*International Journal of Plant Breeding* ©2009 Global Science Books

# Genetics of Stem Rust Resistance in Three Durum Wheat Cultivars

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## ABSTRACT

Durum wheat cultivars 'Kalka', 'Gundaroi' and 'Tamaroi' were crossed with the susceptible landrace 'Bansi' to study inheritance of stem rust resistance. Seedling screening of $F_3$ populations using the *Puccinia graminis* f. sp. *tritici* (Pgt) pathotype 34-1,2,3,4,5,6,7 suggested monogenic control of resistance in cvs. 'Kalka', 'Gundaroi' and 'Tamaroi'. The pathotype 34-1,2,3,4,5,6,7 was avirulent for stem rust resistance genes (*Sr8b*, *Sr9e* and *Sr13*) reported in durum wheat cultivars. Tests with *Sr9e*-virulent and *Sr9e*+*Sr8b*-virulent pathotypes, 40-1,2,3,4,5,6,7 and 40-1,2,3,4,5,6,7,8,9,10,11, respectively, indicated the absence of *Sr9e* and *Sr8b* in these cultivars. Genetic association between stem rust response segregation and the chromosome 6AL located marker gwm427 led us to conclude the presence of *Sr13* in 'Kalka', 'Gundaroi' and 'Tamaroi'. The absence of segregation for susceptibility in the 'Kalka'/'Tamaroi' cross further supported the presence of a common gene for stem rust resistance in these cultivars. 'Gundaroi' and 'Tamaroi' are likely to carry the same resistance gene based on their pedigree. These results indicated a narrow genetic base of stem rust resistance in these durum cultivars. Incorporation of diverse sources of resistance into future durum cultivars is necessary.

**Keywords:** genetics, molecular markers, *Puccinia graminis* f. sp. *tritici*

## INTRODUCTION

Durum wheat (*Triticum turgidum* L. ssp. durum) is an allotetraploid of *T. urartu* (2n = 2x = 14, AA genome) and *Aegilops speltoides* (2n = 2x = 14, SS genome) (Marie *et al.* 2005). It is used for making pasta and semolina. Canada, Europe, America, Turkey and Syria are major producers of durum wheat. In Australia, the area under durum wheat increased since the 1990s because of its better adaptation in some regions, high yield and changes in the industry focus from domestic to export based market. World durum production is around 40 million tonnes annually and Australia contributes about 0.5 million tonnes (www.daff.gov.au).

Stem rust, caused by *Puccinia graminis* f. sp. *tritici* (Pgt), is an important disease of wheat (Roelfs and McVey 1979). A stem rust epidemic in 1904 in the USA caused yield losses up to US$10 million (Campbell and Long 2001), whereas losses in the range of A$200-300 million in south eastern Australia in 1973 were estimated by Watson and Butler (1984). Stem rust affects yield more severely as compared to other wheat diseases (Luig 1983) and has the ability to cause entire crop failures.

McIntosh *et al.* (2008) listed 46 stem rust resistance genes across all three genomes of common wheat. Many of these genes are in common with durum wheat and have been rendered ineffective individually. Exotic introductions, mutation and somatic hybridization are among the possible causes of new pathotypic variation. Detection of the Pgt pathotype Ug99 (TTKSK) in Uganda (Pretorius *et al.* 2000), which acquired virulence for several previously effective stem rust resistance genes including *Sr31*, posed a threat to global wheat production. The TTKSK-derivatives with virulence against stem rust resistance genes *Sr24* and *Sr36* have also been detected (Jin and Singh 2006; Wanyera *et al.* 2006; Jin *et al.* 2009). The A and B genome located genes *Sr13*, *Sr22*, *Sr32*, *Sr35*, *Sr39* and *Sr40* are effective against TTKSK and its derivatives (Jin *et al.* 2007) and the chromosome 2A located stem rust resistance gene *Sr21* was also found to be effective in Kenya (Bariana and co-workers unpublished data).

