Screening wheat (*Triticum aestivum* L.) landraces to use as donor lines of Russian wheat aphid resistance and the application of molecular markers to identify potential high yielding genotypes with minimal linkage drag to undesirable traits

By

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Dedication

Derrick Bapela, you are the most humble, selfless and caring person and I am lucky to be your little sister.

Declaration

I **Bapela Theresa Magabjane** hereby declare that the dissertation **Screening wheat (*Triticum aestivum* L.) landraces to use as donor lines of Russian wheat aphid resistance and the application of molecular markers to identify potential high yielding genotypes with minimal linkage drag to undesirable traits**, which I hereby submit for the degree of **Master of Science in Agriculture** at the University of South Africa, is my own work and has not previously been submitted by me for a degree at this or any other institution.

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I declare that during my study I adhered to the Research Ethics Policy of the University of South Africa, received ethics approval for the duration of my study prior to the commencement of data gathering, and have not acted outside the approval conditions.

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Dissertation summary

Wheat, a staple crop globally, production is constrained by factors such as biotic and abiotic factors, socio-economic factors, decrease in arable land, shift to more profitable crops e.g. maize and soybeans. Therefore, host plant resistance and the use of high yielding cultivars at lower input costs is necessary to meet the local demands for wheat. This study aimed to identify resistance to the multiple South African Russian wheat aphid biotypes and potentially high yielding genotypes. Therefore, 80 wheat donor lines were screened for resistance to RWA biotypes RWASA1, RWASA2, RWASA3 and RWASA4, molecular markers were used on 30 RWASA3, and RWASA4 resistant genotypes. Twenty-five genotypes were resistant to all four RWA biotypes. Almost all 30 genotypes selected contained one gene if not all four tested for yield component traits. These genotypes will be valuable genetic sources for identifying resistance to RWASA5 in the future and for haplotype analysis in molecular breeding.

Kararetšo ya boithuto

Korong sebjalo se se tlwaelegileng lefaseng ka bophara, se angwa gampe ke mabaka a go swana le tše di phelago le tše di sa phelego, maruo le kgolo ya palo ya batho mo Afrika Borwa, ho fokotšega ga mobu o loketšeng temo le temo ya dibjalo tše di nago le poelo e ntši tša go swana le lekgea le dinawa. Ge gole bjalo, go kganya ga semela le temo goba tšhumišo ya korong y abo ba le poelo e tšhi ka ditshenyegelo tša fase go bohlokwa go fihlela ditlhoko tša legae tša korong. Boitutho bjo bo diretšwe le go netefatša go kgetholla kganyetšano mehuta ya dikokonyana tša Afrika Borwa tša go ja korong tše ditšwang Russia le go kgetholla dimela tsa poelo ya godimo. Ka gorialo, dimela tše mashome a seswai di ile tša netefatšwa go bona gore di tla kganyetša mehuta e nne ya dikokonyane, tšona e lego RWASA1, RWASA2, RWASA3 and RWASA4. Maswao a bonolo le a godišitšweng a ile a šomišwa go dimela tše mashometharo le motšo o tee tšeo di šetšego di kganyeditše RWASA3 le RWASA4 go kgetholla dimela tše di ka bontšhago lehlabula goba puno ya godimo. Dimela tše mashomepedi le metso e mehlano di bontšhitše go ganana le dikokonyane tše tše nne kamoka. Enyakile ka moka ga mashometharo ya dimela tse di kgethilwego di kgokagana le e tee ge e se tše nne tša ditšhupetšo tša diphatša tša lehutšo. Dimela tše di bohlokwa le mohola go ka šumišwa kgetholla tše kganyetšang RWASA5 ka moso le go hlahloba katišo ya diphatša tša lehutšo ka moso.

Verhandeling opsommin

Koring is een van die belangrikste graangewasse wêreldwyd en word in Suid-Afrika in drie verskillende produksiestreke verbou. Hierdie kommoditeit word egter beperk deur plae soos Russiese koringluis (RKL), asook deur ander biotiese, abiotiese en sosioekonomiese faktore. In hierdie studie is 80 koringgenotipes vir weerstand teen die vier Suid-Afrikaanse RKL biotipes, oftewel RWASA1, RWASA2, RWASA3 en RWASA4, geëvalueer. Hiervan was slegs 25 genotipes bestand teen al vier biotipes. Hierdie genotipes is derhalwe waardevolle toekomstige bronne vir die identifisering van weerstand teen die nuutste biotipe, RWASA5. Eenvoudige volgorde herhaling (SSR) en gekloofde versterkte polimorfisme volgorde (CAPS) merkers gekoppel aan hoër duisendkorrelmassa en korrelgetalle asook langer korrellengte-gene is op 31 RWASA3- en RWASA4-weerstandige genotipes getoets om nuwe bronne met 'n hoër opbrengspotensiaal te identifiseer. Byna al 31 genotipes wat in hierdie studie vir RKL weerstand gekies is, besit ten-minste een of meer opbrengspotensiaal-gene. Hierdie geselekteerde lyne is waardevolle bronne vir die analise van haplotipes in molekulêre genetika.

Abstract

Wheat is an important staple food produced, consumed and traded globally. South Africa is now experiencing climate change, the emergence of biotypes and pathotypes, increasing human population, as well as a decrease in wheat production due to farmer's transition to more profitable crops like maize and soybeans. Improvement of wheat yield, selection of biotype resistant and high yielding cultivars and wheat production increase is important for meeting the demands of increasing population. This study aimed to select genotypes with resistance to the four South African RWA biotypes and with high yield potential. This was done to contribute to the Agricultural Research Council-Small Grain (ARC-SG) RWA resistance breeding and crop improvement program. This was achieved by screening 80 genotypes with the four RWA biotypes to identify genotypes with unique and stable resistance. Furthermore, by phenotyping the growth period, spike related traits, and screening yield component molecular markers on the RWASA3 and RWASA4 highly resistant plants. RWASA3 was the most damaging biotype while RWASA1 was the least damaging biotype (P<0.0001). After phenotyping the donor lines with the four RWA biotypes, 25 sources of resistance to all four biotypes were identified with comparable resistance to the differential check CItr 2401. New resistance sources to these biotypes were thus found and could help in identifying RWASA5 resistance in the future. Seven new and distinctive resistance patterns from RWASA1 to RWASA4 i.e. RRSR, RSRR, RSRS, RSSR, SRSR, SSRR and SRRS were found on 22 genotypes based on resistance to either one or both RWASA3 and RWASA4. These genotypes add to the ARC-SG host plant resistance pre-breeding. Both stable and mixed reaction exists within the landraces, suggesting the need for continuous selection of useful traits. Therefore, when searching for new germplasm source, landraces are recommended. The spike traits measured on selected RWASA3 and RWASA4 genotypes showed direct and indirect influence on each other. Correlation of coefficient showed strong and positive relationships among the spike traits measured. The tested SSR and CAPS markers were informative whereby almost all genotypes had linkage to one gene if not all four of the yield component genes. Their polymorphism has shown that these markers could be used in different genetic backgrounds.

Keywords: Host plant resistance, Marker assisted selection, Russian wheat aphid, Uniformity, Wheat landraces, Yield potential.

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List of acronyms

AD	Number of days to anthesis
AFLPs	Amplified Fragment Length Polymorphisms
ARC-SG	Agricultural Research Council-Small Grain
ANOVA	Analysis of Variance
CIMMYT	International Maize and Wheat Improvement Centre
CTAB	Cetyl Trimethtyl Ammonium Bromide
DNA	Deoxyribose Nucleic Acid
DAFF	Department of Agriculture Forestry and Fisheries
Dn	*Diuraphis noxia*
EDTA	ethylene-diaminetetraacetate
FAO	Food and Agriculture Organisation
GAIN	Global Agricultural Information Network
CAPS	Cleaved Amplified Polymorphism Sequence
GNS	Grain numbers per spike
GNP	Grain numbers per plant
GWS	Grain weight per spike
GWP	Grain weight per plant
HD	Number of days to heading
KASP	Kompetitive Allele Specific PCR
MAS	Marker-assisted selection
NWIC	National Wheat Improvement Committee
NPGS	National Plant Germplasm System
NSP	Number of spikes per plant
PCR	Polymerase Chain Reaction
PI	Plant Introduction
QTL	Quantitative trait loci
RAPDs	Random Amplification of Polymorphic DNAs
RFLPs	Restriction Fragment Length Polymorphisms
RIL	Recombinant inbred lines
RSA	Republic of South Africa
RWA	Russian wheat aphid
RNA	Ribonucleic acid
SA	South Africa
SADC	Southern African Development Community

SL	Spike length
SNPs	Single Nucleotide Polymorphisms
SSRs	Simple Sequence Repeats
TKW	Thousand-kernel weight
USA	United States of America
USDA	United States Department of Agriculture

Chapter arrangements

Chapter 1 is the summary of the study where background of the study, research problem, aim, research objectives and research motivation are stated.

Chapter 2 provides the review of the study particularly Russian wheat aphid, its origin, evolution, descriptive characteristics, virulence profiles, life cycle, feeding style, damage symptoms and control measures of this pest. This chapter also provides insight into the origin of wheat, its evolution, its genetic resources, an overview of its improvement, traits determining yield and their relationship as well as the use of molecular markers in research studies.

Chapter 3 contains the methodologies, procedures and protocols followed for conducting the Russian wheat aphid phenotyping, yield trait phenotyping and genotyping as well as how data collected were analysed.

Chapter 4 contains all the results obtained from the Russian wheat aphid phenotyping, yield trait phenotyping and genotyping. The results are also discussed and the conclusion is also stated here.

Chapter 5 contains the summary of the research study, research findings, study limitations and contributions, conclusions and recommendations about the entire study.

Chapter 1

1. Background of the study

Wheat (*Triticum aestivum* L.) is one of the most staple crop produced worldwide (Food and Agriculture Organisation (FAO), 2016). Approximately 32 out of 36 South African crop production regions produce wheat (Nhemachena and Kirsten, 2017). The main leading production provinces in SA are Western Cape (winter rainfall region), Free State (summer rainfall region) and Northern Cape (irrigation) (Van Niekerk, 2001; ARC, 2014). This cereal crop ranks the second most important grain crop produced falling between maize and sorghum and second among daily-consumed crops e.g. maize, wheat and rice (FAO Statistics, 2016). Uses of wheat flour include bread making, rolls, biscuits, doughnuts, muffins, pancakes and pasta products such as macaroni, spaghetti, animal feed and ethanol production (Kumar *et al.,* 2011). Wheat production in SA has been fluctuating drastically over the years and it is currently less than half of what the population consumes (Grain SA, 2018; United States Department of Agriculture-Global Agriculture Information Network (USDA-GAIN), 2019). Other factors contributing to wheat production decline include climate change, biotic factors such as biotypes and pathotypes and abiotic factors such as rainfall, temperature and lack of arable land. This had led SA to be a net importer of this commodity. Previous production figures for wheat are estimated at around 1500 000 tons for the 2017/2018 production season and nearly 1700 000 tons for the 2018/2019 production season (USDA GAIN, 2019). This is still below the minimum requirements of 2.8 million tons (Smit *et al.,* 2010; Department of Agriculture Forestry and Fisheries (DAFF), 2016). Therefore, increasing population growth, high food demand and changing lifestyles mean more wheat must be produced. Increased production of wheat at lower input costs through the selection of Russian wheat aphid (RWA) resistant and high-yielding cultivars will stabilise and support the local wheat industry, currently a net importer of this commodity. Continuous research and crop improvement of wheat varieties with resistance to yield and biotypes such as Russian wheat is essential and necessary for food security.

