in our study, it was found to possess the *Lr34* gene as well. Pitana also carries the *Lr13* resistance gene, identified due to the linkage with the gene *Ne2*, producing the hybrid necrosis phenotype (Sousa 1999).

The 1950s

Of the four varieties released in this decade and found to carry the *Lr34* gene using the diagnostic KASP markers, three are descendants from Frontana (Table S1, Figure 1), Veranópolis, Carazinho, and Fortaleza. Veranópolis and Carazinho also carry the *Ne2* gene (Zeven 1973, Zeven 1981) and may carry the *Lr13* resistance gene as well. Descending directly from Mentana, the cultivar BH 1146 was released in 1955 and remained under cultivation for 43 years (Sousa and Caierão 2014). In addition to possessing the *Lr34* resistance allele, BH 1146 was widely used in the development of several other wheat genotypes, primarily due its tolerance to Al³⁺ (Sousa 1998). Our results are in accordance with previous reports in which the presence of the *Lr34* gene was postulated in Veranópolis (Kaur et al. 2000, Kolmer et al 2008), Carazinho (McCallum et al. 2012), Fortaleza (Dakouri et al. 2013) and in BH 1146 (Sousa and Barcellos 2000, Kolmer et al. 2008, McIntosh et al. 2008).

The 1960s

The *Lr34* gene present in the varieties released in the 1960s (Table S1) was probably inherited in one of four ways: 1) Directly or indirectly from Frontana (Figure 1): IAC 3 – descends directly from Frontana; Nova Prata and Cotiporã both descend from Veranópolis, released in 1950 and possessing the *Lr34* gene, as previously discussed in this work. Cotiporã has been previously postulated to carry the *Lr34* gene due to the presence of the *Ltn* gene associated with the leaf tip necrosis (Sousa and Barcellos 2000). Erexim most likely inherited *Lr34* from Frontana via Colotana. Nobre probably inherited *Lr34* from Frontana via Colotana or Yaktana 54. 2) Indirectly from Mentana (Figure 1): IAC 8 – Paraguaçu probably inherited *Lr34* from Mentana via Heana, because Floreana descends directly from Mentana and was detected to possess the *Lr34* gene in this study (Table S1). 3) Indirectly from Polyssú: Patriarca most likely inherited *Lr34* from Minuano, as previously discussed in section **The 1920s and 1930s**. 4) From undetermined sources: Based on our results and on the pedigree analysis, the origin of the *Lr34* gene could not be traced in the following varieties: IAS-C-45 Vila Velha, IAS-C-48 Guarapuava, and IAS 52.

The 1970s

This period was marked by the expansion of wheat acreage in Brazil and by the creation of many research centers for wheat improvement, including the National Wheat Research Center of Embrapa (Embrapa Wheat), located in Passo Fundo. During this decade, a long-lasting collaborative work between Embrapa Wheat and CIMMYT was established. Many varieties from Mexico and some varieties from Paraguay, Argentina, and Uruguay were introduced for cultivation in South Brazil, and these were used in crosses by the local breeding programs as well (Sousa and Caierão 2014). The *Lr34* gene was detected in 19 varieties released in the 1970s (Table S1), where it was probably inherited in one of four ways: 1) Indirectly from Frontana (Figure 1): Encruzilhada most likely inherited *Lr34* from Frontana via Fortaleza, released in 1957 and possessing *Lr34* as discussed previously in this paper. C33 and Vacaria most likely inherited *Lr34* from Frontana via Veranópolis, released in 1950, which descends directly from Frontana and was detected to possess the *Lr34* gene, as discussed previously in this paper. Ciano 67 is one of the Mexican varieties introduced in Brazil in the 1970s and recommended for cultivation in 1976, which carries a combination of adult plant resistance genes to leaf rust and stem rust, as a *Lr13*, *Lr34* and *Sr2* complex (Mishra 1992). It was widely used in crosses by many breeding programs and a number of its descendants released in the 1980s were found to carry the *Lr34* resistance allele (Figure 1), as will be further discussed in this paper. Ciano 67 most likely inherited *Lr34* from Frontana via Chris, a North American variety derivative of Frontana and known to carry *Lr34* (Kolmer et al. 2008). IAC 13-Lorena, released in 1977, descends directly from Ciano 67 and is assumed that they share the same source of the *Lr34* gene. Glória probably inherited *Lr34* from Frontana via Tezanos Pinto Criollo. Although we have not found any paper reporting that Tezanos Pinto Criollo possesses *Lr34*, Selkirk has been characterized as carrying the *csLV34a* allele and a no-*Lr34* variety (Kolmer et al. 2008). Palotina and Nambu probably inherited *Lr34* from Frontana via Tezanos Pintos Precoz, which descends directly from