Breeding for stem rust resistance in Australia was estimated to save A$124 million annually (Brennan and Murray 1988). Genetic control of stem rust is an environment-friendly and economical solution and relies on the availability of genetically diverse sources of resistance. Durum cultivars 'Kalka', 'Gundaroi' and 'Tamaroi' were released for cultivation in South Australia and possess moderate to high levels of resistance against all three rust diseases under Australian conditions. These cultivars showed low seedling stem rust responses when tested against several Australian Pgt pathotypes with virulence for genes commonly detected in durum wheat and common wheat. This investigation was planned to understand inheritance of stem rust resistance in 'Kalka', 'Gundaroi' and 'Tamaroi'.

## MATERIALS AND METHODS

### Host materials

Durum wheat cvs. 'Kalka' (Wollaroi/RH88009), 'Gundaroi' and 'Tamaroi' (both selected from the cross Altar-84/4/Tam-1-B-17/Kamilaroi/3/Wells/56111//Guillemot) were crossed with the susceptible landrace 'Bansi strain 168' (Bansi). 'Kalka' and 'Tamaroi' were crossed to conduct a test of allelism for stem rust resistance gene(s) carried by these cultivars. $F_1$ seeds were grown in the greenhouse to produce $F_2$ populations. Single $F_2$ seeds were sown individually in 10 cm diameter pots filled with potting mix. Pots were placed on benches fitted with capillary mats in a naturally ventilated plastic tunnel-house. Capillary mats were wetted three times daily to provide moisture for plant growth. Plants were harvested and threshed separately to produce $F_3$ families. In the case of the 'Kalka'/'Tamaroi' cross, the generation was advanced only to $F_2$.

Received: 3 July, 2009. Accepted: 19 November, 2009.

Original Research Paper

## Seedling rust tests

Plastic pots of 9 cm diameter were filled with potting mix and fertilized with Aquasol® (25 g/200 pots). Twenty seeds of each $F_3$ family were sown per pot. Parents were also sown with each experiment. An additional dose of nitrogenous fertilizer urea (25 g/200 pots) was applied seven days after sowing. Plants were grown in a controlled-environment microclimate room maintained at 20 ± 2°C. Ten to 12 days old seedlings (two-leaf stage) were inoculated with a suspension of urediniospores of Pgt pathotype 34-1,2,3,4,5, 6,7 (PBI culture no. 103) in Shellsol T® using a hydrocarbon pressure pack®. Inoculated seedlings were incubated under natural light conditions for 48 hours at 18 ± 2°C on water-filled steel trays covered with plastic hoods to maintain 100% relative humidity. Seedlings were then transferred to microclimate room maintained at 25 ± 2°C. Stem rust responses were scored 14 days after inoculation on a 0-4 scale proposed by Stakman *et al.* (1962) and modified by Luig (1983). The mesothetic infection type (IT) X represents the development of various sized pustules on the same leaf including hypersensitive flecks. Parental genotypes were tested against Pgt pathotypes 40-1,2,3,4,5,6,7 (PBI culture no. 383) and 40-1,2,3,4,5,6,7,8,9,10,11 (PBI culture no. 282). $F_3$ families from all three crosses were tested against Pgt pathotypes 34-1,2,3,4,5,6,7 and 40-1,2,3,4,5,6,7. $F_2$ plants from the 'Kalka'/'Tamaroi' cross were tested with the Pgt pathotype 34-1,2,3,4,5, 6,7.

