



Exploring Synthetic Hexaploid Wheat as a source of genetic diversity for rust resistance in wheat

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ABSTRACT

Common wheat (*Triticum aestivum* L.), one of the staple crops today, originates from a **natural hybridization and chromosome doubling** event between *Triticum turgidum* ssp. *durum* Desf. MacKey, a cultivated allotetraploid (2n = 4x = 28, AABB), and *Aegilops tauschii* Coss., a diploid goat grass (2n = 2x = 14, DD). In the evolution of hexaploid wheat, due to the involvement of only a few accessions of both species, the **genetic diversity** of common wheat was largely decreased in comparison with its donor species.

Wild relatives of wheat have long been recognized as a **source of useful genes** for cultivated wheat improvement. One of the wheat breeding programs strategies in exploiting the wild relatives of wheat has been through **synthetic hexaploid wheat (SHW)**.

SHWs have the potential to improve **yield potential**, **tolerance/resistance to biotic stresses** such as rusts, **tolerance/resistance to major abiotic stresses** including drought, high temperature (heat), salinity/sodicity, waterlogging, acidity, cold, and soil macro and micronutrient deficiencies or toxicities.

In this study 13 F2 plants, resulted from the cross of SHW-E1 and double haploid line B2-98, were analyzed regarding the transfer of some rust disease resistance genes. The results highlighted recombinant SHWs lines that cumulated resistance genes (from both parental forms) in different combinations, underlining the importance of SHWs in wheat breeding programs from NARDI Fundulea.

INTRODUCTION

Common wheat (*Triticum aestivum* L.), one of the staple crops today, originates from a **natural hybridization and chromosome doubling event** between *Triticum turgidum* ssp. *durum* Desf. MacKey, a cultivated allotetraploid (2n = 4x = 28, AABB), and *Aegilops tauschii* Coss., a diploid goat grass (2n = 2x = 14, DD). In the evolution of hexaploid wheat, due to the involvement of only a few accessions of both species, the genetic diversity of common wheat was largely decreased in comparison with its donor species.

Wild relatives of wheat have long been recognized as a source of useful genes for cultivated wheat improvement. One of the wheat breeding programs strategies in exploiting the wild relatives of wheat has been through **synthetic hexaploid wheat (SHW)**.

SHW serves as a bridge to broaden the genetic background of wheat, enabling breeders to create high-yielding, resilient cultivars that support global food security.

The SHWs are developed by artificially hybridizing durum wheat (*Triticum turgidum* ssp. *durum*; 2n = 4x = 28, AABB) with accessions of *Ae. tauschii* (2n = 2x = 14, DD), the donor of the D genome of hexaploid bread wheat, in a process analogous to the natural hybridization event that produced hexaploid wheat.

Although, **the primary synthetic wheat** itself cannot be used as a cultivar because of the presence of “wild characters,” or agronomically undesirable characters such as tenacious glumes that causes non-free threshing grains, it can be used in crosses with elite common wheat cultivars for the development of **synthetic derivative lines (SDLs)** or **synthetic backcross-derived lines (SBLs)**.

Rust diseases of wheat pose a significant threat to global wheat production due to highly adaptive nature and evolution of the pathogens, *Puccinia triticina* (leaf rust), *Puccinia graminis* (stem rust), and *Puccinia striiformis* (yellow rust).

This approach is currently used in **NARDI Fundulea wheat breeding program** in order to achieve the transfer of **known resistance genes** from SHW and possibly other **unknown genetic elements** involved in rust resistance/tolerance to new recombinant synthetic wheat lines.

This study was focused on the molecular characterization regarding the transfer of some rust resistance genes in 13 recombinant synthetic hexaploid wheat lines obtained at NARDI Fundulea.

MATERIALS AND METHODS

The plant material consisted of 13 F2 plants resulted from the cross of **synthetic hexaploid wheat E1** and **double haploid line B2-98**. SHW-E1 was obtained by crossing the Romanian cultivar *Triticum durum* - Pandur with *Ae. tauschii* ssp. *tauschii* var. *typica* Iran (2472). B2-98 is a DH line, obtained from the cross of cultivar Izvor and the breeding line F00628-34 (F628), that carries the 1RS:1AL rye translocation.

DNA extraction was carried out from two 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: 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.

RESULTS AND DISCUSSIONS

The short arm of 1R from rye (*Secale cereale* L.) has been used in **translocation lines** due to its favorable agronomic potential, such as resistance to powdery mildew, stem rust, leaf rust, and stripe rust, and also high yield potential. The amplification with SCM9 marker from this study revealed the presence of **1RS:1AL rye-wheat translocation** in 8 out of 13 recombinant SHW lines.

