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Research note

Addition of chromosome 4R from Hungarian rye cultivar Lovászpatonai confers resistance to stripe rust and outstanding end-use quality in wheat

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ABSTRACT

The Hungarian rye (*Secale cereale* L., 2n = 2x = 14, RR) cultivar Lovászpatonai possesses numerous agronomically useful traits that can be exploited in wheat breeding. This study reports on the production, molecular cytogenetic identification, and evaluation of resistance to wheat stripe (or yellow) rust (Puccinia striiformis f. sp. tritici) under field conditions of a newly developed wheat-rye (Mv9kr1-'Lovászpatonai') disomic 4R addition line. Analysis of grain quality properties showed that the transfer of 'Lovászpatonai' chromosome 4R into wheat significantly increased the total arabinoxylan and protein content. This genetic material proved to be resistant to stripe rust in 2014 and 2015 when epidemics broke out in Hungary. It is assumed that this addition line carries an effective, new resistance gene different from that on rye chromosome arm 1RS of the 1RS.1BL translocation. The Mv9kr1-'Lovászpatonai' 4R addition line is a promising pre-breeding material being the first step towards creating translocation lines in order to transfer stripe rust resistance as well as high protein and dietary fibre content into cultivated wheat without deleterious effects on yield and grain quality.

Rye (*Secale cereale* L.) represents a large reservoir of useful agronomic traits which are worth transferring into the genome of cultivated wheat. The most intensively used source of rye chromatin in bread wheat has been the 1RS chromosome arm in the form of 1RS.1BL translocation, because it provided a source of resistance genes against leaf rust (*Lr26*), stem rust (*Sr31*), stripe rust (*Yr9*) and powdery mildew (*Pm8*). Nowadays, the proportion of wheat varieties carrying the 1RS.1BL translocation is decreasing due to the fact that resistance genes *Lr26*, *Pm8* and *Yr9* are no longer effective against new biotypes of diseases. 1RS also contains a complex locus (*Sec-1*) encoding rye storage proteins (secalins). The presence of the secalins, coupled with the loss of the wheat 1BS chromosome arm and, consequently, low-molecular-weight (LMW) glutenin and gliadin proteins, leads to degradation of wheat quality. Production of wheat-rye introgression lines containing rye chromosomes that carry new, effective resistance genes, and do not have unfavourable effect on wheat compositional properties and end-use quality, is of great significance.

The Hungarian rye cultivar Lovászpatonai has good tillering and high protein and dietary fibre (DF) content, and exhibits good resistance to diseases in the field. 'Lovászpatonai' rye was crossed with the wheat line Martonvásári 9 kr1 (Mv9kr1) (Nagy et al., 1998) showing high crossability in interspecific crosses (Molnár-Láng et al., 1996), and, with an intention of producing new recombinant 1RS.1BL translocation, the produced octoploid triticale was crossed with the 1RS.1BL wheat cultivar Mv Matador. Selfed progenies were screened with PCR-based markers (Nagy et al., 2003), and a plant, designated as 04420, was found to possess recombinant 1BL.1RS translocation. After propagation for several years, line '04420' was grown together with the parental genotypes in a pesticide-free nursery and in the Breeders nursery in two consecutive seasons (2013–2014 and 2014–2015). The highly susceptible wheat line Mv9kr1 was sown in the neighbouring plots in order to ensure and easily compare rust infections of these genotypes. In 2014, 2015, Mv Matador and Mv9kr1 suffered severe (evaluated as 4 according to Cobb's scale) attack by stripe rust, while the leaves of the line '04420' did not show any symptoms (Fig. 1a).

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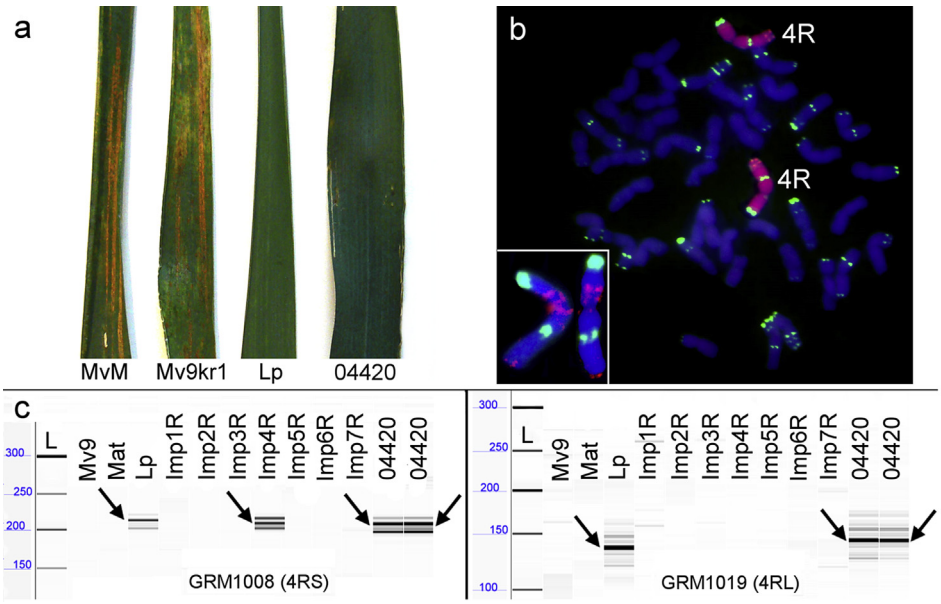

