DNA MARKERS-ASSISTED SELECTION TO PYRAMID RUST RESISTANCE GENES IN WHEAT BREEDING LINES

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Abstract

Rust diseases (leaf, stripe and stem rust) of wheat constitute a major threat to wheat production worldwide including Romania. Durable rusts resistance is a significant component for food security and combining/pyramiding of rusts resistance genes into new wheat cultivars is the main strategy to increase durability of resistance. This work reports a gene pyramiding wheat breeding approach assisted by DNA markers used to develop new breeding rust resistant lines. In this study 60 breeding lines were analyzed for the presence of resistant haplotypes *Lr34*/Yr18//Sr57/*Ltn1*, *Lr37*/Yr17/Sr38, *Lr46*/ Yr29//Sr58/*Ltn2* and *Lr68*/*Ltn4* using DNA markers. The results showed that 17 wheat breeding lines carried the *Lr* genes pyramided in homozygous or heterozygous state, other 13 lines carried only one *Lr* gene, while 30 breeding lines had no resistant alleles *Lr*, from the analyzed *Lr* genes. In homozygous state we found the following combinations: *Lr34+Lr37* (one line); *Lr37+Lr46* (six lines), *Lr37+Lr68* (one line) and only one line, GCO2-12, with three resistant alleles in homozygous state *Lr34+Lr37+Lr46*. This line also carried heterozygous alleles for *Lr68* gene, so, this result suggest that it is possible to obtain a line with four resistance *Lr* alleles (*Lr34+Lr37+Lr46+Lr68*) using markers-assisted selection (MAS). The wheat breeding lines with two, three or four resistance alleles were identified in the F5 generation and will be used to accelerate the rust resistance breeding program at NARDI Fundulea. Furthermore, this study proves the value of MAS breeding strategy, for the acceleration of wheat rusts resistance cultivars development.

Key words: rust resistance, markers assisted selection, wheat breeding, *Lr* genes pyramiding

Wheat (*Triticum aestivum L.*) is one of the most important cereals in the world. In Romania, wheat crop has a big role in the national economy. Among the limiting factors of wheat production, a great threat is represented by the foliar and head diseases such as rusts, powdery mildew, fusarium head blight, smuts, 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 tolerant/resistant cultivars. Incorporating genes that confer tolerance and/or resistance to rusts in modern cultivars is an effective and friendly to environment way of protecting crops against pathogens.

Leaf (brown) rust (LR) caused by *Puccinia triticina* Eriks, stem (black) rust (SR) caused by *Puccinia graminis* Pers. *f.sp. tritici* Eriks. & E. Henn. and stripe (yellow) rusts (YR) caused by *Puccinia striiformis* West. f. sp. *tritici* are diseases that continue to be a problem for wheat production worldwide. Generally, there are two ways to control leaf rust in wheat: chemical and genetic. Genetic control has advantages for environment and economy.

Regarding the genetic resistance there are two classes of genes used by breeders in wheat rusts control. The first class (R genes), is referred to as "race specific resistance", "gene for gene resistance" and "seedling resistance" also called all-stage resistance (ASR).

The second class is called adult plant resistance (APR) because resistance is usually functional only in adult plants and express partial rust resistance. This is characterized by less and slower pathogen growth without a necrotic response (sometimes referred to as "slow rusting") and durable (Lagudah E.S., 2011; Ellis J.G. *et al*, 2014) showed that APR is often insufficient for crop protection during severe pathogen epidemics but is more durable and can offer good protection if more genes are combined. All these things make APR more interesting for wheat breeders. At present, the number of described genes, named *Lr*, involved in wheat leaf rust resistance reached at 80 (Kumar S. *et al*, 2021) and of these only a few have slow rusting effect.

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Among the known genes with slow rusting effect or adult plant resistance (APR) the most common are the genes: *Lr34* (Dyck L.P., 1977), *Lr46* (Singh R.P. *et al*, 1998), *Lr67* (Hiebert C.W. *et al*, 2010; Herrera-Foessel S.A. *et al*, 2011) and *Lr68* (Herrera-Foessel S.A. *et al*, 2012). *Lr34* is one of the well-known and characterized race-non-specific resistance genes. It is located on short arm of the chromosome 7DS (Dyck L.P., 1987) and confers resistance to the adult plant. *Lr34* or genes closely linked was also found to provide resistance to other two rust diseases, *Yr18* and *Sr57* (Singh R. *et al*, 2012) and powdery mildew, *Pm38* (Spielmeyer W. *et al*, 2005; Lillemo M. *et al*, 2008). This locus was also shown to provide resistance to spot blotch caused by *Bipolaris sorokiniana* (Lillemo M. *et al*, 2013). In addition, *Lr34* was reported to be associated with leaf tip necrosis gene *Ltn1* (Singh R.P. 1992) and *Bdv1*. For this reason, *Lr34* is in fact *Lr34/Yr18//Sr57/Pm38/Ltn1/Bdv1*.

