

Bread wheat cultivar Popo harbors QTLs for seedling and adult plant resistance to leaf rust in South African and Argentine environments

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ABSTRACT

Leaf rust, caused by the fungus *Puccinia triticina* Eriks (*Pt*), is a destructive disease affecting wheat (*Triticum aestivum* L.) production in many countries, and a serious threat to food security. As a result, several breeding programs have included leaf rust resistance as an important trait. The discovery and identification of new resistance genes that could aid in incorporating durable or long-lasting leaf rust resistance into wheat is fundamental in these breeding programs. The present study aimed to identify quantitative trait loci (QTLs) for leaf rust resistance in 127 recombinant inbred lines (RIL) developed from the cross between the resistant wheat cultivar Popo and the susceptible cultivar Kariega. The RIL population and parental lines were phenotyped for leaf rust infection type and severity at seedling and adult plant stage, respectively. The former in the greenhouse (in Argentina) and the latter in multiple field test environments comprising 3 locations in South Africa (in Tygerhoek in the Western Cape Province during the 2014, 2015, 2017 and 2018 cropping seasons; Clarens during 2014, 2016 and 2017 cropping seasons and in Bethlehem in the Free State Province during 2017 cropping season) and in 1 location in Argentina (during the 2017 and 2018 cropping seasons in Marcos Juárez, Córdoba Province). The population was genotyped using genotyping-by-sequencing. A total of 12,080 silicoDArT and 2,669 SNP markers were used for QTL analysis. In total, 25 putative QTLs for resistance to leaf rust at seedling and adult plant stages were identified, including 5 QTLs for seedling and 20 QTLs for adult plant resistance (APR). Interestingly, both Popo and Kariega contributed with alleles for resistance. Significant loci for reducing leaf rust infection at seedling stage were designated *QLr.arc-1A*, *QLr.arc-2B*, *QLr.arc-5B*, *QLr.arc-6A* and *QLr.arc-6D*. Three minor QTLs derived from Popo designated as *QLr.arc-1B1*, *QLr.arc-2D* and *QLr.arc-3D* were also detected from the field tests, explaining 5–10%, 10–16% and 5–7% of the phenotypic variance, respectively. The identified QTLs and their closely linked silicoDArT and SNP-based markers can be used for fine mapping and candidate gene discovery in wheat breeding programs targeting durable leaf rust resistance.

Introduction

Leaf rust caused by *Puccinia triticina* Eriks. (*Pt*) is highly diverse and widely distributed across all the major wheat growing regions in the world (Saari and Prescott, 1985; Samborski, 1985; Kolmer, 2013). The disease is considered one of the most destructive fungal diseases of bread wheat (*Triticum aestivum* L.). *P. triticina* is an obligate biotrophic fungus

mainly infecting the leaves of wheat at various stages but can also infect the leaf sheath and glumes. Under conditions favourable for disease development, leaf rust can cause grain yield losses of more than 50 % in susceptible cultivars (Boshoff et al., 2002; Huerta-Espino et al., 2011; El-Orabey and Elkot, 2020; Terefe et al., 2022). In addition to direct grain yield losses, leaf rust causes quality downgrade, and additional costs are also incurred for the control of the disease. Various options are

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available to control *Pt* including biological, cultural, chemical, and host plant resistance. However, limited studies are conducted on the biological and cultural control options, leaving the last two options widely used. Timely and accurate application of fungicides is effective in reducing both the incidence and severity of leaf rust in wheat. Nevertheless, fungicide use is not economically and environmentally sustainable, and can pose health hazards to people and animals, as well as phytotoxicity to the wheat crop (Kolmer et al., 2007). Genetically resistant cultivars are therefore preferred as sustainable and eco-friendly alternative over intensive application of fungicides (Pretorius et al., 2007; Huerta-Espino et al., 2011; Rauf et al., 2022; Bokore et al., 2023; Kokhmetova et al., 2023).

One of the major challenges of using genetic resistance to control leaf rust is that *Pt* frequently acquires new and more aggressive virulence to overcome effective resistance in existing cultivars, in particular when the resistance is conferred by race-specific genes (Kolmer et al., 2007; Terefe et al., 2011; Kolmer, 2013). Given the rapid evolution of new races of *P. triticina*, the discovery of more adult plant resistance (APR) genes for control of leaf rust, and their strategic deployment in breeding programs is of prime importance (Figlan et al., 2020; Kumar et al., 2022). In South Africa (Boshoff et al., 2018; Labuschagne et al., 2021; Terefe et al., 2022) and South America (Germán et al., 2007; Ordoñez et al., 2010; Diéguez et al., 2021) alone, populations of *P. triticina* are highly variable, with many different virulence phenotypes or races detected annually. Current indications are that resistance to leaf rust in many cultivars in South Africa and Argentina is mostly monogenic (Campos and López, 2015; Diéguez et al., 2021; Terefe et al., 2022) and may have limited potential for durability against the disease. For this reason, wheat breeding programs in these two countries and many other wheat-producing regions have included leaf rust resistance as an important trait. Although more than 80 designated leaf rust resistance genes and numerous quantitative trait loci (QTLs) have been characterized and mapped to a chromosome location in wheat (Li et al., 2014; McIntosh et al., 2017; Pinto da Silva et al., 2018, 2020; Kumar et al., 2021; Zhang et al., 2021; Wang et al., 2022), many of these condition effective resistance to specific races of the leaf rust fungus at seedling stage, and relatively few of these genes give resistance to the current *Pt* populations (Kolmer and Rouse, 2022), especially at an adult plant stage or all stages. This is mainly because race-specific genes often lose effectiveness within a few years of deployment in a large area that imposes selection for virulent races of the rust pathogen. Genes that confer for leaf rust resistance at an adult plant stage and provide an incomplete type of resistance to all races (race non-specific) have proven to be more long-lasting or durable (Kolmer et al., 2018a, b, c).