Frontana (GRIS 2019) and was detected to possess the *Lr34* gene (Kolmer et al. 2008, Dakouri et al. 2013). The Mexican variety Jupateco 73 was very popular and achieved large acreage after its release in 1978 (Sousa and Caierão 2014). It has the same *Lr34* resistance haplotype as Mentana and Frontana (the diagnostic 3-bp deletion in exon 11), suggesting the gene has a single origin in these varieties (Krattinger et al. 2009). 2) Directly or indirectly from Mentana (Figure 1): Regarding the variety Palotina, it may also be considered that Mentana could have been the donor of *Lr34* via Nainari 60, known to carry the *csLV34b* allele, the functional form of *Lr34* (Kolmer et al. 2008). The variety Confiança is a Nainari 60 reselection released in 1977, and we assume it inherited *Lr34* from Mentana as discussed. Paraguay 281 is a Paraguayan variety descended from Mentana and recommended for cultivation in 1978. IAC 18-Xavantes descends from BH 1146, released in the 1950s, which was a very important variety in Brazil, as discussed previously in this paper. 3) Sources other than Frontana or Mentana: Some varieties have complex pedigrees, making the source of the *Lr34* gene less than obvious. The Brazilian varieties LA 1434 and PAT 24 most likely inherited *Lr34* from the Mexican varieties Yaqui 48 and Norteño 67, both possessing this resistance gene (Mishra 1992, Kolmer et al. 2008); nevertheless, the source is not clear considering their pedigrees (GRIS 2019). 4) From undetermined sources: Based on our results and on the pedigree analysis, the origin of the *Lr34* gene could not be traced in the following varieties: S 76, Hulha Negra, Peladinho and LA 1549. Itapua 5 is a Paraguayan variety released for cultivation in Brazil in 1979. It possesses a complex pedigree in which Mentana, Ardito, and Frontana are present (GRIS 2019); nevertheless, none of its parental varieties are reported to carry the *Lr34* gene.

