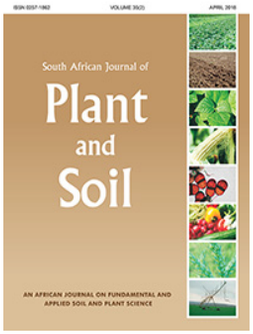

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Detection of rust resistance in selected Zimbabwean and ICARDA bread wheat (*Triticum aestivum*) germplasm using conventional and molecular techniques

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Host resistance is the most effective and economical method to minimise yield losses caused by rusts. The aim of this study was to detect the presence of resistance in 75 wheat genotypes. The presence of the genes *Sr2*, *Sr24*, *Lr34*, *Lr37*, *Lr46* and *Lr68* was investigated using simple sequence repeat and sequence tagged site markers. Quantitative aspects of resistance to leaf rust were assessed through infection response, disease severity, coefficient of infection (CI), disease incidence (DI), leaf tip necrosis (Ltn) and area under disease progress curve (AUDPC) under natural epidemics. Highly significant ($p \leq 0.001$) differences were observed among the genotypes for CI, DI, AUDPC and relative AUDPC (rAUDPC). Twenty genotypes exhibited high levels of adult-plant resistance, showing CI less than 20% and AUDPC less than 300%, with moderately susceptible to susceptible reactions. The most frequently occurring gene was *Lr46* (21%), followed by *Lr68* (20%), *Lr34* (19%), *Lr37* (11%), and *Sr24* (0%). Selection for *Lr34* and *Lr46* based on Ltn alone can sometimes be misleading because of its variable expression in different genetic backgrounds. Cultivars grown in Zimbabwe lacked important rust resistance genes.

Keywords: rust, rust resistance genes, sequence tagged site, simple sequence repeat, wheat germplasm

Introduction

Herrera-Foessel et al. (2012) highlighted three foliar rust diseases caused by *Puccinia graminis* Pers. f. sp. *tritici* (stem rust), *Puccinia triticina* Eriks. (leaf rust) and *Puccinia striiformis* Westend f. sp. (yellow or stripe rust) as the most important biotic constraints to wheat production in the world. Leaf rust and stem rust are capable of causing yield losses of up to 60% and 100%, respectively, under severe conditions (Park 2007).

In Zimbabwe, leaf rust is present in all of the wheat-growing areas, and stem rust is common in the lowveld region (Havazvidi 2008; Mutari et al. 2009, 2010, 2011, 2012). The generation of rust-resistant genotypes and their cultivation is the most effective, economic and environmentally sound method to minimise yield losses caused by fungal diseases (Singh et al. 2005; Herrera-Foessel et al. 2012). Lagudah (2011) proposed the use of slow-rusting adult-plant resistance (APR) genes. Most of the slow-rusting resistance genes, such as *Lr34*, *Sr2* and *Lr46*, have a pleiotropic association with multiple disease resistance genes, making them very valuable in breeding programs (Singh 1992a, 1992b; Mago et al. 2005; Lagudah et al. 2009).

Leaf tip necrosis (Ltn), a morphological marker that is linked with APR genes (*Lr34*, *Lr46* and *Lr67*), has been used by many researchers in predicting the presence of APR genes despite its limitations (Tiwari et al. 2008; Sivasamy et al. 2014). However, the selection of genotypes containing a combination of different rust resistance genes using conventional methods is very time consuming (Mahwish et al. 2012; Parveen et al. 2014). Therefore, it is necessary to complement the evaluation of genotypes for rust resistance genes in the field with molecular characterisation.

In Zimbabwe, most of the old and current commercial wheat cultivars grown are now susceptible to the current races of leaf and stem rust pathogens, although no severe epidemics have been observed previously (Mutari et al. 2009, 2010; Mukoyi et al. 2011; Mutari et al. 2011, 2012; Pretorius et al. 2015). Furthermore, little research has been done so far with respect to evaluation of APR in wheat genotypes. Previous studies done by Pretorius et al. (2015) on some breeding lines and cultivars from Zimbabwe focused on only *Lr34* and *Lr19*.

Therefore, the objectives of the present study were (1) to assess the occurrence of simple sequence repeat (SSR) and sequence tagged site (STS) markers associated with the rust resistance genes *Sr2/Yr30*, *Sr24/Lr24*, *Lr34/Yr18/Sr57*, *Lr37/Sr38/Yr17*, *Lr46/Yr29/Pm39/Sr58* and *Lr68* in advanced breeding lines and wheat cultivars of Zimbabwe; (2) to assess the reliability of SSR and STS markers in predicting the presence of the rust resistance genes *Sr2/Yr30*, *Sr24/Lr24*, *Lr34/Yr18/Pm38/Sr57*, *Lr37/Sr38/Yr17*, *Lr46/Yr29/Pm39/Sr58* and *Lr68* in diverse wheat genotypes; (3) to assess the response of wheat genotypes to natural rust infection; and (4) to assess the reliability of Ltn in predicting the presence of APR genes in diverse wheat genotypes.

Materials and methods

Plant materials and experimental sites

The fieldwork was conducted in 2014 and 2015 at the Save Valley (SVES; 20°48′ S, 33°60′ E; 450 m above sea level [asl]) and Chisumbanje (CES; 20°80′ S, 32°50′ E; 413 m asl) Experimental Stations. Both sites are traditional hot spots for leaf rust disease (Mutari et al. 2009, 2010, 2011, 2012). The molecular study was conducted at the biotechnology laboratory of ICARDA, Morocco, in 2015. Seventy-five genotypes were used in the study (Table 1). A 15 × 5 alpha lattice design with two replications was used. The genotypes were planted in two row plots measuring 1 m in length with inter-row spacing of 0.25 m. Spreader rows of the rust-susceptible genotype 'Morocco' were planted perpendicular to the rows of all entries and around the field.

Phenotypic characterisation for adult plant resistance

Partial resistance behaviour of wheat genotypes was assessed through the infection response (at the adult plant stage), coefficient of infection (CI), area under disease progress curve (AUDPC), relative area under disease progress curve (rAUDPC), disease incidence (DI) and leaf tip necrosis (Ltn). The modified Cobbs' scale by Peterson et al. (1948) was used to record disease severity after the onset of uniform infections in Morocco at 10-day intervals. Five disease severity readings were recorded from 10 pre-tagged plants from each plot per replication. The infection response at the adult plant stage was scored as described by Roelfs et al. (1992).

The infection responses were converted into numeric constant values as described by Roelfs et al. (1992). The CI was calculated in accordance with Roelfs et al. (1992). Disease incidence was calculated as the proportion of infected plants to the total number of plants assessed from each genotype. The AUDPC based on disease severity over time was then calculated for all genotypes using the formula of Jeger and Viljanen-Rollinson (2000).

The rAUDPC was calculated as follows:

$$\text{rAUDPC} = \frac{\text{AUDC of the genotype}}{\text{AUDPC of susceptible genotype ('Morocco')}} \times 100$$

Scores for Ltn were recorded at the soft dough stage to postulate the presence of APR genes using the following scale: 0 = absent, 1 = low, 2 = moderate and 3 = strong (Sivasamy et al. 2014).