Lr21 (1DS) is a gene transferred from an *Aegilops tauschii* accession. Studies showed that **Lr21** gene, beside the known seedling resistance, also confers adult plant field resistance to leaf rust. The molecular results obtained with the KASP marker Lr21-GQ504819-1346-C/T showed that the resistance allele is present in 10 recombinant SHW lines.

Lr23, originally detected in the durum wheat cultivar Gaza, is a leaf rust resistance gene that can provide effective resistance when deployed in combination with other leaf rust resistance genes. The results obtained with KASP marker Lr23-KASP_69462 revealed the presence of the **Lr23** durum resistance allele in all 13 recombinant SHW lines. Furthermore, it was discovered that the parental DH line B2-98 carries the **Lr23** durum resistance allele.

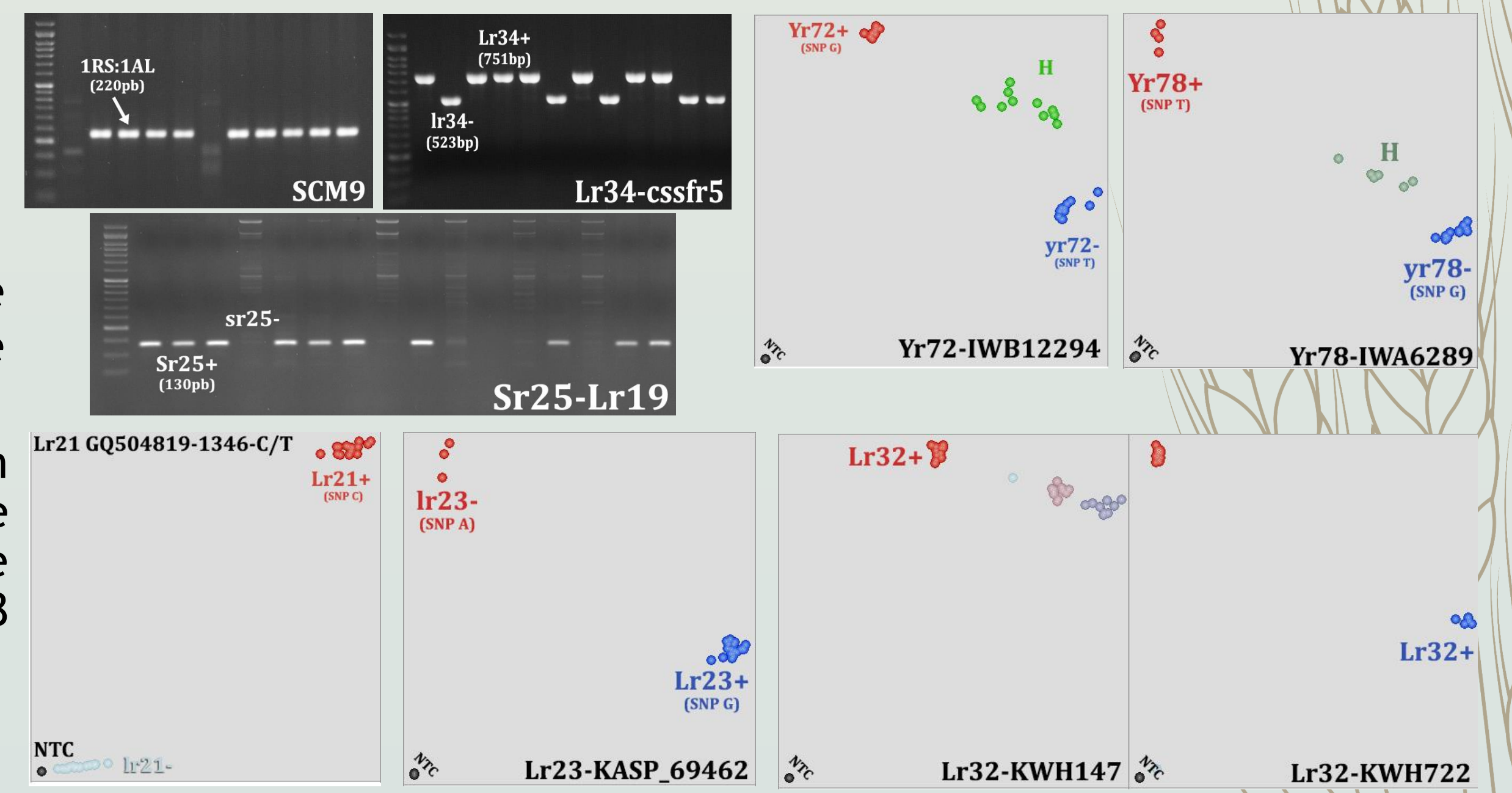
Lr32 gene, located on 3DS chromosome, confers seedling resistance to leaf rust. Results obtained in this study with KASP markers Kwh147 and Kwh722 showed the presence of **Lr32** resistance allele in only two recombinant SHW lines.