To date, much progress has been made in searching for adult plant slow-rusting resistance genes to leaf rust in wheat. Notably, *Lr34* (Dyck et al., 1966), *Lr46* (Singh et al., 1998), *Lr67* (Dyck and Samborski, 1979; Herrera-Foessel et al., 2011; 2014), *Lr68* (Herrera-Foessel et al., 2012), *Lr74* (Chhetri et al., 2016), *Lr75* (Singla et al., 2017), *Lr77* (Kolmer et al., 2018a), and *Lr78* (Kolmer et al., 2018c) are genes that are optimally expressed at an adult plant stage and usually condition an intermediate level of resistance with the production of fewer and smaller uredinia, compared to susceptible genotypes, when present in a single gene wheat line (Kolmer et al., 2018a, b, c). There are over 200 other APR QTLs for leaf rust that have been mapped, distributed throughout all 21 wheat chromosomes (Pinto da Silva et al., 2018; Kumar et al., 2022). Although resistance conferred by minor genes tends to be more durable than major gene resistance, it might also be overcome by slow evolution in the pathogen populations (Pooja et al., 2023; Dracatos et al., 2023; Hadimani et al., 2023; McLaughlin et al., 2023). A single QTL alone rarely confers adequate resistance, especially under high disease pressure. A combination of four or more QTLs or genes is required for a high level of leaf rust resistance (Vanzetti et al., 2011; Tsilo et al., 2014; Silva et al., 2015). Silva et al. (2015) clearly demonstrated that combining the *Lr34* gene with other APR genes, namely *Lr46*, *Lr67*, and *Lr68*, significantly reduced damage from leaf rust. Inheritance studies conducted

using CIMMYT wheat germplasm by Singh et al. (2000) also indicated that combinations of three to five small- to intermediate-effect genes could result in high levels of resistance. Therefore, it is very important to identify new genes in wheat cultivars for controlling leaf rust disease. In view of this, the present study was undertaken to construct a genetic map and determine the number and chromosomal location of QTLs for seedling and adult plant resistance to leaf rust in wheat by analysing 127 recombinant inbred lines (RIL) developed from the cross between the resistant wheat cultivar Popo and the susceptible cultivar Kariega. The RIL population and parental lines were genotyped using genotyping-by-sequencing and phenotyped for leaf rust infection type and severity at seedling and adult plant stage, respectively, in multiple environments from South Africa and Argentina.

Materials and methods

Plant material

For leaf rust phenotyping and QTL mapping, a population of 127 RILs developed from a cross between two spring wheat cultivars Popo and Kariega was used (Figlan et al., 2018). The two cultivars were initially chosen because of their varying levels of resistance to wheat rust, acceptable grain quality, bread making quality and acceptable yield levels in South Africa. Popo (KLEIN-ATLAS/TOBARI-66//CENTRIFEN/3/BLUEBIRD/4/KENYA-FAHARI) is a Kenyan hard red spring cultivar that was released in 1982 and resistant to leaf rust. Kariega (SST-44[CI13523(Agent)/3*T4 (Anza)]/K-4500.2/(SIB)SAPSUCKER) is a South African spring cultivar released in 1993 by the Agricultural Research Council - Small Grain Institute (ARC-SGI) and is susceptible to leaf rust.

Phenotyping of seedling resistance in greenhouse

The evaluation of the resistance response of the Popo/Kariega RIL mapping population at seedling stages was assessed at the Cereal Disease Laboratory at the Argentine National Institute of Agricultural Technology (INTA), Bordenave Experimental Station in 2018. The Popo/Kariega RIL mapping population, together with rust susceptible checks SST88 and McNair were sown in sterilised soil in 10 cm diameter plastic pots under a temperature-controlled seedling room at 20 to 25 °C. Five seeds from each of the genotypes were sown, with three replicates per genotype. After emergence, plants were fertilised twice with 10 g L⁻¹ multi-feed water soluble fertiliser (19:8:16 NPK plus micronutrients).

For inoculation, the urediniospores of leaf rust races were obtained from regularly maintained stocks stored in a –80 °C freezer at INTA. The local leaf rust races MFP 20 (Pt17–30) and KKG 10–20 (Pt17–18) were inoculated on 8-day old seedlings as described by Long and Kolmer (1989). The inoculated plants were incubated in a dark dew chamber overnight at 18 °C and 100 % relative humidity, then moved to the benches in the greenhouse and maintained at 19–22 °C and 75 % relative humidity. Light intensity was supplied at about 7600 lux in a photoperiod of 16 h light and 8 h dark. Seedlings were kept under observation until the development of leaf rust pustules. Leaf rust infection type was evaluated 14 days post inoculation using a scale of 0 to 4, as proposed by Stakman et al. (1962), where the infection types 0, 1, 2, or combinations were considered low infection types, indicating resistance, while 3 and 4 were considered high infection types, indicating susceptibility.

Phenotyping for adult plant resistance

RIL population was evaluated in field conditions over several years at three different locations in South Africa: Tygerhoek in the Western Cape Province (2014, 2015, 2017 and 2018), Clarens in the Free State Province (2014, 2016 and 2017), Bethlehem in the Free State Province (2017) and in one location in Argentina, Marcos Juárez in Córdoba

Province (2017 and 2018). The locations differed in soil conditions, temperature and moisture and are hotspot areas for wheat rust pathogens and are annually surveyed for rust races. Hence, the locations were selected based on the known prevalence of leaf rust disease in the past. In each environment, parents and lines were sown in rows (1 m long). The rust susceptible cultivars Morocco, SST88 and McNair were planted as spreader rows around the experimental area to ensure disease initiation and spread. In Marcos Juárez, spreaders were inoculated with a mix of two isolates (MFP 20 and KKG 10–20), prevalent in the area (isolates with an avirulent/virulent response to differential lines is available on supplementary Table S1). All cultural practices such as fertilisation, irrigation and other management practices were followed according to the recommendation of the specific areas.

Disease severity was recorded as an average value per RIL, using a quantitative scale of 0 to 100 according to the modified Cobb scale (Peterson et al., 1948). A score of 0 to 20 represents highly resistant; 30 is resistant; 40 to 50 is moderately resistant; 60 to 70 is moderately susceptible; 80 is susceptible and 90 to 100 represents highly susceptible, showing 100 % of the leaf or stem area covered with the disease. The disease severity for leaf rust was recorded only once per row in each season when the disease symptoms were fully developed, with the susceptible checks displaying 80 % or higher disease severity. Additionally, the Best Linear Unbiased Estimator (BLUE) was calculated for each RIL using all South African environments (8), all Argentinian environments (2) and for all tested environments (10). The BLUEs were treated as additional environments in the QTL mapping.

