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Characterization of sources of resistance to leaf rust in durum wheat germplasm

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Abstract. A nursery with 184 entries including French, European, North African and CIMMYT/ICARDA lines, was phenotyped for its resistance in field trials inoculated with wheat leaf rust, in 4 locations in France and 2 locations in Mexico, in 2009 and 2010. Moreover, the 184 entries were phenotyped for their resistance to 9 pathotypes in the glasshouse.

Genes *Lr27+31* and *Lr3* were effective in France, but given their breakdown in Mexico, they are unl kely to be durable sources of resistance in France. Genes *Lr61*, *LrCamayo*, *Lr19* and *Lr47* were efficient both in Mexico and in France, and could represent valuable sources of resistance. Some lines displayed a high level of resistance in all locations, l kely due to an unknown major gene. Four French entries, as well as several slow rusting lines from CIMMYT, displayed a good level of partial resistance in all environments tested.

Association mapping, using 1300 DArT markers and 34 variables from the phenotyping studies, revealed two QTLs and one locus corresponding to a major gene: i) on chromosome 2B, a QTL was tagged by wPt-1064, wPt-6477 and wPt-0408 ii) on chromosome 6B, a QTL was tagged by wPt-8059, wPt-7065 iii) on chromosome 7B, a major gene was tagged by wPt-0465, wPt-3700 and wPt-9515, which corresponded to *Lr14a.* This gene is not effective in France, whereas it is still efficient in Mexico.

Keywords. *Puccinia triticina* – Resistance phenotyping – QTL – Association mapping – DArT markers.

Caractérisation des sources de résistance à la rouille brune chez le matériel génétique de blé dur

Résumé. *En 2009 et 2010, 184 accessions de pépinière, incluant des lignées françaises, européennes, nord-africaines et du CIMMYT/ICARDA, ont été phénotypées pour leur résistance en réalisant des essais d'inoculation de la rouille brune du blé au plein champ, sur 4 sites en France et 2 sites au Mexique. De plus, les 184 accessions ont été phénotypées pour leur résistance à 9 pathotypes en serre.*

Vu que les gènes Lr27 + 31 et Lr3 étaient efficaces en France mais déjà contournés au Mexique, il est fort improbable qu'ils constituent une source durable de résistance en France. Les gènes LR61, LrCamayo, Lr19 et Lr47 étaient efficaces au Mexique et en France, et ils pourraient donc représenter des sources de résistance importantes. Certaines lignées ont affiché un niveau élevé de résistance dans tous les endroits, probablement en raison de la présence d'un gène majeur encore inconnu. Quatre accessions françaises, ainsi que plusieurs lignées « slow-rusting » du CIMMYT ont montré un niveau de résistance partielle intéressant dans tous les environnements testés.

La cartographie d'association, réalisée à l'aide de 1300 marqueurs DArT et 34 variables issues des études de phénotypage, a révélé deux QTL et un locus correspondant à un gène majeur : i) sur le chromosome 2B, un QTL a été marqué par wPT-1064, wPT-6477 et wPT-0408 ii) sur le chromosome 6B, un QTL a été marqué par wPT-8059, wPT-7065 iii) sur le chromosome 7B, un gène majeur a été marqué par wPT-0465, wPT-3700 et wPT-9515, qui correspond à Lr14a. Ce gène n'est pas efficace en France, alors qu'il est encore efficace au Mexique.