## Molecular marker analysis

DNA was extracted from 10 days old seedlings of the resistant parents ('Kalka', 'Gundaroi' and 'Tamaroi'), the susceptible parent ('Bansi') and each $F_3$ family according to the procedure described by Doyle and Doyle (1990). In the case of $F_3$ families, care was taken to collect a representative sample from 16 to 20 individual seedlings. Molecular marker gwm427 (forward 5′-AAACTTAGA ACTGTAATTTCAGA-3′ and reverse 5′-AGTGTGTTCATTTGA CAGTT-3′) (S. Chao and E. Lagudah, pers. comm.) observed to be linked with *Sr13* was genotyped on all parents and $F_3$ populations. PCR reactions were performed in 10 µl volume containing 50 ng of genomic DNA, 0.2 mM of each dNTP, 0.5 µM of each SSR primer, 1.5 mM $MgCl_2$, 1X PCR buffer and 0.5 U of *Taq* polymerase. PCR amplification included 42 cycles of the touchdown profile: 30 s at 92°C, 30 s at 57°C and 30 s at 72°C. Following the first cycle, the annealing temperature was reduced by 1°C per cycle for next seven cycles. Amplified products were separated on a 2.5% biotechnology grade agarose (AMRESCO Inc. USA) gel.

## Statistical analysis

Chi-squared ($\chi^2$) analyses were performed to determine the goodness-of-fit of the observed segregation frequencies to the expected genetic ratios. Recombination fractions were estimated by using the maximum likelihood equation (Allard 1956).

# RESULTS

## Parental seedling stem rust responses

Durum cvs. 'Kalka', 'Gundaroi', 'Tamaroi' and the susceptible parent 'Bansi' produced infection types (IT)12=, IT;1C, IT1-N and IT3+, respectively, against the Pgt pathotype 34-1,2,3,4,5,6,7 (**Table 1**). Infection types produced by these parental genotypes did not vary substantially with Pgt pathotypes 40-1,2,3,4,5,6,7 (*Sr9e*-virulent and *Sr8b* and *Sr13*-aviulent) and 40-1,2,3,4,5,6,7,8,9,10,11 (*Sr8b* and *Sr9e*-virulent and *Sr13*-avirulent). These results indicated that cvs. 'Kalka', 'Gundaroi' and 'Tamaroi' did not carry stem rust resistance gene *Sr8b* or *Sr9e* singly, however, the possibility of the presence of *Sr13*, a gene reported to be present in durum wheats, and/or other gene(s) singly could not be discounted based on seedling tests.

## Inheritance studies

Seedling stem rust response variation data from $F_3$ families included three classes: non-segregating resistant (HR), segregating (SEG) and non-segregating susceptible (HS). The HR category included $F_3$ families that expressed seedling responses similar to the resistant parent, the SEG category contained families that included seedlings with both resistant and susceptible responses and $F_3$ families in the HS category produced susceptible responses similar to the susceptible parent. The 'Kalka'/'Bansi'-derived $F_3$ population included 22HR, 48SEG and 25HS families (**Table 2**). Chi-squared analysis of data conformed to the monogenic inheritance of resistance. Similar segregation patterns were observed for $F_3$ families from the other two crosses ('Gundaroi'/'Bansi' and 'Tamaroi'/'Bansi'). Chi-squared analyses of data also supported the monogenic control of stem rust resistance in cultivars 'Gundaroi' and 'Tamaroi'. Stem rust resistance genes carried by 'Kalka', 'Gundaroi' and 'Tamaroi' were temporarily designated as *SrK*, *SrG* and *SrT*, respectively.

$F_3$ families were also tested with the *Sr9e*-virulent Pgt pathotype 40-1,2,3,4,5,7 and segregation behaviour similar to that of pathotype 34-1,2,3,4,5,6,7 was observed for each population. Had *Sr9e* been present singly, all $F_3$ families would have been susceptible. **Fig. 1** illustrates the infection type response variation detected among F3 families. None of the resistant $F_3$ families exhibited ITX produced by *Sr8b*. These results indicated the absence of *Sr9e* and *Sr8b* in cvs. 'Kalka', 'Gundaroi' and 'Tamaroi', suggesting the possible presence of *Sr13* or a yet uncharacterized stem rust resistance gene.