Genotyping

The total genomic DNA of the RIL population and parental cultivars was isolated from young leaves using the modified Cetyltrimethyl Ammonium Bromide (CTAB) method (Dreisigacker et al., 2016). DNA quality, purity and concentration was tested using a NanoDrop ND-1000 UV-vis Spectrophotometer (NanoDrop Technologies, Wilmington, USA). A final volume of 15 ng/μL was sent to Triticarte Pty. Ltd., Canberra, Australia, to be genotyped through the genotyping-by-sequencing (GBS) platform using DArT-seq (Diversity Array Technology) markers (Akbari et al., 2006). The DArT-seq system produces two kinds of markers, classical SNP present in the sequenced fragments, and silico-DArT markers characterised by presence/absence variation (Visioni et al., 2018). For this study, 12,080 polymorphic silicoDArTs and 2669 SNPs were used for genetic linkage map construction and QTL analysis. From a subset of all markers received, a filtering process to remove all redundant and non-informative markers was followed whereby markers that presented multiple genetic positions were removed. Moreover, markers with over 10 % missing data or heterozygous alleles were discarded.

The genetic map was constructed using the R/qtl package (Broman et al., 2003) from the R software (3.3.2). The QTL mapping was conducted using the winQTLcart software (Wang et al., 2012). Specifically, Composite Interval Mapping (CIM) analysis was performed on seedling infection type data and on disease severity (% infected flag leaf area) data for each season separately and for the BLUEs values described before. Threshold LOD value of 2.5 was chosen as a uniform threshold for all analyses. The physical position of the DArTseq SNPs, silicoDArT and *Lr* resistance genes/markers were based on the IWGSC Ref Seq v1.0 genome assembly (Appels et al., 2018).

Statistical analysis

Factorial ANOVA was conducted using the QTL peak marker as class variables in the model, together with all possible three-way interactions. Environments were included as blocks (a random class variable). This analysis was carried out to determine the combined effect of the QTLs detected. Fisher's Least Significant Difference (LSD) was used to make multiple comparisons between the means of severity.

Results

The complete genetic map developed for this population includes 12,080 polymorphic silicoDArTs and 2669 SNPs distributed on 2686 loci across the 21 wheat chromosomes (Table 1). Markers defined 25 linkage groups. Thirteen DArTs markers, however, remained unlinked. The total coverage of the map is 4935 cM, with an average locus spacing of 2.0 cM.

QTLs detected using seedling plant response data

A total of five significant QTLs (LOD > 2.5) were pinpointed across both *Pt* races analysed, as detailed in Table 2. In terms of the MFP 20 race, genetic regions on chromosomes 1AS and 6AL (designated as *Qlr.arc-1A* and *Qlr.arc-6A*, respectively) were linked to resistance. The SNP marker 1007077 positioned on chromosome 1AS with the resistance allele inherited from Kariega accounted for 29 % of the explained phenotypic variation (R^2). Conversely, the R^2 associated with marker 1166019 on chromosome 6AL, originating from the Popo resistance allele, was 7 %. Similarly, concerning the KKG 10–20 race, genomic segments on chromosomes 2BS, 5BL, and 6DL (designated as *Qlr.arc-2B*, *Qlr.arc-5B*, and *Qlr.arc-6D*, respectively) demonstrated associations with resistance. Notably, the R^2 connected to the immune response to KKG 10–20 via marker 1141995 on chromosome 2BS, characterized by the Popo resistance allele was 21 %. Moreover, the R^2 pertaining to the immune response to KKG 10–20 involving marker 4409705 on chromosome 5BL was 8 % and due to Kariega resistance allele, whereas marker 1207192 on chromosome 6DL, also associated with the Kariega resistance allele resulted to 10 % phenotypic variation.

QTLs detected using adult plant response data

Based on the analysis of QTLs in individual environments 20 QTLs, spread on chromosomes 1A, 1B, 2A, 2D, 3A, 3B and 3D, affecting the leaf rust severity for APR were detected (Table 3). It should be noted that only one QTL (*Qlr.arc-1A*) co-located with the race-specific leaf rust resistance genes effective at seedling stage. Among the QTLs detected using adult plant response data, the more stable and significant QTLs across environments and BLUEs were those detected on chromosomes 1BS, 2DS and 3DL. Two QTLs were detected on the 1B chromosome, namely *Qlr.arc-1B1* and *Qlr.arc-1B2*. Since *Qlr.arc-1B2* was detected in only one environment and deserves additional evaluations to be validated, we will focus on *Qlr.arc-1B1* throughout the text. The *Qlr.arc-1B1* QTL whose resistant allele is contributed by the cultivar Popo was significantly detected (LOD > 2.5) in 3 of the 10 tested environments and 1 of the 3 BLUEs (Fig. 1). The peak of the *Qlr.arc-1B1* was mapped at the silicoDArT marker 1252866 (6.6 cM, 57.01 Mb) with a maximum LOD of 5.00. In the factorial ANOVA, silicoDArT marker 1252866 explained 5.0–10.0 % of the observed variation in leaf rust severity. The average difference in severity for this region between Popo and Kariega alleles was 2.04–16.03 % depending on the environment. The QTL located on chromosome 2DS, henceforth *Qlr.arc-2D* whose resistant allele is also contributed by the cultivar Popo, was detected in 4 of the 10 tested environments and 2 of the 3 BLUEs (Fig. 1). The peak of the *Qlr.arc-2D* was mapped at the silicoDArT marker 4993126 (8.3 cM, Un:24.31 Mb) with a maximum LOD of 7.3 (Fig. 1, Table 3). In the factorial ANOVA, silicoDArT marker 4993126 explained 10.0–16.0 % of the observed variation in leaf rust severity. The average difference in severity for this region between Popo and Kariega alleles was 16.05–24.05 % depending on the environment. Finally, the QTL located on chromosome 3DL, henceforth *Qlr.arc-3D* whose resistant allele is also contributed by Popo was detected in 4 of the 10 tested environments and the 3 BLUEs (Fig. 1). The peak of the *Qlr.arc-3D* was mapped at the silicoDArT marker 3534345 (68.6 cM, 149.75 Mb) with a maximum LOD of 5.69. In the factorial ANOVA, silicoDArT marker 3534345 explained 5.0–7.0 % of the observed variation in leaf rust severity. The average difference in severity for this region between Popo

Table 1
Genetic linkage map developed based on GBS markers in Popo/Kariega recombinant inbred line population.