Mots-clés. *Puccinia triticina – Phénotypage de la résistance – QTL – Cartographie d'association – Marqueurs de DArT.*

I – Introduction

Strong leaf rust epidemics, caused by *Puccinia triticina*, regularly occur in the durum wheat growing areas in France and Mexico. Yield losses up to 80% were registered on susceptible cultivars in south-eastern France in 2007, and considerable economic impact was reported in Mexico after the 2001 epidemics (Huerta-Espino *et al.* 2011). Although resistance to this disease has been a concern to breeders globally, the resistance level has to be improved when objectives have been set to curb fungicide use for both environmental and economic reasons. Moreover, most of the resistance sources used in the French germplasm broke down following the evolution of *P. triticina* populations in 2001, and again in 2007 (Goyeau *et al*., 2012).Thus resistance sources should be diversified to respond to a fast changing pathogen population. The objective of this work was to evaluate a collection of selected genotypes with global relevance to wheat leaf rust resistance for i) their seedling reaction against a collection of French and Mexican pathotypes, and ii) their field reaction at adult stage. In addition, the phenotypic data generated was used in combination with DArT genotyping in an association mapping exercise to detect major genomic areas influencing leaf rust reaction in the panel of genotypes.

II – Material and methods

A set of lines and cultivars was selected, including i) breeding lines and cultivars displaying some resistance to wheat leaf rust, ii) lines from CIMMYT/ICARDA germplasm with efficient major genes, or a combination of minor resistance genes, and iii) susceptible germplasm as a control.

1. Phenotyping

Phenotyping was performrd in a greenhouse. Evaluation of the material was conducted by inoculating the set of lines with well-characterized pathotypes individually in separate experiments. In France. The five pathotypes identified up to now in the French wheat leaf rust population (Goyeau *et al.* 2012) were used. In Mexico, pathotypes 61/61 (virulent on *Lr61*), BBG/BP, CBG/BP, BBG/BN (Huerta-Espino *et al*., 2011) were used. Plants were inoculated at the seedling stage by spraying spores suspended in Soltrol® oil, then incubated in a dew chamber at 15-20°C for 24 h, placed in the greenhouse for the next 10 days and assessed for their infection types according to the Stakman *et al*. (1962) scale. In the field, nurseries were sown in France in 4 locations (Lectoure, Montbartier, Castelnaudary and Grisolles) and in Mexico in two.

In France, a mixture of two pathotypes was used, so as to combine the virulences for *Lr14a*, *Lr23* and *Lr72*. In Mexico, a mixture of pathotypes BBG/BP (virulent on *Lr3*) and BBG/BN was used. In France, in each location, the maximum percentage of diseased leaf was assessed independently by two to three people, using the modified Cobb scale (Peterson *et al*., 1948). In Mexico, in each location four to five disease assessments were made, allowing calculation of the area under the disease progress curve (AUDPC).

2. Genotyping

Association mapping was performed for 182 lines or cultivars, using 1300 DArT markers. Analyses were conducted independently by four different collaborators, to compare results obtained with different statistical softwares. Each collaborator used a mixed linear model as described by Yu *et al*. (2006) to calculate the marker-trait association analysis. Mixed linear model can reduce both type I and type II errors as this model simultaneously takes into account population structure and kinship. Significance of associations between loci and traits was described as p-value and the QTL effects level was evaluated by R^2 of the peak marker. All the variables issued from phenotyping were analysed independently, except for one collaborator who grouped highly correlated variables.

III – Results

1. Phenotyping

Cultivars and lines were grouped according to their profiles of infection types against the pathotypes at the seedling stage in the greenhouse. When including information provided by CIMMYT about major *Lr* genes and minor resistance genes identified in the lines, resistance groups could be defined, postulated to differ for the genetic basis of their wheat leaf rust resistance, from information with the French (Table 1) and the Mexican (Table 2) pathotypes. Field Epidemic development was good in the two Mexican locations in 2009 and 2010; in France, it was satisfactory in 2009 in three out of four locations, and in four locations in 2010. In France, a high level of resistance, due to efficient major genes, was achieved in 18 lines from CIMMYT, carrying one of the genes *Lr3*, *Lr19*, *Lr47*, *Lr61* and *LrCamayo*, as well as in Anco Marzo (*Lr27+31*), and in three cultivars (Byblos, Saragolla, and Gaza) postulated to carry unidentified major genes. Quantitative resistance was also expressed: a moderate final disease level (35-60%) was displayed by 39 lines, and 9 cultivars (Acalou, Altar, Arnacoris, Brennur, Lemur, Liberdur, Nautilur, Sachem, and Virgilio); a low level of quantitative resistance, with a final disease level of 60-70%, was displayed by 15 lines and one cultivar (Poulit). Overall, glasshouse and field phenotyping yielded 34 variables (Table 3).