Fig. 1. a: Leaf of the stripe rust sensitive Mv Matador and Mv9kr1, and that of the resistant Lovászpatonai and the line '04420' grown in the pesticide-free nursery in 2015. b: Simultaneous FISH (pSc119.2 – green) and GISH (red) on a complete mitotic metaphase cell of the line '04420'. 4R chromosome pair is enlarged in the bottom left corner (pSc119.2 – green, $(ACC)_5$ – red). c: PCR amplification patterns of the rye 4R chromosome-specific SSR markers GRM1008 (4RS) and GRM1019 (4RL) on the following DNA templates (from left to right): wheat line Mv9kr1 (Mv9), wheat cultivar Mv Matador (Mat), rye cultivar Lovászpatonai (Lp), 'Chinese Spring'-Imperial disomic addition series (Imp1R-7R), and two samples of the line '04420'. PCR products of the markers GRM1008 and GRM1019 are marked with arrows. L: ladder. GISH, FISH and molecular marker analysis were performed as described previously (Schneider et al., 2016). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

A genomic in situ hybridisation (GISH) analysis revealed that the 1BL.1RS translocation chromosome eliminated from this line. Instead, a pair of non-satellite, submetacentric rye chromosomes could be detected beside the complete set of wheat chromosomes. Cytological identification of this chromosome pair was carried out using fluorescence in situ hybridisation (FISH) (Fig. 1b). The FISH probe pSc11.2 (Bedbrook et al., 1980) showed a strong hybridisation signal terminally on the short arm of this chromosome, and a slightly weaker one in interstitial position on the long arm. The repetitive DNA sequence $(AAC)_5$ (Cuadrado et al., 2000) had hybridisation sites in the centromeric region and a single terminal signal on the long arm, thus the added chromosome pair was identified as 4R. GISH analyses revealed that the transmission rate of the 4R chromosome pair was consistently 98% in subsequent generations. Cytogenetic results were confirmed by a simple sequence repeat (SSR) marker analysis (Fig. 1c). 4RS-specific markers GRM0324 and GRM1008 amplified bands both in the 'Chinese Spring' (CS)-'Imperial' 4R addition line (Driscoll and Sears, 1971) used as control and in the line '04420'. 4RL-specific markers GRM0273 and GRM1019 amplified PCR products in 'Lovászpatonai' rye and in the line '04420' but did not produce PCR amplicons in the CS-'Imperial' 4R addition. In a study summarizing the genetic stability of several wheat-rye disomic addition lines, the frequency of progeny plants disomic for 4R ranged from 74% to 93% (Miller, 1984). On the contrary, Szakács and Molnár-Láng (2010) found that the CS-'Imperial' 4R addition line maintained in gene bank was very unstable. Besides disomics (41.9%), plants carrying 4RS isochromosomes (1.3%) and 4RS telocentric chromosomes (9.5%) were identified in its progeny. This suggests that chromosome 4R of the CS-'Imperial' addition line used in the present study lacks its long arm.

Detailed analyses of the physical and compositional properties and determination of bread-making quality characters were carried out according to Rakszegi et al. (2016) to see the effect of chromosome 4R on the grain quality of the wheat. The addition of chromosome 4R resulted in a significant increase in the total protein content, in the ratio of the unextractable polymeric protein (UPP) content and in the total arabinoxylan (TOTAX) content of the wheat (Table 1). Arabinoxylans (AX), the major components of the DF in rye are considered to have positive impacts on human health and they strongly affect wheat functionality during cereal processing, e. g. bread-making. In order to identify the chromosomes responsible for the synthesis of DF and AX in rye, several sets of wheat-rye addition and translocation lines were previously tested.

Table 1
Compositional properties (average ± SD) of the parental rye and wheat genotypes, and the Mv9kr1-'Lovászpatonai' 4R addition line in the pesticide-free nursery (2015).

Genotype	Protein (%)[a]	Glu/Gli[b]	UPP%[b]	HMW/LMW[b]	TOTAX (mg/g)[a]	WEAX (mg/g)[a]
Lovász-patonai	12.70 ± 0.03**	0.35 ± 0.01*	13.48 ± 1.17**	0.81 ± 0.02*	57.51 ± 3.49	23.43 ± 0.94**
Mv Matador	13.90 ± 0.04**	0.77 ± 0.02*	39.21 ± 0.77**	1.11 ± 0.03**	44.4 ± 2.16**	9.25 ± 0.27
Mv9kr1	14.90 ± 0.05**	0.88 ± 0.02	43.18 ± 2.53*	0.78 ± 0.004**	41.39 ± 1.73**	6.44 ± 0.19**
4R disomic addition	21.60 ± 0.07	0.88 ± 0.02	47.59 ± 1.00	0.85 ± 0.007	59.79 ± 2.93	10.25 ± 0.59
$LSD_{5\%}$	0.11	0.031	2.97	0.03	2.70	1.14

*, ** Significantly different at the P < 0.05 level and at the P < 0.01 level from 4R disomic addition line, respectively.
Glu – glutenin; Gli – gliadin; HMW – high molecular weight glutenins; LMW – low molecular weight glutenins; UPP% – unextractable polymeric protein; TOTAX – total arabinoxylan; WEAX – water extractable arabinoxylan.
[a] 2 replications.
[b] 3 replications.