Marker genotyping

A total of 75 genotypes were used (Table 1). The controls were as follows: (1) *Lr37* – Stylet (positive) (Kuchel et al. 2007) and Pavon 76 (negative), (2) *Lr46* – Pavon 76 and Parula (positive) (Singh et al. 1998; Herrera-Foessel et al. 2012) and Stylet and Morocco (negative) (Kuchel et al. 2007), (3) *Lr68* – Parula (positive) (Herrera-Foessel et al. 2012) and Stylet (negative), (4) *Sr2* – Parula and Annuello (positive) (Kuchel et al. 2007; Herrera-Foessel et al. 2012) and Stylet (negative), (5) *Sr24* – Annuello (positive) and Parula (negative) and (6) *Lr34* – Parula (positive) (Herrera-Foessel et al. 2012) and Stylet (negative).

The following markers were used for molecular characterisation: Ventriup-LN2 (Helguera et al. 2003); Sr24#50 (Mago et al. 2005); Xgwm-533 (Spielmeyer et al. 2003); Xgwm-44 (Suenaga et al. 2003); csLV34 (Lagudah et al. 2006) and csGS (Herrera-Foessel et al. 2012). The sequences and other information on the primers are listed in Table 2.

DNA was isolated from each of the 75 genotypes (three-week-old plants) using the cetyltrimethylammonium bromide method as described by Khan et al. (2004). The PCRs were carried out in a 96-well automated thermal cycler (Applied Biosystems 2720). The amplification reaction profiles for the markers Xgwm-533, Xwmc-44, csLV34, Ventriup-LN2, csGS and Sr24#50 were as described by Spielmeyer et al. (2003), Suenaga et al. (2003), Lagudah et al. (2006), Helguera et al. (2003), Herrera-Foessel et al. (2012) and Mago et al. (2005), respectively. The banding patterns were viewed and photographed using a gel documentation system (Bio-Rad Molecular Gel Doc™ XR+).

Table 1: Codes, names, pedigrees and status of the wheat genotypes used in the study. N/A = information not available, ZIMBABWE = sourced from the Crop Breeding Institute in Zimbabwe, ICARDA = sourced from the International Center for Agricultural Research in the Dry Areas (ICARDA) in Egypt

Code	Genotype	Pedigree	Status
G1^{ZIMBABWE}	Dande	CAR422-ANA/SERI//L1555-6/VEE'S-THB'S'	Commercial cultivar
G2^{ZIMBABWE}	Kame	S86481-10H-OH-1C-OH/S89067-OH-OG-7H-OG	Commercial cultivar
G3^{ZIMBABWE}	Kana	FLY CATCHER/S78224//F84042 (BJY/JUP)/F82022(F12.71/COC75)	Commercial cultivar
G4^{ZIMBABWE}	Insiza	VEE'S/SENGWA RES.2	Commercial cultivar
G5^{ZIMBABWE}	Ncema	F01028/SC NDUNA	Commercial cultivar
G6^{ZIMBABWE}	SC Sky	(Nata/W31/89)/(SERI*4//AGA/6*YR/3/SERI)	Commercial cultivar
G7^{ZIMBABWE}	PAN3492	N/A	Commercial cultivar
G8^{ZIMBABWE}	S02006	F01046/INSIZA	Breeding line
G9^{ZIMBABWE}	S02147	S98066-7H-0G-1H-ON/F99012	Breeding line
G10^{ZIMBABWE}	SC Stallion	CP1509/W137.6.3	Commercial cultivar
G11^{ZIMBABWE}	SC Select	N/A	Commercial cultivar
G12^{ZIMBABWE}	SC Smart	NATA/W31/89	Commercial cultivar
G13^{ICARDA}	Attila -7	ND/VG9144//KAL/BB/3/YACO/4/VEE#5	Breeding line
G14^{ICARDA}	Hijee	SAKER/5/RBS/ANZA/3/KVZ/HYS//YMH/TOB/4/BOW'S'/6/PEWIT3/7/ATENA-1	Breeding line
G15^{ICARDA}	Tevee	TEVEE'S'/3T.AEST/SPRW'S'//CA8055/4/PASTOR-2/5/SUNBRI	Breeding line

Table 1: (cont.)

Code	Genotype	Pedigree	Status
G16[ICARDA]	Kadar-1	KADAR-1/4/VAN'3'/CNDR'S'/ANA//CNDR'S'/MUS'S'/5/SOMAMA-3/1356	Breeding line
G17[ICARDA]	Aguilal	AGUILAL/FLAG-3	Breeding line
G18[ICARDA]	Achatar-3	ACHTAR*3//KANZ/KS85-8-4/3/MON'S'/ALD'S'//BOW'S'	Breeding line
G16[ICARDA]	Kadar-1	KADAR-1/4/VAN'3'/CNDR'S'/ANA//CNDR'S'/MUS'S'/5/SOMAMA-3/1356	Breeding line
G17[ICARDA]	Aguilal	AGUILAL/FLAG-3	Breeding line
G18[ICARDA]	Achatar-3	ACHTAR*3//KANZ/KS85-8-4/3/MON'S'/ALD'S'//BOW'S'	Breeding line
G19[ICARDA]	Soonot-10	SAMAR-8/KAUZ'S'//CHAM-4/SHUHA'S	Breeding line
G20[ICARDA]	Sanobar-4	SHA3/SERI//YANG87-142/3/2*TOWPE	Breeding line
G21[ICARDA]	Reyna-28	SAMAR-8/KAUZ'S'//CHAM-4/SHUHA'S	Breeding line
G22[ICARDA]	Fanoos-14	N/A	Breeding line
G23[ICARDA]	Durra-1	FOWS'S'//NS732/HER/3/CHAM-6//GHURAB'S'	Breeding line
G24[ICARDA]	Durra-5	FOWS'S'//NS732/HER/3/CHAM-6//GHURAB'S'	Breeding line
G25[ICARDA]	Marchnough	MARCHOUCH8/5/KAUZ/3MYNA/VUL//BUC/FLK/4/MILAN	Breeding line
G26[ICARDA]	Achatar	ACHTAR*3//KANZ/KS85-8-4/3/ATILA-17/4/MON'S'/ALD'S'//ALDA'S'/IAS58	Breeding line
G27[ICARDA]	Sandal-3	CLEMENT/ALD'S'//ZARZOUR/5/AU//KAL/BB/3/BON/4/KVZ//CNO/PJ6	Breeding line
G28[ICARDA]	Kadar	N/A	Breeding line
G29[ZIMBABWE]	F016-61	N/A	Breeding line
G30[ZIMBABWE]	F016-64	N/A	Breeding line
G31[ZIMBABWE]	F016-67	N/A	Breeding line
G32[ZIMBABWE]	F016-68	N/A	Breeding line
G33[ZIMBABWE]	F016-70	N/A	Breeding line
G34[ZIMBABWE]	F016-71	N/A	Breeding line
G35[ZIMBABWE]	F016-72	N/A	Breeding line
G36[ZIMBABWE]	33ES-F12-17	ATTILA*2/PBW65*2//W485/HD29	Breeding line
G37[ZIMBABWE]	33ES-F12-18	ATTILA*2/PBW65*2/4/BOW/NKT//CBRD/3/CBRD	Breeding line
G38[ZIMBABWE]	S09020	N/A	Breeding line
G39[ZIMBABWE]	S09048	S01072-8H-ON-2H-ON/DANDE	Breeding line
G40[ZIMBABWE]	33ES-F12-13	PBW343*KUKUNA*2//FRJL/PIFED	Breeding line
G41[ZIMBABWE]	33ES-F12-02	ND/VGI1944//KAL//BB/3/YACO'S'/4/VEE#5'S' (PBW343)	Breeding line
G42[ZIMBABWE]	20SA-F12-24	N/A	Breeding line
G43[ZIMBABWE]	S09922	S04281-1H-ON-1H-ON/S02213-7H-ON-2H-ON	Breeding line
G44[ZIMBABWE]	33ES-F12-15	ATTILA*2/PBW65*2//MURGA	Breeding line
G45[ZIMBABWE]	S04020	N/A	Breeding line
G46[ZIMBABWE]	S06051	DANDE/NDUNA	Breeding line
G47[ZIMBABWE]	S03195	N/A	Breeding line
G48[ZIMBABWE]	S06073	KANA/NDUNA	Breeding line
G49[ZIMBABWE]	F07023	N/A	Breeding line
G50[ZIMBABWE]	S04280	N/A	Breeding line
G51[ZIMBABWE]	S03196	N/A	Breeding line
G52[ZIMBABWE]	S05003	S97003-2H-OG-2H-OG/SO1044-1H-ON-2H-ON	Breeding line
G53[ZIMBABWE]	S05004	S01008-12H-ON-1H-ON/S00123-6H-ON-1H-ON	Breeding line
G54[ZIMBABWE]	S06038	S02147-3H-ON-2H-ON/KANA	Breeding line
G55[ZIMBABWE]	F016-57	N/A	Breeding line
G56[ZIMBABWE]	F016-59	N/A	Breeding line
G57[ZIMBABWE]	F016-60	N/A	Breeding line
G58[ZIMBABWE]	F06-62	N/A	Breeding line
G59[ZIMBABWE]	F016-65	N/A	Breeding line
G60[ZIMBABWE]	F016-66	N/A	Breeding line
G61[ZIMBABWE]	F016-69	N/A	Breeding line
G62[ZIMBABWE]	F016-94	N/A	Breeding line
G63[ZIMBABWE]	F016-95	N/A	Breeding line
G64[ZIMBABWE]	F016-96	N/A	Breeding line
G65[ZIMBABWE]	F016-97	N/A	Breeding line
G66[ZIMBABWE]	F016-98	N/A	Breeding line
G67[ZIMBABWE]	F016-99	N/A	Breeding line
G68[ZIMBABWE]	F016-100	N/A	Breeding line
G69[ZIMBABWE]	F016-101	N/A	Breeding line
G70[ZIMBABWE]	S05005	N/A	Breeding line
G71[ZIMBABWE]	SC Shield	SERI*4//AGA/6*YR/3/SERI	Commercial cultivar
G72[ZIMBABWE]	S03197	N/A	Breeding line
G73[ZIMBABWE]	SC Sahai	N/A	Commercial cultivar
G74[ZIMBABWE]	SC Nduna	E16(IN)TP88(1994)	Commercial cultivar
G75[ZIMBABWE]	Morocco	N/A	Susceptible check