RWA *Diuraphis noxia* from the family Aphididae has gained attention in SA as an aggressive pest of wheat. Its first report was dated in 1901 in the Crimea (Kovalev *et al.,* 1991). Later on, it was discovered in South Africa in 1978 (Walters *et al.,* 1980), and United States of America (USA) in 1986 (Stoezel 1987; Webster and Starks, 1987). Australia was known as an RWA free country (Ennahli *et al.,* 2009) until May 2016 (Agriculture Victoria, 2017). Currently, five different RWA biotypes are known and were reported to occur in SA i.e. RWASA1 (Walters *et al*., 1980), RWASA2 (Tolmay *et al*., 2007), RWASA3 (Jankielsohn, 2011), RWASA4 (Jankielsohn, 2014) and RWASA5 (Jankielsohn, 2019). RWA biotypes are resistance-breaking pest populations weakening plants containing specific gene(s) rendering

them susceptible although they were previously resistant (Jankielsohn, 2014). They vary from one another through virulence profiles against different wheat cultivars with different resistant genes (Jankielsohn, 2011; Jankielsohn *et al*., 2016). RWA feeds in the phloem resulting in damage symptoms like leaf rolling, white or yellow longitudinal leaf stripes, purple discoloration, head trapping and underdeveloped growth (Unger and Quisenberry, 1997; Kazemi *et al*., 2001). In SA, chemical control and host plant resistance are used to control RWA in addition to other less used control methods such as cultural and biological control. Globally, chemical control is preferred although it has some disadvantages such as aphid's ability to hide inside rolled leaves and high costs as millions are spent on pesticides thus making chemical control difficult or an expensive approach. In the USA, R1 billion losses from yield reduction and insecticide input have been reported (Morrison and Peairs, 1998). Some chemicals have been banned for use as they threaten the pollination mechanisms from insect pollinators. Therefore, host plant resistance serves as an efficient, environmentally friendly and reliable method of managing the cereal pest in areas prone to RWAs (Marasas *et al.,* 2005). More than 27 wheat cultivars have been released with variable resistance/susceptibility to the four RWA biotypes (Tolmay and van Deventer, 2005; Tolmay *et al*., 2007; Burger and Killian, 2016a,b).

To date, there are 18 identified and reported *D. noxia* resistance genes in different genetic resources including landraces, improved cultivars and, breeding materials. Those *D. noxia* resistance genes include *Dn1* (Du Toit, 1989), *Dn2* (Du Toit, 1989), *dn3* (Nkongolo *et al*., 1991a), *Dn4* (Nkongolo *et al*., 1991b; Saidi and Quick, 1996), *Dn5* (Marais and Du Toit, 1993; Liu *et al*., 2001), *Dn6* (Saidi and Quick, 1996; Liu *et al*., 2002), *Dn7* (Marais *et al*., 1994), *Dn8* and *Dn9* (Liu *et al*., 2001), *Dn10* (Li *et al*., 2018), *Dnx* (Liu *et al*., 2001), *Dny* (Smith *et al*., 2004), *Dn1881* (Navabi *et al*., 2004), *Dn2401* (Dong *et al*., 1997; FazelNajafabadi *et al*., 2014), *Dn2414* (Peng *et al*., 2007), *Dn100695* (Tonk *et al.,* 2016), *Dn225227* (Tolmay *et al.,* 2016) and *Dn626580* (Valdez *et al*., 2012). Either some of these genes are clustered, identical or different alleles located in the same chromosomal region. This include *Dn7* and *Dn2414* resistance genes reported to share similar marker profiles (Peng *et al.,* 2007) and phenotypic profiles with high levels of resistance to all SA RWA biotypes (Jankielshohn, 2014) and all eight USA RWA biotypes (Anderson *et al.,* 2003; Haley *et al.,* 2004: Lapitan *et al.,* 2007; Weiland *et al.,* 2008; Randolph *et al.,* 2009; Mornhinweg 2012; Puterka, 2017). Similar marker profiles for *Dn2401* and *Dn626580* (Valdez *et al.,* 2012; Fazel-Najafabadi *et al.,* 2014) and *Dn1*, *Dn2*, *Dn5*, *Dn6, Dnx*, and *Dn2401* indicates that they may also be allelic or a cluster of genes in the same chromosomal region (Liu *et al.,* 2001; 2002; 2005; Miller *et al.,* 2001; Fazel-Najafabadi *et al.,* 2014). The presence of these clusters is important to molecular breeders interested in those

specific genes. Different biotypes in different locations raise concerns about whether the resistance sources to RWA biotypes in one location may/may not combat RWA biotypes in other locations. *Dn7*, *Dn2401*, and *Dn2414* are the RWA resistance genes resistant to known RWA biotypes globally (Jankielsohn, 2014; Puterka *et al.,* 2014). Both *Dn7* and *Dn2414* have been introduced from 1RS*/*1BL translocation and this translocation is associated with bad dough traits (Graybosch *et al.,* 1990). Moreover, *Dn6* resistance gene is resistant to all SA biotypes and all USA biotypes except RWA2. This leaves us to rely on *Dn2401* for RWA resistance and possibly for commercial deployment because its resistance to all known RWA biotypes (Tolmay and Booyse, 2016).

For a successful RWA and yield pre-breeding process, it is important to identify wheat genotypes with resistance to the latest biotypes and with high yield potential. Furthermore, screening genotypes with more than one biotype in breeding ensures the possibility of incorporating multiple resistances against multiple biotypes. This can be achieved by selecting highly resistant plants and high yielding genotypes from the wheat landraces. Therefore, continuous evaluation, identification, selection, purification and characterization of resistant plants in wheat breeding programs could provide new diverse sources of resistance to multiple biotypes and high yielding germplasm. This study objects: (i) identify new resistant genotypes, new resistance patterns and stable resistance to four South African RWA biotypes i.e. RWASA1, RWASA2, RWASA3, and RWASA4 (ii) to screen previously reported Simple Sequence Repeats (SSR) and Cleaved Amplified Polymorphism Sequence (CAPS) markers linked to high thousand kernel weight (TKW), higher grain numbers and longer grain length on genotypes with resistance to RWASA3 and RWASA4. These genotypes would be valuable sources of resistance in the Agricultural Research Council-Small Grain (ARC-SG) germplasm and crop improvement programs. Moreover, identification of genes potentially linked to traits like higher TKW, higher grain numbers and longer grain length will accelerate multi-gene pyramiding in addition to elucidating the molecular mechanism of how yield is formed.

2. Research problem

RWA poses an enormous threat to the small grain industry globally due to its adaptability to changing environments. Extensive damage caused by the cereal aphid feeding negatively contributes by reducing yield against an increasing population growth while millions are spent on insecticides to control the aphid. Commercial wheat farmers are reluctant to use wheat landraces due to modern techniques not suitable to landraces thus depending on wheat breeders to develop lines with improved traits. However, few breeders are practicing gene combination of resistance to biotypes and pathotypes in genotypes of interest. Moreover, we have an increasing population growth with declining wheat

production. However, the breeding program success depends on the existence of the genetic diversity from which to source the traits. Landraces are a good source of a germplasm with mixed resistance or reaction to a specific biotype. The presence of mixed reaction is due to their multiple traits such as reliability in yield, tolerance to biotic (diseases and pests) and abiotic factors (temperature and drought). Thousands of landraces are kept in many seedbanks worldwide although the majority of these genetic resources are poorly described and little has been done to identify most landraces diversity for effective utilization in pre-breeding programs. Therefore, single plant identification and selection from the landraces for searching useful traits such as highly resistant plants and high yielding plants is the first step forward to a successful pre-breeding process.

3. Aim and objectives

Aim

To contribute to the ARC-SG RWA resistance pre-breeding and crop improvement through selection of genotypes with resistance to the latest South African RWA biotypes RWASA3 and RWASA4 and with high yield potential.

Research objectives

1. To screen wheat donor lines for resistance to the four South African RWA biotypes as already evaluated with the USA biotypes RWA1 and RWA2.
2. To identify high yielding genotypes with resistance to RWASA3 and RWASA4 through use of molecular markers.

4. Research questions

1. How unique and resistant are the wheat donor lines when evaluated against the four RWA biotypes?
2. What resistance patterns do the donor lines possess when evaluated with the four RWA biotypes?
3. How stable or uniform and mixed are the wheat landraces when evaluated against each of the four RWA biotypes?
4. How resistant are the donor lines to the latest RWA biotypes RWASA3 and RWASA4?
5. How variable are the growth periods of the RWASA3 and RWASA4 superior resistant donor lines?
6. Are molecular markers able to identify high yielding genotypes in the selected superior RWASA3 and RWASA4 wheat landraces?

5. Research rationale and motivation

Russian wheat aphid was the choice of this study since is an international pest of small grains which continuously threatens the small grain industry: breeders and farmers. Recently (May 2016), the first RWA biotype was detected in Australia where it was not occurring before therefore, indicating that the pest is still a threat and that a new RWA biotype can emerge anytime in any production region. Acreage of land devoted to wheat production in SA is declining annually due to farmer's transition to more profitable crops like maize and soybeans. Furthermore, property investment, infrastructure and recreation has received much attention through land utilization thus contributing to the continuous decline in agricultural land. The leading wheat breeding and commercialisation companies in SA are Pannar, Sensako and ARC-SG, all striving towards the same goal of addressing wheat agronomic traits such as cultivar adaptability to planting area, revenues and reliability, tolerance to biotic and abiotic stresses and aluminium toxicity. Therefore, there is a need to improve wheat yield potential in order to increase and sustain the agricultural productivity. This can be achieved through utilization of cultivars with high produce, tolerance to biotypes, pathotypes, adaptability to changing temperatures and fluctuating rainfall, nutritional and processing quality. For this reason, rising challenges of poverty, food insecurity, water shortage and instability of prices in the international markets could be addressed. Although the resistance to RWA in SA was found in 1985, during 1998, ARC-SG discarded 80% of wheat germplasm due to RWA susceptibility and it was decided that any further release should contain RWA resistance (https://wheat.pw.usda.gov/ggpages/awn/44/Textfiles/SOAFRICA.html).

