Revisiting Old Landraces of Wheat for Stem Rust Resistance

Bansal UK, Singh D, Miah H, Park RF, Bariana HS

The University of Sydney, Plant Breeding Institute-Cobbitty, Faculty of Agriculture, Food and Natural Resources, PMB11, Camden, NSW 2570, Australia.

INTRODUCTION

Wheat is the world's most important food crop and is grown on over 200m ha globally. There has been very little increase in the area sown to wheat since 1960. However, yield has improved almost three fold during these 40 years. Increased productivity resulted from the introduction of dwarfing genes into wheat making it more responsive to fertilizers, and through improved cultural practices. Sustained wheat production has been and still is hampered by many biotic and abiotic stresses. Of the biotic stresses, rust diseases are of prime importance. Among rust diseases stem rust is reported to be the most damaging. Forty five stem rust resistance genes have been genetically characterised and named[1]. Wheat breeders at the CIMMYT, Mexico combined stem rust resistance *Sr2*, *Sr30* and *Sr31* in widely adapted germplasm and release of countless number of cultivars took place for over 25 years. A new variant of stem rust pathogen of wheat named, Ug99 (TTKS), which was virulent on genotype carrying stem rust resistance gene *Sr31*, was isolated in Uganda[23]. This pathotype has posed a renewed threat to global wheat production. In order to combat this threat, search for new and potentially durable sources of resistance is essential.

This study was planned to screen the Watkins collection of landraces/older cultivars of wheat against stem rust. This paper summarises the seedling and adult plant stem rust response variation among this collection.

MATERIALS AND METHODS

Plant material and field tests

The collection of material used in this study was assembled by the British botanist AE Watkins in the 1930s[4]. The Watkins' collection comprising of 838 wheat genotypes (hexaploids) from 32 countries (Table 1) was evaluated in the field for three years (2005, 2006 and 2007) against *Puccinia graminis* f. sp. *tritici* (Pgt). Data on 776 genotypes were successfully captured. Some genotypes were either very late or were completely killed by other diseases. Test lines were sown as 60 cm rows and each 50 row block was surrounded by susceptible infector row for disease development. The Pgt pathotypes 98-1,2,3,5,6 (PBI accession no. 781219) and 34-1,2,7+*Sr38* (010130) were used to create artificial epidemics during all three years. Adult plant stem rust response assessments were made on a 1 to 9 scale [5].

Multipathotype tests

One hundred and forty genotypes (with field score ≤5) and wheat stem rust differentials were tested in the greenhouse with six Pgt pathotypes 34-2,4,5,7,11[Acc No. 700362] incubated at 20°C and 25°C; 40-1,2,3,4,5,6,7 [383]; 343-1,2,3,,5,6,8,9 [890005]; 98-1,2,3,5,6 [781219]; 34-1,2,3,6,7,8,9 [205]; 34-1,2,3,4,5,6,7 [103]. Infection types (ITs) were recorded 15 days after inoculation on a 0 to 4 scale described by Stakman[6] with slight modifications[7]. Resistance genes were postulated by comparing the ITs produced by an array of pathotypes on test genotypes with those of differential genotypes with known genes. Remaining genotypes from the collection were also tested against three important pathotypes (98-1,2,3,5,6 [781219], 34-1,2,3,4,5,6,7 [103] and 34-1,2,7,+*Sr38* [010130] at the seedling stage in the greenhouse.

Molecular markers

Extraction of DNA was performed from embryo half of four seeds using Matrix Mill and quantified using Nanodrop for PCR analyses. The molecular marker stm560.3tagtg[8], closely linked with the stem rust APR gene *Sr2*, was tested on all 838 genotypes.

RESULTS AND DISCUSSION

The genetically characterised stem rust resistance genes belong to two categories; seedling resistance genes (major genes) and adult plant resistance genes (APR genes). The APR genes also referred to as minor genes, show intermediate responses and often combinations of more than three genes are required to attain commercially acceptable level of resistance.