Table 2: List of markers associated with various leaf and stem rust resistance genes used in the present study. SSR = simple sequence repeat, STS = sequence tagged site

Gene	Marker name	Marker type	Locus (cM)	Primer sequence	DNA marker reference
Sr2	Xgwm-533	SSR	3BS (2)	5′-AAGGCGAATCAAACGGAATA-3′ 5′-GTTGCTTTAGGGGAAAAGCC-3′	Spielmeyer et al. (2003)
Sr24	Sr24#50	STS	3DL	5′-CCCAGCATCGGTGAAAGAA-3′ 5′-ATGCGGAGCCTTCACATTTT-3′	Mago et al. (2005)
Lr34	csLV34	STS	7DS (0.4)	5′-GTTGGTTAAGACTGGTGATGG-3′ 5′-TGCTTGCTATTGCTGAATAGT-3′	Lagudah et al. (2006)
Lr37	Ventriup-LN2	STS	2AS	5′-AGGGGCTACTGACCAAGGCT-3′ 5′-TGCAGCTACAGCAGTATGTACACAAAA-3′	Helguera et al. (2003)
Lr46	Xwmc-44	SSR	1BL (5.6)	5′-GGTCTTCTGGGCTTTGATCCTG-3′ 5′-GTTGCTAGGGACCCGTAGTGG-3′	Suenaga et al. (2003)
Lr68	csGS	STS	7BL (1.2)	5′-AAGATTGTTCACAGATCCATGTCA-3′ 5′-GAGTATTCCGGCTCAAAAAGG-3′	Herrera-Foesselet al. (2012)

Data analysis

Statistical analysis of field data

Analysis of variance (ANOVA) was performed on DI, CI, AUDPC and rAUDPC per season or site using the Genstat® Discovery 14th Edition statistical software package (VSN International, Hemel Hempstead, UK).

SSR and STS marker analysis

The Power Marker 3.2.5 software (Liu and Muse 2005) was used for cluster analysis. The clusters were visually depicted by means of a dendogram. A similarity matrix of 75 wheat genotypes was computed based on Nei's (1973) genetic distances and used to construct a dendrogram with the unweighted pair group method using the arithmetic average (UPGMA) clustering algorithm.

Results

Phenotypic characterisation of rust resistance in the field

Stem rust was not observed during the two seasons of evaluation. During the 2014 season, 19 genotypes (5MR to 80MR) and four genotypes (5R to 10R) exhibited resistance to leaf rust (Table 3). During the same season, 17 genotypes (rated 0) were immune and two genotypes (G43 and G68) exhibited trace-resistant reactions (Tr-R) to leaf rust infection (Table 3). The remaining 17 genotypes were susceptible to leaf rust (5MS to 100S).

In 2015, 17 genotypes (5MR to 80MR) and eight genotypes (5R to 20R) showed resistance to leaf rust (Table 3). Fourteen genotypes (all rated 0) were immune to leaf rust infection during the same season. The remaining 35 genotypes were susceptible to leaf rust (5MS to 100S), with the susceptible check (Morocco) recording the highest severity. Twelve genotypes exhibited an immune reaction (0) to leaf rust during both seasons of evaluation (Table 3).

Results of ANOVA revealed highly significant differences ($p < 0.001$) among the genotypes with respect to DI, AUDPC, rAUDPC and CI during the 2014 and 2015 seasons (Table 3). The CI values for leaf rust ranged from 0 to 100 (G75) during the 2014 season (Table 3). During the 2015 winter season, CI values ranged from 0 to 100 (G75). The AUDPC values ranged from 0 to 2 400 (G75) during the 2014 season (Table 3). During the 2015 winter season, the AUDPC values ranged from 0 to 2 100 (G75). Based on the rAUDPC, the wheat genotypes were categorised into four distinct groups (rAUDPC values of 0%, >0% ≤ 30%, >30% ≤ 70%) (Table 3).

During the 2014 and 2015 seasons, DI ranged from 0% to 100%, with the genotypes G1, G2, G36, G62 and G69 recording the highest DI in 2015 (Table 3). The wheat genotypes showed variable expression with respect to the presence of the phenotypic marker Ltn, ranging from absent (50 genotypes), weak (11 genotypes), moderate (six genotypes) to strong (eight genotypes) (Table 3).