Host plant resistance was the choice of this study because as much as it is a preferred method, in other countries it threatens the pollination mechanisms in pollinators (honey, bumble and flower bees, solitary species, pollen wasps, ants, hoverflies, butterflies and moths). SA relied on pesticides during the first few years of the occurrence of the first RWA biotype RWASA1; however, overtime breeders developed RWA resistant cultivars thus enabling farmers to alternatively adopt host plant resistance approach. Over time, chemical control was seen as an expensive approach leading to reliance to resistant cultivars. This transition from chemical control to host plant resistance saved and will continue to save millions of Rands used to purchase insecticides and pesticides. This research is done to contribute to the ARC-SG pre-breeding program to avail lines with RWA resistance traits. Landraces are valuable donors of specific traits needed by modern wheat breeders. Use of landraces depends on the extensive phenotypic characterization and the knowledge about the existence of the genetic diversity and this requires a precise information to choose

parental lines that can be used for hybridization (cross-pollinating or transferring desirable traits). Studying genetic diversity (molecular markers linked to yield related traits) in landraces is important for conservation and characterisation of valuable genetic sources. Therefore, evaluation of landraces for specific traits forms an integral part of pre-breeding process.

6. Ethical considerations

Ethical approval was obtained from the University of South Africa (UNISA), Faculty of Agriculture and Environmental Sciences, Department of Agriculture and Animal Science. Authorization to conduct the research was also acquired from ARC-SG. Ethical considerations, guidelines, methodologies and protocols adopted from different authors were followed in specific stages of the research.

Chapter 2: Literature review

2.1 Wheat (*Triticum aestivum* L.)

2.1.1 Wheat origin and evolution

Wheat (*T. aestivum* L.) in the family Gramineae is one of the most important staple cereal crops (Figure 1) grown globally (FAO, 2016). However, the information on its domestication is inconclusive. According to Gooding and Davies (1997), wheat is a grassy crop originating in the Middle East and is believed to have been domesticated around 12,000 to 15,000 B.C in the area historians called the Fertile Crescent or the Ancient Middle East (Vavilov and Dorofeev, 1992). The Fertile Crescent was an area extending or passing through Iraq (formerly known as Mesopotamia) and Syria (Shewry, 2009). Its cultivation in the Fertile Crescent began ~10,000 BC at the end of the Neolithic Revolution "stone age" when humans were using tools and weapons made from stones (Harris, 1998). Subsequently, this Neolithic transition from hunting and gathering lifestyle led to stable agriculture. Continuous cultivation and repeated harvesting and planting led to wheat's domestication extending to Africa, Asia and European countries (Kilian *et al.,* 2010).

Figure 1: Wheat (*Triticum aestivum* L.) grains and spikes.

Wheat as an allohexaploid has three genomes A, B and D. This allohexaploid genome (2n=6x=42; AABBDD) originates from hybridization between tetraploid emmer wheat (2n=4x=28; AABB, *T. turgidum*) and diploid goatgrass (2n=14; DD, *Aegilops tauschii* (Figure 2) (Dvorak *et al.,* 2006; Dubcovsky and Dvorak, 2007). The AABB of *Triticum turgidum* was derived from hybridization between *T. urartu* (2n= 14; AA) and

Aegilops speltoides-related species, the donor of the B genome (Salse *et al.,* 2008). It was reported that among other crops, hexaploid wheat (2n=6x= 42, AABBDD) has the largest genome (Gill *et al.,* 2004), which is composed of 80% repetitive sequences (Brenchley *et al.,* 2012) and 70% transposable elements (TEs) (Li *et al.,* 2004).

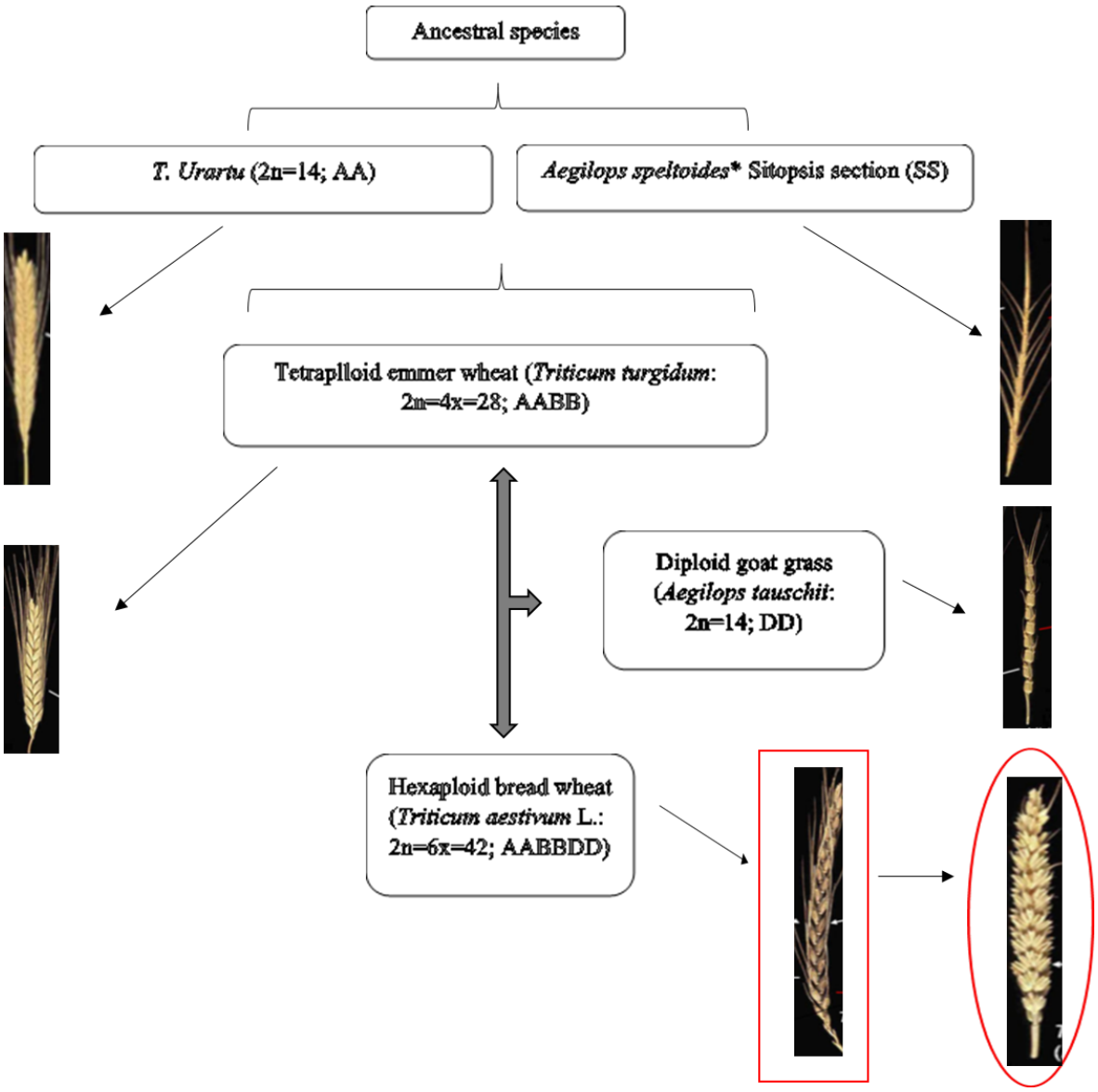

Figure 2: The origin, evolution, domestication, hybridization and genomic relationship between *Triticum aestivum* L. and its hybridization between *Triticum turgidum* and *Aegilops tauschii* [Modified from: Shewry, 2009].

According to Shewry (2009), hexaploid wheat underwent significant changes (Figure 2) during domestication from the hulled form (rectangle) in which glumes stick tightly to the grain, to free/easy threshing naked forms (circle). The author hypothesized that this

hybridization might have occurred on its own over time until the wheat (*Triticum aestivum* L.) was selected by farmers due to its superiority and evolved unique traits (Shewry, 2009).

2.1.2 Wheat growth process

Each wheat component develops at different growth stage and the conditions for each growth stage are different and directly influence the measurable factors of these components (Harasim *et al.,* 2016). Figure 3 is sectioned into four growth phases: tillering, stem elongation, heading and grain filling, and harvesting. These growth phases are involved in the relationship between the wheat components. Typically, it takes six to eight months for a wheat plant to reach maturity depending on the cultivar. The first stage is planting the wheat seed in in the soil followed by fertilizing and watering, to ensure healthy and disease-free environment for the seedling. Few weeks after the seedlings have emerged from the ground, multiple stems called tillers (Figure 3, phase I and II) branch out from the seedlings shoot to form spikes, which later mature to form wheat heads (Phase III), each bearing one to 80 kernels. Grain filling occurs between anthesis and maturity; whereby the dry matter accumulates and its partitioning into grain is determined.

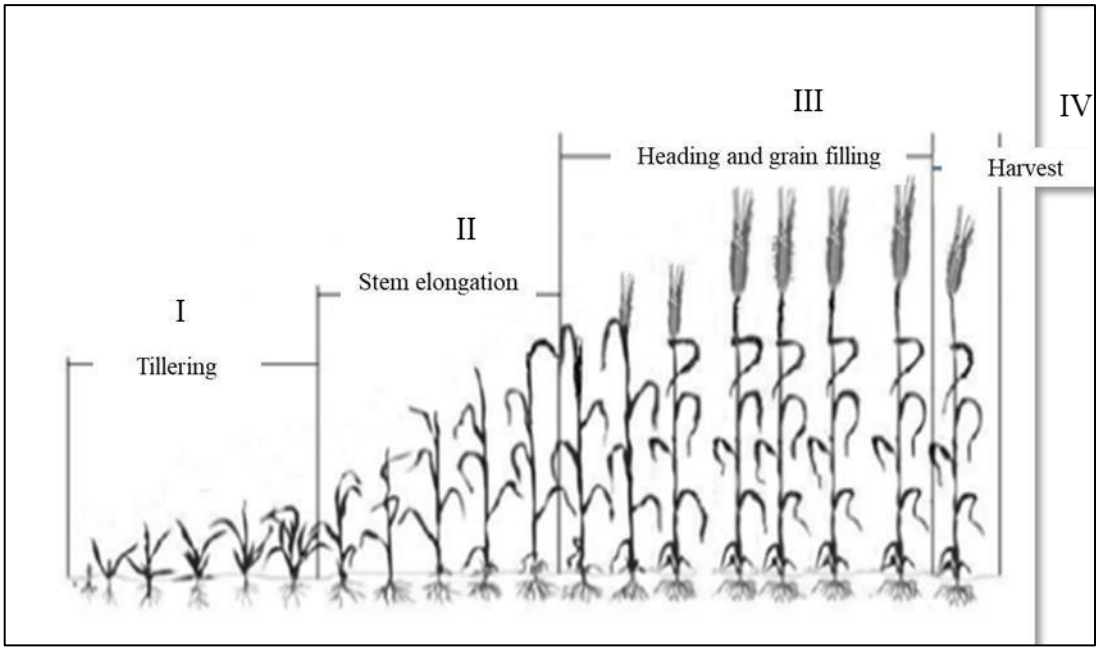

Figure 3: Wheat growth stages (ARC wheat production guide, 2017).

