From Genes to Fields: advancing wheat resistance to fungal diseases

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INTRODUCTION

Wheat yield stability and **grain quality** worldwide are constrained by **fungal diseases** such as **rusts**, **common bunt**, **Fusarium head blight** and **Septoria**. Developing cultivars with **durable and broad-spectrum resistance** is therefore a primary goal in modern wheat breeding programs. While phenotypic selection has traditionally been the cornerstone of resistance breeding, its effectiveness is often limited by environmental variability, pathogen diversity, and the difficulty of identifying resistance alleles expressed only at specific developmental stages or under particular infection conditions.

To address these challenges, **marker-assisted selection (MAS)** has become an indispensable tool for accelerating genetic improvement. Molecular markers enable the precise identification of resistance genes and QTLs directly at the DNA level, facilitating early-generation screening, rapid introgression, and the monitoring of allele transmission through breeding populations. MAS is particularly powerful when targeting multiple resistance loci, as it allows the pyramiding of complementary genes that confer both race-specific and quantitative resistance, thereby enhancing the durability of protection against evolving pathogen populations.

Advances in genotyping technologies—including **diagnostic gene-specific markers, SNP assays,** and **KASP** platforms—have improved the resolution and reliability of detecting key resistance alleles. These tools support the selection of suitable parental lines, the design of targeted crossing schemes, and the efficient tracking of resistance loci in segregating populations.

In this study, we assessed the molecular profiles of a diverse set of parental wheat genotypes (cultivars, breeding lines and synthetic hexaploid wheat) used in a resistance-focused improvement program targeting brown rust, yellow rust, stem rust, common bunt, Fusarium head blight, and Septoria leaf blotch.

Molecular screening revealed substantial allelic variation among the parental forms, allowing the identification of complementary resistance sources. These genotypes were subsequently used in targeted crossing schemes designed to combine multiple resistance loci. The resulting populations were subjected to MAS-based selection to track the presence and accumulation of favorable alleles. Several derived lines successfully pyramided multiple resistance genes, including combinations of three or more rust resistance loci together with genes or QTLs conferring resistance to bunt, Fusarium, and/or Septoria.

By bridging the gap “from genes to fields,” the work highlights the effectiveness of integrating molecular diagnostics with conventional breeding to develop wheat lines with broad-spectrum and potentially durable resistance. This study highlights the critical role of MAS and gene pyramiding in accelerating the development of improved germplasm and supports their continued implementation in wheat disease-resistance breeding programs.

MATERIALS AND METHODS

The biological material consisted in 11 parental forms - four cultivars (Otilia, Pitar, Voinic and Consecvent), three synthetic hexaploid wheat (E1A, E18A and E19A) and four breeding/pre-breeding lines (B2-98, H9G Gri, Amurg and Bogdana) and 23 populations obtained from various crosses between the parental forms.

DNA extraction was carried out from three dry seeds using the NucleoSpin Plant II DNA extraction kit (MACHEREY-NAGEL).

DNA amplification. For PCR reactions, the following kits were used: MyTaq Red DNA Polymerase (Meridian Bioscience) and KAPA2G Fast Multiplex Mix (Sigma-Aldrich). Reactions were performed in an ABI ProFlex™ 3 × 32-well PCR System.

KASP reactions: The competitive allele-Specific PCR was carried out using PACE Genotyping Master Mix from 3CR Bioscience (UK) according to the manufacturer's specifications. The plate fluorescent readings were carried out in a FLUOstar Omega Microplate Reader (BMG LABTECH). The results were processed with KlusterCaller software from LGC Biosearch Technologies.

The molecular assessment aimed to reveal the presence of the following genetic elements:

- Rye-wheat translocations - T1RS:1AL and/or T1RS:1BL;
- Leaf rust resistance genes - *Lr1*, *Lr9*, *Lr17*, *Lr19*, *Lr21*, *Lr32*, *Lr34*, *Lr37*, *Lr46*, *Lr48*;
- Yellow rust - *Yr52*, *Yr72*, *Yr78*, *Qyr.wgp-1B.1*-IWB12603, *QYrsk.wgp-3BS*-IWB1836, *QYrsk.wgp-3BS*-IWB20761, *QYrsk.wgp-4BL*-IWB13720 and the markers AX-94723806-3D and AX-95172478-1A (from marker-trait associations studies);
- Stem rust - AX-94843704-1B (from marker-trait associations studies);
- Septoria resistance genes - *Stb15* and *Stb16q*;
- Common bunt resistance gene – *Bt3*;
- Fusarium head blight – *QFhba-5D.2-1* (AX-110635026).

