Australian Journal of Crop Science AJCS
AJCS 7(5):550-554 (2013) ISSN:1835-2707

Genetic analysis of durable adult plant stripe rust resistance in durum wheat cultivars

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Abstract

The deployment of combinations of resistance genes in commercial cultivars is necessary to achieve sustained disease control. Old American durum cultivars Wells and Leeds as well as Australian cultivars Yallaroi and Wollaroi are susceptible to Australian *Puccinia striiformis* f. sp. *tritici* pathotypes 110 E143A+ and 134 E16A+ at the seedling stage. These cultivars produced low stripe rust responses at the adult plant stage since their release and therefore were chosen for genetic analysis. Genetic analyses of adult plant stripe rust resistance using Wells/Bansi and Leeds/Bansi F3 populations indicated the involvement of two independent genes each in Wells and Leeds. These genes produced intermediate adult plant stripe rust responses when present singly. Absence of segregation among F_3 lines derived from crosses of older north American cultivars Leeds and Wells with Australian cultivars Wollaroi and Yallaroi indicated the presence of common gene(s) for stripe rust resistance. Common parentage of these cultivars supported this conclusion and suggested narrow genetic base of stripe rust resistance among Australian and old American durum wheat cultivars. The effectiveness of both old and modern cultivars over two and four decades, respectively, indicated durable nature of the genes carried by these cultivars.

Keywords: **S**tripe rust, adult plant durable resistance, genetic analysis, genetic diversity.
Abbreviations: Pst_*Puccinia striiformis* f. sp. *tritici*, APR_adult plant resistance, RIL_recombinant inbred line.

Introduction

Durum wheat (*Triticum turgidum* L.) is the most widely cultivated tetraploid wheat species in the world. Although area under durum wheat cultivation is much lower than common wheat, its importance lies in the provision of diverse food products such as pasta, puffed cereals, desserts and noodles (Dick and Matsuo, 1988). The annual global production of durum wheat shows wide fluctuations due to biotic and abiotic stresses. Of biotic stresses, rust diseases caused by *Puccinia* spp. could decrease durum wheat yield significantly. Resistance to rust diseases can be expressed at the seedling or adult plant stage (Zadoks, 1961; Bariana and McIntosh et al., 1995). At present, 53 genes conferring stripe rust resistance to *P. striiformis* f. sp. *tritici* (Pst) have been catalogued (R.A. McIntosh personal communication). A majority of these genes belong to the seedling or overall resistance category. Some resistance genes express only at the post seedling stages and are referred to as adult plant resistance (APR) genes. Several stripe rust resistance genes originated from tetraploid wheats. Genes *Yr15* (Gerechter-Amitai et al. 1989), *Yr24* (McIntosh and Lagudah 2000) and *YrH52* (Peng et al. 1999) for seedling resistance and *Yr36* (Uauy et al. 2005) for APR were derived from tetraploid wheats. The stripe rust resistance gene *Yr7* was originally described in durum cultivar, Iumillo (McIntosh et al., 1995). A minor APR gene *Yr30* was introgressed from Yaroslav emmer into bread wheat along with the stem rust resistance gene *Sr2* (Singh et al., 2001). Durable adult plant stripe rust resistance genes *Yr18, Yr29,* and *Yr46* have been named in hexaploid wheats (Singh, 1992; William et al., 2003; Herrera-Foessal et al., 2011a). *Yr18* (Singh 1992) and *Yr46* (Herrera-Foessal et al., 2011a) are located in chromosomes 7D and 4D, respectively, and therefore cannot be expected in durum cultivars. *Yr29* is located in chromosome 1BL and can be present in durums. Recently, Herrera-Foessal et al., (2011b) reported the presence of *Yr29*/*Lr46* in CIMMYT durum wheat Quetru. North American durum cultivars Wells and Leeds showed adult plant resistance to stripe rust since their release in 1960 and 1966, respectively. Australian cultivars Yallaroi and Wollaroi also maintained commercially acceptable levels of stripe rust resistance since their release in 1987 and 1993, respectively. Considering the high level of resistance over a long period and similar responses of old American and modern Australian cultivars, genetic analysis of resistance in older cultivars was essential. Therefore, the present investigation was planned to understand the genetic basis of durable stripe rust resistance in Wells and Leads and to study allelic relationships of stripe rust resistance among American and Australian durum cultivars.

Results

Inheritance studies

Cultivars Wells, Leeds, Yallaroi and Wollaroi produced susceptible seedlings responses (infection type 3n to 3+) against Pst pathotypes 134 E16A+ and 110 E143A+. Cultivars Leeds, Yallaroi and Wollaroi exhibited a score of 3 at the adult plant stage, whereas Wells was scored 4 (Table 1). The susceptible parent Bansi produced a score of 9. Results of genetic analyses are discussed separately for each cross.