Lr34 has been shown to enhance leaf rust resistance in combinations with other resistance genes (German S.E., Kolmer J.A., 1992; Kloppers F.J., Pretorius Z.A., 1997) such as the race-specific genes *Lr13* and *Lr37*.

Lr37 is located within a segment of *Triticum ventricosum* Tausch, chromosome 2NS, that contains others two rust resistance genes *Yr17* and *Sr38* conferring resistance to stripe rust and stem rust, respectively. This chromosomal fragment from *Triticum ventricosum* was translocated to the short arm of bread wheat chromosome 2AS (Bariana H.S., McIntosh R.A., 1993; McIntosh R.A. *et al*, 1995).

Lr46 was first described in 1998 by Singh R.P. *et al* (1998) in cultivar Pavon 76, and is located on chromosome 1B. The type of resistance conferred by *Lr46* is similar to that of *Lr34*, although with a smaller effect (Martinez F. *et al*, 2001) and is tightly associated with stripe rust gene, *Yr29*, stem rust gene, *Sr58*, powdery mildew gene, *Pm39*, and leaf tip necrosis gene *Ltn2* (William M. *et al*, 2003), so *Lr46* is *Lr46/Yr29//Sr58/Ltn2*.

Lr68 is also an adult plant resistance (APR) conferring slow rusting resistance to wheat leaf rust. The likely origin of *Lr68* is the Brazilian cultivar Frontana (Herrera-Foessel S.A. *et al*, 2012) and the gene is located on chromosome 7BL and also linked with leaf tip necrosis gene *Ltn4* (*Lr68/Ltn4*).

The previous reports reveled in general, that pyramiding of *Lr34*, *Lr46*, *Lr67* and *Lr68* in different combinations within a particular wheat genotype conferred high and/or sustainable level of resistance to wheat leaf rust and also expected to be long-lasting or more durable (Pinto da Silva G.B. *et al*, 2018). These three genes *Lr34*, *Lr46*, and *Lr67*, have been found conferring pleiotropic APR to LR, YR, stem rust (SR), and powdery mildew (PM) diseases, respectively. Combining these pleiotropic APR genes with other minor QTL/genes in wheat has been shown to significantly improve the plant disease resistance (Li W. *et al*, 2018).

Identifying sources of durable resistance against wheat rusts remains a global priority. Marker assisted selection (MAS) which involves indirect selection of traits by selecting the marker linked to the gene of interest is especially advantageous for agronomic traits that are otherwise difficult to select only by phenotypic selection. Leaf tip necrosis (*Ltn*), a morphological marker that is linked with APR genes (*Lr34*, *Lr46* *Lr67* and *Lr68*), has been used by many researchers in predicting the presence of APR genes despite its limitations (Sivasamy M. *et al*, 2014). Selection for *Lr34* and *Lr46* based on *Ltn* alone can sometimes be misleading because of its variable expression in different genetic backgrounds and it is difficult to differentiate resistant lines with major genes vs quantitative genes using only the field data (Mutari B. *et al*, 2018).

However, the selection of genotypes containing a combination of different rust resistance genes using conventional methods is very time consuming (Parveen Z. *et al*, 2014). So, for the best strategy of selection, it is necessary to complement the evaluation of genotypes for rust resistance genes in the field with molecular characterization or vice versa.

Breeding for rust resistance is an integral part of wheat improvement with the challenge to not to compromise yield and quality traits. Deployment of both durable rust resistance genes along with major R genes has been reported as a sound breeding strategy to avoid rust epidemics worldwide (Babu P. *et al*, 2020).

This study aims to highlight the value of a breeding program for diseases resistance, where conventional breeding strategies are integrated with MAS in order to facilitate the pyramiding of rust resistance genes and accelerate the process of developing new and improved cultivars.

MATERIAL AND METHOD

A total of 60 breeding lines, F5 generation, obtained in the wheat breeding program at NARDI Fundulea, were used for *Lr34*, *Lr37*, *Lr46* and *Lr68* genes detection by molecular markers system.