No.	Genealogy	Code
1	E 1A x B2-98	H 401
2	E 18A x B2-98	H 402
3	E 19A x B2-98	H 403
4	E 32A x B2-98	H 404
5	Pitar x B2-98	H 405
6	Voinic x B2-98	H 406
7	Otilia x B2-98	H 407
8	B2-98 x Pitar	H 408
9	B2-98 x Voinic	H 409
10	B2-98 x Otilia	H 410
11	B2-98 x E 1A	H 411
12	B2-98 x E 18A	H 412
13	B2-98 x E 19A (col)	H 413
14	B2-98 x E 19A (norm)	H 414
15	B2-98 x E 28A	H 415
16	B2-98 x E 32A	H 416
17	H9g GRI x Bogdana	H 417
18	H9g GRI x Consecvent	H 418
19	Bogdana x (H9g GRI x Bogdana)	H 419
20	(H9g GRI x BOGDANA) x Bogdana	H 420
21	Consecvent x (H9g GRI x Consecvent)	H 421
22	(H9g GRI X Consecvent) x Consecvent	H 422
23	B2-98 x Amurg	H 423

RESULTS AND DISCUSSIONS

Molecular characterization of parental germplasm is an important step in developing effective breeding strategies for disease resistance. By identifying the specific resistance alleles present in each parental line, breeders can determine the complementarity between genotypes and select the most advantageous combinations for crossing. This approach enables the targeted introgression of desirable genes, avoids redundancy, and maximizes the potential for gene pyramiding.

Accurate allele profiling also supports the detection of rare or high-value resistance loci that may not be fully expressed phenotypically, ensuring their inclusion in breeding schemes. Overall, molecular characterization provides a genetic roadmap for designing informed crosses and increases the efficiency and success rate of developing improved breeding populations.

Molecular screening revealed substantial allelic variation among the parental forms, allowing the identification of complementary resistance sources and also the identification of some new resistance alleles present only in the synthetic hexaploid wheat.

Molecular assessment of the populations demonstrated that the introgression and accumulation of resistance alleles from the parental forms had occurred. The molecular results highlighted genotypes from different population that accumulated genes or QTLs for rusts resistance along with common bunt resistance gene and/or Septoria resistance genes. These results are a part of the ongoing work from the project ADER 311 (2023-2026). Further research and analysis will take place in order to evaluate the best genetic profile through artificial and/or natural infections of the selected genotypes. A selection of genotypes from the populations that accumulated resistance alleles are presented in the following tables.