## Molecular marker analysis

In order to confirm whether stem rust resistance gene carried by 'Kalka', 'Gundaroi' and 'Tamaroi' was *Sr13*, molecular markers reported to be linked with this gene were used. The *Sr13*-linked marker wmc59 (Bhavani *et al.* 2008) was monomorphic among the parents and therefore was not genotyped on $F_3$ populations. Five other SSR markers (gwm169, wmc417, gwm617, gwm642 and wmc580) located in the vicinity of wmc59 in chromosome 6AL (Somers *et al.* 2004) were also found to be monomorphic among parents. Marker gwm427 observed to be linked with *Sr13* (S. Chao and E. Lagudah, pers. comm.) was genotyped on parents. All resistant parents ('Kalka', 'Tamaroi' and 'Gundaroi') amplified a 200-bp fragment, whereas the susceptible parent 'Bansi' produced a 226-bp fragment (**Fig. 2**). This marker was genotyped on all three $F_3$ populations and

**Table 1** Seedling infection types produced by parental genotypes 'Kalka', 'Gundaroi', 'Tamaroi', 'Bansi' and some control genotypes with known stem rust resistance gene(s) against three Pgt pathotypes.

| Wheat cultivars/ lines | 34-1,2,3,4,5,6,7 | 40-1,2,3,4,5,7 | 40-1,2,3,4,5,6,7,8,9,10,11 | Resistance gene(s) |
|---|---|---|---|---|
| Kalka | 12= | 1-N | 1-N | Unknown |
| Gundaroi | ;1C | ;1-N | ;1-N | Unknown |
| Tamaroi | 1-N | 12= | 12= | Unknown |
| Bansi | 3+ | 3+ | 3+ | Nil |
| Emmer | ;;1- | 3+ | 3+ | *Sr9e* |
| Renown | 3+ | 3+ | 3+ | *Sr7b*, *Sr17* |
| Barleta Benvenuto | X | X | 3+ | *Sr8b* |
| Line S | 2- | 12= | 12= | *Sr13*, *Sr17* |
| Morocco | 3+ | 3+ | 3+ | Nil |

**Table 2** Joint analyses of stem rust response and molecular marker segregation data among three $F_3$ families.

| Population | Number of progenies | | | Total |
|---|---|---|---|---|
| | *Xgwm427*$_{200bp}$ | *Xgwm427*$_{200bp/226bp}$ | *Xgwm427*$_{226bp}$ | |
| **Kalka/Bansi** | | | | |
| *SrKSrK* | 18 | 3 | 1 | 22 |
| *SrKsrK* | 1 | 46 | 1 | 48 |
| *srKsrK* | 1 | 6 | 18 | 25 |
| Total | 20 | 55 | 20 | 95 |
| $\chi^2$ 1:2:1 (*SrK* vs. *srK*) = 0.20; $\chi^2$ 1:2:1 (*Xgwm427*-200 vs. *Xgwm427*-226) = 2.37, $\chi^2$ 1:2:1:2:4:2:1:2:1 (*SrK vs. Xgwm427*) =107.53** | | | | |
| **Gundaroi/Bansi** | | | | |
| *SrGSrG* | 17 | 6 | 1 | 24 |
| *SrGsrG* | 7 | 38 | 1 | 46 |
| *srGsrG* | 0 | 4 | 20 | 24 |
| Total | 24 | 48 | 22 | 94 |
| $\chi^2$ 1:2:1 (*SrG* vs. *srG*) = 0.04; $\chi^2$ 1:2:1 (*Xgwm427*-200 vs. *Xgwm427*-226) = 0.13, $\chi^2$ 1:2:1:2:4:2:1:2:1 (*SrG vs. Xgwm427*) =93.57** | | | | |
| **Tamaroi/Bansi** | | | | |
| *SrTSrT* | 22 | 5 | 1 | 28 |
| *SrTsrT* | 3 | 31 | 2 | 36 |
| *srTsrT* | 0 | 7 | 13 | 20 |
| Total | 25 | 43 | 16 | 84 |
| $\chi^2$ 1:2:1 (*SrT* vs. *srT*) = 3.23; $\chi^2$ 1:2:1 (*Xgwm427*-200 vs. *Xgwm427*-226) = 1.98, $\chi^2$ 1:2:1:2:4:2:1:2:1 (*SrT vs. Xgwm427*) =94.92** | | | | |