Genomic DNA was isolated from three seeds, using SDS3 method by Cristina D. *et al* (2017).

DNA amplification. For PCR reactions, two commercial kits were used: KAPA2G Fast Multiplex Mix (Sigma-Aldrich) and DreamTaq Green DNA Polymerase (Thermo Scientific). Reactions were performed in an ABI ProFlex™ 3 × 32-well PCR System. PCR primers and kits used in this study are presented in *table 1*.

Table 1

List of markers used in this study

Genes	Marker	Gel electrophoresis	PCR kit	Reference
Lr34	Functional – cssfr5	1.5%	KAPA2G Fast Multiplex Mix	Lagudah E.S. *et al*, 2009
Lr37	CAPS URIC-LN2/*Dpn*II	2.5%		https://maswheat.ucdavis.edu/protocols/Sr38
Lr46	CAPS csLV46TaqI			Evans Lagudah, CSIRO -Australia, personal communication
Lr68	CAPS cs7BLNLRR/*Hae*III		DreamTaq Green DNA Polymerase	https://maswheat.ucdavis.edu/protocols/*Lr68*

Gel electrophoresis for the separation of the amplicons or digested PCR products (for CAPS-Cleaved Amplified Polymorphic Sequences) was carried out with “routine use” agarose (CleverGEL-Clever Scientific), stained with ethidium bromide and visualized on UV light with Uvidoc HD6 system (Uvitec) (*table 1*).

RESULTS AND DISCUSSIONS

The introduction of molecular characterization of the rust genes *Lr34*, *Lr37*, *Lr46* and *Lr68* alleles status allows a more effective selection, because it can be applied at early plant development stage and in absence of the pathogens, species of *Puccinia*.

MAS approach was applied in winter wheat breeding program at NARDI Fundulea to check the introgression of single and/or multiple leaf rust resistance genes (*Lr34*, *Lr37*, *Lr46* and *Lr68*).

For *Lr34*, the multiplex PCR cssfr5 amplified two products: 523bp representing the susceptible haplotype (*Lr34*-) and 751bp for the resistant haplotype (*Lr34+*). Heterozygous status (H) was indicated by the presence of both products (*figure 1*). The multiplex PCR based on cssfr5 marker for *Lr34* gene showed that 7% of lines carried the resistance allele, 12% are heterozygous lines and 82% carried the susceptible allele. This result showed a significant reduction regarding the frequency of *Lr34* resistant haplotype in the current breeding lines at NARDI Fundulea, compared with the previously reported results, where this haplotype was found with high frequency, 79% and 62%, respectively (Ciuca M. *et al*, 2015; Cristina D. *et al*, 2015).

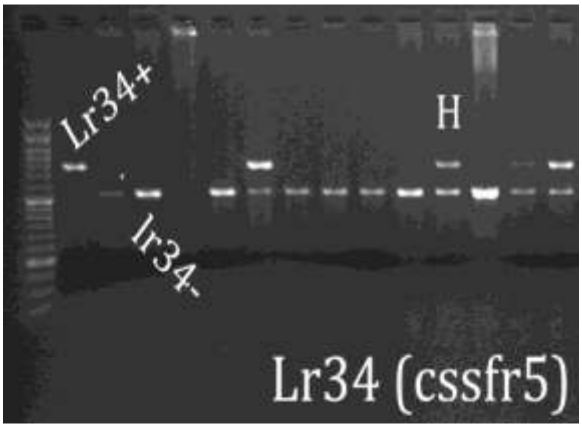

Figure 1 **Electrophoretic pattern obtained with cssfr5 marker (50bp DNA ladder - Cleaver Scientific)**

The second gene, *Lr37*, was detected using CAPS primers URIC and LN2, and the PCR products were cut with *Dpn*II restriction enzyme. The undigested 285bp band PCR product corresponds to the N genome that carry *Lr37* gene from *Triticum ventricosum*, and the 166 + 109 bp fragments corresponded to the digested PCR product of the A genome (*Triticum aestivum L.*) (Helguera M. *et al*, 2003) (*figure 2*). In this study, the results showed that 30% of lines carried the N genome with *Lr37* resistant haplotype (NN), 7% were heterozygous lines (AN) and 63% lines carried the A genome with susceptible haplotype (AA).