Parental germplasm	Rye-wheat translocation (SCM9)	*Lr1*	*LR9*	*LR17*	*LR19 / Sr25*	*Lr21*	*Lr24 / Sr24*	*Lr32*	*Lr34 / Yr18 / Pm38 / Sr57*	*Lr37 / Yr17 / Sr38*	*Lr46 / Yr29 / Pm39*	*Lr48*	*Yr52*	*Yr72*	*Yr78*	*Qyr.wgp-1B.1-IWB12603*	*QYrsk.wgp-3BS-IWB1836*	*QYrsk.wgp-3BS-IWB20761*	*QYrsk.wgp-4BL-IWB13720*	*Yr-AX-94723806-3D*	*Yr-AX-95172478-1A*	*Sr-AX-94843704-1B*	*Stb15*	*Stb16q*	*Bt3*	*Fhb-AX-110635026*
Otilia	-	-	Lr9+	-	-	-	-	-	-	-	-	-	Yr52+	-	-	-	-	-	-	-	G	-	Stb15+	NA	-	C
Pitar	-	-	-	Lr17+	-	-	Lr24+	-	Lr34+	-	-	-	Yr52+	Yr72+	Yr78+	-	-	-	-	T	G	-	-	NA	-	-
Voinic	-	-	Lr9+	-	-	-	-	-	-	-	-	-	Yr52+	-	Yr78+	-	-	-	-	-	G	-	Stb15+	NA	Bt3+	C
Consecvent	T1RS:1AL	-	Lr9+	-	-	-	-	-	-	-	-	-	Yr52+	-	-	-	-	-	-	-	-	-	Stb15+	NA	-	-
E1A	-	Lr1+	-	Lr17+	-	Lr21+	-	Lr32+	-	-	-	-	Yr52+	-	-	-	-	-	-	-	-	-	-	NA	-	-
E18A	-	Lr1+	-*	NA	NA	-	-	Lr32+	-	-	-	-	Yr52+	NA	Yr78+	-	H?	T	-	-	G	A	NA	NA	-	-
E19A	-	-	-*	NA	NA	-	-	-	-	-	-	-	-	NA	-	C	T	-	-	-	-	-	NA	NA	-	-
B2-98	T1RS:1AL	-	-	Lr17+	-	-	Lr24+	-	Lr34+	-	-	-	-	Yr72+	Yr78+	C	-	-	-	T	-	A	-	-	Bt3+	-
H9G Gri	T1RS:1BL	-	-	Lr17+	Lr19+	-	Lr24+	-	Lr34+	-	-**	-	Yr52+	NA	Yr78+	-	T	-	-	T	G	A	-	-	-	C
Amurg	-	Lr1+	-*			-	Lr24+	-	-	Lr37+	Lr46+	Lr48+	Yr52+	NA	Yr78+	-	-	-	A	T	G	-	-	Stb16q+	-	-
Bogdana	-	-	Lr9+	Lr17+?	Lr19+	-	-	-	-	Lr37+	Lr46+	Lr48+	Yr52+	-	-	-	-	-	-	-	G	-	Stb15+	Stb16q+	-	C

Genealogy	Genotype	Rye-wheat translocation (SCM9)	*Lr17*	*Lr21*	*LR24*	*Lr32*	*Lr34*	*Yr72*	*Yr78*	*Bt3*
B2-98 x E1A	411-74-2	T1RS:1AL	Lr17+	Lr21+	Lr24+	-	Lr34+	-	Yr78+	Bt3+
	411-54-2	T1RS:1AL	Lr17+	Lr21+	Lr24+	H	Lr34+	-	-	Bt3+
	411-43-1	T1RS:1AL	Lr17+	Lr21+	-	Lr32+	-	-	Yr78+	Bt3+
	411-43-2	T1RS:1AL	Lr17+	Lr21+	-	Lr32+	-	-	Yr78+	Bt3+
	411-43-4	T1RS:1AL	Lr17+	Lr21+	-	Lr32+	-	-	Yr78+	Bt3+
	411-54-3	T1RS:1AL	Lr17+	Lr21+	Lr24+	H	H	-	H	Bt3+
	411-54-5	T1RS:1AL	Lr17+	Lr21+	Lr24+	Lr32+	H	-	H	-
	411-69-1	T1RS:1AL	Lr17+	Lr21+	-	-	Lr34+	Yr72+	H	H
	411-74-4	T1RS:1AL	Lr17+	Lr21+	H	-	Lr34+	-	Yr78+	H
	411-74-7	T1RS:1AL	Lr17+	Lr21+	H	-	Lr34+	-	Yr78+	H
	411-12/24	-	Lr17+	-	Lr24+	-	Lr34+	H	Yr78+	Bt3+
	411-13/24	-	Lr17+	-	Lr24+	-	Lr34+	H	Yr78+	Bt3+
	411-16/24	-	Lr17+	-	Lr24+	-	Lr34+	H	Yr78+	Bt3+
	411-43-3	T1RS:1AL	Lr17+	Lr21+	-	Lr32+	-	-	Yr78+	H
	411-44-2	T1RS:1AL	Lr17+	Lr21+	-	Lr32+	-	-	Yr78+?	H
	411-69-2	T1RS:1AL	Lr17+	Lr21+	-	-	Lr34+	-	H	Bt3+
	411-74-1	T1RS:1AL	Lr17+	Lr21+	H	-	Lr34+	-	Yr78+	-
	411-74-3	T1RS:1AL	Lr17+	Lr21+	H	-	Lr34+	-	Yr78+	-
	411-74-5	T1RS:1AL	Lr17+	Lr21+	H	-	Lr34+	-	Yr78+	-
	411-74-6	T1RS:1AL	Lr17+	Lr21+	-	-	Lr34+	-	Yr78+	H
	411-74-8	T1RS:1AL	Lr17+	Lr21+	H	-	Lr34+	-	Yr78+	-
	411-54-4	T1RS:1AL	Lr17+	Lr21+	Lr24+	H	H	-	H	H
	411-54-1	T1RS:1AL	Lr17+	Lr21+	Lr24+	H	H	-	H	-
	411-54-6	T1RS:1AL	Lr17+	Lr21+	H	H	H	H	H	H

