



## Diverse sources of resistance to leaf rust in durum wheat

A. N. Mishra\*, K. Kaushal, V. G. Dubey and S. V. Sai Prasad

ICAR-Indian Agricultural Research Institute, Regional Station, Indore 452 001

(Received: December 2014; Revised: May 2015; Accepted: June 2015)

### Abstract

**Inheritance and extent of diversity for leaf rust resistance were seedling studied among five durum genotypes using pathotype 12-2 (1R5). The  $F_2$  and  $F_3$  analyses showed that resistance was controlled by one dominant gene each in B 276 and Guji S and by two dominant genes each in AKDW 4339, CPAN 6118 and VD 2001-14. Allelic tests revealed that these genes were different from each other, except the one common between VD 2001-14 and Guji S. Thus, seven diverse genes for leaf rust resistance were identified among the genotypes studied. The identity of these genes is not known. However, they should be different from *Lr23* which is common in Indian durum germplasm; and from *Lr3*, *Lr14a*, and *Lr27+Lr31* among the *Lr* genes reported in durum wheat, since the pathotype 12-2 (1R5) is virulent to all these genes. The above genotypes can enrich diversity for leaf rust resistance in durum improvement.**

**Key words:** Leaf rust resistance, genetic diversity, *Triticum turgidum* ssp. *durum*, *Puccinia triticina*, *Lr* genes

### Introduction

India produces more than 90 million tons of wheat from an area of more than 25 million hectares, to which the contribution of durum wheat is about 4% and that of dicoccum wheat is about one percent (Singh et al. 2011). However, durum wheat has a special niche in Indian wheat economy as it is purchased at a premium by private trade for value addition; and is preferred over bread wheat for several local food preparations. Durum wheat is mainly grown in the central and southern parts of India, where leaf rust is one of the major disease problems affecting the wheat crop. Broadening of resistance base through utilization of genetically diverse resistance sources is necessary for enhancing the durability of resistance in view of the continued evolution of *Puccinia triticina*, the leaf

rust pathogen. Relatively little work has been done on the inheritance of rust resistance in durum wheat, compared to bread wheat. However, durum wheat generally showed resistance to leaf rust pathotypes virulent to bread wheat, and vice versa (Paradies 1980; Casulli et al. 1983; Pandey and Rao 1984; Sharma et al. 1986; Singh et al. 1992; Huerta-Espino and Roelfs 1992; Honrao and Rao 1996; Mishra 1996; Sharma et al. 1996; Mishra et al. 2001a, 2001b; Singh et al. 2004; Mishra et al. 2009). Results of a study conducted to determine the inheritance and extent of diversity for seedling resistance to leaf rust pathotype 12-2 (1R5) in five durum wheat genotypes viz., AKDW 4339, B 276, CPAN 6118, Guji S and VD 2001-14 are presented here. While CPAN 6118 is an accession of All India Coordinated Wheat Improvement Programme, Guji is likely of Mexican origin. Advance generation durum lines AKDW 4339, B 276, and VD 2001-14 were developed at PDKV-Akola, IARI-New Delhi, and SDAU-Vijapur, respectively.

### Materials and methods

Five durum wheat genotypes viz., AKDW 4339, B 276, CPAN 6118, Guji S and VD 2001-14 showing field resistance to mixtures of important leaf rust pathotypes under heavy inoculum pressure were selected for determining the inheritance and the extent of diversity of leaf rust resistance among them. These genotypes were crossed among themselves without reciprocals, and with susceptible durum varieties Karnataka Local and Local Yellow, which served as female parental lines. The parents,  $F_1$ s,  $F_2$  populations and  $F_3$  families were tested in the seedling stage with the leaf rust pathotype 12-2 (1R5). This was chosen for study as it was observed to be one of the durum-specific (high degree of virulence to durum wheat germplasm)

\*Corresponding author's e-mail: anmishra53@yahoo.co.in

pathotypes (Mishra et al. 2001b, 2009). Its avirulence/virulence characteristics based on seedling tests (S.C. Bhardwaj, *personal communication*) are:

**P** *Lr1*, *Lr2a*, *Lr9*, *Lr10\**, *Lr13\**, *Lr15*, *Lr17a\**, *Lr18\**, *Lr19*, *Lr20*, *Lr24*, *Lr25*, *Lr26*, *Lr28*, *Lr29*, *Lr32*, *Lr36*, *Lr40*, *Lr41*, *Lr42*, *Lr43*, *Lr45/P* *Lr2b*, *Lr2c*, *Lr3*, *Lr11*, *Lr12*, *Lr14a*, *Lr14b*, *Lr14ab*, *Lr16*, *Lr21*, *Lr22a*, *Lr22b*, *Lr23*, *Lr27+Lr31*, *Lr30*, *Lr33*, *Lr34*, *Lr35*, *Lr37*, *Lr38*, *Lr44*, *Lr48*, *Lr49*

\*Temperature-sensitive response: while *Lr10* and *Lr18* showed most effectiveness at 18-20°C, *Lr13* and *Lr17a* expressed best at higher temperatures.