Molecular confirmation of the presence/absence of leaf and stem rust resistance genes

The occurrence of leaf and stem rust resistance genes in the evaluated germplasm is shown in Table 3. The *Sr2* marker data were not reliable because the *Sr2* gene was present in the negative control (Stylet). Therefore, the *Sr2* marker data were not used for interpretation of results. Generally, there was a low frequency of *Lr34* (19%), *Lr46* (21%), *Lr68* (20%) and *Lr37* (11%) genes in the assessed germplasm. The *Sr24* (0%) gene was completely absent from the wheat genotypes. The efficiency of the different markers in predicting the presence of rust resistance genes in a set of genotypes postulated to possess *Lr34*, *Sr2*, *Lr37*, *L46*, *Lr68* and *Sr24* based on published work of other researchers is shown in Table 4. Figures 1 and 2 are examples of gel electrophoresis results of amplification using different SSR and STS markers, respectively.

The highest number of genes (three) was observed in G44 (*Lr34, Lr46* and *Lr68*) and G63 (*Lr34, Lr46* and *Lr37*). In case of the leaf rust resistance gene *Lr37*, the dominant STS marker Ventriup-LN2 produced a specific band of 199 bp in the positive control (Stylet) and in eight genotypes (Table 3). This marker accurately predicted the presence of *Lr37* in 100% of the genotypes postulated to possess the gene (Table 4). The SSR marker Xwmc-44, which is linked to *Lr46/Yr30/Pm39/Sr58*, amplified a 242 bp fragment in the positive controls Parula and Pavon 76, and in 14 genotypes (Table 3). This marker accurately predicted the presence and absence of *Lr46* in 100% of the genotypes postulated to possess the gene (Table 4). Fifteen genotypes, including the positive control (Parula), showed the presence of the

Table 3: Quantitative aspects of resistance to rusts in 75 bread wheat genotypes. * = Control, ** = susceptible check and control, S = susceptible, MR = moderately resistant, MS = moderately susceptible, I = immune, R = resistant, TR = trace to resistant, DS = disease severity, CI = coefficient of infection, AUDPC = area under disease progress curve, rAUDPC = relative area under disease progress curve, DI = disease incidence, Ltn = leaf tip necrosis. Means followed by the same superscript letter are not significantly different (P = 0.05). Parula[a] = genes present (*Sr2*, *Lr34*, *Lr46*, *Lr68*), Annuella[b] = genes present (*Sr2*, *Lr34*, *Lr46*, *Sr24*), Stylet[c] = gene present (*Lr37*), Pavon 76[d] = genes present (*Sr2*, *Lr46*), G75[e] = genes present (none), NT = not tested, NA = no amplification, + indicates presence of the gene, – indicates absence of the gene

