

DETECTION OF SR31 AND SR36 STEM RUST RESISTANCE GENES BY MOLECULAR MARKERS IN WHEAT CULTIVARS REGISTERED IN HUNGARY

AZ SR31 ÉS SR36 SZÁRROZSDA REZISZTENCIAGÉNEK AZONOSÍTÁSA MOLEKULÁRIS MARKEREKKEL MAGYARORSZÁGON ELISMERT BÚZAJAJTÁKBAN

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Abstract: Two hundred and twenty wheat cultivars registered in Hungary in a period of 35 years, from 1970 to 2005, were investigated by molecular markers to determine the frequency of Sr31 and the Sr36 stem rust resistance genes which are very effective in Hungary to date. Among the 156 Hungarian wheats a significant part (32.7%) had the 1BL.1RS wheat-rye chromosome translocation, the source of Sr31 gene, or the *Triticum timopheevi* introgression with Sr36 gene (17.3%). In the 64 foreign cultivars, deriving from 12 countries, only 4.7% had the 1RS.1BL and 10.9% carried the Sr36. The maximal frequency of cultivars with 1RS.1BL reached 47% (in 1994), and those of with Sr36 reached 32% (in 1983 and 1984). The occurrence of above genes in some wheat cultivars developed and produced in Romania was also investigated. Owing to the threat of new pathogen race Ug99 appeared in Uganda and which is virulent to Sr31 stem rust resistance genes there is an urgent need to incorporate several other Sr resistance genes against into the new wheat cultivars.

Kivonat: Az utóbbi 35 évben (1970-től 2005-ig) kétszázhusz Magyarországon elismert búzafajtát vizsgáltunk molekuláris markerekkel, hogy meghatározzuk a Magyarországon mindig nagyon hatásos Sr31 és Sr36 szárrozsdá rezisztencia gének gyakoriságát. A vizsgált 156 magyar búzafajtának jelentős hányada hordozta vagy az 1BL.1RS búzarozs kromoszóma transzlokációt – az Sr31 rezisztenciagén forrását - (a fajták 32,7%-a), vagy a *Triticum timopheevi* fajból származó Sr36 szárrozsdá rezisztencia gént (17,3%). A 64 külföldi eredetű búzafajtából (amelyek 12 országból származtak) csak 4,7% hordozta az 1BL.1RS-t és 10,9% az Sr36 rezisztencia gént. Az összes vizsgált búzafajtára vonatkoztatva az 1BL.1RS maximális gyakorisága 47%-ot (1994-ben) az Sr36 géne pedig 32%-ot (1983 és 1984) ért el. A fenti gének előfordulását néhány Romániában előállított és termelt búzafajtában is megvizsgáltuk. Az Ugandában megjelent új, igen veszélyes, az Sr31 génre is virulens, patogén rassz (Ug99) járványos terjedése miatt, a nemesítés sürgős feladata új rezisztenciagének beépítése a búzafajtákba.

Key words: 1BL.1RS, molecular marker, *Puccinia graminis*, *Secale cereale*, translocation, *Triticum aestivum*, Ug99

Kulcsszavak: 1BL.1RS, molekuláris marker, *Puccinia graminis*, *Secale cereale*, transzlokáció, *Triticum aestivum*, Ug99

INTRODUCTION

The introgression of rye chromatin by chromosome translocations has increased the genetic diversity for several agronomic characters in wheat (*Triticum aestivum*). The most frequently used translocations in wheat cultivar improvement programs is the 1BL.1RS, derived from 'Petkus' rye (*Secale cereale*) via Russian wheat cultivars 'Kavkaz' and Aurora'. 1BL.1RS cultivars are worldwide in their distribution. From 1991 to 1995, 45% of 505 commercial cultivars of bread wheat from 17 countries (RABINOVICH, 1998). In the past forty years, 1BL.1RS translocation has been widely used by breeders to enhance agronomic

performance, particularly grain yield. The 1BL.1RS wheats carry several disease resistance genes like *Sr31*. Another, very effective stem rust resistance gene is the *Sr36*, also derived from alien species *Triticum timopheevi* into common wheat (MCINTOSH and GYARFAS J, 1971) and it spread to several wheat cultivars in the world through cv. 'Arthur'. Isolates of *Puccinia graminis* f. sp. *tritici* with high virulence to *Sr31* were detected (Ug99 race) in Uganda in 1999 (PRETORIUS et al., 2000), and pose a worldwide threat to wheat production in areas where *Sr31* resistance is important.