The 1980s

This decade was considerably prolific, with 103 varieties released by local breeding program (Sousa and Caierão 2014). Thirty-five varieties were found to carry the *Lr34* resistant allele (Table S1), the majority from breeding programs led by Embrapa (varieties Trigo BR), the Agronomic Institute of Campinas (varieties IAC), the Agronomic Institute of Paraná (varieties Iapar) and the former Organization of Cooperatives of the State of Paraná (varieties Ocepar), currently the Cooperative for Technological and Economic Development (Coodetec). It is probable that the *Lr34* gene detected in the varieties released in the 1980s had been inherited in one of three ways: 1) Indirectly from Frontana (Figure 1): Trigo BR 23 most likely inherited *Lr34* from Frontana via Cotiporã, with Veranópolis being a direct descendant from Frontana that carries the *Lr34* gene, as discussed previously in section **The 1950s**. The variety Trigo BR 23 was previously reported to carry the *Ltn* gene causing leaf tip necrosis, a phenotypic marker associated with the gene *Lr34* (Sousa and Barcellos 2000). It also carries the *Lr13* resistance gene, identified due to the linkage with the gene *Ne2* producing the hybrid necrosis phenotype (Sousa 1999). Ciano 67 (Figure 1) and Tezanos Pinto Precoz, both descending from Frontana and possessing *Lr34* (as previously discussed in section **The 1970s**) are most likely the donors of *Lr34* to the following varieties: Pavão, Ocepar 7-Batuíra, Butuí, Trigo BR 11-Guarani, Ocepar 8-Macuco, Ocepar 10-Garça, Ocepar 11-Juriti, IAC 162-Tuiuiú, Trigo BR 17-Caiuá, Trigo BR 18-Terena, Iapar 21-Taquari, Iapar 30-Piratã, Trigo BR 29-Javaé, Ocepar 17, Iapar 1-Mitacoré, El Pato, PAT 72247, Iapar 17-Caeté, Iapar 29-Cacatu, IAC 72-Tapajós, Trigo BR 33-Guará, and Ocepar 16. The pedigree analysis (Table S1) indicates that these two varieties and their descendants, including Bluebird, Aldan Sib, Siskin Sib, Tobari 66, Jaral Sib, and Bluejay (all possessing *Lr34*) participated in high frequency in the crosses originating the above-mentioned varieties. Otherwise, the CIMMYT-derived varieties Calidad and Penjamo 62 also participated in the cross originating Pavão, Iapar 21-Taquari, and IAC 162-Tuiuiú, and therefore are likely donors of *Lr34*. 2) Indirectly from Mentana: Three of the above-mentioned varieties, namely Ocepar 7-Batuíra, IAC 162-Tuiuiú, and Iapar 21-Taquari also could have inherited *Lr34* from Kavkaz, a European winter wheat descending from Ardito (a sib cultivar to Mentana), reported to carry the *csLV34b* allele (Kolmer et al. 2008). Trigo BR 9-Cerrados and Serrano most likely inherited *Lr34* from Mentana via BH 1146, released in the 1950s (Figure 1), as previously discussed. Ocepar 14 probably inherited *Lr34* from Mentana via Almond. Ocepar 14 also has background from Cocoraque 75, a Mexican variety carrying the *csLV34b* allele (Kolmer et al. 2008) released for cultivation in Brazil in 1981 (Sousa and Caierão 2014). 3) From undetermined sources: Based on our results and on the pedigree analysis, the origin of the *Lr34* gene could not be traced in the following varieties: Iapar 3-Aracatu, Jandaia, Iapar 6-Tapejara, IAC 21-Iguaçu, Minuano 82, IAC 23-Tocantins, RS 1-Fênix, RS 4-Ibiraiaras, IAC 27-Pantaneiro, and Trigo BR 35. Trigo BR 35 has been previously postulated to carry a combination of *Lr13+Lr34* resistance genes, due to the presence of the *Ltn* and *Ne2* genes, causing leaf tip necrosis and the hybrid necrosis phenotype, respectively (Sousa 1999, Sousa and Barcellos 2000).