Wells × Bansi

The Wells/Bansi F3 population included 91 non-segregating resistant: 91 segregating: 16 non-segregating susceptible families. Chi-squared analysis of data indicated the presence of two independent genes for adult plant stripe rust resistance in Wells (Table 2). The low stripe rust response in monogenically segregating families varied from 6 to 7. The presence of a high proportion of plants with response 7 to 9 in monogenically segregating F_3 families indicated incomplete dominance of resistance.

Leeds × Bansi

Ninety six F3 families were scored non-segregating resistant, 84 segregating and 12 non-segregating susceptible (Table 2). Statistical analysis of data conformed to segregation at two genetically independent loci. Like Wells/Bansi cross, monogenically families also exhibited incomplete dominance of resistance and low stripe rust response among these families varied from 5 to 6.

Allelic relationship of stripe rust resistance - American vs. Australian durum wheats

Cultivar Wells was a parent of Leeds, which was used to breed Australian cultivar Kamilaroi. Both Yallaroi and Wollaroi carry Kamilaroi Sib in their pedigrees. Based on pedigree analysis, more than 45 years old American cultivar Wells and Leads are expected to share gene(s) for rust resistance with relatively more recent Australian cultivars Yallaroi and Wollaroi. Wells and Leeds were crossed with Yallaroi and Wollaroi in order to test this hypothesis. F_3 populations derived from crosses were screened under field conditions. Assuming the genetic independence of two APR genes carried by each cultivar (Present study; Venkata et al., 2006), tetragenic segregation would be expected among intercross progenies. Considering a population of about 200 F_3 families and 25-30 plant in each family, approximately six monogenically segregating and 16 digenically segregating families would be expected, if genes in respective parents were independent. Alternatively, homozygous susceptible segregates and monogenically segregating families will not be detected. The adult plant stripe rust responses were categorized into three classes, namely; class I (score 3), class II (score up to 4) and class III (score up to 5) (Table 3). Adult plant stripe rust response assessment results on F_3 populations from intercrosses are presented under separate headings.

Leeds × Yallaroi and Leeds × Wollaroi

Both Yallaroi and Leeds showed a low adult plant response of 3. No susceptible segregate was observed among a set of 192 F_3 families (Table 3). Of 192 families, 172 belonged to class I, 20 to class II and none of the families belonged to class III. These results implied that Yallaroi and Leeds shared gene(s) in common. Cultivar Wollaroi also exhibited a low adult plant response of 3 (Table 1). A total of 200 F_3 families belonging to its cross with Leeds were evaluated for adult plant stripe rust response segregation. Like Yallaroi/Leeds cross no susceptible segregate was observed. One hundred and fifty three families belonged to class I, 43 to class II and four to class III. These results suggested that Wollaroi and Leeds also shared at least one gene in common.

Wells × Yallaroi and Leeds × Wollaroi

Cultivars Wells and Yallaroi showed an adult plant response of 4 and 3, respectively (Table 1). F_3 families from the cross between these two cultivars did not segregate for susceptibility (Table 3). Of 184 F_3 families, 164 belonged to class I and 20 to class II indicating the involvement of common gene(s) in controlling APR to stripe rust in these cultivars. Cultivar Wollaroi exhibited a low adult plant response of 3 (Table 3). The F_3 population from the Wollaroi/Wells cross comprised of 197 families and all families produced low adult plant stripe rust scores (Table 3). One hundred and sixty three families were placed in class I and 34 families in class II. These results suggested that Wollaroi also shared gene(s) in common with Wells.

