

MULTIPLEX PCR ASSAY TO DETECT RESISTANCE GENES *Lr26* AND *Lr37*



T. Sumíková, A. Hanzalová

Crop Research Institute, Drnovská 507, CZ-161 06, Prague 6, Czech Republic
sumikova@vurv.cz



AIM

- develop multiplex PCR assay to detect the presence of resistance genes *Lr26* and *Lr37* in 21 winter wheat varieties

MATERIAL AND METHODS

Tested material

- 21 winter wheat cultivars
- presence of the genes *Lr26* and *Lr37* were tested by PCR markers and greenhouse tests



Figure 1: Inoculated plants in the greenhouse

PCR assay

- DNA was extracted from second leaf using Qiagen DNA extraction kit
- The genes *Lr26* and *Lr37* were identified by publish primers marking these genes
- Multiplex PCR assay was developed to detect both genes in one reaction
- The most efficient amplification conditions and concentrations of reaction components are shown in table 1
- The PCR reaction was carried out in thermal cycler Veriti (Applied Biosystems)
- Near isogenic *Triticum aestivum* lines containing the corresponding *Lr* genes in Thatcher background were used as a positive control

Greenhouse tests

- plants were tested at a seedling stage for reaction to 7 leaf rust pathotypes with determined virulence
- inoculation was carried out by rubbing the first leaf with water suspension of urediospores
- inoculated plants were kept in greenhouse in closed glass cylinder for 24 hours at 18-22°C
- infection types were recorded according to Stakman et al. (1962) 14 days after incubation (Fig 1)

Reaction mixture (total volume 25µl)	Amplification conditions	PCR product (bp)	Primers
2.5µl 10 PCR buffer Mg free (Biotools)	94°C – 3 min	412 (<i>Lr26</i>)	SecA2, SecA3 (De Froidmont, 1998)
1.5µl 50mM MgCl ₂	94°C – 30s	262 (<i>Lr37</i>)	Venturip, LN2 (Helguera et al. 2003)
0.5µl 2.5mM dNTPs (Promega)	58°C – 30s	} 35×	
1µl each of 4 nmol primer (SecA2, SecA3, Venturip, LN2)	72°C – 45s		
0.1µl Taq DNA polymerase (Biotools)	72°C – 10 min		
1µl DNA (20ng/µl)			

Table 1

RESULTS AND CONCLUSIONS

- The results of analyses of wheat cultivars by molecular markers for the genes *Lr26* and *Lr37* are shown in Figure 2
- Gene *Lr37* was found in four tested cultivars: Bakfiz, Biscay, Nicol (HE 77/97), Mulan
- Gene *Lr26* was found only in one tested cultivar: Etela
- Both these genes were found in three tested cultivars: Clarus, Orlando, Rapsodia

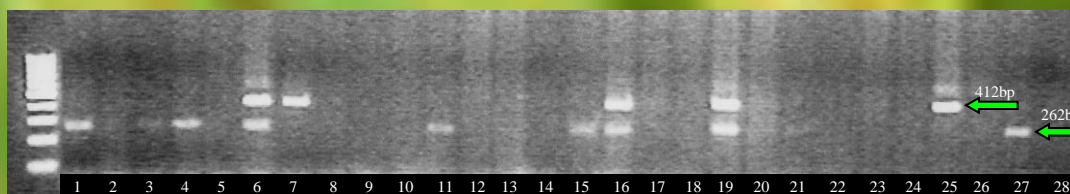


Figure 2: GeneRuler 100bp DNA ladder (Fermentas), 1-Bakfiz, 2-Baletka, 3-Baryton, 4-Biscay, 5-Buteo, 6-Clarus, 7-Etela, 8-Estica, 9-Eurofit, 10-Floret, 11-Nicol (HE 77/97), 12-Helmut, 13-Kodex, 14-Megas, 15-Mulan, 16-Orlando, 17-Pitbull, 18-Raduza, 19-Rapsodia, 20-Simila, 21-Sakura, 22-*Lr1*, 23-*Lr10*, 24-*Lr13*, 25-*Lr26*, 26-*Lr34*, 27-*Lr37*, 28-Water

Reactions of the near isogenic lines possessing *Lr26* and *Lr37* to seven leaf rust pathotypes revealed that especially reactions of the line with *Lr26* can be useful for postulation of the genes analyzed with the markers. Cvs Etela and Clarus had identical reactions with the *Lr26* near isogenic line. Other cultivars that also have *Lr26*, Orlando and Rapsodia were resistant to all tested pathotypes. Obviously, they possess other resistance gene(s) in addition to *Lr26*. Gene *Lr37* is only rarely effective at the seedling stage since it is responsible for the resistance at adult stage.

Large majority of the tested cultivars were resistant to at least to one rust pathotype which indicates that they have other genes for specific resistance alone or in addition to the genes determined by us.

REFERENCES

- Helguera M, Khan IA, Kolmer J, Lijavetzky D, Zhong-gi L, Dubcovsky J (2003). PCR assays for the *Lr37*-*Yr17*-*Sr38* cluster of rust resistance genes and their use to develop isogenic hard red spring wheat lines. *Crop Sci* 43: 1839-1847.
- De Froidmont D (1998): A co-dominant marker for the 1BL/1RS wheat-rye translocation via multiplex PCR. *J Cereal Sci*, 27: 229 – 232.
- Stakman EC, Stewart DM, Loegering WQ (1962): Identification of physiologic races of *Puccinia graminis* var. *tritici*. – U. S. Dept. Agr., Agr. Res. Service, E 617.