Genealogy	Genotype	Rye-wheat translocation (SCM9)	*LR9*	*LR24*	*Lr34*	*Lr37*	*Lr46*	*Lr48*	*Yr78*	*Stb15*	*Stb16q*
Bogdana x (H9g GRI x Bogdana)	419-15-7	-	Lr9+	-	-	Lr37+	Lr46+	Lr48+	-	Stb15+	Stb16q+
	419-1-6	-	Lr9+	H	H	Lr37+	Lr46+	Lr48+	-	H	Stb16q+
	419-2-1	-	Lr9+	-	-	Lr37+	Lr46+	H	H	Stb15+	Stb16q+
	419-8-4	T1RS:1BL	Lr9+	H	-	Lr37+	Lr46+	H	H	-	Stb16q+
	419-10-1	-	Lr9+	-	-	Lr37+	H	Lr48+	H	Stb15+	Stb16q+
	419-11-1	T1RS:1BL	Lr9+	-	-	H	Lr46+	H	-	Stb15+	Stb16q+
	419-12-2	T1RS:1BL	H	-	H	Lr37+	Lr46+	Lr48+	H	H	Stb16q+
	419-3-2-4	-	Lr9+	H	H	Lr37+	Lr46+	Lr48+	-	H	Stb16q+
	419-3-3-10	-	Lr9+	H	H	Lr37+	Lr46+	Lr48+	-	H	Stb16q+
	419-4-2	-	Lr9+	H	-	H	Lr46+	Lr48+	-	Stb15+	H
	419-6/7-5	-	Lr9+	-	-	Lr37+	H	H	-	Stb15+	Stb16q+
	419-6/7-8	-	Lr9+	-	-	Lr37+	H	H	-	Stb15+	Stb16q+
	419-13-1	-	Lr9+??	H	H	H	Lr46+	Lr48+	-	Stb15+	H
	419-14-1	-	Lr9+	H	H	H	Lr46+	Lr48+	-	Stb15+	H
	419-3(G)-8	-	Lr9+	H	-	Lr37+	H	Lr48+	H	Stb15+	H
	419-3(G)-12	-	Lr9+	H	-	Lr37+	H	Lr48+	H	Stb15+	H
	419-3-2-8	-	Lr9+	H	-	H	Lr46+	Lr48+	-	Stb15+	H
	419-1-3	-	Lr9+	H	-	H	Lr46+	Lr48+	-	H	H
	419-3-5	-	Lr9+	-	-	H	Lr46+	H	-	H	Stb16q+
	419-9-7	T1RS:1BL	H	-	H	H	Lr46+	H	-	H	Stb16q+
	419-9-8	T1RS:1BL	H	-	H	H	Lr46+	H	-	H	Stb16q+
	419-3-3-3	-	Lr9+	H	-	H	Lr46+	Lr48+	-	H	H
	419-3-3-8	T1RS:1BL	H	H	H	H	H	H	-	H	Stb16q+
	419-3-4(S)-2	-	Lr9+	H	-	Lr37+	H	H	-	H	H
	419-3-4(S)-4	-	H	H	H	H	Lr46+	H	H	Stb15+	H
	419-5-1	-	H	-	H	H	H	Lr48+	H	H	H