The 1990s

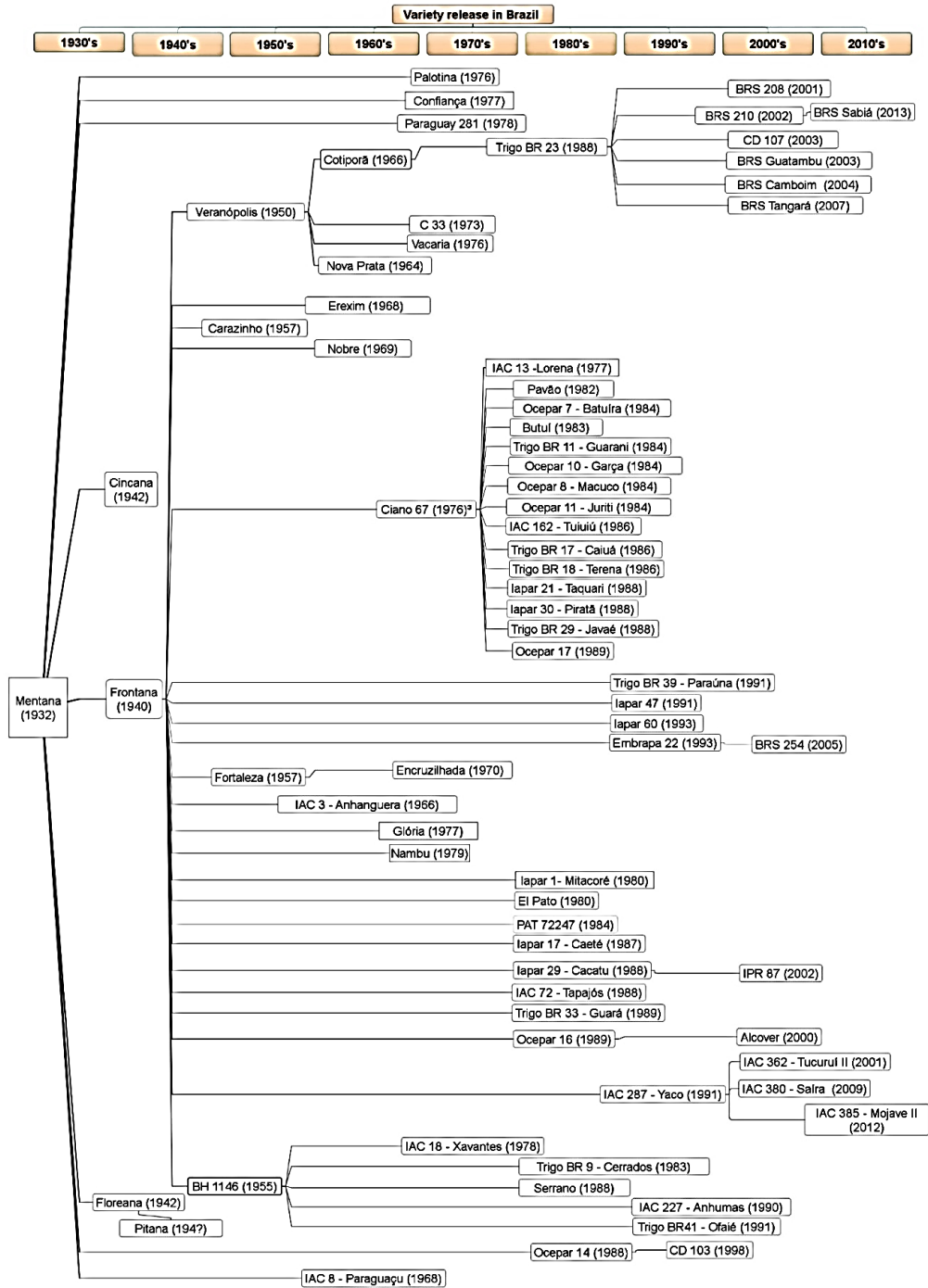


Figure 1. Likely origin and inheritance of the *Lr34* resistance allele in wheat varieties released in Brazil from 1922 to 2016.