Seedlings were tested in a glasshouse at 20°C ± 2°C using standard glasshouse procedures (Roelfs et al. 1992). The test seedlings were raised in clay pots of 10 cm diameter. Seedlings with primary leaf fully expanded and second leaf just emerged (generally 8-10 days old) were spray inoculated with aqueous suspension of uredospores of the test pathotype, freshly collected from the actively sporulating pots of Agra Local maintained in isolation in the glasshouse. Agra Local served as susceptible check. Inoculated pots were incubated in moist chambers for 16-24 h, and were then transferred to glasshouse benches. Infection types (ITs) on the seedlings were recorded 12-15 days after inoculation on a 0-4 scale. The Infection Types (ITs) 3, 3<sup>+</sup>, 34 and 4 produced by a pathotype on a host line indicated latter's susceptibility to that pathotype, whereas lower ITs (0; 1, 2 and X) indicated resistance (Roelfs et al. 1992).

The F<sub>2</sub> plants were grouped in to resistant (R) and susceptible (S) classes to determine the F<sub>2</sub> ratios. The F<sub>3</sub> families were classified as homozygous resistant (HR), segregating (SEG), or homozygous susceptible (HS), based on the presence of exclusively resistant plants, both resistant and susceptible plants, and exclusively susceptible plants, respectively. The chi-squared test was used to test the goodness-of-fit of the observed F<sub>2</sub> and F<sub>3</sub> ratios to the expected ones on the basis of Mendelian segregation.

## Results and discussion

The F<sub>1</sub>s from all of the susceptible parent/resistant parent crosses were almost as resistant as the resistant parent, indicating dominant inheritance of seedling resistance to leaf rust pathotype 12-2 (1R5) (Table 1). A single dominant resistance gene was identified in B 276 and Guji S [3R:1S F<sub>2</sub> plants (P=0.29-0.72); 1HR:2SEG:1HS F<sub>3</sub> families (P=0.39-0.90)]

**Table 1.** Infection types (ITs) of the parental lines and their F<sub>1</sub>s in response to seedling tests with leaf rust pathotype 12-2 (1R5)

Wheat genotypes and the respective F <sub>1</sub> s	Seedling ITs
AKDW 4339, KL/AKDW 4339, LY/AKDW 4339	;2, ;2 <sup>+</sup> , ;2 <sup>+</sup>
B 276, KL/B 276, LY/B 276	X, X, X
CPAN 6118, KL/CPAN 6118, LY/CPAN 6118	;3, ;3 <sup>+</sup> , ;3 <sup>+</sup>
Guji S, KL/Guji S, LY/Guji S	0;1, ;1, ;1
VD 2001-14, KL/VD 2001-14, LY/VD 2001-14	;1N, ;2N, ;2N
Karnataka Local (KL), Local Yellow (LY)	34, 34

ITs on 0-4 scale; IT '34' indicates susceptibility, others indicate resistance; N = pronounced necrosis

(Table 2). Two independent dominant genes each conditioned resistance in AKDW 4339, CPAN 6118 and VD 2001-14 [15R:1S F<sub>2</sub> plants (P=0.06-0.35); 7HR:8SEG:1HS F<sub>3</sub> families (P=0.19-0.90)] (Table 2). Among resistant parents' inter-crosses, no susceptible segregants were observed in F<sub>2</sub> population derived from the Guji S/VD 2001-14 cross (Table 3) showing that one of the genes in VD 2001-14 was common with that of Guji S. With the exception of B276/VD 2001-14 cross, the remaining crosses showed F<sub>2</sub> segregation for the expected ratio of 15R:1S, 63R:1S, or 255R:1S (Table 3), based on the inheritance respectively of two, three, or four independent dominant genes, showing that the genes involved were different from each other. However, number of susceptible plants was higher than the expected in B276/VD 2001-14 cross (Table 3), for which we do not find any explanation. Anyway, diversity of resistance genes involved is obvious from segregation for susceptible plants in the above cross. Thus, a total of seven diverse dominant genes were identified for seedling resistance to the leaf rust pathotype 12-2 (1R5) among the five genotypes studied.

