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Molecular Detection of the Adult Plant Leaf Rust Resistance Gene *Lr34* in Romanian Winter Wheat Germplasm

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Wheat continues to be one of the most cultivated cereals in the world, and also in Romania. Leaf rust caused by *Puccinia triticina* reduces the wheat yield and grains quality worldwide. In the context of climate change, leaf rust has become a more important problem for both wheat growers and breeders in our country. Use of genetic resources, carrying rust resistance genes, play an important role in breeding programs leading to resistant varieties, which can have positive impact on environment and economy. Therefore, the identification of resistance genes in modern wheat cultivars and breeding lines, and then selection of the best resistance genes combination(s) are the first steps for a successful breeding program. At present, one of the best known and studied adult plant leaf rust resistance gene is *Lr34* that contributes significantly to durable leaf rust resistance. The functional markers that enable early detection of this gene are a major advantage in the wheat breeding.

The aim of this study was to evaluate the presence of the slow rusting resistance gene *Lr34* in Romanian wheat germplasm, using cssfr4 and cssfr5 molecular markers. Screening of 47 winter bread wheat cultivars and 47 breeding lines with these markers showed the presence of the *Lr34* resistant haplotype in 62% (homozygous genotypes) of the total genotypes. A high frequency (79%) of *Lr34* resistance allele was found among 47 breeding lines, suggesting that maintenance of a high frequency of this allele represents a real advantage for the development of adult plant resistance in Romanian breeding programs.

Keywords: molecular detection, molecular markers, adult plant leaf rust resistance, *Lr34* gene

Introduction

Wheat is an important cereal in the world. In Romania wheat plays an important role in the national economy, being grown on about 2 million hectares and its production was 7.4 million tons in 2013 (Anonymous 2014). A limiting factor in the world wheat production is represented by diseases, such as rusts, smuts, powdery mildews, etc. In the context of climate change, at present, in Romania the rusts continue to be a problem for wheat production and so, a renewed challenge for breeders is to obtain new rust resistant cultivars,

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because growing of tolerant/resistant cultivars is an effective way of protecting crops against pathogens. Among rusts (leaf, yellow and stem rust), the leaf rust or brown rust, caused by *Puccinia triticina* Eriks., is currently one of the most frequent wheat diseases.

At present over 70 leaf rust resistance genes *(Lr)* have been mapped in wheat (Singh et al. 2013). Most of them confer race-specific resistance, acting in "gene for gene" manner as seedling plant resistance genes, but the resistance provided by these genes can be short-lived as new races of the pathogen appear. There are a few *Lr* genes that confer non-race-specific resistance, acting at the adult plant stage (APR – Adult Plant Resistance), as partial resistance. This small group of leaf rust resistance genes is named "slow-rusting" group; although, their effect is smaller than that of the race-specific genes they had remained durable in time (Singh et al. 2003; Lagudah 2011). The effect of slow-rusting genes is also more dependent on environmental conditions. The best known slow-rusting *Lr* genes are *Lr34* (Dyck 1977), *Lr46* (Singh et al. 1998), *Lr67* (Hiebert et al. 2010; Herrera Foessel et al. 2011) and *Lr68* (Herrera Foessel et al. 2012).

One of the well-known and characterized race-non-specific resistance genes is the adult plant leaf rust resistance gene *Lr34.* This gene was described by Dyck (1977), as *LrT2,* in the cultivar Frontana and was located on short arm of the chromosome 7DS (Dyck 1987). *Lr34* or closely linked genes was found to provide resistance to two other rust diseases (Singh et al. 2012) and powdery mildew (Spielmeyer et al. 2005; Lillemo et al. 2008). Recently, this locus has been also shown to provide resistance to spot blotch caused by *Bipolaris sorokiniana* (Lillemo et al. 2013). In addition, *Lr34* was reported to be associated with LTN (*Ltn1;* Singh 1992) and *Bdv1*. Another important reported feature of *Lr34* is that this gene combined with *Lr* genes such as *Lr13, Lr37* (German and Kolmer 1992; Kloppers and Pretorius 1997) and *lm* (lesion mimic) enhances wheat resistance to leaf rust (Li et al. 2012).

Resistant and susceptible haplotypes of the *Lr34* gene have been identified (Krattinger et al. 2009). The resistant haplotype of *Lr34* is characterized by a 3 bp deletion (TTC) in exon 11 and a SNP (T to C) in exon 12. At present, several molecular markers for alleles of *Lr34* gene are available (Lagudah et al. 2009; Dakouri et al. 2010), and can be used in marker assisted selection in breeding programs.