Genealogy	Genotype	Rye-wheat translocation (SCM9)	*Lr34*	*Lr37*	*Lr46*	*Lr48*	*Yr52*	*QYRSK-WGP-4BL-IWB13720*	*Sr-AX-94843704 (1B)*	*STB16q*	*Bt3*
B2-98xAmurg	423-17	T1RS:1AL	-	-	Lr46+	Lr48+	Yr52+	H	-	Stb16q+	Bt3+
	423-337	T1RS:1AL	Lr34+	H	H	H	Yr52+	-	A	Stb16q+	Bt3+
	423-341	T1RS:1AL	H?	Lr37+	H	Lr48+	Yr52+	A	A	-	H
	423-338	T1RS:1AL	H?	Lr37+	H	Lr48+	Yr52+	A	A	H	H
	423-61	T1RS:1AL	H?	Lr37+	H	H	H	A	A	H	Bt3+
	423-216	T1RS:1AL	-	Lr37+	-	Lr48+	Yr52+	H	H	-	Bt3+
	423-233	T1RS:1AL	H	Lr37+	H	H	H	A	H	Stb16q+	Bt3+
	423-238	T1RS:1AL	-	Lr37+	-	Lr48+	-	-	A	Stb16q+	H
	423-249	T1RS:1AL	H	Lr37+	Lr46+	H	Yr52+	H	A	H?	H
	423-277	T1RS:1AL	H?	Lr37+	H?/-	Lr48+	H	A	A	-	H
	423-308	T1RS:1AL	H?	Lr37+	H	H	Yr52+	-	A	H	Bt3+
	423-321	-	H?	Lr37+	Lr46+	H	H	A	A	H	Bt3+
	423-325	T1RS:1AL	-	Lr37+	H	Lr48+	Yr52+	A	H	H	H
	423-328	-	H?	Lr37+	Lr46+	H	H	A	A	H	Bt3+
	423-335	T1RS:1AL	Lr34+	Lr37+	-	Lr48+	-	-	A	H	H
	423-357	T1RS:1AL	Lr34+	Lr37+	-?	-	H	H	A	-	Bt3+
	423-21	T1RS:1AL	H	Lr37+	H	H	Yr52+	A	H	H	H
	423-44	T1RS:1AL	H?	Lr37+	-	H	H	H	H	Stb16q+	Bt3+
	423-51	-	-	H	-	Lr48+	Yr52+	H	A	Stb16q+	-
	423-56	T1RS:1AL	H?	H	H	-	-	-	A	Stb16q+	Bt3+
	H423-87	T1RS:1AL	-	H	H	Lr48+	-	A	-	Stb16q+	H
	423-131	-	H?	-	Lr46+	H	Yr52+	H	A	Stb16q+	-
	423-140	T1RS:1AL	H	-	H	-	Yr52+	A	H	Stb16q+	H
	423-145	T1RS:1AL	H	H	-	H	H	A	A	-	Bt3+
	423-153	T1RS:1AL	H?	H	Lr46+	H	H	A	-	Stb16q+	H
	423-385	T1RS:1AL	-	H	-	H	Yr52+	A	A	H	H
	423-386	T1RS:1AL	-	H	-	H	Yr52+	H	A	Stb16q+	H
	423-360	T1RS:1AL	H	H	H	H	Yr52+	H	A	H	H

Conclusions

The integration of molecular characterization proved highly effective in identifying valuable resistance alleles and guiding their introgression into new wheat populations.

Profiling the parental germplasm enabled the selection of complementary sources of resistance, while genotyping of segregating populations facilitated the precise tracking and accumulation of multiple resistance genes. Several derived lines successfully combined important loci for resistance to rusts, common bunt and/or Septoria, demonstrating the efficiency of marker-assisted selection. Overall, these results highlight the importance of molecular tools in accelerating the development of wheat lines with broad-spectrum and potentially durable resistance, reinforcing the value of MAS-driven approaches in modern breeding programs.

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