Chr	SNPs	DArTs	#Markers	#Loci	Length (cM)	Avg. spacing (cM)	Max. spacing (cM)
1A	127	350	477	113	208,7	1,9	58,4
1B.1	167	1697	1864	126	138,1	1,1	10,5
1B.2	155	606	761	49	40,1	0,8	3,6
1D.1	63	261	324	60	112,5	1,9	19,7
1D.2	10	159	169	16	13,3	0,9	3,4
2A	161	420	581	110	303,0	2,8	102,4
2B	124	821	945	192	280,5	1,5	70,1
2D	33	403	436	78	248,4	3,2	70,7
3A	178	384	562	136	272,7	2,0	48,7
3B	226	1021	1247	232	275,5	1,2	8,3
3D	12	99	111	62	267,1	4,4	54,0
4A	129	613	742	152	230,3	1,5	17,9
4B	37	176	213	62	136,7	2,2	31,6
4D	6	52	58	29	54,8	2,0	4,6
5A	198	470	668	159	262,0	1,7	16,7
5B	179	798	977	208	296,2	1,4	20,6
5D	24	104	128	50	184,0	3,8	43,3
6A	124	428	552	112	229,1	2,1	42,6
6B	183	823	1006	153	219,5	1,4	25,8
6D	46	251	297	89	362,6	4,1	134,5
7A.1	155	606	761	117	214,5	1,8	25,4
7A.2	60	239	299	68	130,5	1,9	12,2
7B	231	1077	1308	205	225,8	1,1	11,1
7D.1	39	197	236	91	206,6	2,3	59,6
7D.2	2	12	14	8	10,2	1,5	3,2
Unl	0	13	13	9	12,3	1,5	5,3
ABD Avg.	102,7	464,6	567,3	103,3	189,8	2,0	34,8
Σ	2669	12,080	14,749	2686	4935		

Table 2
Composite interval mapping of leaf rust resistance in the Popo/Kariega recombinant inbred lines at seedling stage in the greenhouse.

Leaf rust race	Chromosome	QTL name	Peak marker ¹	Position (cM)	LOD	Resistance donor	Resistant allele variant ²	Add effect	R2 (%)	Marker interval (± 1 LOD)	Position (cM)
MFP 20	1AS	<i>Ql.r. arc-1AS</i>	1007077*	13,91	12,9	KARIEGA	C	-0,278	29	1204785*-1083062	13,8–14,6
MFP 20	6AL	<i>Ql.r. arc-6AL</i>	1166019	218,51	3,7	POPO	0	-0,1357	7	1234390-3533961*	218,1–218,6
KKG 10–20	2BS	<i>Ql.r. arc-2BS</i>	1141995	1,01	8,1	POPO	0	-0,2316	21	3942819- 987112	0,0–4,8
KKG 10–20	5BL	<i>Ql.r. arc-5BL</i>	4409705	81,11	3,4	KARIEGA	1	-0,1418	8	4395235*-2323591	79,4–81,6
KKG 10–20	6DL	<i>Ql.r. arc-6DL</i>	1207192	356,61	4,4	KARIEGA	1	-0,1617	10	1695538-1115058	356,1–357,1

¹ * denotes DArT SNP Marker; without asterisk silicoDArT marker.

² SNP Marker = base detected in the resistant allele; silicoDArT marker = 1 presence and 0 absence of sequence fragment.

and Kariega alleles was 9.73–15.69 % depending on the environment. The three-way ANOVA interaction between the peak markers of the main QTLs did show significant *P* values between *Ql.r. arc-2D***Ql.r. arc-3D* (*P* < 0.0021) and for the triple interaction *Ql.r. arc-2D***Ql.r. arc-3D* (*P* < 0.0138), suggesting some epistatic interactions between the QTLs as analysed in the section below. A QTL on chromosome 3B (*Ql.r. arc-3B*) was also found to be significant (LOD > 2.5) and consistent in 3 environments (Marcos Juárez 2017, 2018 and Clares 2016), with a resistant allele contributed by Popo. The 3BS QTL explained 3–9 % of the observed variation in leaf rust severity.

Our analysis revealed that Kariega may contribute with resistant alleles effective to rust races present in South Africa. We detected one QTL on chromosome 2A, which was detected in only one environment and would deserve additional studies to be validated. The second QTL on chromosome 3A was observed more consistently in South African environments. This region explained 4–11 % of the observed variation in leaf rust severity.

Epistasis analysis

Significant epistatic interactions were identified for leaf rust severity

and infection response. Interactions were averaged across seasons. As an example, based on QTL haplotype simulations, RILs that carried resistant alleles on *Ql.r. arc-1B1*, *Ql.r. arc-2D* and *Ql.r. arc-3D* and all possible combinations of these QTLs, were identified (Fig. 2; Table S2). Generally, RILs carrying the resistant alleles from the parent Popo on all three loci had significantly less disease severity compared with the lines with the allele from Kariega. Also, the mean leaf rust severity was numerically lower in all tested environments with the combination of the *Ql.r. arc-2D* and *Ql.r. arc-3D* resistant alleles inherited from Popo as compared to alleles contributed by Kariega (Fig. 3).

Discussion

Recently, leaf rust has become one of the most serious diseases resulting in big losses in wheat production around the world. With the global climate change, the meteorological conditions are becoming more suitable for the development and prevalence of leaf rust, and this is projected to cause more serious damage in the future. Identification of leaf rust resistance genes, especially of adult plant slow rusting nature, is therefore needed for wheat improvement to design breeding strategies based on a pyramidal approach to provide increased resistance to the

Table 3
Composite interval mapping of leaf rust resistance in the Popo/Kariega recombinant inbred lines in ten test environments.