Grain yield is determined by multiple components which are influenced by plant growth, development and its interaction with the environmental factors such as temperature, soil type and rainfall. (Mohammadi *et al.,* 2011).

2.1.3 Wheat climatic requirements

The wheat climatic requirement is dependent upon the cultivar used. Wheat can be classified into spring and winter cereal crop whereby its growth period is dependent upon climate, seed type, and soil conditions (DAFF, 2016). Spring wheat requires warm temperatures ranging from 22-34°C while winter wheat requires temperatures ranging from five to 25°C (DAFF, 2016) or vernalisation in cases of glasshouse/greenhouse experiments. South Africa produces wheat in both summer (dryland and irrigation conditions) and winter rainfall (dryland conditions) regions respectively (Nhemachena and Kirsten, 2017). To successfully plant wheat, cool and moist season, followed by a warm, dry season for harvesting are needed (DAFF, 2016). The cereal crop is planted mainly between mid-April and mid-June in the western and southern Cape (winter rainfall areas) while in the eastern Free State (summer rainfall areas) it is planted between mid-May and the end of July (DAFF, 2016).

2.1.4 Wheat production to date

Jan van Riebeeck's introduction of wheat in SA laid a foundation for all wheat production and consequent breeding programs existing to date. The three companies involved in wheat improvement in SA include Sensako, ARC-SG and Pannar, established in 1958, 1992, and 1990s respectively, although Sensako formed part of Monsanto from 1999. All these sectors strive towards the same goal of addressing wheat agronomic traits such as cultivar adaptability to different environments, yield and reliability, tolerance to biotic and abiotic stresses and aluminum toxicity (Smit *et al.,* 2010). Globally, SA is the 37th wheat-producing country, fourth largest in Africa and the largest in the Southern African Development Community (SADC) region (FAO, 2016). Figure 4 shows the South African wheat production trend for nearly three decades. According to Figure 4, wheat production is SA has been fluctuating from 1996 to 2018 due to farmers shift to more profitable crops like maize and soybean. Moreover, farmer's ability to retain seeds from their planting makes the purchase of seeds unnecessary thus leading to plant breeders and seed companies less likely to invest in the development of new cultivars due to costs involved in developing and registering new cultivars. The current production is still below the minimum requirement of 2.27 million tons and below what the country consumes (Nhemachena and Kirsten, 2017). A decrease in agricultural land and water, increasing population growth, changing lifestyles, increased meat and dairy consumption and biofuel consumption raises significant demands and threats to crop production (Godfray *et al.,* 2010; Tilman *et al.,* 2011).

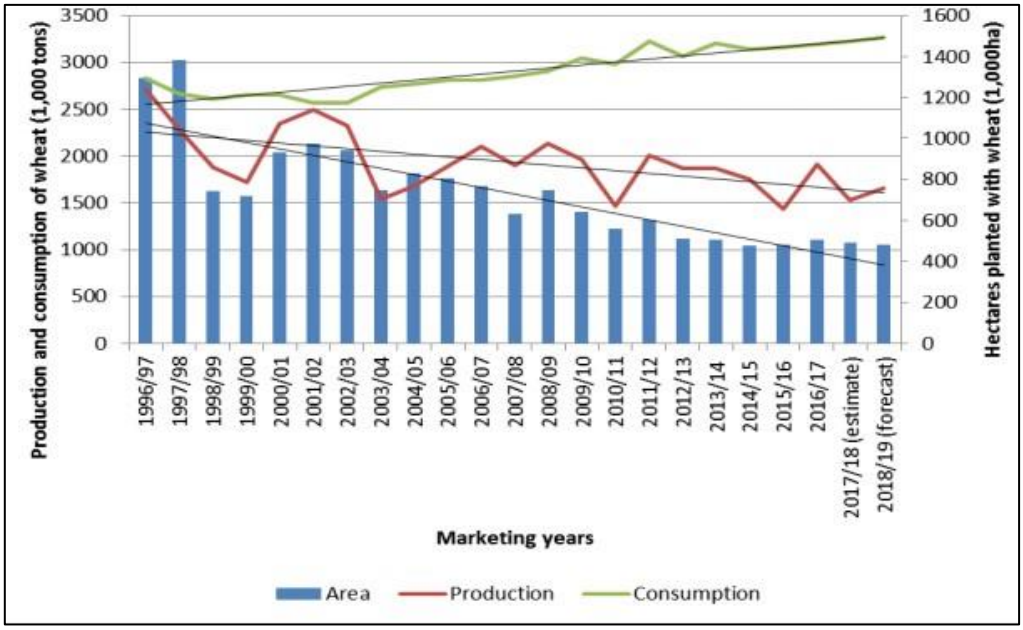

Figure 4: South African wheat production trend from 1996/1997 to 2018/2019 (USDA GAIN, 2019).

Global human population is predicted to reach up to approximately 9 billion by 2050 and 11.2 billion by 2100 (United Nations Department of Economic and Social Affairs, Population Division, 2015). In order to meet these projections, improvement of wheat yield and food production must increase by 70% by 2050 (FAO, 2009; Hunter *et al*., 2017) where 12% is for cereals (Linehan *et al*., 2012). However, with the current production decrease rate, it is uncertain that this will keep up with this increasing population growth. Therefore, the need for crop improvement and doubling production is crucial to address hunger issues and demands that may arise with the increasing population. Moreover, improved cultivars and management techniques e.g. irrigation, fertilizers, pesticides, and fungicides applications are important techniques for increased grain yields (FAO, 2010).

2.1.5 Uses of wheat

Wheat demand and use are dependent upon population type and amount, taste and preferences (USDA, 2016). Gluten (protein) and starch are two properties making wheat useful in food and non-food products and industrial applications. The ability of the gluten to be elastic, bind water and form films that can be stabilized with heat makes the gluten unique thus rendering wheat gluten useful for adhesives, coatings, polymers and resins preparations. Bread and durum wheat are used to make a variety of food products. This include (i) bread wheat processed into leavened and unleavened bread, biscuits, cookies, and noodles (ii) durum wheat used to make pasta products such as macaroni and spaghetti mainly in industrialised countries, however, in developing countries it is used to make bread,

couscous and bulgur freekeh, puffed cereals, hot cereal, desserts and filler for pastries. (Worldgrain.com, 2018). In SA bread wheat is produced in larger quantities. In addition to wheat uses, wheat more than any other food crop had played a significant role in religion as part of Holy Communion during the Passover celebrations (Shewry, 2009).

2.1.6 Wheat allergies

Gluten intolerance known as non-celiac gluten sensitivity (NCGS) is an emerging new entity and the clinical features of gluten intolerance include symptoms such as abdominal pains, dermatitis, headache, fatigue, and irritable bowel symptoms which occur soon after gluten has been ingested and rapidly disappear once the patient is on a gluten-free diet (Rathi and Zanwar, 2016). Celiac disease is a small intestinal disease caused by wheat gluten (Shewry *et al.,* 2003). Baker's asthma, an allergy associated with inhalation of cereal flours was reported a serious occupational disease negatively affecting workers in the baking industry (Barber *et al*., 1989). Is has been reported that wheat antigens are the most reactive allergens and these allergens which are related to the salt-soluble fractions/proteins of flour dust (Gomez *et al*., 1990). Generally, flour contains many potential allergens such as flour contaminants contributing to the problems associated with the flour inhalation (Baatjies and Jeebhay, 2002, 2013). Flour additives like enzymes and fungal α-amylases also contribute to occupational respiratory diseases during flour manipulation (Ngahane *et al*., 2015).

2.1.7 Genes associated with grain yield in wheat

Wheat has a large genome size of 17 Gb (Mayer *et al.,* 2014). This however makes molecular approaches such as map-based cloning difficult and time-consuming. Comparative genomics have shown that wheat and rice chromosomes exist collinearly (Ma *et al*., 2016). Therefore, homology-based cloning serves as one of the most effective approaches for isolating genes associated with higher yield such as TKW, higher grain numbers and longer grains in wheat. The genetic factors controlling these traits are complex and may differ in different genetic backgrounds.

A few wheat genes have been cloned by comparative genomics such as *TaG5* for TKW (Ma *et al*., 2016), *TaGW2* for grain weight and grain size (Su *et al.,* 2011; Yang *et al,* 2012; Qin *et al*., 2014), *TaSus1* and *TaSus2* for TKW (Hou *et al*., 2014; Jiang *et al*., 2015). There are more other genes linked to the above traits contributing to higher yield in wheat. These include *TaCWI-4A* and *TaCWI-5A* for TKW (Jiang *et al.,* 2015), *TaTGW7A* for TKW (Hu *et al.,* 2016), *TaGASR7-A1* for grain length and yield (Dong *et al.,* 2014), *TaAAP6-3B* which regulates grain protein content (Jin *et al.,* 2018), *TaGS-D1* for TKW and grain length (Zhang *et al.,* 2014), *TaTGW6* for grain weight and grain size (Hu *et al.,* 2016; Hanif *et al.,*

2016) and *TaGS1a* for mineral nutrient and grain size (Guo *et al.,* 2013). Wheat domestication resulted from mutations that gave rise to traits such as rachis fragility and falling over of glumes. There are domesticated genes that have been reported to influence domesticated traits of wheat. These include *Q*, compactum (*C*) and sphaerococcum (*S1*) located on chromosome 5A, 2D and 3D, each affecting specific yield-related traits. *Q* gene pleiotropically affects plant height, spike length, rachis fragility and falling over of glumes (Simons *et al.,* 2006; Sormacheva *et al.,* 2014). *C* gene affects spike morphology, grain size, shape and numbers per spike (Johnson *et al.,* 2008). *S1* gene determines flag leaf shape, dense spike, seed shape and glumes (Salina *et al.,* 2000).