Previous research identified the presence of *Lr34* resistance allele in some Romanian cultivars, using PCR markers, noting that this allele is very frequent in Romanian wheat germplasm (Purnhauser et al. 2011). In this paper, we aimed to characterize a larger collection of Romanian winter wheat cultivars and breeding lines for allelic variation at *Lr34* locus, using both the tightly linked marker csLV34 and functional molecular markers.

Materials and Methods

Plant material

Ninety-four Romanian winter wheat genotypes, including forty-seven bread wheat cultivars released in Romania during the last 81 years (Table 1) and forty-seven breeding lines, were included in this study. Three wheat genotypes, namely Chinese Spring

Table 1. Molecular markers characterization of leaf rust resistance gene *Lr34* in Romanian winter wheat cultivars

Nr. crt.	Wheat variety	Genealogy	Released (Year)	Molecular markers	
				csLV34	Cssfr5
1	A15	Sel Tenmarq	1933	a	Lr–
2	Odvos 241	Sel Champlain	1933	a	Lr–
3	Bucuresti 1	Kanred/Tiganesti	1962	a	Lr–
4	Dacia	Bucuresti1/Skorospelka3	1971	a	Lr–
5	Ceres	Miciurinka/Bezostaya1	1974	a	Lr–
6	Diana	Fiorello/Bezostaya1	1976	a	Lr–
7	Doina	Et.Choisy/Monon	1977	a	Lr–
Nr. crt.	Wheat variety	Genealogy	Released (Year)	Molecular markers	
				csLV34	Cssfr5
8	Fundulea 29	Aurora/Riley67	1979	a	Lr–
9	Fundulea 133	SWW7/PRIBOI	1985	a	Lr–
10	Simnic 30	Sel Diana	1987	a	Lr–
11	Trivale	Er397H66/DACIA	1991	a	Lr–
12	Apullum	Odesskaia75/Bezostaya1	1992	a	Lr–
13	Fundulea 4	Fundulea29/2*Lovrin32	1994	a	Lr–
14	Turda 95	F199I1-2/T6-80-86	1995	a	Lr–
15	Zimbru	Fundulea 4Sib/F154I1-1//Aniversar/Roxana	1998	a	Lr–
16	Gasparom	Sv 9710-79/Fundulea 4	1998	a	Lr–
17	Esential	Sv2946-86/Sv9710-78	2001	a	Lr–
18	Dumbrava	603106/Flamura85//F2416W2-12/Fundulea4	2003	a	Lr–
19	Crisana	Fundulea4/Atlas66	2005	a	Lr–
20	Miranda	ERYT26221/96869G1-1//GLOSA	2010	a	Lr–
21	Otilia	F96052G16-2/FAUR	2014	a	Lr–
22	Suceava 84	Bezostaya1/F208	–	a	Lr–
23	Excelsior	Bucuresti1/Skorospelka3	1971	H	H
24	Favorit	Bezostaya1/Odvos241	1971	H	H
25	Iulia	Bezostaya1/Beloterkovsk198	1974	H	H
26	Simnic 50	Lovrin32/Fundulea29/F553D4-22/3/1502W23-1/4/Donskaia Polukarlikovaya	2004	H	H
27	Lovrin 34	Ranniaia12/Nadadores63//Lovrin 12	1981	b	Lr+
28	Transilvania 1	US(60)43/Avrora//T141-65	1981	b	Lr+
29	Ariesan	Rubin/T141	1985	b	Lr+
30	Aniversar	Lv11/F53-67	1986	b	Lr+
31	Flamura 85	JUWEL/Lv32A//2*FL80	1989	b	Lr+
32	Rapid	Juwel/Lv32A//2*FL80	1992	b	Lr+
33	Dropia	COLOTANA/2120W1	1993	b	Lr+
34	Alex	Flamura80/Fundulea29	1994	b	Lr+
35	Ardeal 1	F48212-112/F2098W1	1999	b	Lr+
36	Boema 1	F308O2-20/DROPIA	2000	b	Lr+
37	Crina	F309O3-0/4141W1-1	2001	b	Lr+

Table 1 (cont.)