Environment	Chromosome	QTL name	Country	Peak marker ¹	Position (cM)	LOD	Resistance donor	Resistant allele variant ²	Add effect	R2 (%)	Marker interval (\pm 1 LOD)	Position (cM)
MsJz_18	1AS	<i>Qlr.arc-1A</i>	AR	1005431*	11,9	8,81	KARIEGA	G	-16,00	1	5367440-4262943	11.3-12.6
MsJz_17	1B.1S	<i>Qlr.arc-1B1</i>	AR	3935951	7,1	3,11	POPO	1	-16,36	7	10592726-4989415	5.8-8.9
MsJz_18	1B.1S	<i>Qlr.arc-1B1</i>	AR	1252866	6,6	5,00	POPO	1	-10,72	5	10592813-3935951	0.0-7.1
Bethelhem_17	1B.1S	<i>Qlr.arc-1B1</i>	SA	10592726	5,8	5,80	POPO	1	-2,04	7	3945460-4991011	3.3-6.2
BLUE_AR	1B.1S	<i>Qlr.arc-1B1</i>	AR	1252866	6,6	5,00	POPO	1	-15,57	1	3945460-4991011	3.3-6.2
Tygerhoek_18	1B.2L	<i>Qlr.arc-1B2</i>	SA	1241732	10,8	9,57	POPO	1	-10,15	8	3953130-1233997	9.8-11.2
BLUE_ALL	1B.2L	<i>Qlr.arc-1B2</i>	SA+AR	3953130	9,8	5,00	POPO	0	-10,02	9	1206482-1152951	6.2-15.2
BLUE_AR	1B.2L	<i>Qlr.arc-1B2</i>	AR	1089721	2,9	5,20	POPO	1	-9,57	3	1128983-1397574	2.5-3.5
Tygerhoek_15	2A	-	SA	1021498	177,1	3,62	KARIEGA	1	-14,57	4	3027296-2322321	170.3-179.8
BLUE_SA	2A	-	SA	1682733	187,8	3,52	KARIEGA	1	-8,14	5	1280732-1230135	186.3-109.8
Tygerhoek_14	2DS	<i>Qlr.arc-2D</i>	SA	4993126	8,4	3,33	POPO	1	-15,31	12	1107483-1126200	7.9-12.8
Tygerhoek_15	2DS	<i>Qlr.arc-2D</i>	SA	4993126	8,4	3,48	POPO	1	-24,05	11	3938920-1126200	3.4-12.8
Tygerhoek_17	2DS	<i>Qlr.arc-2D</i>	SA	4993126	8,4	5,48	POPO	1	-22,95	16	3938920-1,126200	3.4-12.8
BLUE_SA	2DS	<i>Qlr.arc-2D</i>	SA	4993126	8,4	7,35	POPO	1	-12,46	11	3938920-1126200	3.4-12.8
Clarens_17	2DS	<i>Qlr.arc-2D</i>	SA	1101647	13,4	7,55	POPO	1	-12,18	10	1126200-7348277	12.8-16.1
BLUE_ALL	2DS	<i>Qlr.arc-2D</i>	SA+AR	7348277	16,1	6,52	POPO	1	-12,73	13	1126200-1127473	12.8-86.5
Clarens_14	3A	-	SA	4993047	125,7	4,18	KARIEGA	1	-13,86	9	1771416*-1151102	122.1-151.7
Tygerhoek_18	3A	-	SA	4911104	200,2	3,11	KARIEGA	1	-12,94	4	1207283-2254520	198.1-201.2
Bethelhem_17	3A	-	SA	3937462*	248,2	3,20	KARIEGA	G	-2,13	11	3954624-1375542	243.6-251.6
MsJz_17	3B	-	AR	1317824	23,0	3,00	POPO	0	-14,53	5	1268868-3955311	21.5-24.2
MsJz_18	3B	-	AR	1317824	23,0	2,63	POPO	0	-8,46	3	1378816-1076070	20.4-32.8
Clarens_16	3B	-	SA	1317824	23,0	6,25	POPO	0	-19,19	9	1321223-3955311	22.6-24.2
MsJz_17	3DL	<i>Qlr.arc-3D</i>	AR	3534345	68,6	3,54	POPO	0	-15,69	5	1114702-3534345	61.6-68.6
MsJz_18	3DL	<i>Qlr.arc-3D</i>	AR	3534345	68,6	4,21	POPO	0	-11,76	6	5332507-4538282	67.9-69.7
Tygerhoek_17	3DL	<i>Qlr.arc-3D</i>	SA	1235264	64,5	3,26	POPO	1	-13,55	5	3532920-3534345	63.2-68.6
BLUE_AR	3DL	<i>Qlr.arc-3D</i>	AR	3534345	68,6	5,69	POPO	0	-14,06	7	5332507-4538282	67.9-69.7
BLUE_ALL	3DL	<i>Qlr.arc-3D</i>	SA+AR	3534345	68,6	3,54	POPO	0	-9,73	7	5332507-4538282	67.9-69.7
Tygerhoek_14	3DL	<i>Qlr.arc-3D</i>	SA	3935748	81,5	2,79	POPO	1	-12,98	5	3950372-3934704	71.3-84.2
BLUE_SA	3DL	<i>Qlr.arc-3D</i>	SA	3935748	81,5	2,68	POPO	1	-9,96	7	1155336-1124782	73.4-83.4

¹ * denotes DARt SNP Marker; without asterisk silicoDARt marker.

² SNP Marker = base detected in the resistant allele; silicoDARt marker = 1 presence and 0 absence of sequence fragment. AR = Argentina; SA = South Africa.

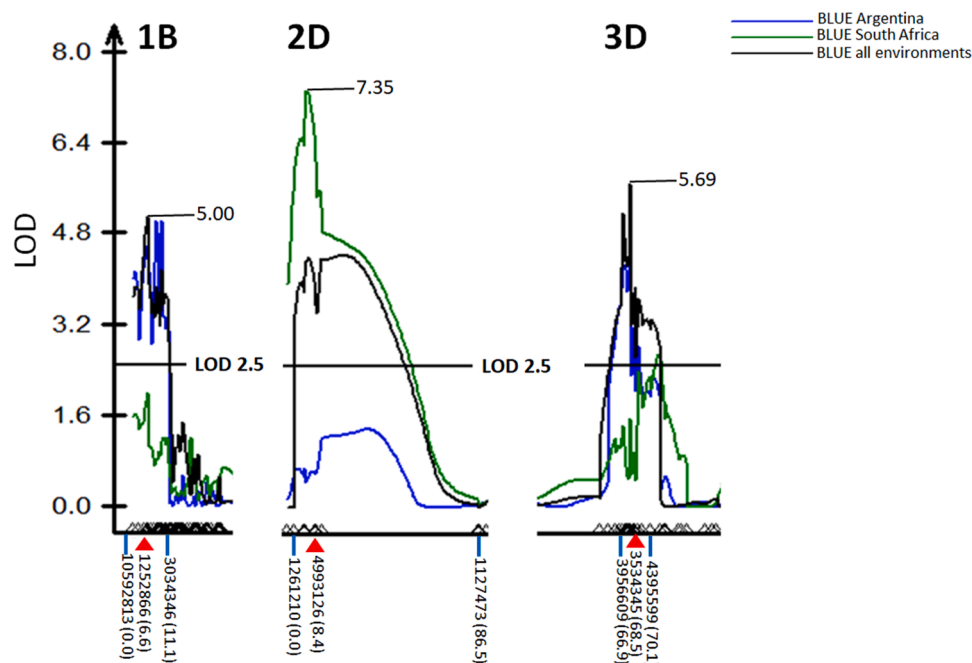


Fig. 1. Stable QTLs for leaf rust severity at adult plant stage identified from the Popo/Kariega recombinant inbred line population on chromosomes 1B, 2D, and 3D. The highest peak LOD scores among the BLUE values are indicated in each plot, along with the horizontal line indicating the threshold LOD (2.5). The three plots are in the same scale to facilitate comparisons among genes. Lines of different colours indicate different BLUEs. Names of the peak markers (red triangle) and their flanking markers are listed below their positions (cM).