2.1.8 Wheat genetic resources

2.1.8.1 Wheat landraces

Landraces are unique genotypes with an extensive genetic pool and can therefore provide valuable traits important for conventional and molecular breeding (Dotlačil *et al.,* 2010). Before the Green Revolution, most farmers' e.g. smallholder farmers continued to cultivate them without scientific breeding due to their adaptation to climatic conditions, agronomic traits, quality, and suitability for home use (Jaradat, 2013). After centuries of development of landraces through the natural and human selection to meet various environmental, social, economic and cultural needs (Zeven, 1999; Jaradat, 2011; 2013), this led to genotypes with a combination of traits (Masood *et al.,* 2005; Dwivedi *et al.,* 2016). The traits/characteristics include disease, pest and drought tolerance, stable and intermediate levels of yield. The traditional methods to produce wheat landraces included low input agricultural systems e.g. the utilization of internal production inputs, hand planting and harvesting (Zeven, 1999; 2002). Genotypes with required or acceptable traits like plant height (non-lodging), adaptive traits suited for various environments (flowering time) and traits facilitating harvesting were selected when recognized (Peng *et al.,* 2011). However, during domestication, complex morphological, physiological, and genetic traits changed and this change was termed as 'domestication syndrome'. Evolution traits of wheat under domestication include loss of spike shattering at maturity while maintaining spike threshability, changes in the plant structure, changes in the yield and yield components e.g. spike and kernel size and loss of seed dormancy (Shewry, 2009; Peleg *et al.,* 2011).

A set of examples of landraces are listed below. 'Turkey Red' was widely grown in the United States, the central Great Plains during the late 19th and early 20th century due to its resistance to winter conditions thus transforming Nebraska into a winter producing region (Olmstead and Rhode, 2002). Moreover, 'Cheyenne' a selection from landrace Crimea was

used for germplasm improvement in Nebraska. Furthermore, a Japanese variety 'Norin10' originating from a Japanese landrace Shiro Daruma had the *Rht* dwarfing genes (*Rht1*, *Rht2*) which may be dominant or semi-dorminant (*Rht-Blb* and *Rht-Dld*) (Swaminathan, 2014) and with reduced plant height of 60-110 cm compared to other cultivars with plant height taller than 150 cm (Lumpkin, 2015). Since then, those dwarfing genes were utilised by Dr Norman Borlaug in developing high yielding semi-dwarf wheat's that were widely utilized during the Green Revolution (Swaminathan, 2014). A Chinese landrace Pingyuan 50 was a leading cultivar in the 1950s chosen based on adult plant resistance to stripe rust and powdery mildew (Lan *et al.,* 2010; Asad *et al.,* 2014). An Iranian bread wheat landrace PI 137739 was the first source of resistance to RWA in SA (Du Toit, 1987). Recently, various studies have reported different resistance sources originating from wheat landraces. Tolmay and Booyse (2016) reported different levels of phenotypic resistance (moderately resistant and resistant) conferred by wheat landraces to RWA biotypes (RWASA1 to RWASA4). Furthermore, Kertho *et al.,* (2015) reported seedling resistance from wheat landraces to leaf rust race (THBL, MCDL, TDBG and MFPS) and stripe rust race (PSTv-37). Adhikari *et al.* (2012) found resistance to bacterial leaf streak and spot blotch in spring wheat landraces.

These findings indicate how valuable the landraces can be as donors of specific traits needed by wheat breeders. Use of landraces depend on the extensive phenotypic characterization and the knowledge about the existence of the genetic diversity and this requires precise information to choose parental lines that can be used for hybridization (cross-pollinating or transferring desirable traits). Studying genetic diversity in landraces is important for conservation and characterisation of valuable genetic sources. Therefore, evaluation of landraces for specific traits forms an integral part of the pre-breeding process. Most of the wheat landraces are no longer cultivated because they are not suited to modern production methods. For this reason, farmers prefer the use of pure or improved seeds thus resulting with many landrace seeds stored in worldwide whereby breeders or scientists have to source and improve. This in turn results in a reduction or loss of wheat diversity when using pure genotypes (Taghouti *et al.,* 2014). Moreover, in most cases little has been done to describe or understand their traits e.g. yield potential, performance, and genetic diversity for effective exploitation in plant breeding (Dos Santos *et al.,* 2009). Therefore, landraces could serve as sources of genes and alleles that can be utilized in novel breeding programs that are aiming at crop improvement. This in turn benefits the commercial farmers who rely on improved cultivars for production. However, caution is a pre-requisite to ensure access to important genetic diversity with a minimum/no linkage to undesirable traits when using landraces in breeding programs (Manickavelu *et al.,* 2014).

2.1.8.2 Improved and breeding lines

The focal point and the goal of most farmers is sustainable yield. However, for them to successfully plant cultivars of choice, they rely on researchers and breeders to develop cultivars with yield potential and resistance to biotic and abiotic stresses and adaptation to changing climatic conditions. However, potential lines need to be identified from mixed germplasm collections, phenotyped for important agronomic traits and resistance to pathogens such as rust, Fusarium head blight and powdery mildew and biotypes such as RWA and then undergo single plant selection (donor parent). The donor parent provides the trait of interest and may not perform well like a breeding line or elite line in other regions. Therefore, breeding for cultivars resistant to diseases and pests, tolerant to higher temperatures and drought, positive and promising traits for better performance need to be recognized and transferred to the breeding lines (Lopes *et al.,* 2015). A widely used method of transferring the trait of interest is through the backcross method. A donor parent is crossed with the recurrent parent (breeding line) for five to six generations or even more until almost complete homozygous genotypes are created except for small regions (2% of genome size). The F_1 are crossed with the recurrent parent to develop the BC1 population. When this is successful, they are then characterised and utilized in pre-breeding programs.

The Green revolution initiated between the 1940s and 1960s encouraged cereal production due to significant yield increase through a combination of different traits existing in wheat cultivars e.g. high yielding and semi-dwarf wheat's. These dwarfing genes were associated with reduced plant height, lodging resistance, higher grain numbers per spike or unit area and higher harvest index (Shearman *et al.,* 2005). In SA, the release of the first RWA resistant cultivar by ARC-SG in 1992 led to an increase from 3% to 70% in area planted with RWA resistant cultivars (Marasas *et al.,* 1997; 2005). Hernandez *et al*. (2012) evaluated multi-trait resistance (stripe rust, leaf rust, tan spot and Karnal bunt) in a recombinant inbred line (RIL) from a cross between HD29/WH542 and this analysis identified a combination of multiple disease resistance. The combination of RWA and stem rust resistance through gene pyramiding was successful (Amulaka *et al.,* 2013). Recently, Tolmay *et al*. (2016) successfully combined RWA resistance and rust resistance (stem, leaf and stripe rust) in five spring wheat lines to result with an improved genotype. These study types that are aimed at combining multiple traits in an individual accession are of economic importance and should be continuously and effectively adopted by many breeders and researchers to help smallholder farmers and commercial farmers to use them.

2.2 Russian wheat aphid '*Diuraphis noxia*'

2.2.1 Descriptive features and characteristics of Russian wheat aphid

The RWA is a small lime green insect, spindle-shaped and spineless-bodied. It is characterized by shortened antennae and reduced cornicles at the end of its abdomen including a supracaudal (double tail-like) structure (Figure 5) on adult aphids (Hodgson and Karen, 2008). For this reason, they are easy to distinguish from other cereal aphids by not visible tubes or pores in the abdomen known as siphunculi, functioning for excreting waxy defensive fluids (Kazemi *et al.,* 2001). The RWAs are mostly found in the upper leaf surfaces of young growing host plant while feeding (Akhtar *et al.,* 2010). They prefer this feeding section because it provides large amount of mineral nutrients from the phloem tissues (Macedo *et al.,* 2009).

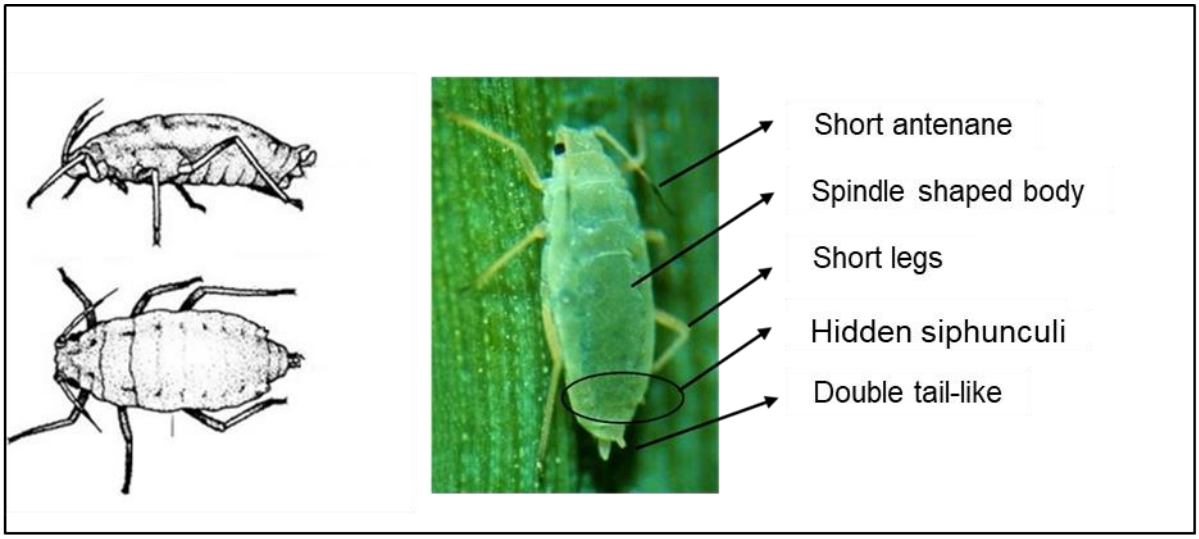

Figure 5: Fundratrix (stem mother) adult Russian wheat aphid, double tail like and with the hidden siphunculi (Modified from Grain Research and Development Corporation, 2019).

2.2.2 Origin and geographic distribution of Russian wheat aphid

The translation of Russian literature of RWA into English by Poprawski (Poprawski *et al*., 1992) from Grossheim (1914) made the pest information accessible to scientists whereby different names of the RWA have come to light since then. This include *Brachycolus korotnewi* (by Mordviko in 1900), *Brachycolus noxius* (by Kurdjumov as barley aphid in 1912) to current *Diuraphis noxia* (by Aizenberg and Mordvillko) (Robinson, 1994). It is a devastating cereal pest reported to occur in all global cereal production areas. Its first report was dated in 1901 in the Crimea (Kovalev *et al.,* 1991), from Crimea then reported in the former Soviet Union in 1912 (Moldova and Ukraine), Turkey in 1959 (Tuatay and

Remaudiere, 1964), China in 1975 (Zhang *et al.,* 1999), SA in 1978 (Walters *et al.,* 1980), Mexico in 1980 (Gilchrist *et al.,* 1984), USA in 1986 (Stoetzel 1987; Webster and Starks,1987), Canada in 1988 (Kindler and Springer, 1989), Czech Republic in 1993 (Starý, 1996), Kenya in 1995 (Macharia *et al.,* 1999). Australia was known as an RWA free country (Ennahli *et al.,* 2009) until May 2016 (Agriculture Victoria, 2017). Reports of new biotypes in SA took place in 2005 for RWASA2 (Tolmay *et al.,* 2007), RWASA3 in 2009 (Jankielsohn, 2011), RWASA4 in 2011 (Jankielsohn, 2014) and RWASA5 in 2019 (Jankielsohn, 2019). Annually, Dr Astrid Jankielsohn in SA monitors the influence of environmental changes on aphid distribution of all four biotypes in the Western Cape, Free State, and Northern Cape. The author's results reported dominance of RWASA1 during 2010 and 2011, the dominance of RWASA3 during 2012 and 2013 and dominance of RWASA4 from 2014 to 2016 in the Free State. Furthermore, consistent dominance of RWASA1 in Western Cape from 2010-2016 and the Northern Cape from 2011-2016 was reported (Jankielsohn, 2017).