Nr. crt.	Wheat variety	Genealogy	Released (Year)	Molecular markers	
				csLV34	Cssfr5
38	Delabrad2	308O2-20/DROPIA	2002	b	Lr+
39	Dor F	F151M1-1/F2076W12-11// F4105W2-1	2002	b	Lr+
40	Faur F	F307P2-1101/F135U2-1	2004	b	Lr+
41	Gruia	AF93-2/F135U2-1	2005	b	Lr+
42	Glosa	F135U2-1/F508U1-1BUCUR	2005	b	Lr+
43	Izvor	KARL/F201R2-111//F508U1-1	2008	b	Lr+
44	Litera	ERYT26221/F96869G1-1// GLOSA	2009	b	Lr+
45	Pajura	IZVOR/F96012G2-2//GLOSA	2014	b	Lr+
46	Ostrov	IZVOR/F95272G1-11	under official testing	b	Lr+
47	Pitar	02555GP2/00099GP2	under official testing	b	Lr+

Notes: H heterozygous/heterogenic; Lr+ *Lr34* resistant allele; Lr– *Lr34* susceptible allele

(Lr34+), nulli-tetrasomic 7D7A (N7DT7A) line of Chinese Spring and Thatcher (non-*Lr34*), were used as controls, in order to confirm the presence or absence of specific *Lr34* alleles using molecular markers.

DNA extraction and PCR analysis for Lr34 alleles

For extraction of genomic DNA, 10 embryos rescued from each tested genotype were ground with 1 ml extraction buffer (sorbitol 2.5%, sarkosyl 1%, sodium chloride 5%, sodium-EDTA 0.8%, CTAB 2%, all reagents were dissolved into Tris 0.2 M, pH 8) preheated at 65 °C. The ground material was transferred into 2 ml tubes; after that, the tubes were incubated in water bath at 65 °C, for one hour. Then, the tubes were cooled at room temperature and 1 ml chloroform: isoamyl alcohol (24:1) was added; the tubes were mixed by inversion 1–2 min and centrifuged 10 min at 8000 rpm. After that, the supernatant was transferred into a new tube and treated with chloroform:isoamyl alcohol (24:1) and centrifugation were repeated. After centrifugation the supernatant was transferred into a new tube and sodium chloride 5 M at 0.25 M final concentration and 2.5 vol. of cold absolute ethanol were added. The tubes were centrifuged 5 min at 13000 rpm and after that the supernatant was discarded and DNA pellet was washed with 400 µl wash buffer (76% absolute ethanol and 10 mM ammonium acetate). The tubes were again centrifuged 3 min at 14,000 rpm, the supernatant was discarded and the pellet was dried and 100 µl TE (Tris 10 mM and EDTA 1 mM) was added in tube. DNA was checked in agarose gel 0.8% and with a spectrophotometer (DU730-Beckman Coulter). Genomic DNA was diluted to 20 ng/µl for each tested genotype.

For PCR analysis regarding the *Lr34* alleles, we used 2 functional markers and one STS – csLV34 marker by Lagudah et al. (2006) and Lagudah et al. (2009): multiplex cssfr4

Table 2. PCR markers used in this study

Primers name	Markers-common name	Primers sequence	Size of PCR product (bp) *Lr34+*	Size of PCR product (bp) non-*Lr34*
L34MINUSR	Cssfr4	5'TTGATGAAACCAGTTTTTTTTCTA 3'	–	523
L34DINT9F (cssfr2)		5'TATGCCATTTAACATAATCATGAA3'		
csLV34-F		5'GTTGGTTAAGACTGGTGATGG3'	150	229
csLV34-R		5'TGC TTG CTA TTG CTG AAT AGT3'		
L34DINT13R2	Cssfr5	5'ACTTTCCTGAAAATAATACAAGCA 3'	751	–
L34SPF		5'GGGAGCATTATTTTTTTCCATCATG 3'		
L34MINUSR		5'TTGATGAAACCAGTTTTTTTTCTA 3'	–	523
L34DINT9F (cssfr2)		5'TATGCCATTTAACATAATCATGAA 3'		

(cssfr2 (L34DINT9F/L34MINUS) with csLV34 marker) and multiplex cssfr5 (cssfr2 with L34SPF/L34DINT13R2) (Table 2).

All amplification reactions were carried out in a 25 µl volume containing 1× buffer (AmpliTaq 360 DNA polymerase kit), 0.2 mM dNTPs, 0.4 µM for primers: L34DINT9F/L34MINUS and L34SPF/L34DINT13R2 and 0.2 µM for primers of csLV34 marker, 0.625U of DNA polymerase, 1.5 mM magnesium chloride (1.8 mM magnesium chloride for cssfr5 marker), 100 ng genomic DNA and 0.8 µl of 360 GC enhancer solution. The following amplification parameters were used: initial denaturation at 94 °C–3 min, and then 35 cycles of 94 °C–1 min, 58 °C–1 min, 72 °C–2 min and with final extension 72 °C–10 min. PCR was performed in Gene Amp PCR system 9700 thermal cycler. The PCR products were separated on 1.5% agarose for routine use, in 0.5× TBE buffer, stained with ethidium bromide and photographed under ultraviolet light with Vilber Lourmat system.