In this study, we aimed to determine the frequency of *Sr31* and the *Sr36* gene, among wheat cultivars registered in Hungary in the past 35 years period.

MATERIALS AND METHODS

Two hundred and twenty wheat entries were tested, all of them registered in Hungary from 1970- 2005. Most of the varieties originated from the two main Hungarian wheat breeding institutions: Cereal Research Institute (or its later name Cereal Res. Ltd. Co.), Szeged (GK) and the Agricultural Research Institute of the Hungarian Academy of Sciences, Martonvasar (Mv). Rest of the cultivars originated from other Hungarian breeding sites or European countries: Austria (AT), Croatia (CRO), Czech Republic (CZ), France (FR), Germany (DE), Netherlands (NE), Romania (RO), Russia (RUS), Serbia (SRB), Slovakia (SLK) and Ukraine (UA).

Molecular markers were used to detect the presence of *Sr36* as well as the wheat-rye translocation, 1BL.1RS carrying *Sr31*. Genomic DNA samples were extracted from a bulk of leaves from 3 plants according to the CTAB method essentially as described by (Rogers and Bendich, 1985). The presence of rye translocation was tested by the OPH20 RAPD marker (Francis et al., 1995), which is able to detect, however, can not distinguish any rye chromosome in wheat. To distinguish 1BL.1RS translocation from 1AL.1RS cultivars samples were further tested by the SCM9 microsatellite marker (WENG et al., 2007). The gwm271 microsatellite marker (BARIANA et al., 2001) was used, with some modifications, to identify the *Sr36* stem rust resistance gene RAPD amplifications were assayed by agarose (1.5%) gel electrophoresis, stained with ethidium bromide, and the amplified samples of microsatellite reactions were separated on a 5% denaturing polyacrylamide gel than visualized by silver staining.

RESULTS AND DISCUSSION

Among the 220 cultivars deriving from Hungary and 12 other countries, 54 (24.5%) showed 1BL.1RS translocation and evidently the presence of *Sr31* gene. Among the 156 Hungarian wheats 32.7% had the 1RS.1BL translocation and in the 64 foreign cultivars only 4.7% had the 1RS.1BL. In Hungary, the first 1RS.1BL cultivars were Aurora and Kavkaz in the seventies. In short time after their registration, in 1970, they became extremely popular and by 1974 their planting area reached practically half of the total wheat acreage of the country, although they represented only the 14% of wheat cultivars registered at that time (Fig. 1). However, these cultivars disappeared from the production as fast as they came. From the advent of the second generation 1BL.1RS cultivars starting with cultivar GK Sagvari, from 1982, their proportion rose continuously up to their maximal value (49%) in 1994. After this peak, their proportion decreased steadily to 14% until 2005.

In contrast to the *Sr31*, the ancestors of *Sr36* (i.e. Arthur) was not represent in the wheat production of Hungary. The first registered cultivars carrying *Sr36* were Baranjka and Super Zlatna from Croatia (1980) which were followed by two Hungarian varieties: Mv 10 in 1981 and GK Kincső in 1983. Out of the 220 tested entries, 34 cultivars (15.5%) carried the *Sr36* gene (Fig. 1.) – the maximal frequency of cultivars with *Sr36* reached 32% (in 1983 and

1984). Among the foreign cultivars *Sr36* occurred only in the Croatians – all seven Croatian cultivars investigated contained this gene.

Considering the occurrence of the two main stem rust resistance gene together, a large portion of cultivars registered in the middle of 90s, contained at least one of the *Sr31* or *Sr36* resistance genes, so their joint frequency and their share in sowing area reached 60% (from 1993 to 1995, and in 1998, respectively) among other cultivars (data not shown).

Among the two cultivars originated from Romania (cv. Alex and cv. Fatima 2 – later was developed in a cooperation between Martonvasar, Hung. and Fundulea, Rom.), only the *Sr31* resistance gene occurred (in Fatima 2). Investigating further 10 cultivars produced in Romania, but were not registered in Hungary, non of them carried *Sr36* but four of them (Dor, Delia, Fundulea 4 and Lovrin 41) had the *Sr31* resistance gene.