Adult plant screening

A similar stem rust response distribution of genotypes over three years indicated consistent genetic variation among the collection (Fig. 1). Although all response scores were represented among the population, a high proportion of genotypes with intermediate stem rust responses were observed. Greenhouse screening against three important pathotypes, including those two used in the field, at the seedling stage indicated that 126 genotypes were susceptible in the seedling stage and produced adult plant stem rust response score varying from 2 to 7 at the adult plant stage. Some of these genotypes carried adult plant stem resistance gene *Sr2*-linked pseudo black chaff (PBC). These results suggested the involvement of APR genes in conditioning low stem rust response of 126 genotypes.

Mutipathotype testing

Genotypes showing an adult plant response score of ≤5 were subjected to multipathotype tests in the seedling stage. Stem rust resistance genes *Sr6, Sr8a, Sr8b, Sr9g, Sr12, Sr17* and *Sr30* were postulated (Table 2). These genes were present either singly or in combinations. The presence of seedling chlorosis indicated the presence of APR gene *Sr2* in some genotypes. Twenty three genotypes showed low ITs against all the Pgt pathotypes tested and no postulations could be made. These genotypes appeared to carry uncharacterized seedling resistance gene (s). These genotypes have been crossed with the stem rust susceptible cultivar Yitpi to understand inheritance of resistance and to produce stem rust resistant Yitpi-derivatives. A set of genotype carrying uncharacterized seedling and adult plant resistance genes will be tested in Kenya during the 2008 crop season to understand their responses against the Ug99 and related pathotypes.

Table 1 Geographical distribution of Watkins' collection

Country	No. of lines	Country	No. of lines
Afghanistan	32	Iran	53
Algeria	9	Iraq	9
Armenia	4	Italy	14
Australia	32	Morocco	22
BGR [a]	15	Palestine	2
Brazil	1	Poland	18
Burma	4	Portugal	39
China	90	Rumania	6
Cyprus	3	Syria	5
Egypt	4	Tunis	14
ESP [a]	98	Turkey	18
Finland	1	Turkistan	43
France	21	USA	8
Greece	38	Yugoslavia	49
Hungry	7	32 [b]	1
India	141	33 [b]	37

[a] Full form was not available, [b] No explanation available about these locations.

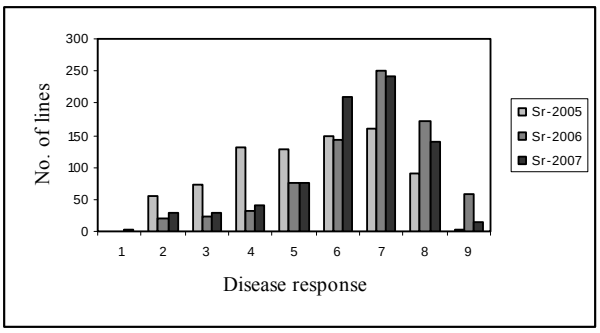

Fig. 1 Frequency distribution of Watkins' collection for stem rust response over three years

Marker based detection of the APR gene *Sr2*

The APR gene *Sr2* is considered of high value in wheat improvement because of its effectiveness since 1926[9,10]. The *Sr2*-linked marker stm560.3tagtg was used to determine the presence of *Sr2*. Marker data revealed the presence of *Sr2* in 159 of 838 genotypes. The absence of *Sr2* in some genotypes that were seedling susceptible and adult plant resistant indicated the presence of additional APR.