The 1990s were equally prolific regarding the number of varieties released (Sousa and Caierão 2014). However, there was decrease in the number of varieties detected to carry the *Lr34* gene (11 varieties, Table S1), probably inherited in one of three ways: 1) Indirectly from Frontana (Figure 1): Trigo BR 39-Paraúma, Iapar 47, Iapar 60, and IAC 287-Yaco have pedigrees that trace back to the cultivars Ciano 67, Chris, and Tezanos Pintos Precoz, which were important sources of the *Lr34* gene in the varieties released in the 1970s, as previously discussed in this paper. The Mexican variety Jupateco 73 (see section **The 1970s**) is the most likely donor of *Lr34* to Embrapa 22 and is possibly the source of *Lr34* in Iapar 60. IAC 287 may also have inherited *Lr34* from Cocoraque 75, which also carries the *csLV34b* allele (Kolmer et al. 2008) as previously discussed in section **The 1980s**. 2) Indirectly from Mentana: The above-mentioned variety Iapar 47, alternatively, may have inherited *Lr34* from Mentana via Kavkaz, as previously discussed in section **The 1980s**. IAC 227-Anhumas and Trigo BR 41-Ofaé most likely inherited *Lr34* from Mentana via BH 1146 (Figure 1), as previously discussed in the section **The 1950s**. CD 103 probably inherited *Lr34* from Ocepar 14, released in 1988, which pedigree has been discussed in the corresponding section. 3) From undetermined sources: Based on our results and on the pedigree analysis, the origin of the *Lr34* gene could not be traced in three varieties: RS 8-Westphalen, Manitoba 97, and Iapar 40-Mirim. Kolmer et al. (2008) included IAC 5-Maringá in the group of varieties possessing the *csLV34b* allele, and then this variety could be considered as the donor of *Lr34* to Iapar 40. However, in our study, IAC 5 was not found to possess the diagnostic deletion that unambiguously identify the presence of *Lr34*.

The 2000s

We tested 87 varieties released for cultivation between 2000 and 2009. Of these, 19 were found to possess the *Lr34* allele (Table S1) all of which were probably inherited in one of three ways: 1) Indirectly from Frontana (Figure 1): The variety Trigo BR 23 was released in the 1980s (please refer to this section for pedigree analysis) and was widely cultivated in the 1990s (Sousa and Caierão 2014). It is the most likely donor of the *Lr34* gene to BRS 208, BRS 210, CD 107 BRS Guatambu, BRS Camboim, and BRS Tangará. Otherwise, CD 107 may have inherited *Lr34* from Cocoraque 75 or from Trigo BR 35 (see section **The 1980s**). Regarding the variety BRS Camboim, an alternative origin of the *Lr34* allele may be the Argentinian variety Klein Cartucho, reported to carry the *csLV34b* allele (Kolmer et al. 2008), presumably inherited from Penjamo 62, Jupateco 73) or Calidad. The varieties BRS 254, IPR 87, Alcover, IAC 362-Tucuruí II and IAC 380-Saíra are descendants from varieties released in the 1980s and 1990s (Embrapa 22, Iapar 29, Ocepar 16 and IAC 287-Yaco) which have pedigrees that trace back to *Lr34*-carrying varieties Tezanos Pintos Precoz, Jupateco 73, Ciano 67 and Chris (see section **The 1970s**). 2) Indirectly from Mentana: BRS 296 probably inherited *Lr34* from the Australian variety Cook, a derivative of *WW15 = Anza* (McIntosh et al. 1995). *WW15* and *Anza* served as key donors of *Lr34* in the Australian wheat gene pool and were originated from the CIMMYT variety Lerma Rojo, which is derivative of crosses with Mentana (GRIS 2019) and was found to carry the *csVL34b* allele (Kolmer et al. 2008). 3) From undetermined sources: Based on our results and on the pedigree analysis, the origin of the *Lr34* gene could not be traced in the following varieties: CD 106, IAC 364-Tucuruí III, CD 109, BRS Camboatá, BRS Reponte, BRS Guamirim and IPR 129.