Genotype	DS		CI		AUDPC		rAUDPC (%)		DI (%)		Ltn	*Lr34*	*Lr46*	*Sr2*	*Lr68*	*Sr24*	*Lr37*
	2014	2015	2014	2015	2014	2015	2014	2015	2014	2015	2015						
Parula[a]*	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	+	+	+	+	–	–
Annuello[b]*	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	+	+	NT	+	–
Stylet[c]*	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	–	–	+	–	–	+
Pavon 76[d]*	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	+	+	NT	–	–
G1	60S	80S	60^{ef}	80^{ef}	1 325^{klmn}	1 700^{s}	55.4^{klmn}	80.9^{t}	95^{ik}	100^{o}	0	–	–	+	–	–	–
G2	40S	60S	40^{cde}	60de	1 050^{ij}	1 450^{qr}	43.7^{ij}	69.1^{rs}	75^{hi}	100^{o}	0	–	–	+	–	–	–
G3	80MR	10MS	32^{bcd}	8^{ab}	700^{g}	425^{hi}	28.7^{fg}	20.2^{hi}	35de	90^{mno}	0	–	+	+	–	–	–
G4	60MR	5MS	24^{abcd}	4^{a}	500^{f}	225^{ef}	20.5^{ef}	10.7^{efg}	8^{ab}	55^{ghi}	1	–	–	+	+	–	–
G5	20S	10S	20^{abc}	10^{ab}	750^{g}	400^{ghi}	31.4^{gh}	19.0^{hi}	95^{ik}	95no	1	–	–	+	–	–	–
G6	60S	5S	60^{ef}	5^{a}	1 500^{nop}	225^{ef}	62.8^{mno}	10.7^{efg}	45^{ef}	75^{iklm}	0	–	–	+	–	–	–
G7	80S	20S	80^{fg}	20^{abc}	1 500^{nop}	850^{lmn}	62.8^{mno}	40.5^{mno}	90^{ik}	75^{iklm}	0	NA	–	+	–	–	–
G8	10MS	40MR	8^{ab}	16^{abc}	400^{ef}	300^{fg}	16.8^{de}	14.6^{gh}	15^{bc}	60^{ghij}	0	–	NA	+	+	–	–
G9	0d	5MR	0^{a}	2^{a}	0^{a}	30^{ab}	0.0^{a}	1.4^{ab}	0^{a}	75^{iklm}	0	+	–	+	+	–	–
G10	5MS	60MR	4^{a}	24^{abc}	200^{bcd}	400^{ghi}	8.4^{abcd}	19.3^{hi}	20^{bcd}	15^{abc}	2	–	+	+	–	–	–
G11	10MR	20Re	4^{a}	4^{a}	100^{abc}	125^{bcde}	4.3^{ab}	6.0^{bcdef}	75^{hi}	55^{ghi}	3	+	–	+	+	–	–
G12	20S	10S	20^{abc}	10^{ab}	800^{gh}	450^{hij}	33.6^{gh}	21.5^{ij}	55^{fg}	95no	2	–	–	+	–	–	–
G13	40S	80MS	40^{cde}	64de	1 250^{kl}	950no	52.3^{jkl}	45.2^{op}	95^{ik}	45^{efg}	0	–	–	+	–	–	–
G14	5S	40MS	5^{a}	32^{bc}	200^{bcd}	800^{lm}	8.4^{abcd}	38.3^{mn}	95^{ik}	15^{abc}	1	–	–	+	–	–	–
G15	20S	40S	20^{abc}	40^{cd}	800^{gh}	1 150^{p}	33.6^{gh}	55.0^{q}	35de	95no	0	–	–	+	–	–	–
G16	10MS	40S	8^{ab}	40^{cd}	400^{ef}	1 350^{q}	16.6^{de}	64.6^{r}	65^{gh}	75^{iklm}	0	–	–	+	–	–	–
G17	10MS	20S	8^{ab}	20^{abc}	400^{ef}	850^{lmn}	16.8^{de}	40.5^{mno}	75^{hi}	90^{mno}	1	+	–	+	–	–	–
G18	5MR	60MR	2^{a}	24^{abc}	50^{ab}	400^{ghi}	2.0^{ab}	19.3^{hi}	15^{a}	70^{ijkl}	3	–	–	+	–	–	+
G19	10MR	5MS	4^{a}	4^{a}	200^{bcd}	200^{def}	8.3^{abcd}	9.6^{defg}	35de	45^{efg}	0	–	–	+	+	–	–
G20	0	5R	0^{a}	1^{a}	0^{a}	30^{ab}	0.0^{a}	1.4^{ab}	0^{a}	20^{bcd}	1	–	–	+	–	–	–
G21	60MS	40MR	48de	16^{abc}	1 250^{kl}	225^{ef}	52.1^{jkl}	10.8^{fg}	95^{ik}	100^{o}	0	–	NA	+	–	–	–
G22	40MR	20MS	16^{abc}	16^{abc}	500^{f}	550^{jk}	20.7^{ef}	26.4^{jk}	55^{fg}	55^{ghi}	0	–	–	+	+	–	–
G23	0	0	0^{a}	0^{a}	0^{a}	0^{a}	0.0^{a}	0.0^{a}	0^{a}	0^{a}	0	–	–	+	–	–	+
G24	0	0	0^{a}	0^{a}	0^{a}	0^{a}	0.0^{a}	0.0^{a}	0^{a}	0^{a}	0	+	–	+	+	–	–
G25	10MR	5MR	4^{a}	2^{a}	100^{abc}	30^{ab}	4.1^{ab}	1.4^{ab}	15^{bc}	50^{fgh}	1	–	NA	+	–	–	+
G26	40S	60S	40^{cde}	60de	1 300^{klm}	1 650^{s}	54.4^{klm}	78.9^{t}	90^{ik}	85^{lmno}	0	–	–	+	–	–	–
G27	80MR	10MS	32^{bcd}	8^{ab}	400^{ef}	375^{ghi}	16.7^{de}	17.8^{hi}	65^{gh}	34de	0	–	–	+	+	–	–
G28	40MS	20MR	32^{bcd}	8^{ab}	1 200^{jk}	125^{bcde}	50.3^{jk}	6.0^{bcdef}	95^{ik}	15^{abc}	0	–	–	+	+	–	–
G29	20MS	10MR	16^{abc}	4^{a}	800^{gh}	125^{bcde}	35.6^{ghi}	5.9^{bcdef}	70^{h}	5^{ab}	0	–	–	+	–	–	+
G30	0	10R	0^{a}	2^{a}	0^{a}	100^{abcd}	0.0^{a}	4.9^{abcde}	0^{a}	25^{cd}	0	+	+	+	–	–	–
G31	0	0	0^{a}	0^{a}	0^{a}	0^{a}	0.0^{a}	0.0^{a}	0^{a}	0^{a}	3	–	–	+	–	–	–
G32	60MR	10MS	24^{abcd}	8^{ab}	350^{def}	425^{hi}	14.5^{cde}	20.2^{hi}	95^{ik}	95no	0	–	+	+	–	–	–
G33	40MS	10MR	32^{bcd}	4^{a}	1 050^{ij}	225^{ef}	43.9^{ij}	10.8^{fg}	25^{cd}	70^{ijkl}	2	–	–	+	–	–	+
G34	0	10R	0^{a}	2^{a}	0^{a}	55^{ab}	0.0^{a}	2.6^{abc}	0^{a}	5^{ab}	0	–	–	+	+	–	+
G35	5MR	10MR	2^{a}	4^{a}	50^{ab}	130^{bcde}	2.0^{ab}	6.3^{bcdef}	55^{fg}	35^{def}	0	–	+	+	+	–	–
G36	20MR	40MS	8^{ab}	32^{bc}	125^{abc}	1 050^{op}	5.2^{ab}	50.0^{pq}	85^{ij}	100^{o}	2	–	+	+	–	–	–
G37	80MS	40S	64^{ef}	40^{cd}	1 450^{mnop}	1 500^{r}	60.6^{lmno}	71.4^{s}	100^{k}	70^{ijkl}	1	–	–	+	–	–	–
G38	20S	100MS	20^{abc}	80^{ef}	800^{gh}	1 125^{p}	35.6^{ghi}	53.8^{q}	100^{k}	90^{mno}	0	–	–	+	–	–	–
G39	10R	0	2^{a}	0^{a}	50^{ab}	0^{a}	2.1^{ab}	0.0^{a}	8^{ab}	0^{a}	0	–	–	+	–	–	–
G40	0	0	0^{a}	0^{a}	0^{a}	0^{a}	0.0^{a}	0.0^{a}	0^{a}	0^{a}	0	–	–	+	–	–	–
G41	60MS	20S	48de	20^{abc}	1 600^{pq}	850^{lmn}	67.0^{op}	40.5^{mno}	45^{ef}	100^{o}	0	–	–	+	–	–	–
G42	10MS	40MR	8^{ab}	16^{abc}	400^{ef}	350^{gh}	16.8^{de}	16.8^{hi}	65^{gh}	65^{hijk}	0	–	NA	+	–	–	–
G43	Tr–R	5R	1^{a}	1^{a}	25^{ab}	30^{ab}	1.1^{a}	1.4^{ab}	15^{bc}	80^{klmn}	0	–	–	+	–	–	–
G44	0	0	0^{a}	0^{a}	0^{a}	0^{a}	0.0^{a}	0.0^{a}	0^{a}	0^{a}	1	+	+	+	+	–	–
G45	80MR	40MS	32^{bcd}	32^{bc}	400^{ef}	775^{l}	16.7^{de}	36.9^{lm}	55^{fg}	90^{mno}	3	–	+	+	–	–	–
G46	80S	60S	80^{fg}	60de	1 750^{q}	1 850^{t}	73.1^{p}	88.2^{u}	95^{ik}	85^{lmno}	1	–	–	+	–	–	–
G47	5R	0	1^{a}	0^{a}	50^{ab}	0^{a}	2.0^{ab}	0.0^{a}	15^{bc}	0^{a}	1	–	–	+	–	–	–
G48	0	0	0^{a}	0^{a}	0^{a}	0^{a}	0.0^{a}	0.0^{a}	0^{a}	0^{a}	0	–	–	+	–	–	–
G49	100S	20S	100^{g}	20^{abc}	2 200^{r}	900^{mn}	92.0^{qr}	43.0^{no}	95^{ik}	75^{iklm}	0	–	–	+	–	–	–
G50	60MS	10S	48de	10^{ab}	1 550^{op}	450^{hij}	64.6^{nop}	21.5^{ij}	90^{ik}	100^{o}	0	–	–	+	–	–	–
G51	80S	60S	80^{fg}	60de	2 200^{r}	1 650^{s}	92.0^{qr}	78.6^{t}	45^{ef}	85^{lmno}	0	–	–	+	–	–	–
G52	0	10R	0^{a}	2^{a}	0^{a}	55^{ab}	0.0^{a}	2.6^{abc}	0^{a}	35^{def}	0	–	–	+	–	–	–

Table 3: (cont.)