The results of our study indicated that both *Sr31* and *Sr36* genes had widely spread in wheat cultivars registered in Hungary in the last 35 year period. However, great difference in the distribution of *Sr31* and *Sr36* depending on breeding programs and time course was also observed. The definite and steady decline in the frequency of 1BL.1RS cultivars, from very beginning of this decade, is probably due to the deliberate breeding activity to improve bread making quality of wheat cultivars, rather than to the risk of stem rust epidemic, as *Sr31* is still efficient in Europe. Although, about one third of cultivars investigated originated from foreign countries almost none of them carried (only cv. Balada, besides Kavkaz and Auora) *Sr31*. It is might be due to that most of these countries are North or West to Hungary where climatic condition does not favor for the stem rust, or they are still protected by *Sr36* (cultivars from Croatia, the Southern neighbor of Hungary). The high occurrence of *Sr31* or *Sr36* in Hungarian gene pool and the *Sr36* in the Croatian one prove the efficiency of their conventional program for stem rust resistance.

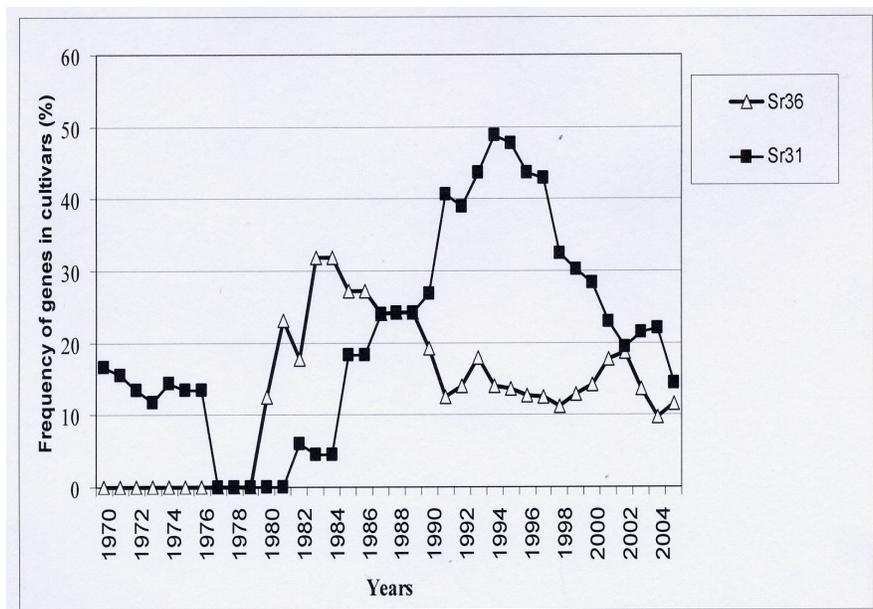


Figure 1. Occurrence of 1BL.1RS translocation (*Sr31*) and the *Sr36* stem rust resistance gene in wheat cultivars registered in Hungary from 1970 to 2005

In Hungary, both *Sr31* and especially the *Sr36* still provide an effective protection against stem rust infection (CSÓSZ et al. 2001), and cultivars having neither the *Sr31* nor the *Sr36*, with few exceptions, proved very susceptible to stem rust in trials using artificial inoculation (unpublished results). However, stem rust race Ug99, which has a high virulence to *Sr31*, was detected in Uganda in 1999 (PRETORIUS et al., 2000), and pose a worldwide threat to wheat production in areas where *Sr31* resistance is important.

It seems that the introgression of alien chromosome translocations did not increase the diversity in stem rust resistance gene pool in wheat cultivars registered in Hungary, as the use of efficient resistance genes became very biased. The narrowed diversity may increase genetic vulnerability. It might open the door to new races of pathogens, i.e. TTKS (Ug99) race for *Sr31*. There is an urgent need to incorporate several resistance genes against major diseases into the new wheat cultivars. Marker assisted selection along with precise artificial field tests provide new and efficient possibilities for geneticists and breeders to achieve these goals.

ACKNOWLEDGEMENTS

This work was supported by Hungarian grant GAK (lr9ylr29, contract No.: OMF 00950/2005) and a grant provided by the European Union to enhance cross-border co-operations between Hungary and Romania (INTERREG IIIA grant: HU-RO-SCG-1/206). We also thank for the cooperation of the Romanian project leader Prof. Gallia Butnaru from the USAMVB, Timisoara, and to Timea Lajtár and Andrea Csukás for their excellent technical assistance.

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