The 2010s

Despite the great progress of wheat breeding observed in the current decade, when 62 varieties were released by 11 public and private companies, there was a steep decrease in the number of varieties carrying the *Lr34* (only 7), the least since the landmark represented by Fontana. This reduction may be because the *Lr34*-mediated resistance imposes high energetic demands that lead to the induction of multiple metabolic responses to support cellular energy requirements (Bolton et al. 2008). The energetic cost of the resistance provided by the *Lr34* gene may probably affect the yield so as to negatively affect the selection of lines carrying the *Lr34* locus. Furthermore, the selection pressure for cultivars with better grain quality could have decreased the intensity of selection for resistance to rust. In the varieties released in the 2010s in which the *Lr34* gene was found, inheritance may have occurred in one of two ways: 1) Indirectly from Frontana (Figure 1): BRS Sabiá and IAC 385-Mojave descend from varieties BRS 210 (released in 1988) and IAC 287-Yaco (released in 1991), respectively. In our study, both BRS 210 and IAC 287-Yaco were found to carry the *Lr34* gene with origin traced back to Frontana, as discussed in the respective sections (see sections **The 1980s** and **The 1990s**). 2) From undetermined sources: Based on our results and on the pedigree analysis, the origin of the *Lr34* gene could not be traced in the following varieties: TBIO Selete, LG Prisma, and FPS Certero. Although BRS Marcante and LG Supra most probably inherited *Lr34* from BRS Guamirim (released in 2004), nevertheless, the source of the resistant allele in this variety could not be traced in our work.

Adult plant field resistance

The resistance phenotype of *Lr34* is characterized by an increased latent period, reduced infection frequency, smaller uredinia, and a decreasing gradient of uredinia from leaf base to tip (Bolton et al. 2008). Genetic analysis of Frontana and many CIMMYT wheats showing high levels of partial resistance to leaf rust worldwide indicates that this adult plant resistance is based on the additive interaction of *Lr34* and two or three additional slow rusting genes (Singh and Rajaram 1992). In Mexico, leaf rust severity on most cultivars can be related to the number of slow rusting genes they carry. While susceptible cultivars can show 100% of leaf rust severity, cultivars carrying *Lr34* alone can display 50% of rust severity; cultivars with *Lr34* plus one or two minor genes display up to 30% of severity; and cultivars with *Lr34* and two or three additional genes show a stable response across environments, with final leaf rust ratings lower than 10% (Singh and Rajaram 2002). In Brazil, wheat-producing areas are in environments very conducive to leaf rust epidemics and prevalent races change dramatically over time according to the resistance genes present in the commercial cultivars (German et al. 2007). In our work, the susceptible varieties displayed high levels of severity, mostly 80% to 100%, while the *Lr34*-carrying genotypes (Brazilian varieties and *Lr34*-genetic stocks) showed reactions compatible with the phenotypes described above (Table 1). Among the varieties, Pitana and BH 1146 likely possess the *Lr34* gene alone, because the leaf rust reaction ranged from 15% to 50% of severity with susceptible infection types. These varieties have been reported to carry additional *Lr* genes (Table 1); however, they are not effective against the prevalent races occurring in the Southern Cone of America (German et al. 2011). The field reaction of Cincana, Patriarca, Minuano 82, Trigo BR, 18-Terena, Trigo BR 23, Trigo BR 35, Embrapa 22, CD 103, BRS 208, IPR 87, BRS Guatambu, BRS Camboatá, BRS 254, and IAC 385-Mojave ranged from 5S to 30MSMR (Table 1), compatible with the presence of *Lr34* associated with one or two additional resistance genes. The field reaction of RS 8-Westphalen, BRS 210, BRS Guamirim, BRS Tangará, BRS 296, BRS Marcante and BRS Sabiá ranged across years from 1S to 20MR, suggesting that, in these varieties, the resistance probably is due to the presence of *Lr34* and two, three, or more additional genes. The *Lr34*-carrying genetic stocks displayed low levels of disease severity (Table 1) ranging from 3S to 20SMS in RL 6050, RL 6114, RL 6058, Chris,

Table 1. Adult plant rust reaction under field conditions of Brazilian *Lr34*-carrying wheat varieties, *Lr34*-carrying genetic stocks and susceptible varieties