Genotype	DS		CI		AUDPC		rAUDPC (%)		DI (%)		Ltn	*Lr34*	*Lr46*	*Sr2*	*Lr68*	*Sr24*	*Lr37*
	2014	2015	2014	2015	2014	2015	2014	2015	2014	2015	2015						
G53	20MR	20MS	8^{ab}	16^{abc}	250^{cde}	475^{ij}	10.5^{bcd}	22.6^{ij}	45^{ef}	65^{hijk}	0	–	+	+	–	–	–
G54	80S	40S	80^{fg}	40^{cd}	$1\,550^{op}$	$1\,500^{r}$	64.8^{op}	71.8^{s}	65^{gh}	85^{lmno}	3	–	–	+	–	–	–
G55	5MR	10R	2^{a}	2^{a}	55^{ab}	75^{abc}	2.3^{ab}	3.6^{abc}	85^{ij}	45^{efg}	0	+	+	+	–	–	–
G56	20MS	20MR	16^{abc}	8^{ab}	800^{gh}	15^{cde}	33.2^{gh}	8.3^{cdef}	35^{de}	55^{ghi}	2	–	–	+	–	–	+
G57	60S	20S	60^{ef}	20^{abc}	$1\,400^{lmno}$	900^{mn}	58.6^{klmno}	43.0^{no}	75^{hi}	100^{o}	0	–	–	+	–	–	–
G58	0	0	0^{a}	0^{a}	0^{a}	0^{a}	0.0^{a}	0.0^{a}	0^{a}	0^{a}	0	–	–	+	–	–	–
G59	5MR	10MR	2^{a}	4^{a}	35^{ab}	225^{ef}	1.4^{ab}	10.8^{fg}	100^{k}	95^{no}	0	+	+	+	–	–	–
G60	0	0	0^{a}	0^{a}	0^{a}	0^{a}	0.0^{a}	0.0^{a}	0^{a}	0^{a}	3	–	–	+	–	–	–
G61	20R	5MR	4^{a}	2^{a}	150^{abc}	55^{ab}	6.2^{abc}	2.6^{abc}	55^{fg}	15^{abc}	0	–	–	+	–	–	–
G62	5S	20S	5^{a}	20^{abc}	180^{abcd}	850^{lmn}	7.6^{abcd}	40.7^{mno}	65^{gh}	100^{o}	0	–	–	+	–	–	–
G63	0	0	0^{a}	0^{a}	0^{a}	0^{a}	0.0^{a}	0.0^{a}	0^{a}	0^{a}	0	+	+	+	–	–	+
G64	0	0	0^{a}	0^{a}	0^{a}	0^{a}	0.0^{a}	0.0^{a}	0^{a}	0^{a}	1	+	+	+	–	–	–
G65	10MR	40MR	4^{a}	16^{abc}	105^{abc}	375^{ghi}	4.4^{ab}	18.1^{hi}	45^{ef}	45^{efg}	3	–	NA	+	–	–	–
G66	0	0	0^{a}	0^{a}	0^{a}	0^{a}	0.0^{a}	0.0^{a}	0^{a}	0^{a}	0	–	–	+	–	–	–
G67	80MR	20MS	32^{bcd}	16^{abc}	400^{ef}	450^{hij}	16.7^{de}	21.6^{ij}	95^{jk}	70^{ijkl}	0	+	NA	+	–	–	–
G68	Tr-R	10R	1^{a}	2^{a}	20^{ab}	55^{ab}	0.9^{a}	2.6^{abc}	20^{bc}	65^{hijk}	0	+	+	+	–	–	–
G69	20MR	10MS	8^{ab}	8^{ab}	250^{cde}	375^{ghi}	10.4^{bcd}	18.0^{hi}	90^{jk}	100^{o}	2	+	–	+	–	–	–
G70	40MS	10MR	32^{bcd}	4^{a}	800^{gh}	200^{def}	39.6^{hi}	9.6^{defg}	35^{de}	75^{jklm}	0	–	–	+	+	–	–
G71	80S	60S	80^{fg}	60^{de}	$2\,050^{r}$	$1\,500^{r}$	85.4^{q}	71.6^{s}	95^{jk}	95^{no}	0	–	–	+	–	–	–
G72	0	0	0^{a}	0^{a}	0^{a}	0^{a}	0.0^{a}	0.0^{a}	0^{a}	0^{a}	0	–	–	+	–	–	–
G73	10R	5MR	2^{a}	2^{a}	105^{abc}	100^{abcd}	4.4^{ab}	7.7^{abcd}	100^{k}	75^{jklm}	0	+	–	+	+	–	–
G74	60MR	20MS	17^{abc}	16^{abc}	950^{hi}	650^{k}	39.6^{hi}	31.1^{kl}	65^{gh}	95^{no}	3	–	–	+	+	–	–
G75e**	100S	100S	100^{g}	100^{f}	$2\,400^{s}$	$2\,100^{u}$	100.0^{qr}	100.0^{v}	100^{k}	100^{o}	0	NT	–	NT	NT	NT	NT
Trial mean			21.7	17.5	579.7	492.9	24.20	23.5	48.4	55.7							
$LSD_{(0.05)}$			26.1	24.3	189.2	119.9	9.20	5.9	13.9	15.7							
SE			13.1	12.2	94.9	60.2	4.6	2.9	7.0	7.9							
F pr			***	***	***	***	***	***	***	***							

*** $p \leq 0.001$

Table 4: Efficiency of SSR and STS markers in predicting the presence or absence of rust resistance genes in genotypes of known rust resistance status. KS = known status, AF = amplified fragment, NT = not tested, + indicates positive, – indicates negative. References from which presence/absence status was sourced: William et al. (1997), Singh et al. (1998, 2005), Kuchel et al. (2007), Herrera-Foessel et al. (2009, 2012), Khan et al. (2013)

Marker	Parula		Stylet		Annuello		Pavon 76		Morocco		Efficiency in predicting (%)
	KS	AF	KS	AF	KS	AF	KS	AF	KS	AF	
csLV34	+	+	–	–	+	NTc	–	NT	NT	NT	100
Ventriup-LN2	–	–	+	+	–	–	–	–	NT	NT	100
Xwmc-44	+	+	–	–	+	NT	+	+	–	–	100
csGS	+	+	–	–		NT		NT	NT	NT	100
Sr24#50	–	–	–	–	+	+	–	–	NT	NT	100
Xgwm-533	+	+	–	+	+	+	+	+	NT	NT	75

leaf rust resistance gene *Lr68* based on the marker band size of 385 bp (Table 3). This marker accurately predicted the presence of *Lr68* in 100% of the genotypes postulated to possess the gene (Table 4).

A 150 bp fragment associated with the presence of the gene complex *Lr34/Yr/18/Sr57/Pm38* was amplified by the co-dominant bi-allelic marker csLV34 in the positive control (Parula) and in 14 genotypes (Table 3). In addition, a 229 bp fragment associated with the recessive allele at the locus *Lr34* was amplified in the negative control (Stylet) and in 58 genotypes. This marker accurately predicted the presence or absence of *Lr34* in 100% of the genotypes postulated to possess the gene (Table 4). Among the 13 commercial cultivars, *Lr34* was only present in two genotypes, G11 and G73. A single fragment of 200 bp (*Sr24*#50) specific to *Sr24/Lr24* was never amplified in all genotypes, except for the positive control (Annuello) (Table 3). Of the 25 wheat genotypes that expressed *Ltn1*, 12 genotypes carried either one or more of the four leaf rust resistance genes viz. *Lr34*, *Lr46*, *Lr37* and *Lr68* (Table 3). The remaining 13 genotypes did not possess any of the above-mentioned genes.