2.2.3 Virulence profiles of the Russian wheat aphid biotypes existing in South Africa

Biotype in entomology refers to an individual or population that that can be differentiated from the rest of its species by criteria apart from morphology (Maxwell, 1980). They are insect populations with the ability to survive, reproduce and cause symptoms on resistant genotypes that are known to be resistant to insect populations of the same species (Shufran and Payton, 2009). Furthermore, infraspecific groups, similar morphologically, and variation in expressed biological characteristics can be used to further describe what biotypes are (Shufran and Payton, 2009). RWA biotypes are also resistance-breaking pest populations injuring/weakening plants containing specific gene(s) rendering them susceptible although they were previously resistant (Jankielsohn, 2014). They vary from one another through virulence profiles against different wheat cultivars with different resistant genes (Jankielsohn, 2011; Jankielsohn *et al*., 2016). The term virulence originates from pathology describing the disease-producing ability of the organism (Steinhaus and Martignoni, 1970). Over time, it has evolved to fit even in entomology. Shaner *et al.* (1992) gave a classical definition of virulence as the relative capability to damage the host.

Five biotypes are known to occur in SA i.e. RWASA1-RWASA5 (Du Toit, 1987, Tolmay *et al*., 2006; Jankeilsohn, 2011, 2014, 2019) while eight biotypes RWA1-RWA8 occur in USA (Puterka *et al.,* 2014), and Kenya has two biotypes (Malinga'a *et al.,* 2007). The differentiation of biotypes was achieved through screening different biotypes and assessing their feeding damage on genotypes containing reported *D. noxia* resistance genes i.e. *Dn1* to *Dn9* (Burd *et al.,* 2006; Weiland *et al.,* 2008) *Dn1* to *Dn9*, *Dnx* and *Dny*

(Jankielsohn, 2014; Puterka *et al.,* 2014). Puterka *et al*. (2014) reported biotypic variation with USA biotype RWA1 virulent to genotypes containing *Dn8* and *Dn9* and *Dn1-Dn7* genotypes resistant to this biotype. RWA2 is virulent on *Dn1-Dn6, Dn8* and *Dn9* sources ad only *Dn7* sources are resistant to this biotype. RWA3 and RWA4 virulent on *Dn1-Dn5*, *Dn8* and *Dn9* donors and only *Dn6* and *Dn7* sources are resistant to these biotypes. This reaction to RWA3 and RWA4 was expressed by two genotypes CO960223 and 94M370 reported to carry *Dn6* and *Dn7*. Therefore, the genetics of each line is different as the *Dn6* donor was bread wheat while *Dn7* donor was from rye. Table 1 provides virulence profiles of the four RWA biotypes described in SA.

Table 1: Virulence profiles of different RWA biotypes existing in South Africa and their references.

Biotype	Year detected	Virulent against	Ineffective against
RWASA1	1978 (Walters *et al*., 1980)	*Dn2* and *dn3* (Jankielsohn, 2014; 2016)	*Dn1*, *Dn4*, *Dn5*, *Dn6*, *Dn7*, *Dn8, Dn9, Dnx, Dny* (Jankielsohn, 2014; 2016) and *Dn2401* (Tolmay and Booyse, 2016)
RWASA2	2005 (Tolmay *et al*., 2007)	*Dn1*, *Dn2*, *dn3*, *Dn8* and *Dn9* (Jankielsohn, 2014; 2016)	*Dn4*, *Dn5*, *Dn6*, *Dn7, Dnx, Dny* (Jankielsohn, 2014, 2016) and *Dn2401* (Tolmay and Booyse, 2016)
RWASA3	2009 (Jankielsohn, 2011)	*Dn1*, *Dn2*, *dn3*, Dn*4*, *Dn8*, *Dn9* and *Dny* (Jankielsohn, 2014; 2016)	*Dn5*, *Dn6, Dn7, Dnx* (Jankielsohn, 2014, 2016) and *Dn2401* (Tolmay and Booyse, 2016)
RWASA4	2011 (Jankielsohn, 2014)	*Dn1*, *Dn2*, *dn3*, *Dn4*, *Dn5, Dn9* and *Dny* (Jankielsohn, 2014; 2016)	*Dn6*, *Dn7*, *Dn8, Dnx* (Jankielsohn, 2014; 2016) and *Dn2401* (Tolmay and Booyse, 2016)
RWASA5	2019 (Jankielsohn, 2019)	*Dn1, Dn2, dn3, Dn4, Dn5, Dn7* (Jankielsohn, 2019) *Dn6, Dn8, Dn9 Dnx2006, Dny2006* and *Dn2401* (Jankielsohn, 2019)	

2.2.4 Life cycle, host plants and reproduction rate of Russian wheat aphid

In SA, RWA biotypes survival ability on alternative hosts varies between biotypes (Jankielsohn, 2013). Primary hosts for full life cycle (egg-adult) of RWA are wheat, barley and grass species such as goat-grass and wheatgrass (Stoetzel, 1987, Kindler and Springer, 1989). Secondary hosts for enabling adult RWAs to complete maturity feeding and final instar nymphs to complete development to adult stage are oats and rye (Kindler and Springer, 1989). Both holocyclic and anholocyclic reproduction is known to occur in RWA (Kiriac *et al.,* 1990). A holocyclic reproduction is also known as cyclical parthenogenesis

(possess an egg-laying stage), whereby aphids undergo asexual (in summer) to sexual reproduction (in autumn) to produce eggs that can survive in cold environments. The laying of eggs may be on the same host (monoecious) or different hosts (heteroecious) and this reproduction type normally occurs in severe temperate environments. Contrariwise, in anholocyclic (sexual) reproduction also known as obligate parthenogenesis, aphids reproduce parthenogenetically throughout the year overwintering as adults and this is common in tropics and mild temperate climates. These two principles vary geographically, therefore, enabling them to adapt to changing environments (Dixon, 1985). Kiriac *et al.* (1990) studied several morphs collected from different locations in the Soviet Union and Northwestern USA and found that Jordanian, Syrian, French, Turkish and Kyrgyz RWAs produced no sexual forms meaning they were anholocyclic. However, in the same study, the author found that Moldavian and Crimean populations produced sexual forms. Holocyclic reproduction of RWAs was reported in the USA (Puterka *et al.,* 2012), Hungary and Russia (Basky and Jordaan, 1997) and China (Zhang *et al.,* 2012). RWA was reported to be anholocyclic in SA where individual RWA females can produce 40 to 50 nymphs in 40 days of their lifespan, and the nymphs take approximately 7-10 days to reach the adult stage and start reproduction (Aalbersberg *et al.,* 1987). Field infestation of 20% to 80% can occur within 2 weeks (Schultz, 2014). Environmental factors such as temperature, humidity and light intensity usually influence the RWA feeding, dispersal, reproduction and development. This was recorded in SA with mean temperatures of 17:25°C (night:day)affecting the development of nymphal instars at different stages while lesser mean temperature of 13°C resulting in a high developmental rate except in the 3rd instar (Aalbersberg *et al.,* 1987). This correlates with the studies of Akhtar *et al.* (2010) in Pakistan with greater reproduction rates of nymphs at low temperatures, resulting in aphid population decline although reproduction was associated with high temperatures. However, Qureshi and Michaud (2005) found faster nymphal development of RWA at higher temperatures and with low amplitude.

2.2.5 Russian Wheat Aphid feeding style and damage symptoms

There are three different behaviors conducted by aphids on the host plant before feeding. First is the pre-alighting behaviour where the aphid randomly selects a host plant to land and feed on, secondly, plant surface exploration behavior where the aphid wanders around/over the host plant leaves probing for appropriate leaf area to feed on and lastly nutrients search behavior particularly in the phloem for ingestion (Caillaud *et al.* 1995; Botha *et al.,* 2005). RWA feeds through inserting their piercing and sucking mouthparts called stylets (Goggin, 2007) into the leaf tissue, carefully moving intercellularly until the vascular bundle is reached. There are two saliva kinds involved during host-aphid interaction. Gelling saliva is a salivary type that forms a shielding layer (sheath) around the stylet to enhance

effective stylet insertion. Watery saliva is delivered is delivered into the cell in which the aphid feeds and is involved in manipulating host cell processes. Usually, watery saliva serves to prevent sieve tubes from clogging or sealing off the phloem. The phloem section provides phloem-mobile nutrients such as amino compounds, simple sugars, secondary metabolites and carbohydrates (Douglas, 1993; Macedo *et al.,* 2009; Züst and Agrawal, 2016). Susceptible and resistant wheat varieties react differently upon RWA attack (Haile *et al.,* 1999). Plant development, biology, physiology and morphology when combined are the main factors involved in plant susceptibility to RWA infestation (Macedo *et al.,* 2003). RWA feeds on leaves, stems and developing kernels resulting in damage symptoms such as rolled leaves around the aphid colonies, chlorosis (Figure 6A) in the form of white to yellow longitudinal streaks (Figure 6C) (Unger and Quisenberry, 1997; Kazemi *et al.,* 2001).