Table 2 Summary of Seedling and adult plant stem rust resistance genes postulated in Watkins' collection

Postulated genes	No. of genotypes
Sr2	4
Sr6	2
Sr8a	2
Sr9b	1
Sr12	16
Sr12+	8
Sr30+	4
Sr2, Sr12	1
Sr2, Sr30	4
Sr6, Sr30	1
Sr8b, Sr30	2
Sr12, Sr30	8
Sr9g, Sr12+	1
Sr9g Sr30	4
Sr17, Sr30+	1
Sr2, Sr12, Sr30+	2
Sr9g, Sr12, Sr30,	1
Uncharacterized seedling resistance	23
APR	126

CONCLUSIONS/FUTURE DIRECTIONS

Seedling stem rust resistance genes *Sr6, Sr8a, Sr8b, Sr9g, Sr12, Sr17* and *Sr30* were postulated among 140 genotypes. Two putatively new sources of seedling stem rust resistance genes were identified in 23 genotypes.

Genotypes carrying varying levels of APR were identified. Some of these genotypes carried the APR gene *Sr2*. These genotypes will be tested in Kenya against Ug99 and its derivatives to confirm their effectiveness.

Some genotypes carrying putatively new sources of seedling resistance and APR were crossed with the stem rust susceptible cultivar Yitpi for genetic analysis and improvement of its resistance.

Genotypes carrying moderate levels of resistance to all three rust diseases have been identified (data not

presented). These genotypes will be tested against other biotic and abiotic stresses through collaboration with scientists from different institutions.

A collaborative approach for molecular mapping of confirmed new sources of multiple resistances will be adopted.

ACKNOWLEDGEMENTS

We thank GRDC Australia for financial assistance through the Australian Cereal Control Program. We thank Greg Grime and Michael Mackay, Australian Winter Cereal Collection, Tamworth for supplying seed. We thank Prof. John Snape, John Innes Centre, Norwich, UK, for discussions related to the Watkins' collection.

REFERENCES

1. McIntosh RA, Yamazaki Y, Devos KM, Dubcovsky J, Rogers WJ, Appels R (2003) Catalogue of Gene Symbols for Wheat. Proceedings of the 10th International Wheat Genetics Symposium. pp55-60
2. Pretorius ZA, Singh RP, Wagoire WW, Payne TS (2000) Detection of Virulence to Wheat Stem Rust Resistance Gene *Sr31* in *Puccinia graminis*. f. sp. *tritici* in Uganda. Plant Dis. 84:203
3. Singh RP, Hodson DP, Jin Y, Huerta-Espino J, Kinyua MG, Wanyera R, Njau P, Ward RW (2006) Current status, likely migration and strategies to mitigate the threat to wheat production from race Ug99 (TTKS) of stem rust pathogen. CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources. pp13
4. Miller TE, Reader SM, Ambrose MJ (2000) The Watkins wheat collection. Annual Wheat Newsletter 46: 172.
5. Bariana HS, Miah H, Brown GN, Willey N, Lehmensiek A (2007a) Molecular mapping of durable rust resistance in wheat and its implication in breeding. In: H.T Buck, J.E. Nisi, N.Salomón (Eds.), Wheat Production in Stressed Environments. Developments in Plant Breeding, 12: 723-728
6. Stakman EC, Stewart DM, Loegering WQ (1962) Identification of physiological races of *Puccinia graminis* var *tritici*. Agricultural Research Service E617. (United States Department of Agriculture: Washington DC)
7. Luig NH (1983) A survey of virulence genes in wheat stem rust, *Puccinia graminis* f. sp. *tritici*. Advances in plant breeding, Supplement 11 to Journal of Plant Breeding, Paul Parey, Berlin
8. Hayden MJ, Stephenson P, Logojan AM, Khatkar D, Rogers C, Elsden J, Koebner RMD, Snape JW, Sharp PJ (2006) Development and genetic mapping of sequence-tagged microsatellites (STMs) in bread wheat (*Triticum aestivum* L.) Theor Appl Genet 113:1271-1281
9. Mcfadden ES (1930) A successful transfer of emmer characters to *vulgare* wheat. Agron J 22:1020-1034
10. Bariana HS, Brown GN, Bansal UK, Miah H, Standen GE, Lu M (2007b) Breeding triple rust resistant wheat cultivars for Australia using conventional and marker-assisted selection technologies. Aust J Agric Res 58:576-587