Lr34 gene (7DS), also known as **Yr18** and **Pm38**, is one of the most well studied genetic locus in wheat that provides durable, broad-spectrum resistance against multiple fungal pathogens, including leaf rust, stripe rust, and powdery mildew. The results obtained with PCR marker *cssfr5* showed the presence of the resistance allele (homozygous form) in 5 recombinant SHW line, one heterozygous SHW line and 7 SHW lines with the susceptible allele.

Yr72 is a yellow rust resistance gene from 2BS chromosome. The molecular results obtained with KASP marker IWB12294 revealed the presence, in heterozygous form, only in 3 SHW lines.

Yr78, located on 6BS chromosome, is a yellow rust resistance gene that confers resistance to adult plant. The results obtained with KASP marker showed the presence of the resistance allele (homozygous form) in 5 SHW lines, 7 heterozygous lines and one line with the susceptible allele.

Sr25 is a stem rust resistance gene first transferred from *Thinopyrum ponticum* Barkworth and Dewey. **Sr25** is **linked** with leaf rust resistance gene **Lr19** (leaf rust resistance gene). Results from this study, obtained with the dominant PCR marker Sr25-Gb, showed the presence of **Sr25** resistance allele in 7 SHW lines.



No.	Genotype	Rye translocation	Lr21	Lr23-Dur	Lr32	Lr34	Yr72	Yr78	Sr25
P1	B2-98	1RS:1AL	-	Lr23D+	-	Lr34+	Yr72+	Yr78+	-
P2	SHW-E1	-	Lr21+	Lr23D+	Lr32+	-	-	-	Sr25+
1	411-1	-	Lr21+	Lr23D+	-	-	-	Yr78+	-
2	411-2	-	-	Lr23D+	-	-	-	-	Sr25+
3	411-3	1RS:1AL	Lr21+	Lr23D+	Lr32+	-	-	H	Sr25+
4	411-4	1RS:1AL	Lr21+	Lr23D+	Lr32+	-	-	H	Sr25+
5	411-5	1RS:1AL	Lr21+	Lr23D+	-	H	-	H	-
6	411-6	-	Lr21+	Lr23D+	-	-	-	Yr78+	Sr25+
7	411-7	-	-	Lr23D+	-	Lr34+	H	Yr78+	-
8	411-8	1RS:1AL	Lr21+	Lr23D+	-	Lr34+	H	H	-
9	411-9	-	-	Lr23D+	-	Lr34+	-	Yr78+	-
10	411-10	1RS:1AL	Lr21+	Lr23D+	-	Lr34+	H	H	Sr25+
11	411-11	1RS:1AL	Lr21+	Lr23D+	-	Lr34+	-	Yr78+	-
12	411-12	1RS:1AL	Lr21+	Lr23D+	-	-	-	H	Sr25+
13	411-13	1RS:1AL	Lr21+	Lr23D+	-	-	-	H	Sr25+

CONCLUSIONS

Gene pyramiding, the process of combining multiple genes that contribute to desirable traits, is one important approach in modern wheat breeding. This study highlighted recombinant synthetic hexaploid wheat lines that carry gene combination such as: T1RS:1AL + Lr21 + Lr23D + Lr32 + Sr25 + Yr78 (heterozygous), T1RS:1AL + Lr21 + Lr23D + Sr25 + Yr78 (heterozygous), T1RS:1AL + Lr21+ Lr23D + Lr34 + Yr72 (heterozygous) + Yr78 (heterozygous), T1RS:1AL + Lr21 + Lr23D + Lr34 + Sr25 + Yr72 (heterozygous) + Yr78 (heterozygous), T1RS:1AL + Lr21 + Lr23D + Lr34 + Yr78, T1RS:1AL + Lr21 + Lr23D + Lr34 (heterozygous) + Yr78 (heterozygous), Lr21 + Lr23D + Yr78 + Sr25, Lr23D + Lr34 + Yr78 + Yr72 (heterozygous).

These results underline the importance of **SHWs in wheat breeding programs**, as a **genetic resource**, for the development of **new genotypes with improved genetic diversity**.

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