Use of marker-assisted selection (MAS) for pyramiding leaf rust resistance genes (*Lr9*, *Lr24*, *Lr22a*) in wheat

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Abstract

Two leaf rust resistance genes, *Lr9* and *Lr24*, have been pyramidised through the use of simple sequence repeats (SSR) markers. The obtained wheat lines which carry the two resistance genes are indeed resistant to the leaf rust races currently present in Switzerland and have very good baking quality. However, the lines can only be considered as a first step, since it was difficult to reach acceptable uniformity. Furthermore, sufficient yield for commercial success could not be achieved. However, the lines have been used as parents to cumulate other resistance genes (*Lr22a*). This first cycle of 'pyramidisation' allowed us to evaluate the costs for marker-assisted selection (MAS). MAS proved to be an efficient tool in a breeding program. It is yet necessary to integrate this benefit into the global context of yield, resistance and quality required for the release of commercially successful wheat varieties.

Key words

leaf rust, MAS, molecular markers, PCR, resistance breeding, SSR, wheat

Introduction

Leaf rust is an important foliar disease of wheat. Growing resistant cultivars is probably the most efficient, cost-effective and environment-friendly method to control this disease (SINGH et al. 2004). More than 70 specific leaf rust resistance genes are known (KOMUGI 2009). Many of them have been introgressed into wheat from wild relatives as *Lr9* from *Aegilops umbellulata*, *Lr24* from *Agropyron elongatum* or *Lr22a* from *Aegilops tauschii*. However the ability of the pathogen to adapt to new resistances by single step mutation constitutes a never-ending challenge for breeders. Frequently, the pyramiding strategy, combining several resistance genes into one cultivar, has been proposed to enhance the durability of resistances (PEDERSEN and LEATH 1988). Combining two or more resistance genes using classical host-parasite infection methods is highly time-consuming and needs specific virulent pathotypes that are often not available or too risky to use. Molecular biology and marker-assisted selection (MAS) offers the possibility to trace resistance genes in cultivars in an easier and more efficient way. At least 33 molecular markers linked to *Lr* resistance genes have been described at present (KOMUGI 2009). When the project started, no virulence was found for *Lr9* or *Lr24* in the leaf rust populations in Switzerland and, worldwide, no virulence was reported for the combination *Lr9* and *Lr24* (SCHACHERMAYR et al. 1995). Molecular markers and plant material for these genes were also available. Similar studies have been done by other groups in Europe (VIDA et al. 2005, NOCENTE et al. 2007) but only little information is available on the lines and their commercial outcome. In this study we evaluate the pyramiding of two leaf rust resistance genes by MAS in a small breeding program.

Materials and methods

Plant material

The *Lr9* and the *Lr24* resistance gene donors (Transfer/6* Thatcher and Agent/6*Thatcher) were backcrossed seven times to susceptible Swiss winter wheat cultivar Arina and selfed to produce the F_8 generation. NILs (near isogenic lines) containing *Lr9* were crossed with the *Lr24* one. Three F_1 populations were crossed with four advanced lines giving 765 F_2 progenies on which, marker assisted selection (MAS) was used to select 194 lines containing both resistance genes. After 6 years of classical breeding, MAS was applied to confirm the presence of *Lr9* and/or *Lr24* in 30 of the F_8 remaining lines.

Disease evaluation

The lines were evaluated for leaf rust and other diseases in separate nurseries using artificial infection with mixtures of isolates collected in Switzerland as described by MICHEL (2001).

Homogeneity

The lines' homogeneity was evaluated comparing plant height, spike and leaves morphology and colour of 30 head-to-row lines. Lines with insufficient uniformity were selfed one to tree more generations before testing them in yield trials.

Field trials and bread making quality

Small-plot (7m²) trials with 2 replications have been carried out in 4 locations during one year. Samples collected from yield trials were used for quality parameters evaluation

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Table 1: **Sequence of primers for STS locus linked to the *Lr9* and *Lr24* resistance genes**

Gene	Name	Primer sequence (5' - 3')	Reference
Lr9	J13	F - CCA CAC TAC CCC AAA GAG ACG	SCHACHERMAYR et al. 1994
		R - TCC TTT TAT TCC GCA CGC CGG	
	SCS5	F - TGC GCC CTT CAA AGG AAG	GUPTA et al. 2005
		R - TGC GCC CTT CTG AAC TGT AT	
Lr24	J09	F - TCT AGT CTG TAC ATG GGG GC	SCHACHERMAYR et al. 1995
		R - TGG CAC ATG AAC TCC ATA CG	
	H05	F - AGT CGT CCC CGA AGA CCC GCT GGA	DEDRYVER et al. 1996
		R - TCG TCC CCT GAT GCC ATG TAA TGT	

involving protein content and Zeleny sedimentation value (ICC 1999).