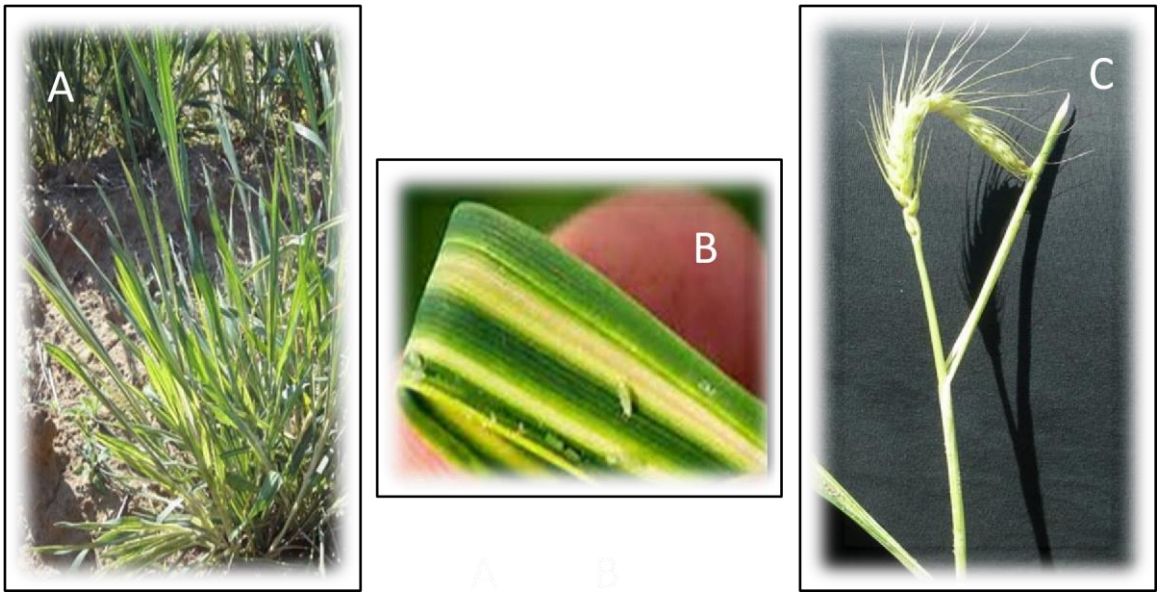

Figure 6: Damage symptoms caused by Russian wheat aphid feeding on wheat seedling leaves attributed by leaf chlorosis and stunting (A), leaf streaking (B) and head trapping (C). (Source: [1]Dr Vicki Tolmay, Dr Astrid Jankielsohn and Dr Gary Puterka)

Saheed *et al*. (2007) reported leaf rolling, chlorosis and necrosis with aphid xylem sucking while feeding on the cell sap. Rolled or curled leaves create an enclosure that protects the aphids from insecticides and natural enemies. Mikak *et al*. (2004) observed

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purple discoloration of leaves under cold temperatures. Stunted growth (Figure 6A) or prostrate tillers have been observed in young plants under heavy infestation (Khan *et al*., 2009). In the results of Akhtar *et al*. (2010) aphids rolled the flag leaf trapping the emerging heads and awns. Up to 34% yield losses were also reported (Akhtar *et al*., 2010). Considerable yield losses of up to 90% have been reported in susceptible cultivars (Du Toit and Walters, 1984). Yield losses of up to 100% are also possible under heavy infestations (Burd *et al.,* 2006). In resistant cultivars, damage symptoms are characterised by small chlorotic spots and blotches on leaves (Tolmay and Booyse, 2016). Smaller yield losses have been reported in resistant cultivars (Tolmay *et al.,* 2005).

2.2.6 Possible control measures for Russian Wheat Aphid

2.2.6.1 Chemical control

Application of chemicals has been in use since the 1970s (Du Toit, 1987). Insecticides such as systemic and contact insecticides were reported effective towards this pest when mixed (Du Toit, 1989). Systemic insecticides are still the most applied method for reducing pest populations in order to counteract the ability of aphids to hide inside rolled leaves, which reduces the effectiveness of the contact insecticides. Contact insecticide is any insecticide that kills the potential target through 'cuticle absorption' rather than being 'ingested' by the target. Systemic insecticide refers to a chemical that is soluble in water and can be absorbed by the plant and translocated to other plant parts/tissues (Bennett, 1957). Grain yield of RWA resistant and susceptible cultivars in SA was high due to seed treatment with imidacloprid (Tolmay *et al.,* 1997). An important consideration when using this method includes economic thresholds, biotype insecticide resistance, natural and beneficial enemies (Farm Biosecurity, 2016).

2.2.6.2 Host plant resistance or use of resistant cultivars

Host plant resistance is the host plant ability to survive insect damage or become less damaged through the presence of unknown or designated genes (Smith, 1989; Jankielsohn *et al.,* 2016). Resistance breeding has been the focal point of many seed companies to develop insect-resistant cultivars (De Vos and Van Doorn, 2013). To date, the use of resistant cultivars serves as the most cost-effective, economical and reliable method of controlling *D. noxia* populations and heavy infestations (Ennahli *et al.,* 2009). Economic benefits of the host plant resistance are reduced insecticides usage and reduced aphid populations (Smith and Clement, 2012). Another advantage of resistant cultivars is that they open more opportunities for increased breeding research studies (Turanli *et al*., 2012) such as development of molecular markers and mapping of resistance genes (Liu *et al*., 2001, 2002). The RWA resistant wheat does not exhibit the same damage symptom as susceptible

wheat and the level of resistance may differ increasing the need to categorize these resistance mechanisms (Randolph *et al.,* 2005). There are three reported categories of resistance/defense mechanisms used by plants upon RWA attack i.e. antibiosis, antixenosis, and tolerance. Painter (1951) first described these three categories. Antibiosis refers to the host adversely affecting insect biology e.g. development, growth and reproduction resulting in reduced insect growth rate, body weight, and fecundity. Antixenosis is defined as the host plant`s ability to serve as a non-preferred host due to toxic substances or lack of an attractant thus resulting in less aphid infestation and less plant damage during the attack. Furthermore, tolerance is reported as the host plant ability to withstand aphid's attack, therefore, resulting with no reduced plant height or biomass (Painter, 1951; Smith *et al.,* 1992; Smith, 2005; Smith, 2006). Consequently, the resistance mechanisms have been well experimented and documented (Smith *et al.,* 1992; Lage *et al.,* 2004; Randolph *et al.,* 2005; Tolmay, 2006; Ennahili *et al.,* 2009; Khan *et al.,* 2009; Lazzari *et al.,* 2009; Smith *et al.,* 2012). Wheat cultivars containing the *Dn4* resistance gene have been reported to exhibit antibiosis, antixenosis, and tolerance (Hawley *et al.,* 2003; Miller *et al.,* 2003). The wheat line CItr 2401 containing *Dn2401* exhibited tolerance and antixenosis to RWA1 (Voothuluru *et al*., 2006) while 94M370 containing *Dn7* exhibited antibiosis as resistance mechanism (Lizzari *et al*., 2009).

Plants are known to use mechanisms of resistance to defend themselves during insect pest attack. For almost five decades now, breeders across the globe have been searching, identifying and using genetic sources of resistance to control RWA. Globally the first report about genetic resistance to RWA was from in SA with two hard white bread wheat genotypes (PI 137739 and PI 262660) evaluated at ARC-SG, against RWASA1 (Du Toit, 1987) furthermore, Tugela-*Dn* containing the *Dn1* resistance gene was the first RWA resistant cultivar released in SA in 1992 resulting in more than 70% of wheat-producing area planted with RWA resistant cultivars (Marasas *et al.,* 1997). This genetic resistance results opened doors for increased research regarding the investigation of genes present and controlling resistance in PI 137739 and PI 262660 lines. Du Toit (1989) investigated this phenomenon and concluded that the resistance in these genotypes are controlled by different genes and assigned them to be *Dn1* in PI 137739 and *Dn2* in PI 262660. Nkongolo *et al*. (1991a) identified a recessive gene in *Triticum tauschii* SQ24 line, different from *Dn1* and *Dn2* and was designated to carry the *dn3* gene. The accession PI 372129 was the first genotype reported resistant to RWA1 in the US in 1987 (Quick *et al*., 1991). The *Dn4* gene was the resistance controlling factor in this genotype (Saidi and Quick 1996; Ma *et al.,* 1998) and a few years later, the first *D. noxia* resistant cultivar containing the *Dn4* gene "Halt" was released in North America (Quick *et al.,* 1996). The CItr 2401 genotype has been reported to carry two resistance genes: one allelic to *Dn4* on chromosome 1DL, and *Dn2401* on

chromosome 7D (Dong *et al.,* 1997). In wheat–rye translocation (1BL/lRS), the short arm of the rye chromosome 1R replaces the long arm of the wheat chromosome 1B (Zhao *et al.,* 2012). The first RWA gene located on the short arm of a rye-chromosome 1R was reported in 94M370 containing the gene *Dn7* (Marais *et al*., 1994). The *Dn2414* gene is located on the short arm of rye chromosome 1R and long arm of wheat chromosome 1B (Peng *et al.,* 2007). Furthermore, 02 Altus 034 (Porter *et al.,* 2005) is also known to be linked to *Dn7* gene. Dough derived from lines with 1B/1R translocation has a poor bread-making quality known as a sticky dough (Martin and Stewart, 1990). Identification of these resistant genes influenced other research institutes/stations across the world to continue searching for resistance genes in different genetic backgrounds. To date, a total of 18 resistance genes including these aforementioned ones have been documented (Table 2), mostly mapped on chromosome 1 and 7 respectively (Anderson *et al.,* 2003; Liu *et al.,* 2001; 2002; Peng *et al.,* 2007; Lapitan *et al.,* 2007).

Table 2: Russian wheat aphid resistance genes, their sources, chromosomal locations and linked markers.

Gene	Source	Chromosomal Location	References
Dn1	PI 137739	7DS	Du Toit, 1989; Ma *et al.,* 1998; Liu *et al.,* 2001
Dn2	PI 262666	7DS	Du Toit, 1989; Liu *et al.,* 2001
dn3	*Aegilops tauschii* line SQ24	Recessive gene	Nkongolo *et al.,* 1991a
Dn4	PI 372129	1DS	Nkongolo *et al.,* 1991b; Saidi and Quick 1996; Liu *et al.,* 2002
Dn5	PI 294994	7DS	Marais and Du Toit 1993; Liu *et al.,* 2001
Dn6	PI 243781	7DS	Saidi and Quick, 1996; Liu *et al.,* 2002
Dn7	Rye accession	1RS:IBL translocation	Marais *et al.,* 1994
Dn8	PI 294994	7DS	Liu *et al.,* 2001
Dn9	PI 294999	1DL	Liu *et al.,* 2001
Dn10	PI 682675	7DL	Li *et al.,* 2018
Dnx	PI 220127	7DS	Liu *et al.,* 2001
Dny	Stanton (PI 220350)	-	Smith *et al.,* 2004
Dn1881	1881	7BS	Navabi *et al.,* 2004
Dn2401	CItr 2401	7DS	Dong *et al.,* 1997; Fazel-Najafabadi *et al.,* 2014
Dn2414	ST-ARS 02RWA241411 (2414-11)	1RS:IBL translocation	Peng *et al.,* 2007
Dn100695	*IG 100695*	7DS	Tonk *et al., 2016*
Dn225227	PI 225227	-	Tolmay *et al.,* 2016
Dn626580	PI 626580	7DS	Valdez *et al.,* 2012

Dn1, *Dn2* and *Dn8* (Liu *et al*., 2001), *Dn10* (Li *et al*., 2018), *Dnx* (Liu *et al*., 2001), *Dn2401* (Dong *et al*., 1997; Fazel-Najafabadi *et al*., 2014), *Dn2414* (Peng *et al*., 2007), *Dn100695* (Tonk *et al.,* 2016), and *Dn626580* (Valdez *et al*., 2012) are located on chromosome 7DS while *Dn4* and *Dn9* are located on chromosome 1DS and 1DL respectively (Ma *et al.,* 1998; Arzani *et al.,* 2004; Liu *et al.,* 2001; 2002). *Dn1881* is located on the short arm of chromosome 7BS (Navabi *et al.,* 2004). The chromosomal location of *Dn2* (Ma *et al.,* 1998) and *Dn5* (Marais and Du Toit, 1993) genes on chromosome 7D have been debated across a few authors. Through aneuploidy analysis and restriction fragment length polymorphism markers (RFLPs), *Dn2* was mapped on the long arm of chromosome 7DL. Through telosomic analysis, Du Toit *et al.,* (1995) located the *Dn5* gene on the long arm of chromosome 7DL. Later on, Liu *et al*., (2005) through 'mapped microsatellite markers' suggested that both genes are on the short arm of chromosome 7DS. However, Heyns *et al.,* (2006) through mapped microsatellite markers and 'endopeptidase' proved that the *Dn5* gene is located on the long arm of chromosome 7DL aligning with the findings of (Du Toit *et al.,* 1995). *Dn7* and *Dn2414* are located on chromosome 1RS/1BL (Marais *et al.,* 1994; Marais *et al.,* 1998; Peng *et al.,* 2007). Only the *dn3* gene has not been located chromosomally. There are some conflicting reports about the possibility of two genes conferring resistance in CItr 2401. Dong *et al.,* (1997) suggested that it was *Dn4* until proven invalid by Haley *et al.,* (2004).