Results

The multiplex PCR based on primers combination cssfr4 with csLV34 and cssfr2 (L34DINT9F/L34MINUS) (Lagudah et al. 2009) amplified three products; the 150 bp product is the csLV34 "b" allele that is diagnostic of the *Lr34* resistant haplotype, indicating the likely presence of the *Lr34* resistance allele in genotypes carrying this gene. Two other products 229 bp and 523 bp are amplified, indicating genotypes not carrying the resistance allele of the *Lr34* gene. The 229 bp fragment is the csLV34 "a" allele and the 523 bp fragment belongs to the susceptible haplotype that lacks *Lr34.*

In genotypes that are heterozygous all three fragments are present, while in genotypes that are homozygous for resistance allele only one fragment (150 bp – csLV34 "b") is present and in genotypes that are homozygous for susceptible allele two fragments (229 bp – csLV34 "a" and 523 bp) are present (Fig. 1).

The result of the second multiplex PCR based on primer combination cssfr5 (L34SPF/L34DINT13R2 and L34DINT9F/L34MINUS), consisted of two products: 751 bp product

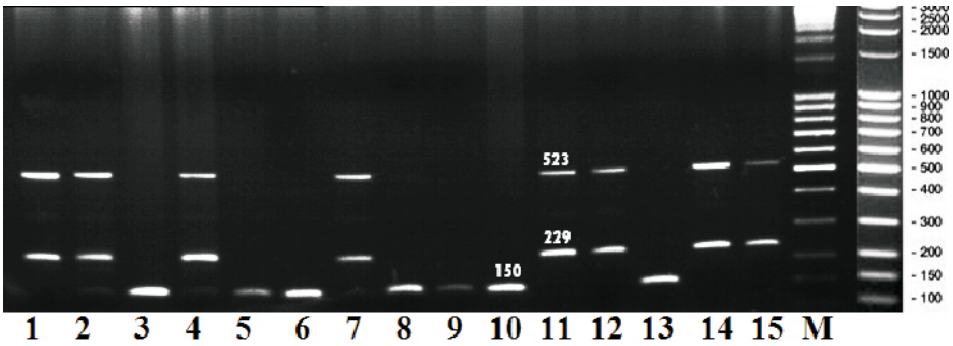

Figure 1. Electrophoretic pattern obtained with cssfr4 marker. 1. Thatcher; 2. A15; 3. Dropia; 4. Ceres; 5. Izvor; 6. Glosa; 7. Bucuresti1; 8. Litera; 9. Pitar; 10. Partener; 11. Miranda; 12. Otilia; 13. Chinese Spring; 14. Diana; 15. Fundulea 4. M – 100 bp-DNA-Ladder extended (Roth)

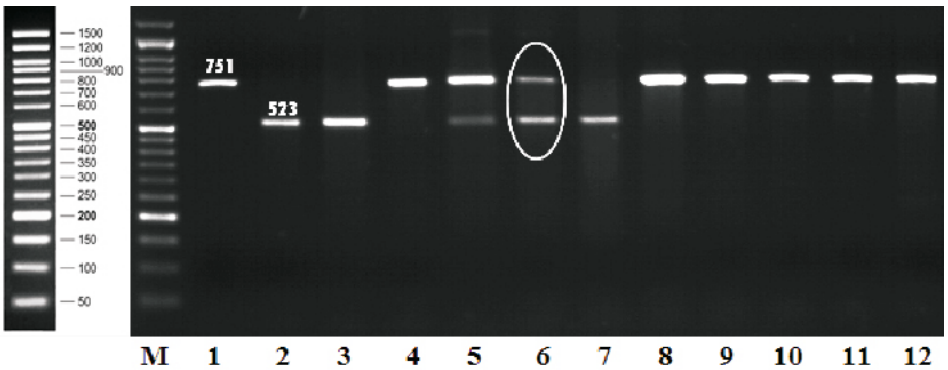

Figure 2. Electrophoretic pattern obtained with cssfr5 marker. M – 50 bp (GeneDirex); 1. Chinese Spring; 2. Thatcher; 3. Dacia; 4. Boema 1; 5. Favorit; 6. Line 08145G; 7. Doina; 8. Rapid; 9. Lovrin34; 10. Flamura 85; 11. Alex; 12. Dor F

specific for *Lr34* resistant haplotype and 523 bp, aforementioned, for *Lr34* susceptible haplotype (Fig. 2).

The csLV34 marker is tightly linked to *Lr34* gene, about 0.4 cM distance (Lagudah et al. 2006) and the cssfr2 and cssfr5 are functional markers that were developed around the sequence changes in exon 11 (Lagudah et al. 2009).