DNA extraction

Genomic DNA from the 765 F_2 populations was isolated from leaves tissue according to LAGUDAH and APPELS (1991). For the 30 advanced lines, the DNA was extracted with a quick and efficient method. Two young leaves were grown in 2 ml of extraction buffer (Tris-HCl 50 mM pH 8, EDTA 50 mM ph8, sucrose 15% (w/v), NaCl 250 mM). After centrifugation (5 min at 6000 x g), the supernatant was removed and the pellet, suspended in 340 μl of Tris-HCl 20 mM pH8, EDTA 10 mM pH8 and SDS 1.2% (w/v), was incubated for 15 min at 70°C. Then 150 μl of 7.5 M ammonium acetate were added, the mixture incubated on ice for 30 min and centrifuged at 16000 x g for 15 min. The DNA from the supernatant was concentrated by ethanol precipitation, washed with 75% (v/v) ethanol and dissolved in TE at a concentration of 25 ng/μl.

PCR amplification

Polymerase chain reaction (PCR) was performed in 10 μl volumes with 25 ng of template, Qiagen PCR buffer and Q-solution as recommended by the manufacturer, 0.2 mM dNTPs, 1 μM of each primer, 1.5 mM $MgCl_2$ and 0.35 U HotStar Taq DNA polymerase (Qiagen). Amplifications were performed in an Hybaid PX2 thermocycler programmed at 95°C for 15 min, followed by 35 cycles at 94°C for 1 min, at 64°C for 1 min 30 sec and at 72°C for 2 min 30 sec. The extension of amplified fragment was achieved at 72°C for 10 min. The sequences of the specific primers for *Lr9* and *Lr24* are shown in *Table 1*.

Electrophoresis

Amplification products were separated by electrophoresis on 1.5% agarose gel in 1 X TAE buffer at 100 V for 3 h and visualized under UV light after ethidium bromide staining.

Results and discussion

Homogeneity

At F_7, only 14.3% of the 98 F_7 '*Lr9/Lr24*' lines reached a sufficient uniformity to be tested in yield trials and to start the maintenance breeding compared to 20.6% for the 'normal' lines.

Leaf rust markers and disease resistance

The MAS at F_2 with dominant PCR-based markers discard the plants without *Lr* genes but cannot sort heterozygous from homozygous plants. Lines without the genes continue to appear after self-pollination of heterozygous plants. The second PCR was performed with accurate concentration of DNA isolated from 6 to 10 plants. For the heterozygous lines, the band intensity was lower than the one obtained with homozygous lines. The heterozygosity of these lines was confirmed by analysing 6 plants separately. For the 30 lines tested in yield trials, 6 lines have markers for *Lr9* and *Lr24*, 10 lines only for *Lr24*, 4 lines only for *Lr9* and 10 lines have no markers for neither resistance genes. In fact, the susceptibility to leaf rust confirmed the role of *Lr9* and *Lr24* in the resistance response. Even though the virulence *Lr9* is now frequently observed in Switzerland (F. MASCHER, unpublished) lines possessing *Lr9* show few or no symptoms. On the other hand, some lines possessing *Lr24* markers display low symptoms even if virulence *Lr24* has not been reported in Switzerland. The few lines with both *Lr* markers have absolutely no symptoms (*Figure 1*). Arguably the pyramidisation of both resistance genes is feasible and the results obtained are efficient but the durability of the resistance has still to be proved. The mean resistance of these lines against other important diseases is very good for stripe rust (score 2.0), *Septoria nodorum* blotch (index leaf 79, index spike 85) and good for *Septoria tritici* blotch (index 91), powdery mildew (score 2.4) and Fusarium head scab (score 3.2).

Yield trial

The 30 lines mean yield is only 90% of the usual standard cultivars, hence, insufficient for a commercial cultivar. Even if the number of lines is certainly insufficient to compare with confidence the yield between the lines with or without Lr markers or between the lines from different crosses we observe that the relative yield for the 6 lines with *Lr9* and *Lr24* markers is 94.2% (86.5% to 100.9%) compared to 88.8% (77.7% to 97.4%) for the 10 lines without *Lr* markers. The 8 lines issued from the cross with the best parent have a 94.1% relative mean yield compared to 80.8% for the 4 lines issued from the lowest yielding parent.