In all, 72 leaf rust resistance genes have so far been catalogued (McIntosh et al. 2013; Herrera-Foessel et al. 2014). Until recently, only *Lr14a* derived from *Triticum turgidum* subsp. *dicoccum* cv. Yaroslav and *Lr23* derived from *Triticum turgidum* subsp. *durum* cv. Gaza were known to be of tetraploid (AABB genomes) origin (McIntosh et al. 1995). The gene *Lr23* is long known to occur in CIMMYT durum cultivar Altar C84 (Nelson et al. 1997). Presence of *Lr14a* in any durum lines (Chilean durum cultivar Llareta INIA and CIMMYT

**Table 2.** Segregation for seedling resistance to leaf rust pathotype 12-2 (1R5) in F<sub>2</sub> plants/F<sub>3</sub> families derived from crosses of resistant parents with susceptible parents Karnataka Local (KL) and Local Yellow (LY)

Cross	No. of F <sub>2</sub> plants		$\chi^2$	P	No. of F <sub>3</sub> families			$\chi^2$	P
	R	S			HR	SEG	HS		
KL/AKDW 4339	106	11	1.99 (15:1)	0.16	34	40	6	0.23 (7:8:1)	0.89
LY/AKDW 4339	124	14	3.57 (15:1)	0.06	33	37	8	2.14 (7:8:1)	0.34
KL/B 276	122	33	1.14 (3:1)	0.29	15	45	20	1.87 (1:2:1)	0.39
LY/B 276	131	41	0.12 (3:1)	0.72	20	41	18	0.21 (1:2:1)	0.90
KL/CPAN 6118	159	17	3.49 (15:1)	0.06	38	33	8	3.31 (7:8:1)	0.19
LY/CPAN 6118	163	16	2.20 (15:1)	0.14	37	38	5	0.21 (7:8:1)	0.90
KL/Guji S	123	37	0.30 (3:1)	0.58	18	38	22	0.46 (1:2:1)	0.79
LY/Guji S	109	43	0.88 (3:1)	0.35	24	35	20	1.43 (1:2:1)	0.49
KL/VD 2001-14	149	13	0.87 (15:1)	0.35	42	35	3	2.82 (7:8:1)	0.24
LY/VD 2001-14	152	14	1.35 (15:1)	0.24	41	35	3	2.47 (7:8:1)	0.29

R = Resistant; S = Susceptible; HR = Homozygous resistant; SEG = Segregating; HS = Homozygous susceptible

**Table 3.** Segregation for seedling resistance to leaf rust pathotype 12-2 (1R5) in F<sub>2</sub> populations derived from inter-crosses of resistant parents

Cross	R	S	$\chi^2$	P value
AKDW 4339/B 276	459	9	0.40 (63R:1S)	0.53
AKDW 4339/CPAN 6118	538	4	1.67 (255R:1S)	0.19
AKDW 4339/Guji S	442	5	0.57 (63R:1S)	0.45
AKDW 4339/VD 2001-14	505	3	0.53 (255R:1S)	0.47
B 276/CPAN 6118	481	12	2.44 (63R:1S)	0.12
B 276/Guji S	438	25	0.57 (15R:1S)	0.45
B 276/VD 2001-14	504	18	12.05 (63R:1S)	0.0005
CPAN 6118/Guji S	497	12	2.10 (63R:1S)	0.15
CPAN 6118/VD 2001-14	517	2	0.0 (255R:1S)	0.98
Guji S/VD 2001-14	487	0	7.73 (63R:1S)	0.005

R = Resistant; S = Susceptible

derived durum Somateria) was reported later (Herrera-Foessel et al. 2008a). Linked markers suggest that *Lr14a* has been present in about 95% CIMMYT lines developed since 2001 (Loladze et al. 2014). The gene *Lr23* has commonly been postulated in Indian durum germplasm through comparison of seedling infection types with lines carrying known *Lr* genes (Nayar et al. 2001; Bhardwaj 2013). However, the identified leaf rust resistance genes are different from *Lr23* and *Lr14a* as both of them are ineffective against the test pathotype 12-2. Some *Lr* genes known to be present