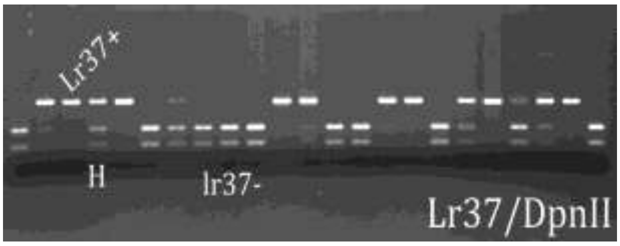

Figure 2 **Electrophoretic pattern obtained with CAPS marker URIC-LN2 (*Lr37*)**

Regarding the *Lr46* gene detection, molecular assay with CAPS marker revealed a ~1200bp PCR product that was digested with the restriction enzyme *Taq*I. After digestion, resistance allele (*Lr46*) was identified based on the electrophoretic profile consist from fragments of ~90+140+310+700bp, while for the susceptible allele (*lr46*) the fragments resulted were ~230+310+700bp (*figure 3*).

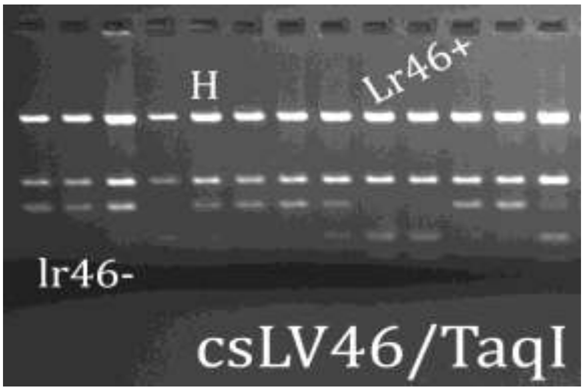

Figure 3 **Electrophoretic pattern obtained with CAPS marker csLV46 (*Lr46*)**

Result obtained with csLV46 marker on the analyzed germplasm revealed that 20% of the lines were homozygous resistant, 10% heterozygous and 70% homozygous susceptible.

For the last gene, *Lr68*, a CAPS marker was also used to amplify the DNA, cs7BLNLRR, followed by the digestion of the amplified product with *Hae*III restriction enzyme.

cs7BLNLRR marker yields a fragment of 738 bp in lines with *Lr68* resistance allele, and two bands of 270 and 478 bp in lines with *Lr68* susceptible allele (Herrera-Foessel S.A. *et al*, 2012) (*figure 4*).

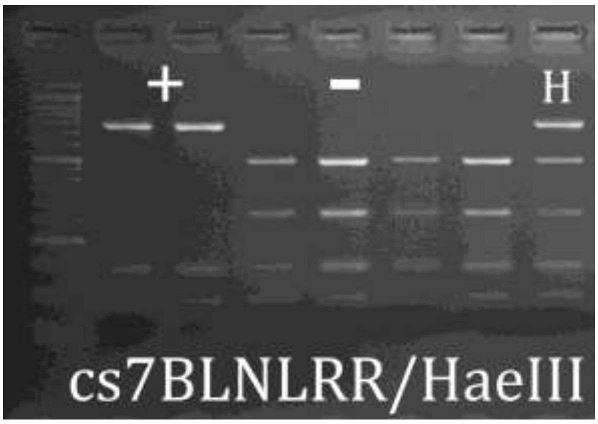

Figure 4 **Electrophoretic pattern obtained with CAPS marker cs7BLNLRR (*Lr68*)**

The results of marker assisted selection for *Lr68* gene, revealed that, in the studied germplasm, only 2% of the lines carried the resistance allele *Lr68* in homozygous state, 3% heterozygous (both alleles) and 95% with the susceptible allele in homozygous state.

MAS proved to be a powerful tool that facilitated the identification of 17 breeding lines with pyramided *Lr* genes in the analyzed germplasm, lines that carry two, three or four genes, in homozygous and/or heterozygous state.

Based on molecular assays, one line was identified with both resistance alleles for *Lr34* and *Lr37*, six lines with *Lr37+Lr46*, one line (GDD4-7) with *Lr37+Lr68* (homozygous form). This line, GDD4-7, also carried *Lr46* in heterozygous state (*Lr46lr46*), meaning that there is an opportunity to select wheat homozygous lines with *Lr37+Lr46+Lr68* combination.

Another interesting line was GCO2-12 with three resistant alleles in homozygous state *Lr34+Lr37+Lr46* and *Lr68* in heterozygous (*Lr68lr68*) state, suggesting that it is possible to obtain a line with four resistant *Lr* alleles (*Lr34+Lr37+Lr46+Lr68*) using MAS (*table 2*).