Table value of $\chi^2$ at *P = 0.05* and *2 d.f. = 5.99* and *P = 0.01* and *2 d.f.= 9.21* and at *P=0.05* and *8 d.f.= 15.507* and *P = 0.01* and *8 d.f = 20.0;* ** Highly significant

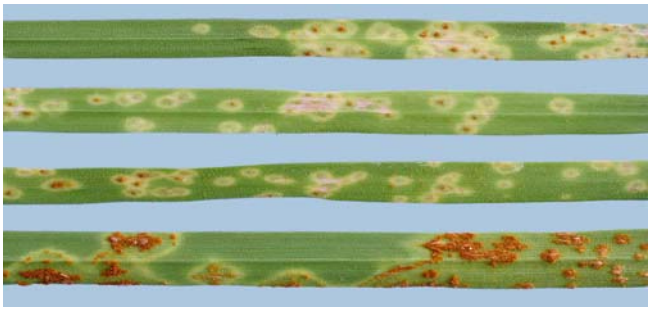

**Fig. 1 Infection type variation observed among $F_3$ families derived from the 'Kalka'/'Bansi' cross.**

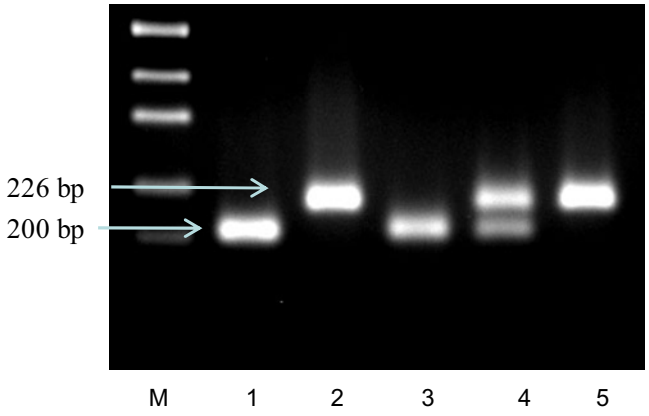


**Fig. 2 PCR amplification of SSR marker gwm427 on a selected set of genotypes.** M = pUC19/*Msp1*, 1 = 'Tamaroi', 2 = 'Bansi', 3 = homozygous resistant F3 family, 4 = segregating F3 family, and 5 = homozygous susceptible F3 family.

showed a 1:2:1 segregation with $\chi^2$ values ranging from 0.13 to 2.37 (**Table 2**). Highly significant $\chi^2$ values (**Table 2**) for joint segregation analyses in all $F_3$ populations indicated genetic association between *Xgwm427* and stem rust response segregations conditioned by *SrK*, *SrG* and *SrT*. Recombination frequencies of 8.1 ± 3.0, 10.7 ± 3.4 and 11.3 ± 3.1% between the marker gwm427 and stem rust resistance genes *SrK*, *SrG* and *SrT*, respectively, were computed. The previously reported locations of gwm427 and *Sr13* in chromosome 6AL and their genetic association in 'Kalka'/'Bansi', 'Gundaroi'/'Bansi' and 'Tamaroi'/'Bansi' crosses enabled us to conclude the presence of *Sr13* in these cultivars.

## Test of allelism

'Gundaroi' and 'Tamaroi' are sister lines from the same cross and therefore were not crossed with each other. 'Tamaroi' was crossed with 'Kalka'. One hundred and thirteen $F_2$ seedlings from the 'Kalka'/'Tamaroi' cross were tested against the Pgt pathotype 34-1,2,3,4,5,6,7. All seedlings expressed infection type IT;1- indicating presence of the same gene in these cultivars.