highly variable and dynamic leaf rust pathogen. Previously, the only known loci involved in leaf rust adult plant resistance included *Lr12* on chromosome 4BL (Singh and Bowden, 2011), *Lr13* on 2BS, *Lr11/LrBP2* (Darino et al., 2015), *Lr22* (allele a and b) and *LrSV1* (Ingala et al., 2012) on 2DS, *LrSV2* on 3BS (Ingala et al., 2012), *Lr34* on 7DS (Dyck, 1977; 1987; Lagudah et al., 2009), *Lr35* on 2BS, *Lr37* on 2AS, *Lr46* on 1BL (Singh et al., 1998), *Lr48* on 2BS, *Lr49* on 4BL, *Lr67* on 4DL (Dyck and Samborski, 1979), *Lr68* on 7BL (Herrera-Foessel et al., 2012), *Lr74* on 3BS (Chhetri, 2016), *Lr75* on 1BS (Singla et al., 2017), *Lr77* on 3BL (Kolmer et al., 2018a) and *Lr78* on 5DS (Kolmer et al., 2018b). The current contribution of some of the mentioned genes to the global leaf rust protection is limited, especially under high disease pressure (Yuan and Chen, 2011). Leaf rust genes like *Lr13* (from a Brazilian cultivar Frontana) was reported to be an important gene for resistance in the past and continues to contribute to resistance in some regions of the world, whereas in other areas like South America and South Africa, the gene was reported to be completely ineffective. This is due to the race-specific nature of the gene, though it was originally and continues to be reported to confer adult plant resistance (McIntosh et al., 2008; Singh and Bowden, 2011). The need for additional leaf rust resistance genes therefore underlines the importance of research to identify and incorporate durable resistance sources – especially APR genes – into wheat cultivars. Genetic dissection of complex traits including rust resistance through QTL mapping will be important in designing appropriate breeding strategies through MAS. In the current study, phenotyping of 127 RILs from the Popo/Kariega population suggested the presence of a wide variability of resistance to leaf rust. Overall, 25 QTLs for resistance to leaf rust at seedling and adult plant stages were identified. The 5 mapped QTLs represent seedling resistance whilst the maximum number of 20 QTLs were detected using field adult plant response data. Popo contributed more leaf rust resistance QTLs (17) as compared with eight coming from Kariega, consistent with the field observation that Popo displayed more resistance compared to Kariega. The significant loci for reducing leaf rust severity at seedling stage were designated *Qlrc-arc-1A*, *Qlrc-arc-2B*, *Qlrc-arc-5B*, *Qlrc-arc-6A* and *Qlrc-arc-6D*, explaining 29 %, 7 %, 21 %, 8 % and 10 %, respectively of the phenotypic variation. The

leaf rust resistance in Popo in field tests was attributed to QTL located in 1BS, 2DS and 3DS, found to be more stable and significant across environments and BLUEs (Fig. 1). The three QTLs were designated as *Qlrc-arc-1B1*, *Qlrc-arc-2D* and *Qlrc-arc-3D*, respectively. Data show that the effect of the combination of the three QTL alleles inherited from Popo condition low infection “<20” response to leaf rust infection in the field (Fig. 2). According to the data, the three QTLs on chromosome 1B, 2D and 3D were only detected in 4 of the 10 environments and of minor effect, explaining 5–16 % of the phenotypic variance. The other QTLs detected in this study were non-significant and remain unexplained.

Significant QTLs identified in seedling tests

Qlrc-arc-1A

Several genes and QTLs for leaf rust have been mapped to chromosome 1A, including *Lr10* (cloned by Feuillet et al., 2003), *Qlrc-B22-1A* (Naz et al., 2008), *Qlrc.ccsu-1A.1* and *Qlrc.ccsu-1A.3* (Kumar et al., 2013), *Qlrc.cim-1AS* (Lan et al., 2015), *Qlrc.cau-1AS* (Du et al., 2015), *Qlrc.apr-1A* (Kokhmetova et al., 2023), *Qlrc.iau-1A-1*, *Qlrc.iau-1A-2* and *Qlrc.iau-1A-3* (Talebi et al., 2023), and *Qlrc.hbau-1A* (Zhou et al., 2023), but all, except for *Lr10* are at different positions from *Qlrc-arc-1A* detected in this study. Schachermayr et al. (1997) confirmed that the *Lr10* gene and it is located on the distal region of the short arm of chromosome 1A. In our study, *Qlrc-arc-1A* was mapped on chromosome 1AS (13.91cM) with the resistant allele being contributed by Kariega and is shown to confer resistance at both seedling and adult plant stages, specifically to the Argentina leaf rust race MFP 20. The presence of *Lr10* in Kariega was confirmed by submitting a query sequence using BLASTN 2.12.0+ on NCBI wheat Kariega v1 database. The best match (dc-megablast: e-value of 0, query coverage of 100 %) on chromosome 1A validated the detection of our QTL on 1A, which has been confirmed to be *Lr10*. However, our results show that the R^2 % value of *Qlrc-arc-1A* was lower in the field at 1 % compared to the 29 % at seedling stage. The variation might be caused mainly by the difference in spatial uniformity of disease pressure between greenhouse and field trials. For instance, in the greenhouse, temperature and humidity were controlled to be suitable