in bread wheat like *Lr3* (Herrera-Foessel et al. 2007), and *Lr27+Lr31* (Huerta-Espino et al. 2009) were also found in durum wheat. The identified genes should be different from these as well since pathotype 12-2 is virulent to them. Resistance in Australian durum cultivar Wollaroi (Singh et al. 2010) and Italian durum Trinakria (Gireesh et al. 2014) was controlled by a dominant gene which was thought to be either *Lr52* or its allele or a closely linked gene. A partially dominant leaf rust resistance gene mapped to the chromosome arm 6BS in Chilean durum Guayacan INIA and its sister line Guayacan 2 was named *Lr61* (Herrera-Foessel et al. 2008b). Presence of recently designated gene *Lr72* was confirmed in Mexican durum cultivar Atil C2000 (Herrera-Foessel et al. 2014). 'Trinakria' showed seedling resistance to pathotype 12-2 (Gireesh et al. 2014). However, 'Wollaroi' and lines carrying *Lr61* or *Lr72* have not been tested with Indian leaf rust pathotypes or their allelic relationship with the identified genes is not known. It was assumed that a combination of *Lr14a* and *Lr72* provided resistance to durum wheat in India (Loladze et al. 2014) based on no published report on combined virulence for these genes (Loladze, *personal communication*). Occurrence of *Lr72* and of *Lr61* in Indian germplasm is possible since CIMMYT durum lines have frequently been used in Indian durum improvement programme. Presuming the presence of *Lr61* and *Lr72* as well as of the Trinakria/Wollaroi gene/s (*Lr52/Lr52* allele/a

closely linked gene) in some of the genotypes studied, at least three to four of the identified genes should be different ones. Thus, the reported genotypes can contribute to broaden the base and prolong durability of leaf rust resistance in durum wheat. Leaf rust resistance genes identified in the present study need to be characterized, and their allelic relationship with *Lr52*, *Lr61* and *Lr72* needs to be studied.

### Acknowledgements

Receipt of the rust inoculum from Directorate of Wheat Research, Regional Station, Flowerdale, Shimla is gratefully acknowledged. We thank the Director, IARI, New Delhi for providing facilities.