Discussion

This study demonstrated the presence of two independent genes for adult plant stripe rust resistance in cultivars Leeds and Wells. Individual components of resistance carried by Leeds and Wells conferred intermediate responses. The combined effect of two genes resulted in lower responses characteristics of the resistant parents. Venkata et al., (2006) concluded the presence of two adult plant stripe rust resistance genes in Australian durum cultivars Kamilaroi, Wollaroi and Yallaroi against Pst pathotype 134 E16A+. Ma et al. (1997) reported the presence of two genes at the seedling growth stage and at least one additional gene at the adult plant stage in CIMMYT durum wheat genotypes. Ideotype breeding and limited number of ancestors led to limited genetic diversity among durum cultivars (Autrique et al., 1996). Estimates of genetic diversity can be obtained from phenotypic polymorphism (Bar-Hen et al., 1995; Dillmann et al., 1997; Rebourg et al., 2001), detailed pedigree records (Falconer, 1989) and molecular polymorphism (Stachel et al., 2000). Segregation analysis of rust response variation among progenies of intercrosses of resistance sources can also predict the extent of diversity. Absence of susceptible segregates among crosses of Australian durum cultivars Yallaroi and Wollaroi with old American cultivars Leeds and Wells demonstrated the presence of common gene(s). Although Leeds was not crossed with Wells, the pedigree information (Table 1) suggests that Leeds and Wells could carry adult plant stripe rust resistance gene(s) in common. In addition to the common parent, Ld357, shared by Leeds and Wells, Wells was also a parent of Leeds. Venkata et al. (2006) failed to detect any susceptible segregates among F_2 populations derived from Yallaroi/Kamilaroi and Wollaroi/Kamilaroi crosses. Both Yallaroi and Wollaroi have Kamilaroi sib in their pedigree. Absence of segregation among American and Australian durum wheat intercross F_3 populations in the present study was also supported by pedigree relationships. Kamilaroi Sib, a parent of both Australian durum cultivars, was derived from the Durati Sib/Leeds cross. These results pointed to the narrow genetic base of adult plant stripe rust resistance. Similar observation was made by Ma et al. (1997). Bhavani (2006) reported the presence of two seedling stripe rust resistance genes in Australian durum cultivar Arrivato. One of the genes was proved to be *Yr24* and identity of the second gene remained unresolved. Based on adult plant stripe rust responses of F_3 families lacking seedling stripe rust resistance, he also showed the presence of an APR in Arrivato. QTL analysis of adult plant stripe rust response variation among doubled haploid or recombinant inbred line (RIL) populations from crosses studied in this investigation would determine chromosomal location of APR genes. The location of APR to stripe rust in a synthetic hexaploid was reported on chromosome 1BL (Zwart et al. 2010).

This QTL is located in the *Yr29* region. *Yr29* has also been

Table 1. Pedigree details and stripe rust responses of durum cultivars.

Cultivar	Pedigree	Adult plant response[1]
Wells	Sentry // Ld357 / Ld379 [Lakota sib]	4
Leeds	Ld357*4 // St464 / Ld357 / 3 / Wells	3
Yallaroi	Guillemot selection no. 3/ Kamilaroi Sib [Durati Sib/Leeds]	3
Wollaroi	Tam-1B-17/Kamilaroi sib//Rokel selection/Kamilaroi Sib	3
Bansi	Landrace	9

[1]based on Bariana et al. (2007) against Pst pathotype 134 E16A+ under field conditions.

Table 2. Adult plant stripe rust response distribution of F_3 families from two crosses, when inoculated with a mixture of Pst pathotypes 134E16A+ and 110 E143A+.

Cross	No of lines				$\chi^2_{7:8:1}$	Number of genes
	Non-segregating resistant	Segregating	Non-segregating susceptible	Total		
Wells x Bansi	91	91	16	198	1.93	2
Leeds x Bansi	96	84	12	192	3.21	2

Table value of χ^2 at P=0.05 and 2 d.f. is 5.99 and at P = 0.01 and 2 d.f. is 9.21.

reported in CIMMYT durum wheat Quetru (Herrera-Foessal et al. 2011b). Preliminary QTL analysis of APR to stripe rust in Wollaroi/Bansi-derived RIL population using DArT markers suggested the involvement of three chromosomes (Bansal and Bariana unpublished results). These chromosomal regions are currently being enriched using SSR markers to confirm preliminary DArT based results. Despite the fact that stripe rust resistance in cultivars included in this study showed limited genetic diversity, the effectiveness of Wells-derived resistance in Australian cultivars Yallaroi and Wollaroi for almost a decade demonstrated durability of resistance genes involved.

Materials and methods

Plant materials

Durum cultivars Wells (AUS3529 - the Australian Winter Cereals Collection accession number) and Leeds (AUS10943) were crossed with the susceptible landrace Bansi Strain 168 (AUS1866). The susceptible parent was used as pollen parent. F_{1s} were grown and harvested as single plants. F_2 seeds from a single F_1 plant in each cross were space planted 10 cm apart with the precision seeder (Nodat) under rust free conditions in the field at Liverpool Plains Field Station, Breeza, NSW, Australia. F_2 plants were carefully harvested and threshed as single plants. Australian durum cultivars Yallaroi (AUS23825) and Wollaroi (AUS25926) reported to carry two independent stripe rust resistance genes by Venkata et al., (2006). These cultivars were crossed with the American cultivars Wells and Leeds as pollen parent to study allelic relationship of stripe rust resistance carried by older American and modern Australian durum wheat cultivars. F_3 populations from a single F1 plant progenies of these crosses were developed.