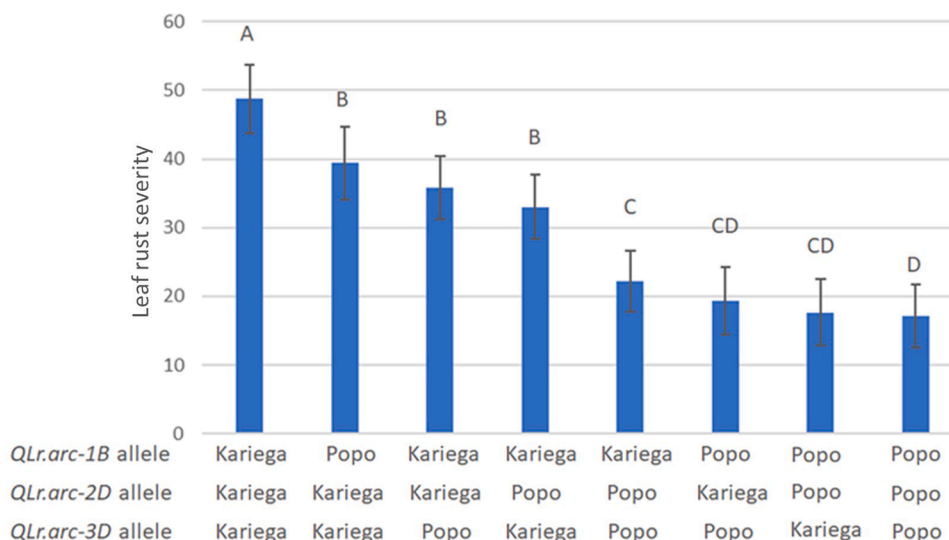


Fig. 2. The effects of different QTL and combinations on leaf rust severity. The letters on the bars refer to the mean comparison test (LSD). Bar heights are averages over all seasons.

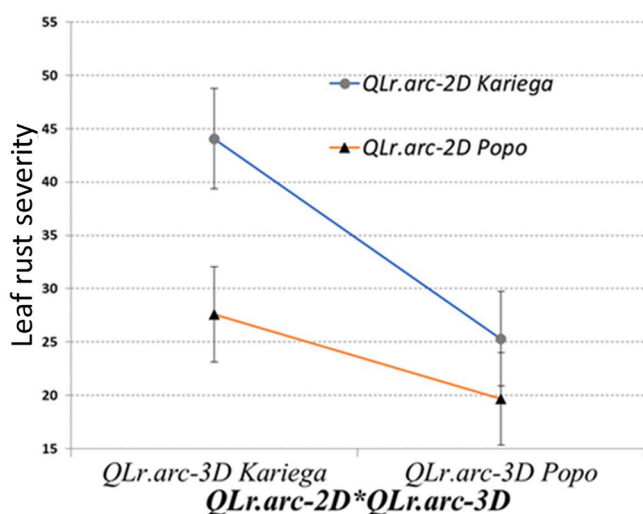


Fig. 3. Epistatic interaction between the 2 QTLs, *QLr.arc-2D* and *QLr.arc-3D* based on field tests.

for leaf rust, and fans were frequently used to facilitate even distribution of urediniospores for re-infection. The manipulation of greenhouse conditions could increase test accuracy and the effect of *QLr.arc-1A* could be better observed compared with the trial in Marcos Juárez in 2018. The environmental conditions are usually not consistent across seasons, with sporadic rainfall, and in some seasons the urediniospores were not adequate enough for establishing a good spatially uniform distribution and re-infection. The lower uniformity might increase experimental error and consequently the effect of *QLr.arc-1A* could not be fully expressed. According to these results and observations, it can be concluded that *QLr.arc-1A* was inconsistent but effective across diverse environments, and its effectiveness could be better revealed in a trial of high and uniform infections.

QLr.arc-2B

Chromosome 2B is a resistance-rich region and is known to possess many disease resistance genes (Li et al., 2014; Wu et al., 2017; Pinto da Silva et al., 2018). Leaf rust, powdery mildew, and stripe rust resistance all have at least two QTL clusters on 2BS, and one each for leaf rust and powdery mildew resistance on 2BL. *QLr.arc-2B* mapped on the short arm

(1.01cM), close to *Lr16* located at the distal end of chromosome 2BS (McCartney et al., 2005). The 2BS QTL derived from Popo explained 21 % of the phenotypic variation for resistance to race MFP 20. The pathotype MFP 20 used in the seedling tests is avirulent to *Lr16*, indicating that the gene present in Popo could be *Lr16*. Further screening of the RILs using the *Lr16* genetic marker will be necessary to confirm our hypothesis.

QLr.arc-5B, QLr.arc-6a and QLr.arc-6D

Two known leaf rust genes, *Lr18* (5BL) and *Lr52* ((5BS), have previously been reported on chromosome 5B (McIntosh, 1983; Hiebert et al., 2005). Carpenter et al. (2017) reported that gene *Lr18* was likely flanked by two 5BL QTLs, *QLr.vt-5B1* and *QLr.vt-5B2* which were identified in cultivar Jamestown, positioned between 113.89–145.90cM which is distant to 81.1cM location of *QLr.arc-5B*. Therefore, it can be concluded that 5BL QTL is not *Lr18*. Regarding the 6DL QTL, apart from *Lr38* which was previously located on the same chromosome arm, no other designated leaf rust genes have been identified in this region. No close linkage between *QLr.arc-6D* and *Lr38* was found in this study. Overall, the QTLs *QLr.arc-5B*, *QLr.arc-6A* and *QLr.arc-6D* mapping on chromosome 5BL (81.1cM), 6AL (218cM) and 6DL (336.6cM), respectively, have chromosome locations that – to our knowledge – have not been previously implicated in leaf rust resistance, therefore these are potentially new unexploited genes for leaf rust resistance in wheat.

Significant QTLs identified in field test environments

QLr.arc-1B1

Popo's *QLr.arc-1B1* QTL (6.5 cM) mapped in close proximity to the 8 cM target region of the slow rusting or APR gene, *Lr75*, located on the distal end of the short arm of chromosome 1B. The only other reported leaf rust resistance gene present on chromosome 1BS closer to *QLr.arc-1B1* is *Lr71* (Singh et al., 2013). The *Lr71* gene was mapped between markers *Xgwm18* and *Xbarc187*, with linkage distance of 1.0 and 1.3 cM, respectively. Observations from the deletion bin mapping conducted by Singh et al. (2013) using the SSR markers *wmc230* and *swm271* mapped *Lr71* towards the centromere on chromosome 1BS. Therefore, our 1BS QTL is distant from the *Lr71* region and there's a possible question of allelism of *QLr.arc-1B1* and *Lr75* based on gene location and action. Nevertheless, the arising question proves the difficulty to directly compare results from different biparental mapping experiments since different susceptible parents are often used and the size of the RIL

populations vary significantly. The criteria and methods used for evaluation of leaf rust resistance in the segregating RILs and mapping of the quantitatively expressed resistance are also quite varied.