Of the 94 Romanian investigated genotypes, 29 showed the presence of the 229 bp and/or 523 bp in both multiplex reactions; therefore, the 29 genotypes are homozygous for *Lr34* susceptible allele. Molecular detection showed 65 genotypes with *Lr34* resistance allele. These 65 genotypes were divided into three groups:

– 6 genotypes were heterozygous (or heterogenic because the DNA isolation was made from 10 seeds) according to both multiplex reactions;

– one genotype line 08145G was heterozygous for cssfr5 multiplex reaction (Fig. 2) but not for csLV34 marker from cssfr4 multiplex reaction. This line, showed only two bands (150- and 523-bp), and no 229-bp band for csLV34 "a" allele (Fig. 3); This result suggested a recombination between *Lr34* gene and csLV34 marker locus for one chromosome.
– 58 genotypes homozygous for *Lr34* resistance allele, according to both markers; 150 bp and 751 bp bands were present.

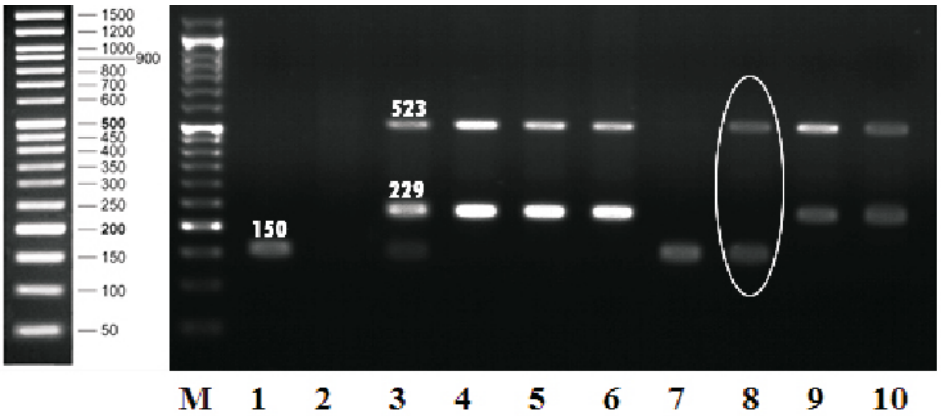

Figure 3. Electrophoretic pattern obtained with cssfr4 marker. M – 50 bp (GeneDirex); 1. Chinese Spring; 2. N7DT7A; 3. Favorit; 4. Simnic 30; 5. Trivale; 6. Apullum; 7. Izvor; 8. line 08145G; 9. Turda 95; 10. Zimbru. The circle shows the recombination between csLV34 marker and *Lr34* gene

Molecular detection, using molecular markers, in the 94 analyzed Romanian genotypes, identified 58 genotypes homozygous for *Lr34* resistant haplotype, representing a frequency of 62%. Regarding the Romanian cultivars analyzed, the homozygous *Lr34* resistance allele was present in 21 cultivars, representing a frequency of 45% (Table 1).

Molecular analyses showed that the frequency of the resistance allele of the gene *Lr34,* among 47 Romanian winter wheat cultivars, released since 1933, increased from 0%, for the cultivars released before the establishment of the Fundulea Institute (in 1957), to 10% in cultivars released before 1989, and to 90% in modern cultivars, including the cultivars currently grown. Among the 47 analyzed Romanian winter wheat breeding lines 37 (79% frequency) were found homozygous for *Lr34* resistance allele, 3 lines were heterozygous or heterogenic and only 7 lines carried the *Lr34* susceptibility allele. It is interesting to note that the frequency of the resistance allele of *Lr34* in the breeding lines is higher than 75%, which suggests that the selection pressure favored the maintenance of a high frequency of this allele.

Discussion

The introduction of molecular characterization of the gene *Lr34* allele status allows a more effective selection, because it can be applied at the early plant development stage and in absence of *Puccinia triticina.* The mostly widely used molecular marker for evaluation and selection of *Lr34* gene has been csLV34 (Kolmer et al. 2008; Vanzetti et al. 2011; Kadkhodaei et al. 2012), but functional markers tend to replace this marker (Karelov et al. 2011; McCallum et al. 2012; Dakouri et al. 2013). Our results also suggest that functional markers are more informative than csLV34 marker.

Because the resistance allele of *Lr34* gene is present in almost all cultivars released in recent years, at present more than 60% of the area planted with wheat in Romania has been in recent years partially protected against the leaf rust attack by a gene that has proven to be durable (Table 3). The high frequency of the resistance allele of the *Lr34* gene can be explained on the basis of pedigrees of cultivars created at Fundulea (Fig. 4). The cultivars Lovrin 32 and Flamura 80, selected from the cross between the Russian variety Ranniaia 12 and Nadadores 63 a Mexican variety, both carrying the resistance allele, was the main source of this allele.