Seedling rust tests

Nine centimetre diameter plastic pots were filled with a potting mixture containing 80% composted pine bark and 20% coarse sand. The pots were fertilised with a water soluble fertiliser Aquasol® at a rate of 30g/10L of water for 200 pots. After about 20 minutes seeds were sown and covered with potting mix. Pots were transferred to a microclimate room in the greenhouse maintained at 20°C. A second application of the nitrogenous fertilizer Nitram® was applied at the same rate as Aquasol® to the one week old seedlings. The Pst pathotypes 110 E143A+ (861725) and 134 E16A+ (021510) were used to assess seedling responses of durum cultivars. Inoculations were carried out on 12 day-old seedlings. Urediniospores suspended in light mineral oil (Isopar L®), at a rate of 5 mg spores per 10 ml of oil for 200 pots, were sprayed with a hydrocarbon propellant pressure pack sprayer. The inoculation equipment was thoroughly washed with 70% alcohol and rinsed under tap water to prevent any contamination between two successive inoculations. Inoculated seedlings were kept overnight in an airconditioned room at 9-12°C on trolleys filled with water and covered with plastic hoods to generate high levels of humidity. The material was then transferred to greenhouse microclimate room maintained at 17-18°C. Stripe rust responses were scored 14 days after inoculation on a 0 - 4 scale devised by Gassner and Straib in 1932 (cited from McIntosh et al., 1995), with some modifications suggested by Wellings (1986), where IT “0” = no visible signs of infection, IT “;”= necrotic flecks, IT “;N” = necrotic areas without sporulation, IT “1” = necrotic and chlorotic areas with restricted sporulation, IT “2” = moderate sporulation with necrosis and chlorosis, IT “3” = sporulation with chlorosis, IT “4” abundant sporulation without chlorosis. Infection types of 3 or higher were regarded as susceptible, whereas ITs lower than 3 were regarded as resistant.

Adult plant rust tests

F_3 families from Wells/Bansi, Leeds/Bansi, Leeds/Yallaroi, Leeds/Wollaroi, Wells/Yallaroi and Wells/Wollaroi were sown as 60 cm rows at a rate of 25-30 seeds in field. The infector rows were planted after every two experimental blocks. Parental cultivars were sown as controls. The recommended dose of fertiliser was applied to a well prepared field to provide essential nutrients for proper germination and growth. The entire field was sprayed with herbicide Glean® (chlorsulfuron) after sowing at a rate of 1g/20L of water as a pre-emergence treatment to control a range of weeds. The field was irrigated with a sprinkler irrigation system as and when required to enhance crop growth and to favour environmental conditions for rust development. The Pst pathotypes 110 E143A+ and 134 E16A+ were used for the field study. The entire experiment was inoculated with urediniospores suspended in light mineral oil Isopar L® 3440 using an ultra-low-volume applicator (Microfit®, Micron Sprayer Ltd.) three times at weekly intervals starting from the early jointing stage. Field assessments of adult plant stripe rust response variation were based on a 1-9 scale (Bariana et al., 2007), where 1 = very resistant, 2 = resistant, 3 = resistant to moderately resistant, 4 = moderately resistant, 5 = moderately resistant to

Table 3 Adult plant stripe rust response variation among F_3 lines from crosses of durum wheats against a mixture of Pst pathotype 134 E16A+ and 110 E143A+.

Cross	No. of lines			Predicted genetic ratios[a]	Partitioning of families			Total
	Non-segregating resistant	Segregating	Non-segregating susceptible		Class I[b]	Class II	Class III	
Leeds × Yallaroi	192	0	0	223:32:1	172	20	0	192
Leeds × Wollaroi	200	0	0	223:32:1	153	43	4	200
Wells × Yallaroi	184	0	0	223:32:1	164	20	0	184
Wells × Wollaroi	197	0	0	223:32:1	163	34	0	197

[a] based on presence of two independent APR genes in each cultivar

[b]Class I= Plants with response of 3 (resistant to moderately resistant), Class II = Plants with responses upto 4 (moderately resistant), Class III= Plants with responses upto 5 (moderately resistant to moderately susceptible)

moderately susceptible, 6 = moderately resistant, 7 = moderately susceptible to susceptible and 8 = susceptible and 9 = very susceptible. Rust response assessments were made when the susceptible parent Bansi exhibited a score of 9. The whole experiment was scored twice and terminal ratings were used for analyses.

Statistical analysis

F_3 families were classified as non-segregating resistant (no plant with rust response equivalent the susceptible parent), segregating (one or more plants with rust responses equivalent the susceptible parent) and non-segregating susceptible (all plants with rust responses equivalent the susceptible parent) and Chi-square analysis was used to predict the number of genes involved in controlling low stripe rust response (Table 2). The non-segregating resistant class included lines that showed adult plant stripe rust responses less than the susceptible parent. On the other hand, the segregating class had at least one plant with stripe rust response level equivalent to the susceptible parent. This class was presumed to contain monogenically and digenically segregating families. The non-segregating susceptible class included plants with response level equivalent to the susceptible parent Bansi.

Acknowledgements

The first author thanks the University of Sydney for the award of International Post Graduate Research Scholarship. We thank GRDC, Australia for financial support through the Australian Cereal Rust Control Program.

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