Table 2
List of wheat breeding lines with pyramided Lr genes

No.	Line	*Lr34*	*Lr37*	*Lr46*	*Lr68*
1	GDD4-6	h	+	+	-
2	GDD4-7	-	+	h	+
3	GDD6-18	h	+	h	-
4	GDD8-18	h	+	+	-
5	GDD8-19	h	-	+	-
6	GDD8-22	h	h	-	-
7	GDD14-5	-	h	h	-
8	GDD14-7	-	+	+	-
9	GDD15-15	-	h	h	-
10	GDD15-16	-	h	h	-
11	GDD15-17	-	+	+	-
12	GDD15-19	h	-	-	h
13	Bogdana	-	+	+	-
14	GCO-2-12	+	+	+	h
15	GCO-2-15	h	+	h	-
16	GCO3-8	+	+	-	-
17	GCO3-24	-	+	+	-

"+" resistance allele; "-" susceptible allele; h-heterozygous

Based on these results, future selection with molecular markers on the heterozygous plant material will make possible to choose the following combinations: *L34+Lr37+Lr46* (GDD4-6, GDD6-18, GDD8-18 and GCO2-15) and *Lr34+ Lr68* (GDD15-19). Phenotypic testing in different environmental conditions is required to validate the most efficient and durable *Lr* gene combinations, highlighted in this study.

Worldwide, based on conventional and molecular techniques, breeding lines with three or four rust resistance genes were reported. Mutari B. *et al*, (2018) reported two breeding line with three

APR gene combinations (*Lr34+Lr46+Lr68* and *Lr34+Lr46+Lr37*). Other four wheat genotypes, that displayed strong and high levels of adult plant resistance, of the leaf and stripe rusts, were reported in 2021. This level of resistance was explained as a result of the four pyramided resistance genes, *Lr34+Lr37+Lr46+Lr67*. Also, in the same study, other two breeding lines were found with three rust resistance genes pyramided (*Lr34+Lr37+Lr67*) (Omara R.I. *et al*, 2021).

Results from previous studies and also, the results from this study, can be used to design crossing program as well as strategies to highlight different resistance genes combinations to prolong the wheat effective resistance and to face the new virulent races.

CONCLUSIONS

The results showed that the pyramiding of *Lr34* and *Lr46* genes, conferring pleiotropic APR to leaf rust (LR) yellow rust (YR), stem rust (SR) and powdery mildew (PM) diseases, was achieved. Furthermore, lines that cumulated these two pleiotropic APR genes with other ASR genes were identified. The subsequent marker assisted selection (MAS) on the heterozygous lines allow obtaining other *Lr* genes combinations.

This study proves the value of MAS breeding strategy, for the acceleration of wheat rusts resistance cultivars development.

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REFERENCES

Babu P., Baranwal D.K., Harikrishna D.P., Bharti H., Joshi P., Thiyagarajan B., Gaikwad K.B., Bhardwaj S.C., Singh G.P., Singh A., 2020 – *Application of genomics tools in wheat breeding to attain durable rust resistance*. Frontiers in Plant Science, 11. https://doi.org/10.3389/fpls.2020.567147

Bariana H.S., McIntosh R.A., 1993 – *Cytogenetic studies in wheat. XV. Location of rust resistance genes in VPM1 and their genetic linkage with other disease resistance genes in chromosome 2A*. Genome, 36(3), pp.476-482.

Ciuca M., Cristina D., Turcu A.G., Contescu E.L., Ionescu V., Saulescu N.N., 2015 – *Molecular detection of the adult plant leaf rust resistance gene Lr34 in Romanian winter wheat germplasm.* Cereal Research Communications, 43(2), pp.249-259.

Cristina D., Ciuca M., Cornea C.P., 2017 – *Comparison of four genomic DNA isolation methods from single dry seed of wheat, barley and rye.* AgroLife Scientific Journal, 6(1), pp.84-91.

Cristina D., Turcu A.G., Ciuca M., 2015 – *Molecular detection of resistance genes to leaf rust Lr34 and Lr37 in wheat germplasm.* Agriculture and Agricultural Science Procedia, 6, pp.533-537.

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 whea*t. Genome 29:467–469.

Ellis J.G., Lagudah E.S., Spielmeyer W., Dodds P.N., 2014 – *The past, present and future of breeding rust resistant wheat*. Frontiers in plant science, 5, p.641.

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.

Helguera M., Khan I.A., Kolmer J., Lijavetzky D., Zhong-Qi L., Dubcovsky J., 2003 – *PCR assays for the Lr37-Yr17-Sr38 cluster of rust resistance genes and their use to develop isogenic hard red spring wheat lines.* Crop Science, 43(5), pp.1839-1847.