Wheat variety (Year of release)	Adult plant rust reaction in the field *						Genetic background **
	2006	2007	2008	2009	2010	2013	
<i>Lr34</i>-carrying varieties							
Frontana (1940)	5S	10SMS	10SMS	10S	15SMS	15MS	<i>Ne2, Ne2m, Ltn, LrT2, LrT3, Lr13, Lr34-csLV34b, Lr1, Lr3, Lr10, Lr13, Lr15, Lr20, Lr22, Lr28, Sr2, Bdv1</i>
Cincana (1942)	20S	---	---	10S	---	30MS	NA
Pitana (195?)	15S	5S	20S	50S	---	30MS	<i>Ne2, Lr13</i>
BH 1146 (1955)	15S	20S	50S	45S	50S	40S	<i>Ltn, Lr13, Lr34, csLV34b, Lr14a, Lr27, Sr2</i>
Cotiporã (1965)	---	---	---	---	20SMS	---	<i>Lr34, Ltn</i>
Patriarca (1969)	5MSMR	---	30SMS	---	20MS	30MS	NA
Hulha Negra (1977)	5S	---	5MSMR	10SMS	---	15MR	NA
Minuano 82 (1982)	20S	---	30S	20MSMR	---	20MRMS	<i>Lr34, Ltn</i>
Trigo BR 18-Terena (1986)	30MSMR	5MS	20S	---	25SMS	20RMR	<i>Ne2, Lr13</i>
Trigo BR 23 (1988)	20S	30S	25S	25S	10SMS	30SMS	<i>Ne2, Ltn, Lr13, Lr34</i>
Trigo BR 35 (1989)	20S	5SMS	35S	15SMS	30S	30SMS	<i>Ne2, Ltn, Lr13, Lr34</i>
RS 8-Westphalen (1991)	1MS	---	5SMS	15MS	---	5MR	<i>Lr34, Ltn</i>
Embrapa 22 (1993)	30S	5MS	15SMS	---	15SMS	---	NA
CD 103 (1998)	15MS	---	---	25SMS	---	20S	NA
BRS 208 (2001)	10SMS	15S	25S	15S	20S	1MS	NA
BRS 210 (2002)	15MSMR	5MSMR	15SMS	15MS	---	---	NA
IPR 87 (2002)	---	15S	5S	---	---	---	NA
BRS Guatambu (2003)	20SMS	---	---	1S	5S	---	NA

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BRS Camboatá (2003)	---	10MS	25S	---	25S	---	NA
BRS Guamirim (2004)	5SMS	1SMS	15S	5MSMR	5S	---	NA
BRS 254 (2005)	10S	5MS	15SMS	25MSMR	---	---	NA
IPR 129 (2006)	30S	1MS	30S	---	---	---	NA
BRS Tangará (2007)	10MSMR ¹	5S	10S	15MSMR	5S	---	NA
BRS 296 (2009)	5SMS ²	TMS ²	---	8MS	1S	15MS	NA
BRS Marcante (2012)	---	---	---	---	3MS ³	20MR	NA
IAC 385-Mojave (2012)	---	---	---	---	---	30SMS	NA