Using molecular marker csLV34, Kolmer et al. (2008) found the origin of the *Lr34* gene in wheat cultivars from North and South America, CIMMYT, Australia and Russia was tracked back to the cultivars Mentana and Ardito developed in Italy by Nazareno Strampelli in the early 1900s. Both Ranniaia 12 and Nadadores 63 have Mentana in their

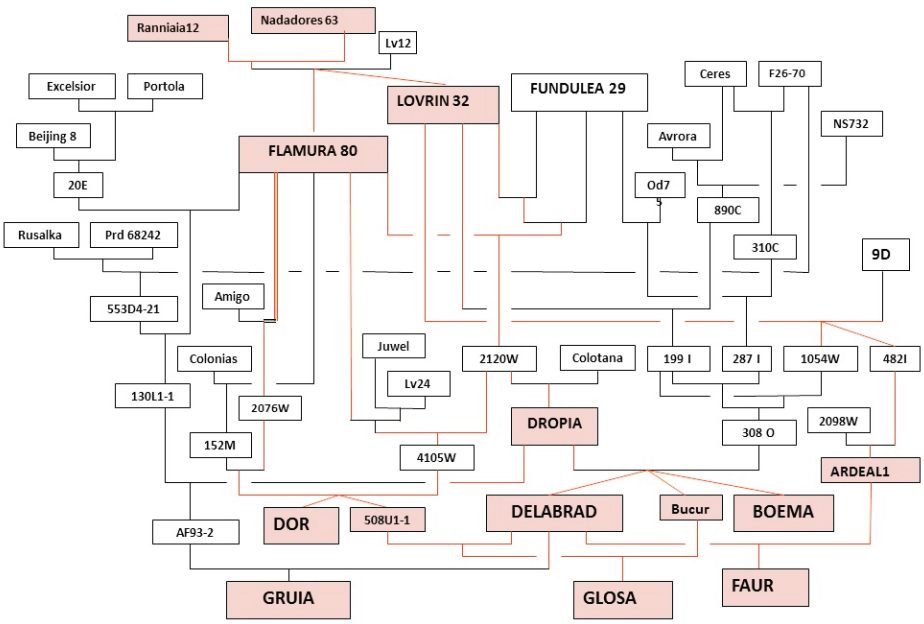

Figure 4. Origin of *Lr34* gene in Romanian winter wheat cultivars

Table 3. Share of cultivars created at NARDI Fundulea, carrying the leaf rust resistance gene *Lr34+* (% of total wheat area in Romania)

Year	Flamura 80	Flamura 85	Dropia	Rapid	Ardeal 1	Boema 1	Crina	Delabrad	Dor	Faur	Glosa	Gruia	Izvor	Litera	Total *Lr34+* carriers
1984	0.1														0.1
1985	0.8														0.8
1986	1.9														1.9
1987	3.2														3.2
1988	4.3														4.3
1989	5.3														5.3
1990	6.6	0.4													7.0
1991	9.1	2.6													11.7
1992	5.5	8.2													13.7
1993	1.0	14.0	1.0	0.2											16.2
1994		18.0	5.0	1.0											24.0
1995		19.4	9.6	3.0											32.0
1996		23.0	12.0	4.0											39.0
1997		20.0	15.0	4.0											39.0
1998		20.0	20.0	4.0											44.0
1999		20.0	20.0	4.0											44.0
2000		24.0	30.0	4.0											58.0
2001		25.6	34.8	3.5	0.0	0									63.9
2002		22.8	35.4	2.0	0.2	0.1	0.0								60.5
2003		16.6	32.3	0.7	0.7	0.3	0.1								50.7
2004		17.0	32.0	0.7	0.5	2.0	1.0								53.2
2005		17.0	32.0	0.6	0.5	5.0	1.5		0						56.6
2006		14.8	32.0	0.5	0.4	7.8	1.7	0.0	0.2		0.0				57.4
2007		14.8	30.9	0	0.4	8.8	1.7	0.1	0.2		0.1				57.0
2008		8.4	20.6		0.2	20.4	2.3	1.3	0.6	0.1	3.8	0			57.7
2009		4.74	19.9		0.3	19.8	2.1	2.5	0.8	0.1	7.3	0.5	0		57.96
2010		0.1	15.7		0.0	23.4	2.1	1.3	1.9	0.4	15.8	2.0	0.2		62.9
2011		0.2	9.1			20.1	0.7	1.1	1.5	0.5	26.2	1.3	0.9	0.0	61.6
2012		0.2	5.4			17.5	0.2	0.3	0.3	0.9	32.1	0.5	5.1	0.2	62.7
2013			4.0			15.3	0.1	0.1	0.1	0.9	33.5	0.5	7.6	2.4	64.5

ancestry. The progenies of Flamura 80 and Lovrin 32 have been extensively used in the breeding program especially at Fundulea, as a source of genes for semi-dwarf trait and good grain filling.