Bread making quality

The 30 lines Zeleny mean value (60.2 ml; 43.7-73.5 ml) and the protein content mean values (13.5%; 11.7-15.0%) indicate a good to very good bread making quality for the
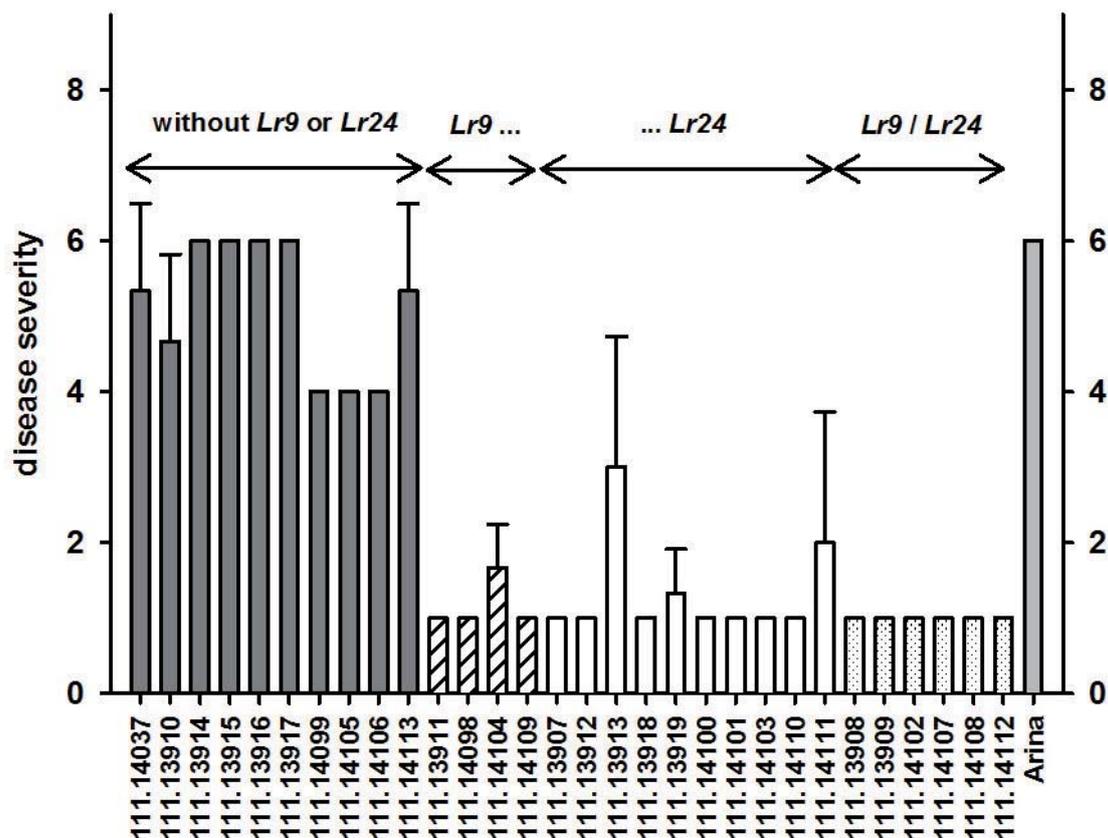
Figure 1: **Evaluation of leaf rust resistance (1: no symptom; 9: fully susceptible) of F$_8$ lines derived from the project of pyramidisation of *Lr9* and *Lr24*.**

lines compared with the quality standards Arina (52.3 ml, 13.8%) and Runal (65.2 ml, 14.3%).

Costs

If we don't include costs for markers development and field trials cost (sowing, treatments, sampling), we need now, in our conditions, 1 person, two weeks and a cost of 515.- € for analyzing 1000 samples.

Conclusions and perspectives

The lines herein obtained, even though displaying good resistances and excellent bread making quality, had low yield and more difficulties to reach uniformity. They could not be developed as commercial cultivars and are used as genitors. The low yield and low uniformity might be unwanted effects of *Lr* genes and of 'drag genes' unintentionally introduced from the wild relative. This is especially valid for the *Lr24* donor where a large segment has been translocated (SCHACHERMAYR et al. 1995). It might be also caused by the genetic value of the advanced lines used as genitors in this experiment. The number of lines tested here is too small to have clear evidences for one or the other hypothesis. Some of the best lines have been crossed with more yielding lines and with lines with other *Lr* genes especially adult plant resistance genes *Lr22a* to combine different kinds of resistance as proposed by HIEBERT et al. (2007). The MAS was effective for pyramiding two resistance genes but the investment was important just for one of the six important diseases we breed for. Even if the cost of MAS has dropped dramatically during the last decade, it is still a challenge to find the best way to use it in a breeding program with the aim of producing cultivars not only resistant against one single disease but aiming at an optimal combination between yield, resistance and quality.

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