QLr.arc-2D

Eight designated *Lr* genes and 23 QTLs have been identified on chromosome 2D. The *Aegilops tauschii*-derived *Lr22a* and *T. aestivum*-derived *Lr22b*, both located on 2D confer race-specific APR (McIntosh et al., 1995; 2008). The most effective QTLs identified on 2D include *QLr.lp.osu-2DS* (wheat breeding line CI 13,227) and *QLr.inru-2D* (cultivar Balance), which explained 34.6 to 48.2 % and 4.4 to 46.4 % of the phenotypic variation, respectively (Azzimonti et al., 2014; Xu et al., 2005). Li et al. (2017) also identified a slow-rusting QTL designated as *QLr.hwwg-2DS* located on the short arm of chromosome 2D (14.4 cM). The location of *QLr.hwwg-2DS* was found to overlap that of *QLr.lp.osu-2DS* detected by Xu et al. (2005), so it was concluded that the two QTLs are identical. *Lr39/41* was also mapped on chromosome 2DS, however this gene is highly effective in seedlings. In our study, both MFP 20 and KKG 10–20 are *Lr39*-avirulent, but *Lr41*-virulent. Our results may suggest that *QLr.arc-2D* is allelic to *Lr41*. Further screening of the RILs using the *Lr41* genetic marker will be necessary to confirm or dispute this hypothesis. The QTL detected in our study is also closer in proximity to *Lr2a* and *QLr.hwwg-2DS*, though *QLr.hwwg-2DS* appears to be distal to the 2DS loci of the present study. *Lr2a* is a strong major gene and confers high levels of resistance in the field, and on the other hand, *QLr.arc-2D* appears to be of minor slow-rusting effect explaining 10.0–16.0 % of the phenotypic variation. Therefore, it was concluded that the two loci result to different phenotypes, hence cannot be the same. The *LrSV1* APR gene mapped by Ingala et al. (2012) on 2DS is also excluded since it was found to be allelic to *Lr22a*. All the other resistance loci (*Lr2*, *Lr2a*, *Lr2b*, *Lr2c* and *Lr15*) mapping closer to *QLr.arc-2D* are excluded because of their seedling resistance nature (Tsilo et al., 2014; McIntosh et al., 1995). It should be noted that *QLr.arc-2D* was consistently observed in South African environments, but not in Argentina. This observation suggests differences in virulence among rust races present in both areas.

QLr.arc 3D

The last stable QTL detected in the present study, *QLr.arc-3D*, was mapped on the long arm of chromosome 3D at 68.6 cM. Very few leaf rust resistance QTLs have been mapped on 3D, and *QLr.cim-3D* is the most effective leaf rust APR locus on this chromosome, but maps on the short arm. *QLr.cim-3D* explained 17.8 to 25.4 % of the phenotypic variation in wheat cultivar Francolin#1 (Lan et al., 2014). *QLr.tam-3D*, co-locating with the yellow rust QTL *QYr.tam-3D* was also detected on the short arm of chromosome 3D, but explained only 4 to 7.1 % of the phenotypic variation in the wheat line Quaiu 3 (Basnet et al., 2014). Among designated *Lr* genes, the seedling gene *Lr24* derived from *Agropyron elongatum* (3DL/3Ag translocation) is known to be located on the long arm of chromosome 3D, near *LrHR122* and tightly linked with the stem rust resistance gene *Sr24* (McIntosh et al., 1977; Schachermayr et al., 1995; Dedryver et al., 1996). The *QLr.arc-3D* QTL in our study inherited from Popo, mapped at 68.6 cM which was outside the interval of *Lr24*. The source parent Popo is also not genetically related to *A. elongatum*, indicating the QTL is different from *Lr24*. It is therefore possible that this is a novel QTL since it doesn't share a region with any of the previously detected QTL or gene.

Effect of different QTL combinations

Analysis of the effect of different combinations revealed that lines with favourable resistance alleles (contributed by Popo) at all three stable QTLs, namely *QLr.arc-1B1*, *QLr.arc-2D* and *QLr.arc-3D* had significantly lower leaf rust severity than lines that had susceptible alleles (contributed by Kariega) at the three loci (Fig. 2). Our observations of epistatic interactions of *QLr.arc-1B1*, *QLr.arc-2D* and *QLr.arc-3D* appears to be previously unreported. The results demonstrate that epistasis

plays a significant role in controlling the expression of complex rust resistance. These allelic combinations need to be further validated in independent populations and a representative range of diverse field environments. Popo harbours several quantitative genomic regions that contribute minor to major effects for leaf rust APR that can be used as a resistance donor to develop resistant cultivars.

Conclusion

For a deeper understanding of the efficacy of the rust resistance genes, the use of multi-environment, multi-season and multi-region trials is recommended. Moving from this consideration, in the present study we tried to dissect the genetic control of the leaf rust resistance trait by employing a RIL population replicated in different seasons and environments. By testing the same bread wheat population in different environments where different races of the pathogen were present, we expected to unveil "hidden" resistance genes of potential interest. Along this reasoning, we showed that closely examining epistatic interactions in order to comprehend gene-by-gene interactions and optimize resistance via marker-assisted selection is crucial. Among the QTLs detected in this study, three stable leaf rust resistance QTLs on chromosome 1B, 2D and 3D were some of the regions identified for further investigation, and they were all contributed by the spring wheat cultivar Popo. The uniqueness of these QTLs remains to be determined, as markers linked to the loci would need to be placed on a consensus map and then integrated into a meta-analysis with QTLs from other studies. Popo is a useful source of adult plant resistance especially when combined with other cultivars or breeding lines that have known genes or QTLs that condition durable leaf rust resistance.

CRedit authorship contribution statement

Sandiswa Figlan: Conceptualization, Project administration, Writing – original draft, Software, Methodology, Investigation, Formal analysis, Data curation. **Tsepiso Hlongoane:** Writing – review & editing, Investigation. **Carlos Bainotti:** Writing – review & editing, Investigation. **Pablo Campos:** Writing – review & editing, Investigation. **Leonardo Vanzetti:** Writing – review & editing, Software, Formal analysis. **Gabriela Edith Tranquilli:** Writing – review & editing, Resources, Investigation, Funding acquisition, Conceptualization. **Toi John Tsilo:** Writing – review & editing, Supervision, Resources, Project administration, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.stress.2024.100570.

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