Table 1. Resistance profiles of the lines and cultivars, combining information from i) infection types from the seedling tests in the greenhouse using 5 French pathotypes and ii) presence of known *Lr* genes or minor genes based on information from CIMMYT. Infection types after Stakman *et al*., (1962).

RESISTANCE FROUP	Pathotype (see Goyeau *et al*., 2012)					
	no vir.	vir 23, Altar	vir 14a	vir14a, 23	vir Altar, 23, (Gaza)	Number of lines.
No effective major gene	3+	3+	3+	3+	3+	40
Lr14a only	X++	X	3+	3+	X++	38
CIMMYT lines with minor genes	Y++	3+	Y++	X++3	3+	13
Lr23	12	3+	X-	X++3	3+	4
Lr72	1	3+	;	X++3	3+	19
Lr14a + unidentified major gene	;	X++	X++	X++3	X--	10
Lr14a + *Lr72*	0;	X-	;--	X++3	X++	28
Unidentified major gene	X+	Y+	X++	X+	X+	7
Lr14a + *Lr72* + unidentified major gene	;--	;1+	;	X++	X++	2
Unidentified major gene (Gaza), *Lr61* (Guayacan Inia)	;	;	;12	;12	X++	4
Lr72+ unidentified major gene	;-	X--	;1	X-	X-	4
Saragolla	;12	;12	;12	;1+	;1	1
Lr_{Camayo}	;	;12+	;	;	;12	3
Lr3/*Lr19*/*Lr47* or unidentified major gene	;-	;	;-	;	;-	10
Byblos	X	;-/X++	;--/X++	;--/X++	0;	1
					TOTAL	184

Table 2. Resistance profiles of the lines and cultivars, combining information from i) infection types from the seedling tests in the greenhouse using 4 Mexican pathotypes and ii) presence of known *Lr* genes or minor genes based on information from CIMMYT. Infection types after Stakman *et al*., (1962).

	A	B	C	D	Lines No.
Lr72 but *Lr14a* positive	x	3+	3+	3+	2
Lr61	3+	;1=	;1=	;1=	2
Lr27+31	;1	33+	33+	1++	3
Lr3	0;	0;	33+	0;	6
Undecided/lost/ inconclusive	-	-	-	-	13
Lr72	x	3+	3+	3+	22
Uncharacterised Seedling Resistance	;1=	x	x	x	26
No detectable seedling resistance	33+	33+	33+	33+	29
Lr14a (based on the marker)*	1=	x=	;1=	x=	81

A = Race BBG/BP vir Lr10,23,61
B =Race CBG/BP vir Lr10,11,23,27+31,72
C = Race BBG/BP virLr3,10,11,23,27+31,72
D = Race BBG/BN vir Lr10,11 23 72
**could be with or without Lr72 or any other gene*

Table 3. Phenotyping variables included in the association mapping analyses.