The genotypes that did not have any gene or genotypes with *Lr46/Yr29* were grouped in cluster C in the dendogram (Figure 3). Most of the commercial cultivars (69.2%) were included in this group. Cluster A consisted of G34, G33, G56, G18, G23, G25 and G29, which only carried *Lr37/Sr38/Yr17*. Cluster B was made up of nine genotypes that only had *Lr68*. Cluster D consisted of 14 genotypes that carried many rust resistance genes in the following

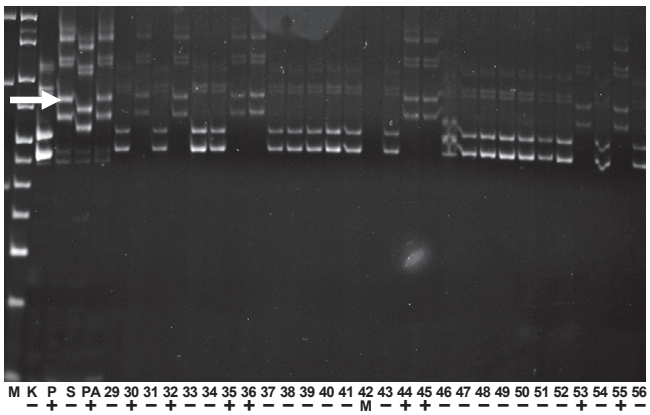

Figure 1: Amplification of the SSR marker Xwmc-44 linked to the *Lr46/Yr29* gene in 28 wheat genotypes. Lane M is a molecular weight marker – 25 bp DNA ladder; lane K is a negative control (Morocco − *Lr46*); lane P is a positive control (Parula + *Lr46*); lane S is a negative control (Stylet − *Lr46*); lane PA is a positive control (Pavon 76 + *Lr46*); lanes 29–56 are wheat genotypes; + indicates the possible presence of the *Lr46* resistance gene, – indicates the absence of the *Lr46* resistance gene, M indicates no amplification. The arrow indicates the *Lr46*$^{+Lr46}$ (242 bp) band

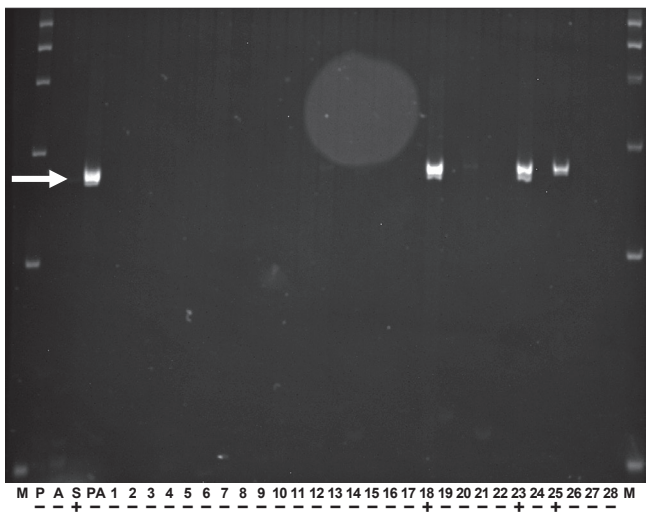

Figure 2: Amplification of the STS marker Ventriup-LN2 specific to the *Lr37/Sr38/Yr17* gene in 28 wheat genotypes. Lane M is a molecular weight Bench Top PCR Marker; lane P is a negative control (Parula − *Lr37*); lane A is a negative control (Annuello − *Lr37*); lane S is a positive control (Stylet + *Lr37*); lane PA is a negative control (Pavon 76 − *Lr37*); lanes 1–28 are wheat genotypes; + indicates presence of the *Lr37* resistance gene, – indicates absence of the *Lr37* resistance gene. The arrow indicates the *Lr37*$^{+Lr37}$ (259 bp) band

combinations: *Lr34/Lr46/Lr68*, *Lr34/Lr68*, *Lr34/Lr46* and *Lr34* (Figure 3, Table 3).

Discussion

Phenotypic characterisation of rust resistance in the field

Stem rust was not observed during the period of assessment, even at the Chisumbanje Experimental Station (a hot spot for rust diseases). This suggested that the prevalence and occurrence of stem rust varies from season to season, depending on the presence of the pathogen and environmental conditions. Genotypes that showed rAUDPC, CI and DI values of 0% could have a combination of 4–5 APR genes or major gene-based resistance. However, in order to discriminate major genes vs APR, seedling host responses should be scored, in addition to screening with known races of the leaf rust pathogen in the field. Singh (2012) reported that near immunity (trace to 5% severity) can be achieved even under high disease pressure by combining 4–5 slow-rusting genes.

The genotypes that showed reactions of MS to S, AUDPC values of less than 300, CI values of less than 20, and rAUDPC values of less than 30% during both seasons are good candidates for further APR studies. Safavi et al. (2010) and Sharma and Sharma (2014) reported similar results. The rAUDPC values greater than 80% observed in some genotypes may indicate the absence of slow-rusting resistance. The genotypes that displayed high final leaf rust severity values (≥70%) can be regarded as susceptible and lacking slow-rusting resistance genes and/or major genes. These results corroborate findings by Singh et al. (2004, 2005).

Genotyping of rust resistance genes

The present study revealed that very few cultivars of wheat grown in Zimbabwe carry APR genes, suggesting that most of the current cultivars may be susceptible to leaf rust as they are protected by very few resistance genes. A narrow genetic base for resistance to rusts is not desirable because of the increased vulnerability to attack by the evolving races of rust. The detected genes were mostly present in breeding lines although at low frequencies (*Lr46*: 21%, *Lr68*: 20%). Madenova et al. (2015) observed relatively similar findings. In their study, *Lr68* had the highest frequency of 29% compared with *Lr34* and *Lr37*.

In the present study, almost all of the genotypes lacking *Lr34*, *Lr37*, *Lr46* and *Lr68* originated from the national wheat breeding program, suggesting that local wheat breeding programs previously focused on increasing grain yield, with little effort on improving rust resistance. The two genotypes G44 (*Lr34*, *Lr46* and *Lr68*) and G63 (*Lr34*, *Lr46* and *Lr37*), which carried the highest number (three) of rust resistance genes, were introduced from CIMMYT-Mexico. Similarly, in a study by Dadrezaei et al. (2013), most of the studied Iranian cultivars, which contained *Lr34*, originated from CIMMYT. Among the 13 commercial cultivars evaluated, only G73 (SC Sahai) and G11 (SC Select) carried *Lr34.* These results concur with findings by Pretorius et al. (2015) in which only four genotypes (SC Sahai, SC Scan, Non-Sprout and W2486/6/18) out of 50 genotypes from Zimbabwe tested positive for *Lr34*.

The frequency of *Sr24/Lr24* (0%) observed in the present study was similar to that reported by Urbanovich et al. (2006) and Sharma et al. (2015). The frequency of *Sr24/Lr24* among the genotypes assessed in their studies was 0% using the J09/1J09/2 and Sr24#/12 markers, respectively. However, Mago et al. (2005) reported that the *Sr24/*