In this way adult plant resistance given by *Lr34* gene could be fixed in the breeding program by continuous selection for a good behavior against leaf rust attack in the field, and also by the presence of phenotypic markers "leaf tip necrosis", associated with this leaf rust resistance allele.

Conflict of interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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References

Anonymous 2014. Producția agricolă vegetală la principalele culturi, în anul 2013 (Agricultural production of main crops in 2013). http://www.insse.ro/cms/files/statistici/comunicate/com_anuale/Prod_veg/prod_veg_r13 (in Romanian)

Dakouri, A., McCallum, B.D., Walichnowski, A.Z., Cloutier, S. 2010. Fine-mapping of the leaf rust *Lr34* locus in *Triticum aestivum* (L.) and characterization of large germplasm collections support the ABC transporter as essential for gene function. Theor. Appl. Genet. **121**:373–384.

Dakouri, A., McCallum, B.D., Radovanovic, N., Cloutier, S. 2013. Molecular and phenotypic characterization of seedling and adult plant leaf rust resistance in a world wheat collection. Mol. Breeding **32**:663–677.

Dyck, P.L. 1977. Genetics of leaf rust reaction in three introductions of common wheat. Can. J. Genet. Cytol. **19**:711–716.

Dyck, P.L. 1987. The association of a gene for leaf rust resistance with the chromosome 7D suppressor of stem rust resistance in common wheat. Genome **29**:467–469.

German, S.E., Kolmer, J.A. 1992. Effect of gene *Lr34* in the enhancement of resistance to leaf rust of wheat. Theor. Appl. Genet. **84**:97–105.

Herrera-Foessel, S.A., Lagudah, E.S., Huerta-Espino, J., Hayden, M.J., Bariana, H.S., Singh, D., Singh, R.P. 2011. New slow-rusting leaf rust and stripe rust resistance genes *Lr67* and *Yr46* in wheat are pleiotropic or closely linked. Theor. Appl. Genet. **122**:239–249.

Herrera-Foessel, S.A., Singh, R.P., Huerta-Espino, J., Rosewarne, G.M., Periyannan, S.K., Viccar, L., Calvo-Salazar, V., Lan, C., Lagudah, E.S. 2012. *Lr68:* A new gene conferring slow rusting resistance to leaf rust in wheat. Theor. Appl. Genet. **124**:1475–1486.

Hiebert, C.W., Thomas, J.B., McCallum, B.D., Humphreys, G.D., DePauw, R.M., Hayden, M.J., Mago, R., Schnipenkoetter, W., Hayden, M. 2010. An introgression on wheat chromosome 4DL in RL6077

(Thatcher*6/PI 250413) confers adult plant resistance to stripe rust and leaf rust *(Lr67).* Theor. Appl. Genet. **121**:1083–1091.

Kadkhodaei, M., Dadkhodaie, A., Taghi Assad, M., Heidari, B., Mostowfizadeh-Ghalamfarsa, R. 2012. Identification of the leaf rust resistance genes *Lr9, Lr26, Lr28, Lr34,* and *Lr35* in a collection of Iranian wheat genotypes using STS and SCAR markers. J. Crop Sci. Biotech. **15**:267–274.

Karelov, A.V., Pirko, Ya.V., Kozub, N.A., Sozinov, I.A., Pirko, N.N., Litvinenko, N.A., Lyfenko, S.F., Koliuchii, V.T., Sozinov, A.A. 2011. Identification of the allelic state of the *Lr34* leaf rust resistance gene in soft winter wheat cultivars developed in Ukraine. Cytology and Genetics **45**:271–276.

Kloppers, F.J., Pretorius, Z.A. 1997. Effects of combinations amongst genes *Lr13, Lr34* and *Lr37* on components of resistance in wheat to leaf rust. Plant Pathol. **46**:737–750.

Kolmer, J.A., Singh, R.P., Garvin, D.F., Viccars, L., William, H.M., Huerta-Espino, J., Ogbonnaya, F.C., Raman, H., Orford, S., Bariana, H.S., Lagudah, E.S. 2008. Analysis of the *Lr34/Yr28* rust resistance region in wheat germplasm. Crop Sci. **48**:1841–1852.