## DISCUSSION

Inheritance studies suggested the presence of a single dominant gene in durum cvs. 'Kalka', 'Gundaroi' and 'Tamaroi' and the genes involved were temporarily designated as *SrK*, *SrG* and *SrT*, respectively. Infection types IT;1C, IT1-N and IT12= produced by 'Gundaroi', 'Tamaroi', and 'Kalka', respectively, were similar to that produced by stem rust resistance genes *Sr9e* and *Sr13*, commonly present in durum cultivars (Luig 1983). Tests with *Sr9e* and *Sr8b*-virulent pathotypes eliminated the possibility of the presence of these genes in 'Kalka', 'Gundaroi' and 'Tamaroi' singly and suggested the possible involvement of *Sr13* or an uncharacterized resistance gene. Genetic association of the chromosome 6AL located marker gwm427 and stem rust resistance genes *SrK*, *SrG* and *SrT* indicated the involvement of this chromosome in controlling stem rust resistance in 'Kalka', 'Gundaroi' and 'Tamaroi', respectively.

Stem rust resistance genes *Sr8a*, *Sr8b*, *Sr13* and *Sr26* are also located on chromosome 6AL (McIntosh *et al.* 2008). Pathotypes used in this study were virulent on *Sr8a*. *Sr8b* produces ITX (McIntosh *et al.* 1995) and absence of expression of such infection type by parental cultivars and $F_3$ families did not support the presence of this gene in cvs. 'Kalka', 'Gundaroi' and 'Tamaroi'. Moreover, these cultivars showed low stem rust responses against the *Sr8b*-virulent Pgt pathotype 40-1,2,3,4,5,6,7,8,9,10,11 (**Table 1**). *Sr26* is derived from *Agropyron intermedium* (McIntosh *et al.* 1995) and therefore was not expected to be present in durum wheat. These results conclusively supported the presence of *Sr13* in cvs. 'Kalka', 'Gundaroi' and 'Tamaroi'.

Knott (1962) reported the presence of *Sr13* in cvs. 'Khapstein' and 'Maddhen' which were derived from 'Khapli emmer'. This gene was reported in North American durum cultivars (Luig 1983) and an Australian durum cv. 'Arrivato' (Bhavani *et al.* 2008). *Sr13* produces infection type IT2= (Knott 1989; McIntosh *et al.* 1995; Bhavani *et al.* 2008). Inconsistency in infection type was observed for *Sr13*, especially at high temperature (Roelf and Mcvey 1979). In the present study infection type exhibited by the three durum cultivars (IT;1, IT1-N and 12=) was slightly

lower than the usual infection type produced by *Sr13*-carrying genotypes (IT2= to 2-). *Sr13* imparts partial resistance; however, it is reported to provide better protection when present in combinations with other stem rust resistance genes (Bariana *et al.* 1996). It is effective against prevalent Australian Pgt pathotypes. 'Kalka', 'Gundaroi' and 'Tamaroi' produced moderately susceptible responses when tested in Kenya against Ug99 (Bariana and Park unpublished data) confirming the expression of intermediate level of resistance conditioned by *Sr13*.

Based on the genomic location through association of marker gwm427 and field stem rust response data from Kenya, we concluded the presence of *Sr13* in these cultivars. The presence of *Sr13* in parents used in the breeding of these cultivars (H.S. Bariana unpublished results) also supports this conclusion. This conclusion drew our attention to the narrow genetic base of stem rust resistance in durum cultivars. Efforts need to be directed to increase genetic diversity for stem rust resistance. A chromosome 7D located stem rust and leaf rust resistance gene combination, *Sr25/Lr19*, has been transferred to durum wheat (Zhang *et al.* 2005). Adult plant stem rust resistance detected in tetraploid landraces (Toor *et al.* 2009) will be useful in increasing genetic diversity for stem rust resistance. These genotypes are being genetically characterised. Identification of markers linked with adult plant resistance genes would ensure their pyramiding with seedling stem rust resistance genes in future durum wheat cultivars to achieve durability.

## ACKNOWLEDGEMENTS

The first author thanks the Higher Education Commission (HEC) of Pakistan for financial assistance. We thank GRDC Australia for funding through the Australian Cereal Rust Control Program.

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