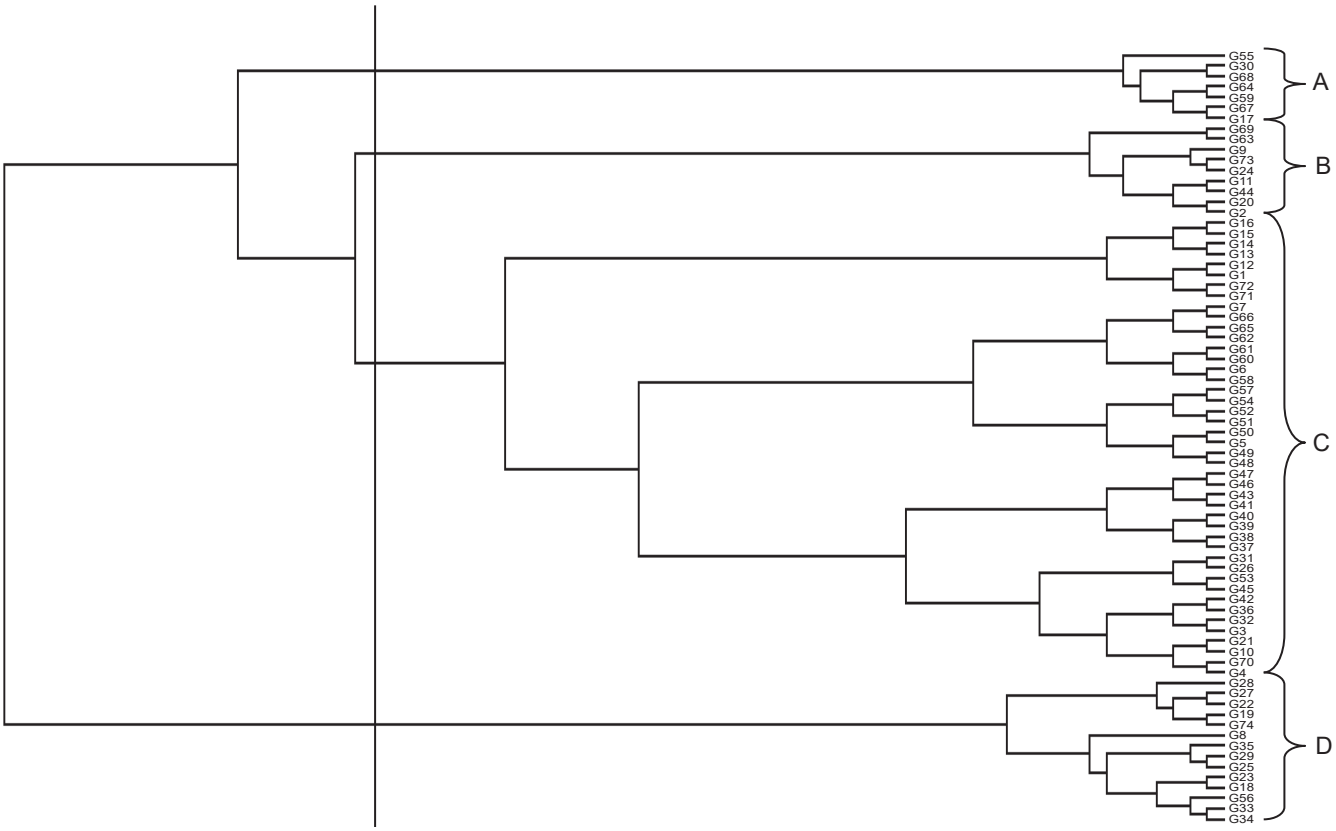

Figure 3: Dendrogram constructed from molecular data for 74 bread wheat genotypes based on Nei's (1973) genetic distance

Lr24 gene is extensively deployed in Australian wheat cultivars. The frequency of *Lr37* observed in the present study was lower than that reported previously by Cristina et al. (2015) who observed a frequency of 40%. The low frequency of *Lr37* observed in the present study indicated that most of the tested genotypes lack the *Triticum ventricosum* fragment, which was introduced into *Triticum aestivum* from *Aegilops ventricosa* Tausch. As reported by Bariana and McIntosh (1993), the *Lr37* gene is located in a 2NS–2AS translocation, implying that genotypes carrying both alleles (2NS and 2AS) could not be identified in the present study.

Although some of the clustering was in accordance with pedigree data, many genotypes, such as G23 and G24, which had the same or common parents were grouped into different clusters. This scenario could be explained in terms of Mendel's law of independent assortment. The molecular markers csLV34, csGS, Xwmc-44, Ventriup-LN2 and Sr24#50 could be diagnostic and completely linked with their respective genes. This is because their prediction efficiency in genotypes postulated to possess leaf and stem rust resistance genes based on published work of various researchers was 100%. The SSR marker Xgwm-533, which is linked to *Sr2*, gave unreliable results. In studies by Singh (1998), Spielmeyer et al. (2003), Mahwish et al. (2012) and Malik et al. (2013), some non-carriers of the *Sr2* gene produced the 120 bp fragment, which is associated with the presence of *Sr2*. Such mismatches could be a result of incomplete linkage between the molecular marker and the gene (Stepien et al. 2003).

Comparison of molecular data with field data

When the field data were compared with molecular data, two contrasting observations were made. The first set of observations included those genotypes whose molecular data for the presence of *Lr34*, *Lr37*, *Lr46* and *Lr68* corresponded well with the expression data in the field. For example, G44 showed the combination of *Lr34/Yr18*, *Lr46/Yr30* and *Lr68* together with an immune response to infection. In a study by Hussain et al. (2015), the marker data for *Lr46* and *Lr34* corresponded well with the field data. In contrast, in the present study, disease severity (MR – MS) and subsequently CI (range of 10 to 15.5) was significantly higher among genotypes when *Lr34*, *Lr46* and *Lr68* were present in individual forms. These results validated previous findings in which APR genes have been reported to express resistance in a quantitative manner (Singh et al. 2005).

The genotype G10 exhibited moderate levels of Ltn, and the SSR marker Xwmc-44 confirmed the presence of the leaf rust resistance gene *Lr46* in this genotype. In addition, the genotype G18 exhibited high levels of Ltn and marker analysis confirmed the presence of the *Lr34* and *Lr68* genes in this genotype. These findings indicate that Ltn is quantitatively expressed and the degree of Ltn could be correlated with the number of genes present (Singh 1992a; Sivasamy et al. 2014).

The scenario in which some genotypes exhibited immune reactions to infection despite not carrying a leaf rust resistance gene could be attributed to the presence of additional minor and major rust resistance that was not tested in this study. In the case of dominant markers,

such as csGS for *Lr68* and Ventriup-LN2 for *Lr37*, this discrepancy could be a result of PCR failure to generate bands during amplification. In the second set of observations, the marker data did not correspond with the disease resistance field screening data. For example, the genotypes G67 and G69 showed the presence of a 250 bp band for the *Lr34* gene but in the field these genotypes exhibited susceptible reactions to leaf rust infection. Parveen et al. (2014), Sharma and Sharma (2014) and Hussain et al. (2015) obtained similar results. The absence of Ltn in some genotypes that carried *Lr34* and *Lr46* validates previous reports by Singh (1992a), Rosewarne et al. (2006) and Sivasamy et al. (2014).

Conclusions

The most frequently occurring rust resistance gene among the evaluated wheat genotypes was *Lr46* (21%) followed by *Lr68* (20%), *Lr34* (19%), *Lr37* (11%) and *Sr24* (0%). The molecular markers csLV34, csGS, Xwmc-44, Ventriup-LN2 and Sr24#50 accurately (100%) predicted the presence or absence of rust resistance genes in diverse wheat genotypes. Data obtained with linked DNA markers such as Xgwm-533 may only be reliable if accompanied by comparison of the marker results with field response to infection. Therefore, the genotypes need to be tested using other reliable *Sr2* markers, which should also be supported by field data on stem rust reaction. Selection for *Lr34* and *Lr46* based on Ltn alone can sometimes be misleading because of its variable expression in different genetic backgrounds. The genotypes that were immune despite not carrying a leaf rust resistance gene could have carried major rust resistance genes. It is difficult to differentiate resistant lines with major genes vs quantitative genes using only the field data.

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