Name	location	year	Variable
V1, V2, V3	Castelnaudary	2009	Final % of diseased flag leaf, assessed by 3 people
V4, V5, V6	Castelnaudary	2010	Final % of diseased flag leaf, assessed by 3 people
V7, V8, V9	Montbartier	2009	Final % of diseased flag leaf, assessed by 3 people
V11, V10, V12	Montbartier	2010	Final % of diseased flag leaf, assessed by 3 people
V13, V14	Lectoure	2009	Final % of diseased flag leaf, assessed by 2 people
V15, V16	Lectoure	2010	Final % of diseased flag leaf, assessed by 2 people
V17	Grisolles	2010	Final % of diseased flag leaf
V18, V19	Obregon	2009	Final % of diseased flag leaf, RAUDPC
V20, V21	Obregon	2010	Final % of diseased flag leaf, RAUDPC
V22, V23	Batan	2009	Final % of diseased flag leaf, RAUDPC
V24, V25	Batan	2010	Final % of diseased flag leaf, RAUDPC
V26, V27, V28, V29, V30	GH France	2009 - 10	Infection types to 5 pathotypes
V31, V32, V33, V34	GH Mexico	2009 - 10	Infection types to 4 pathotypes

GH = Greehouse.

2. Genotyping

Independent analyses by four collaborators yielded similar results. The very few markers identified as significant by only one collaborator were dropped, so as to keep markers significant for at least two collaborators and two variables. A first analysis detected 37 DArT markers, corresponding to at least 3 chromosomal regions (2B, 6B, and 7B). On the chromosome 2B, markers wPt-1064, wPt-6477, and wPt-0408 were significant, with a low effect, and for four variables only (final disease scoring for one location one year in France, and two French pathotypes in the glasshouse). On the chromosome 6B, markers wPt-8059 and wPt-7065 were significant, with a low effect, and for nine variables only (two French field locations in 2009 and one in 2010). On the chromosome 7B, markers wPt-0465, andwPt-9515 were significant in the field in Mexico in 2009 and 2010; marker wPt-3700 was significant in the field in France and in Mexico, in 2009 and 2010. These three latter markers were also significant in the greenhouse with the four Mexican pathotypes, and with two French pathotypes. The corresponding QTL has a strong effect, particularly in Mexico (45% of the phenotypic variance). Comparison of mapping with DArT markers used in the present study and SSR markers performed at CIMMYT established that this QTL corresponded to gene *Lr14a*.

Haplotype 011 for markers wPt-0465, wPt-3700, and wPt-9515, respectively, was associated to an increased resistance level in Mexico, whereas it was associated to an increased susceptibility in France. A second analysis was performed, dropping lines with haplotype 011 to check whether gene *Lr14a* could mask the expression of other QTLs. For the 80 lines left, 23 DArT markers were significant; however, most of these markers were not mapped.

IV – Discussion and perspectives

The present study brought information on the effectiveness and the diversity of sources of resistance to wheat leaf rust in durum germplasm. Combined greenhouse and field phenotyping of lines and cultivars allowed detection of useful efficient major genes. However, breeding cultivars with single major genes should be avoided, as they have frequently proven to be quickly overcome, as for *Lr3* (race CBG/BP) and *Lr27+31* (race BBG/BP) in Mexico (Huerta-Espino *et al*., 2011) and *Lr14a* in France (Goyeau *et al*., 2010). A number of lines, carrying minor resistance genes, displayed an interesting level of quantitative resistance in the field. Phenotyping also brought valuable information about the diversification level of the resistance sources investigated, yielding a classification in different groups of resistance. However, genotyping is necessary to determine whether the genetic basis is indeed diversified, and to identify markers useful for marker-assisted selection. Association mapping revealed three chromosomal regions (2B, 6B, and 7B) involved in the resistance, as well as other interesting markers, which should be further investigated using a map with a higher density of markers. The bimodal distribution of French lines when dropping lines carrying *Lr14a*, suggested another major gene in this germplasm, for which we did not have close DArT markers. Moreover, our analysis revealed an increased susceptibility of lines carrying *Lr14a* in French field trials which raises the question of a deleterious effect of this gene on the resistance level. Another hypothesis could be that, given its efficiency in Mexico, and its efficiency in France before 2000, lines and cultivars with *Lr14a* could not be evaluated for their quantitative resistance, and may lack any QTL, when lines without *Lr14a* could have been selected for their good level of quantitative resistance.

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