Wheat variety (Year of release)	Adult plant rust reaction in the field *						Genetic background **
Lr34-carrying genetic stocks and genotypes							
RL 6050	10S	---	20SMS	10SMS	---	---	<i>Lr13+Lr34</i>
RL 6114	10SMS	---	10S	10MS	---	---	<i>Lr13+Lr34</i>
RL 6058	10SMS	---	10S	10MS	---	---	<i>Lr34</i>
Chris	5MS	---	---	10S	---	---	<i>Lr10, Lr13, Lr27, Lr34-csLV34b</i>
Cocoraque 75	20R10S	---	---	---	---	---	<i>Ne2, Ltn, Lr12, Lr13, Lr34-csLV34b, Lr3, Lr14a, Lr17, Lr17a, Lr27, Lr31</i>
Coker 797	15R/5S	---	---	15R	---	---	<i>Lr10, Lr11, Lr13, Lr14a, Lr34, Lr18+</i>
Buck Buck	15MRMS	---	5MS	15MSMR	---	---	<i>Lr13, Lr16</i>
Era	5S	---	---	60S	---	---	<i>Ltn, Sr2, Lr13, Lr34-csLV34b, Lr10, Lr12, Lr23, Lr27</i>
Esmeralda 86	20S	---	---	25SMS	---	---	<i>Ltn, Sr2, Lr13, Lr34-csLV34b, Lr10, Lr14a, Yr18, T1BL.1RS, Bdv1</i>
Glenlea	8S	30S	---	35S	---	---	<i>Lr1, Lr13, Lr34-csLV34b, lrl2, Yr18, Lr46</i>
Pampa INTA	15MRMS	---	15S	10MS	---	---	<i>Rht2, Lr28, Lr34.</i>
Parula	5MS	---	3S	---	---	---	<i>Ltn1, Ltn2, Lr3b, Lr13, Lr34/Yr18/Sr57/Pm38-csLV34b Lr46/Yr29/Sr58/Pm39, Lr68, T1BL.1RS, Bdv1, Sr2/Yr30</i>
Tonichi 81	5SMS	---	15MS	10SMS	---	---	<i>Ne2, Ltn, Lr12, Lr13, Lr34, csLV34b, Lr1, Lr10, Lr14a, Lr27, Lr31, Lr49, Sr2, Bdv1</i>
Susceptible varieties (not carrying Lr34)							
Anahuac 75 (1981)	80S	20MS	25S	80S	100S	50S	NA
OR 1 (1996)	80S	80S	45S	100S	100S	---	NA
CD 104 (1999)	80S	80S	80S	80S	90S	80S	NA
MGS 1-Alliança (1999)	80S	40S	80S	50S	100S	---	NA
IPR 85 (1999)	80S	20S	70S	20S	75S	80S	NA
BRS 194 (2000)	80S	50S	---	---	90S	30S	NA
Ônix (2001)	80S	50S	30S	60S	100S	30S	NA
BRS 209 (2002)	80S	30S	---	---	100S	---	NA
Safira (2004)	50S	25S	30S	70S	90S	---	NA
Fundacep Nova Era (2004)	70S	25S	20S	90S	70S	---	NA
Fundacep 52 (2005)	20MS	70S	50S	---	90S	40S	NA
Abalone (2006)	20S	50S	50S	---	90S	80S	NA
Quartzo (2007)	---	---	60S	70S	75S	80S	NA

* Rust reaction scored as the severity (percentage of leaf area affected by leaf rust) and the infection type: 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; T = traces of infection, meaning less than 1% of the leaf area affected by leaf rust (Roelfs et al. 1992). ** According to the Genetic Resources Information System for Wheat and Triticale (GRIS) <http://wheatpedigree.net/>. NA: Not available. Tested as advanced lines: ¹ PF 003295-A/B, ² PF 990283, ³ PF 080310, ⁴ IWT 08111

Cocoraque 75, Coker 797, Buck Buck, Esmeralda 86, Glenlea, Pampa INTA, Parula and Tonichi 81. Esmeralda 86 and Glenlea showed maximum leaf rust ratings of 25SMS and 35S, respectively. The results obtained in this work confirm the presence of the *Lr34* resistant allele in many Brazilian wheat varieties, which has been mostly inherited directly and indirectly from Mentana and Frontana and the genetic variability observed in the wheat germplasm in Brazil is equally distributed across the local wheat breeding programs as described by Scherlosky et al. (2018). It can be noticed that this important locus has been maintained over time, even though unconsciously during the cycles of phenotypic selection. Additionally, our results confirm the high level and stability of the partial resistance conferred by *Lr34* across years under a very conducive environment, and across a wide diversity of genetic backgrounds, as reported by Lillemo et al. (2011).

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