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., Viccars L., Calvo-Salazar V., Lan C., Lagudah E.S., 2012 – *Lr68*: *a new gene conferring slow rusting resistance to leaf rust in wheat*. Theoretical and Applied Genetics, 124(8), pp.1475-1486.

Hiebert C.W., Thomas J.B., McCallum B.D., Humphreys D.G., DePauw R.M., Hayden M.J., Mago R., Schnippenkoetter W., Spielmeyer W., 2010 – *An introgression on wheat chromosome 4DL in RL6077 (Thatcher* 6/PI 250413) confers adult plant resistance to stripe rust and leaf rust (Lr67)*. Theoretical and Applied Genetics, 121(6), pp.1083-1091.

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.

Kumar S., Bhardwaj S.C., Gangwar O.P., Sharma A., Qureshi N., Kumaran V.V., Khan H., Prasad P., Miah H., Singh G.P., Sharma, K., 2021 – *Lr80: A new and widely effective source of leaf rust resistance of wheat for enhancing diversity of resistance among modern cultivars*. Theoretical and Applied Genetics, 134(3), pp.849-858.

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*. TAG Theoretical and Applied Genetics. 119:889-898

Leaf rust resistance gene Lr68, available online at: https://maswheat.ucdavis.edu/protocols/Lr68

Li W., Song G. Q., Zhang R. Z., Li Y. L., Zhang S. J., Gao J., Li G. Y., 2018 – *Present situations and prospects for several pleiotropic disease* *resistance genes in wheat*. J. Triticeae Crops 38:791-797.

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/Yr29are 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.

Martinez F., Niks R.E., Singh R.P., Rubiales D., 2001 – *Characterization of Lr46, a gene conferring partial resistance to wheat leaf rust*. Hereditas, 135(2-3), pp.111-114.

McIntosh RA, Wellings CR, Park RF., 1995 - *In: Wheat Rusts, an Atlas of Resistance Genes* Melbourne, Australia, CSIRO.

Mutari B., Udupa S.M., Mavindidze P., Mutengwa C.S., 2018 – *Detection of rust resistance in selected Zimbabwean and ICARDA bread wheat (Triticum aestivum) germplasm using conventional and molecular techniques*. South African Journal of Plant and Soil, 35(2), pp.101-110.

Omara R.I., Shahin A.A., Ahmed S.M., Mostafa Y.S., Alamri S.A., Hashem M., Elsharkawy, M.M., 2021 – *Wheat Resistance to Stripe and Leaf Rusts Conferred by Introgression of Slow Rusting Resistance Genes*. Journal of Fungi, 7(8), p.622. https://doi.org/10.3390/jof7080622

Parveen Z., Iqbal N., Rahman S., Younis M., Nawaz M., Raza S.H., Iqbal M.Z., 2014 – *Rust resistance evaluation of advanced wheat (Triticum aestivum L.) genotypes using PCR-based DNA markers.* Pakistan Journal of Botany 46: 251–257

Pinto da Silva G.B., Zanella C.M., Martinelli J.A., Chaves M.S., Hiebert C.W., McCallum B.D., Boyd L.A., 2018 – *Quantitative trait loci conferring leaf rust resistance in hexaploid wheat*. Phytopathology, 108(12), pp.1344-1354.

Rusts resistance gene Lr37-Yr17-Sr38, available online at: https://maswheat.ucdavis.edu/protocols/Sr38

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: 13th Cereal Rust and Powdery Mildew Conference, August 28–September 1, 2012. Friendship Hotel, Beijing. 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.* Phytopathology, 88(9), pp.890-894.

Sivasam M., Aparna M., Kumar J., Jayaprakash P., Vikas VK., John P., Nisha R., Srinivasan K., Radhamani J., Jacob S.R., Kumar S., Satyaprakash S., Punniakotti K., Tyagi E., Bansal K.C., 2014 – *Phenotypic and molecular confirmation of durable adult plant leaf rust resistance (APR) genes Lr34+, Lr46+ and Lr67+ linked to leaf tip necrosis (LTN) in select registered Indian wheat (T. aestivum L.) genetic stocks.* Cereal Research and Communications 42: 262–273.

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.

William M., Singh R.P., Huerta-Espino J., Islas S.O., Hoisington D., 2003 – *Molecular marker mapping of leaf rust resistance gene Lr46 and its association with stripe rust resistance gene Yr29 in wheat.* Phytopathology, 93(2):153-159.