### References

- Bhardwaj S. C. 2013. *Puccinia* - *Triticum* interaction : an update. Indian Phytopathol., **66**: 14-19.
- Casulli F., Siniscalco A. and Tommasi F. 1983. Behaviour of some varieties of durum and bread wheat to some physiologic races of *Puccinia recondita* f. sp. *tritici*. Phytopath. Medit., **22**: 147-151.
- Gireesh C., Vinod, Sharma J. B. and Prabhu K. V. 2014. Inheritance and molecular mapping of leaf rust resistance in *Triticum turgidum* var. *durum* cv. Trinakria. Indian J. Genet., **74**: 10-15.
- Herrera-Foessel S., Singh R., Huerta-Espino J., William H., Garcia V., Djurle A. and Yuen J. 2008a. Identification and molecular characterization of leaf rust resistance gene *Lr14a* in durum wheat. Plant Dis., **92**: 469-473.
- Herrera-Foessel S., Singh R., Huerta-Espino J., William H. M., Rosewarne G., Djurle A. and Yuen J. 2008b. Molecular mapping of a leaf rust resistance gene on the short arm of chromosome 6B of durum wheat. Plant Dis., **92**: 1650-1654.
- Herrera-Foessel S., Singh R. P., Huerta-Espino J., William H., Rosewarne G., Djurle A. and Yuen J. 2007. Identification and mapping of *Lr3* and a linked leaf rust resistance gene in durum wheat. Crop Sci., **47**: 1459-1466.
- Herrera-Foessel S. A., Huerta-Espino J., Calvo-Salazar V., Lan C. X. and Singh R. P. 2014. *Lr72* confers resistance to leaf rust in durum wheat cultivar Atil C2000. Plant Dis., **98**: 631-635.
- Honrao B. K. and Rao V. S. P. 1996. Sources of resistance to race 77 of leaf rust in durum wheat. 1. Seedling resistance. Cereal Rusts & Powdery Mildews Bull., **24**: 39-43.
- Huerta-Espino J. and Roelfs A. P. 1992. Leaf rust on durum wheats. Vortr. Pflanzenzuchtg., **24**: 100-102.
- Huerta-Espino J., Singh R., Herrera-Foessel S., Perez-Lopez J. and Figueroa-Lopez P. 2009. First detection of virulence in *Puccinia triticina* to resistance genes *Lr27* + *Lr31* present in durum wheat in Mexico. Plant Dis., **93**: 110.
- Loladze A., Kthiri D., Pozniak C. and Ammar K. 2014. Genetic analysis of leaf rust resistance in six durum wheat genotypes. Phytopathology, **104**: 1322-1328.
- McIntosh R. A., Wellings C. R. and Park R. F. 1995. Wheat rusts: an atlas of resistance genes. CSIRO Publishing, Melbourne, Australia.
- McIntosh R. A., Yamazaki Y., Dubcovsky J., Rogers J., Morris C., Appels R. and Xia X. C. 2013. Catalogue of Gene Symbols for Wheat. 12th International Wheat Genetics Symposium, 8-13 September 2013, Yokohama, Japan. <http://www.shigen.nig.ac.jp/wheat/komugi/genes/macgene/2013/GeneSymbol.pdf> [accessed 15 December, 2014].
- Mishra A. N. 1996. Genetics of leaf rust resistance in durum wheat. Unpublished Ph.D. thesis, University of Minnesota, St. Paul, MN 55108, U.S.A.
- Mishra A. N., Kaushal K., Jain S. K. and Pandey H. N. 2001a. 'Thatcher'- avirulent leaf rust pathotypes from India. Wheat Inf. Serv., **93**: 32-34.
- Mishra A. N., Kaushal K. and Pandey H. N. 2001b. Appropriate pathotypes of stem rust and leaf rust for evaluating resistance in durum wheat and bread wheat. Wheat Inf. Serv., **93**: 38-39.
- Mishra A. N., Shirsekar G. S., Yadav S. R., Dubey V. G., Kaushal K., Sai Prasad S. V. and Pandey H. N. 2009. Protocols for evaluating resistance to leaf and stem rusts in durum and bread wheats. Indian Phytopathol., **62**: 461-468.
- Nayar S. K., Nagarajan S., Prashar M., Bhardwaj S. C., Jain S. K. and Datta D. 2001. Revised catalogue of genes that accord resistance to *Puccinia* species in wheat. Research Bulletin No. 3, Directorate of Wheat Research, Regional Station, Flowerdale, Shimla.
- Nelson J. C., Singh R. P., Autrique J. E. and Sorrells M. E. 1997. Mapping genes conferring and suppressing leaf rust resistance in wheat. Crop Sci., **37**: 1928-1935.
- Pandey H. N. and Rao M. V. 1984. Differential behaviour of *aestivum* and *durum* wheats to races 77 and 106 of leaf rust (*Puccinia recondita* Rob. Ex Desm.). Wheat Inf. Serv., **58**: 34-35.
- Paradies M. 1980. Standard races and variants (biotypes) of *Puccinia recondita* f. sp. *tritici* identified in Italy in 1977. Effectiveness of some resistance genes and greenhouse tests of durum wheat (*Triticum durum* Desf.) and bread wheat (*Triticum aestivum* L.). Cereal Rusts Bull., **8**: 3-9.

- Roelfs A. P., Singh R. P. and Saari E. E. 1992. Rust Diseases of Wheat: Concepts and Methods of Disease Management. CIMMYT, Mexico, D.F.
- Sharma D. L., Saini R. G., Gupta A. K. and Gupta S. 1986. Diversity for resistance to leaf rust in *Triticum durum* (Desf.). Cereal Rusts Bull., **14**: 53-57.
- Sharma S. C., Saini R. G. and Goel R. K. 1996. Diversity for new leaf rust resistance genes in some macaroni wheat accessions. Cereal Rusts and Powdery Mildews Bull., **24**: 35-38.
- Singh B., Bansal U. K., Forrest K. L., Hayden M. J., Hare R. A. and Bariana H. S. 2010. Inheritance and chromosome location of leaf rust resistance in durum wheat cultivar Wollaroi. Euphytica, **175**: 351-355.
- Singh H., Dhaliwal H. S. and Gill K. S. 1992. Diversity for leaf rust resistance in *Triticum durum* germplasm. Cereal Rusts and Powdery Mildews Bull., **20**: 62-67.
- Singh R. P., Huerta-Espino J., Pfeiffer W. and Figueroa-Lopez P. 2004. Occurrence and impact of a new leaf rust race on durum wheat in northwestern Mexico from 2001 to 2003. Plant Dis., **88**: 703-708.
- Singh S. S., Sharma R. K., Singh G. and Tyagi B. S. (Eds). 2011. 100 Years of Wheat Research in India – A saga of distinguished achievements. Directorate of Wheat Research, Karnal 132 001. pp. 191-192.