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Identification of molecular markers for an efficient leaf rust resistance gene (*Lr29*) in wheat

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ABSTRACT The aim of this study was to find molecular markers (RAPD and SCAR) for the wheat leaf rust resistance gene Lr29. Among 81 RAPD primers tested, only one (OPY10) detected an additional band in the resistant NIL of Lr29. The genetic linkage of this molecular marker to Lr29 was tested on a segregating F_2 population derived from a cross between the leaf rust resistant line and the susceptible parent GK Délibáb. This marker was closely linked to the Lr29 gene. The polymorphic band was cloned and sequenced. Specific primers (SCAR) were synthesized and after amplification only resistant lines showed an amplified product. A second SCAR primer for another Lr29 RAPD fragment (UBC219, Procunier et al., 1995) was also designed and tested.

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KEY WORDS

RAPD
SCAR

Leaf rust caused by *Puccinia recondita f. sp. tritici* is one of the most important fungal diseases of wheat in Hungary. Breeding for resistance is considered to be the most economical and environmentally appropriate strategy to reduce damages due to this disease. To date more than 40 leaf rust resistance genes have been characterized (McIntosh et al. 1995). The traditional way of transferring one or more resistance genes to a single wheat cultivar relay on field and greenhouse screening with different races, which is very laborious and time consuming process. In recent years, DNA-based markers have shown great promise in lessening the time and expense for pyramiding resistance genes. So far more than 20 molecular markers have been reported which are closely linked to Lr genes (reviewed by Gupta et al., 1999). A RAPD/DGGE marker ($UBC219_{1000}$) linked to the Lr29 have been described (Procunier et al. 1995) In this study a search for RAPD and SCAR markers linked to Lr29 leaf rust resistance gene was carried out by comparing the NIL Lr29 and its recurrent parent Thatcher variety.

Materials and Methods

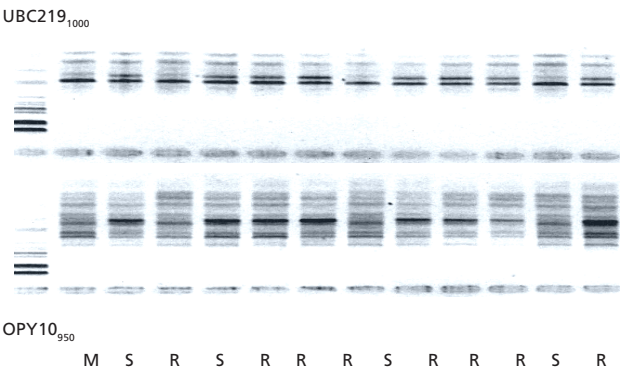
The NIL Lr29 and its recurrent parent Thatcher obtained from J. Kolmer, Canada (Win.), were used to identified RAPD markers linked to the resistance gene Lr29. For linkage analysis Lr29 NIL was crossed to a wheat cultivar GK Délibáb susceptible for leaf rust. A F_2 population (204 plants) were grown in a glasshouse and tested for the presence of molecular markers linked to Lr29 and in paralell, for their resistance or susceptibility to *P. recondita* in pathogenic tests.

DNA was isolated from 10-day-old glasshouse-grown seedling by CTAB method as described by Rogers and Bendich (1985) and was used for RAPD analysis (Williams et al. 1990). Approximately 2ng of DNA was used as template in 20µl reaction volume that contained 1x Taq buffer (Gibco), 1,5mM $MgCl_2$ (Gibco), 200 µM each dNTP (Fermentas) 1U Taq DNA polymerase (Gibco) and 0,7 µM primer (Operon Technologies, Alameda, Calif.). Amplifications were performed in a Perkin Elmer GeneAmp PCR System 9700 for 40 cycles. After an initial denaturation of 2 min at 94ºC, each cycle consisted of 10 s at 94ºC, 30 s at 36ºC, and 2 min at 72ºC. The 40 cycles were followed by a 10 min final extension step at 72ºC.

The RAPD bands ($OPY10_{950}$ and $UBC219_{1000}$) were excised from the agarose gel and extracted using QIAGEN Extraction Kit. The recovered DNA fragments were cloned and sequenced by Éva Ádám and Anikó Páy at the Biological Research Center, Szeged. The sequence information of the cloned polymorphic bands, $OPY10_{950}$ and $UBC219_{1000}$, was used to design forward and reverse SCAR primers.

Results and Discussion

A total of 81 RAPD primers were screened to identify polymorphisms between the resistant Lr29 NIL wheat line and its recurrent parent *Thatcher*. Only one of these primers, the OPY10 generated a polymorphic band (950 bp DNA



M=DNA size marker, R=resistnat, S=susceptible

Figure 1. Linkage analysis of the Lr29 gene and the $OPY10_{950}$ and $UBC219_{1000}$ markers

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fragment) specific for Lr29. Another Lr29 specific RAPD primer the UBC219 (Procunier at al., 1995) was also checked on (Fig 1.). These polymorphic markers, $OPY10_{950}$ and $UBC219_{1000}$, showed a linkage of 11,5 cM and 12,5 cM, respectively, to the resistant phenotype.

The SCAR fragments derived from $OPY10_{950}$ and $UBC219_{1000}$ resulted single bands and proved to be dominant markers and followed the same segregation pattern as it was observed for the RAPD analysis.

The information provided by these two PCR markers would be very useful in breeding programs to select resistant wheat cultivars for the leaf rust.

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