Krattinger, S.G., Lagudah, E.S., Spielmeyer, W., Singh, R.P., Huerta-Espino, J., McFadden, H., Bossolini, E., Selter, L.L., Keller, B. 2009. A putative ABC transporter confers durable resistance to multiple fungal pathogens in wheat. Science **323**:1360–1363.

Lagudah, E.S. 2011. Molecular genetics of race non-specific rust resistance in wheat. Euphytica **179**:81–91.

Lagudah, E.S., Krattinger, S.G., Herrera-Foessel, S., Singh, R.P., Huerta-Espino, J., Spielmeyer, W., Brown-Guedira, G., Selter, L.L., Keller, B. 2009. Gene-specific markers for the wheat gene *Lr34/Yr18/Pm38* which confers resistance to multiple fungal pathogens. Theor. Appl. Genet. **119**:889–898.

Lagudah, E.S., McFadden, H., Singh, R.P., Huerta-Espino, J., Bariana, H.S., Spielmeyer, W. 2006. Molecular genetic characterization of the *Lr34/Yr18* slow rusting resistance gene region in wheat. Theor. Appl. Genet. **114**:21–30.

Li,T., Bai, G.H., Gu, S.L. 2012. A combination of leaf rust resistance gene *Lr34* and lesion mimic gene *lm* significantly enhances adult plant resistance to *Puccinia triticina* in wheat. Chin. Sci. Bull. **57**:2113–2119.

Lillemo, M., Asalf, B., Singh, R.P., Huerta-Espino, J., Chen, X.M., He, X.H., Bjornstad, Å. 2008. The adult plant rust resistance loci *Lr34/Yr18* and *Lr46/Yr29* are important determinants of partial resistance to powdery mildew in bread wheat line Saar. Theor. Appl. Genet. **116**:1155–1166.

Lillemo, M., Joshi, A.K., Prasad, R., Chand, R., Singh, R.P. 2013. QTL for spot blotch resistance in bread wheat line Saar co-locate to the biotrophic disease resistance loci *Lr34* and *Lr46*. Theor. Appl. Genet. **126**:711–719.

McCallum, B.D., Humphreys, D.G., Somers, D.J., Dakouri, A., Cloutier, S. 2012. Allelic variation for the rust resistance gene *Lr34/Yr18* in Canadian wheat cultivars. Euphytica **183**:261–274.

Purnhauser, L., Fónad, P., Kótai, E., Csősz, M. 2011. Disease resistance of Hungarian and Romanian wheat cultivars observed by field and laboratory methods. "Strong wheat" Proc. Closing Conference. Timisoara, Romania, pp. 11–16.

Singh, D., Mohler, V., Park, R.F. 2013. Discovery, characterisation and mapping of wheat leaf rust resistance gene *Lr71.* Euphytica **190**:131–136.

Singh, R., Herrera-Foessel, S.A., Huerta-Espino, J., Bariana, H.S., Bansal, U., McCallum, B.D., Hiebert, C.W., Bhavani, S., Singh, S., Lan, C., Lagudah, E.S. 2012. *Lr34/Yr18/Sr57/Pm38/Bdv1/Ltn1* confers slow rusting, adult plant resistance to *Puccinia graminis tritici.* In: Proc. 13th Cereal Rust and Powdery Mildew Conference. Beijing, China, p. 173.

Singh, R.P. 1992. Association between gene *Lr34* for leaf rust and leaf tip necrosis in wheat. Crop Sci. **32**:874–878.

Singh, R.P., Mujeeb-Kazi, A., Huerta-Espino, J. 1998. *Lr46:* A gene conferring slow-rusting resistance to leaf rust in wheat. Phytopathol. **88**:890–894.

Singh, R.P., Huerta-Espino, J. 2003. Effect of leaf rust resistance gene *Lr34* on components of slow rusting at seven growth stages in wheat. Euphytica **129**:371–376.

Spielmeyer, W., McIntosh, R.A., Kolmer, J., Lagudah, E.S. 2005. Powdery mildew resistance and *Lr34/Yr18* genes for durable resistance to leaf and stripe rust cosegregate at a locus on the short arm of chromosome 7D of wheat. Theor. Appl. Genet. **111**:731–735.

Vanzetti, L.S., Campos, P., Demichelis, M., Lombardo, L.A., Aurelia, P.R., Vaschetto, L.M., Bainotti, C.T., Helguera, M. 2011. Identification of leaf rust resistance genes in selected Argentinean bread wheat cultivars by gene postulation and molecular markers. Electronic J. Biotechnology http://www.ejbiotechnology.info DOI: 10.2225/vol14-issue3-fulltext-14.