Consequently, some studies suggest that these genes are either a cluster, identical or different alleles located in the same chromosomal region. This includes the *Dn7* and *Dn2414* resistance genes since they have been reported to share similar marker profiles i.e. *Xrems1303* and *Xiag95* (Peng *et al.,* 2007) and phenotypic profiles with high levels of resistance to all eight USA biotypes (Anderson *et al.,* 2003; Haley *et al.,* 2004; Lapitan *et al.,* 2007; Weiland *et al.,* 2008; Randolph *et al.,* 2009; Mornhinweg 2012; Puterka, 2017). However, bad linkage drag from the 1RS translocation is associated with poor bread-making quality due to the sticky dough trait (Graybosch *et al.,* 1990). Similar marker profiles *Xgwm473* and *Xbarc214* for *Dn2401* and *Dn626580* also suggested that the genes are either identical or different alleles located at the same locus (Valdez *et al.,* 2012; Fazel-Najafabadi *et al.,* 2014). Moreover, *Dn1*, *Dn2*, *Dn5*, *Dn6, Dnx*, and *Dn2401* may also be allelic or a cluster of genes linked to the same marker *Xgwm111* in the same chromosomal region (Liu *et al.,* 2001; 2002; 2005; Miller *et al.,* 2001; Fazel-Najafabadi *et al.,* 2014).

2.2.7 Molecular markers in marker assisted selection (MAS)

Markers are used as effective tools "tags or signs" for detecting the presence or absence or potential linkage to a gene of interest. There are three groups of markers:

morphological, biochemical and molecular markers (Winter and Kahl, 1995; Jones *et al*., 1997). Morphological markers are visual traits such as seed size and shape, colour of leaves and growth habit. Biochemical or isozyme markers are enzyme differences distinguished through gel electrophoresis and staining. Molecular markers are markers that reveal variation in the deoxyribonucleic acid (DNA) of genotypes. DNA is the main genetic molecule containing all the genetic information within chromosomes about any individual or specie (Watson and Crick, 1953). Markers that reveal genetic differences between genotypes are called polymorphic markers while markers that do not reveal genetic difference between genotypes are called monomorphic markers (Collard *et al*., 2005). DNA markers vary depending on the polymorphism techniques used. For example, polymerase chain reaction (PCR) based markers are the most used markers as they enable visualisation of the extent of DNA among organisms. This is achieved by screening the gene of interest with molecular markers linked to the trait of interest and viewing the PCR product on an agarose gel (Mullis and Faloona, 1987). To date, numerous markers have been developed and are used in different crops for different purposes globally. This includes RFLPs (Anderson *et al*., 2003), Random Amplification of Polymorphic DNAs (RAPDs; Fukuoka *et al*., 1992; Tehrim *et al*., 2012), Amplified Fragment Length Polymorphisms (AFLPs; Eivazi *et al*., 2008), SSRs or microsatellites (Liu *et al*., 2001; Bernado *et al*., 2013), Single Nucleotide Polymorphism (SNPs; Khlestkina and Salina, 2006) and Kompetitive Allele Specific PCR (KASP; Yang *et al*., 2018). Table 3 provides a comparison of different markers used in different genetic backgrounds.

Table 3: Comparison of different molecular markers used crop genetics, their advantages and disadvantages [Adopted from Collard *et al*., 2005; ISAAA, 2017].

Molecular markers					
RELPs	**RAPDs**	**AFLPs**	**SSRs**	**SNPs**	**KASP**
High DNA quality, reliable, transferable across samples, inexpensive, easy to use, reliable, low levels of polymorphism Co-dominant	Small amounts of DNA required, high DNA quality, PCR-based, easy to use, inexpensive, low levels of polymorphism generated, unreliable, dominant	Moderate DNA quality, PCR-based, high levels of polymorphism generated, easy to use, inexpensive, reliable large amount of DNA required, dominant	Small amounts of DNA required, moderate DNA quality, PCR-based, easy to use, reliable, low polymorphism levels, expensive, Co-dominant	Small amounts of DNA required, high DNA quality, PCR- based, easy to use, reliable, low polymorphism levels, Co- dominant, transferable across chromosomes	Large amount of DNA required, high DNA quality, PCR-based, high levels of polymorphism, easy to use, reliable, Co- dominant

Chapter 3

The following studies have received ethical clearance Ref 2017/CAES/167

3. Methodology of the study

3.1 Russian wheat aphid phenotyping

3.1.1 Aim

To identify resistance from the tested landraces by using the four RWA biotypes to contribute to the ARC-SG RWA pre-breeding programs. Identification, selection, purification and characterisation could provide new diverse sources of resistance for RWA wheat pre-breeding programs.

3.1.2 Material and methods

3.1.2.1 Site description

The study was conducted in the entomology laboratory and glasshouses (planting, RWA infestation and evaluation) of ARC-SG near Bethlehem (28°10'S, 28°18'E).

3.1.2.2 Plant material

Wheat landraces used in this study were imported from the USA National Plant Germplasm System (NPGS) (www.ars-grin.gov) as already evaluated with US RWA biotypes RWA1 and RWA2. The collection was composed of 74 donor lines and six breeding lines from Afghanistan (38), Iran (22), Pakistan (11), United States (4), South Africa (2), Turkey (1), Georgia (1) and Egypt (1). However, there is no pedigree information available for these lines since they are landraces. The five differential checks Gariep (*Dn1*), Yumar (*Dn4*), PAN 3144, CItr 2401 (*Dn2401*) and Hugenoot (Tomay and Booyse, 2016) were used. The differential checks were obtained from the ARC-SG pre-breeding program. Three differential checks Gariep, Hugenoot, PAN 3144 are South African cultivars. CItr 2401 is a landrace from Tajikistan and Yumar is a winter wheat, RWA resistant cultivar from Colorado in the USA. They were used to confirm that the correct biotype is used for the experiment. Gariep is moderately resistant to RWASA1 and susceptible to RWASA2, RWASA3 and RWASA4. Yumar is moderately resistant to RWASA1 and RWASA2 but susceptible to RWASA3, and RWASA4. PAN3144 is resistant to RWASA1, RWASA2 and RWASA3 although it is susceptible to RWASA4. Only CItr 2401 is resistant to all four biotypes and Hugenoot is susceptible to all four biotypes (Tolmay and Booyse, 2016).

3.1.2.3 Trial establishment

A RWA resistance screening bioassay consisting of three sets of 27 genotypes and five differential checks was conducted in a glasshouse in (28°09′55.12″ S, 28°18′32.97″ E),

Free State Province of SA. The genotypes were screening using the method of Tolmay and Booyse (2016). A seedling tray consisting of 98 cones (Figure 7), each measuring at 40 x 40 x 95 mm containing a Professional growing mix® (http://www.culterra.co.za) and watered with KynoPop™ (http://www.kynoch.co.za).

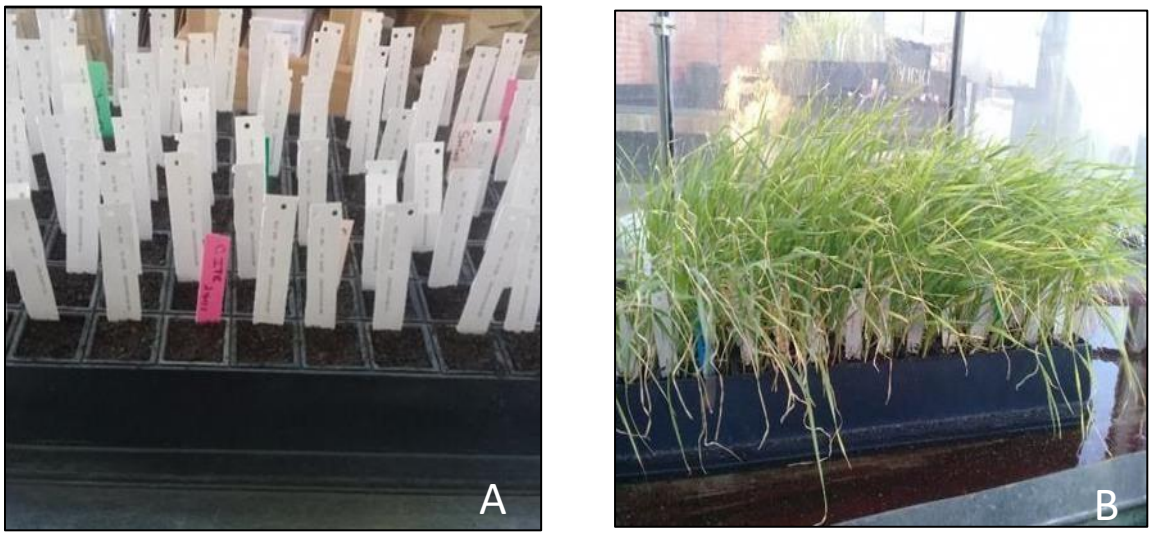

Figure 7: Depiction of wheat genotypes (before germination) and after 21 days of Russian Wheat Aphid infestation. A: Five seeds planted per cone in 98 cone seedling trays covered with a Professional growing mix®. B: RWA infested plants after 21 days.

To avoid cubicle size limitations and screening problems, the bioassays of the three sets were done approximately 2 to 6 weeks apart. A randomised complete block design was used consisting of 27 test entries and five differential checks with three replicates of five seeds each, for each biotype RWASA1, RWASA2, RWASA3, and RWASA4. Eight hundred and sixty-four plants were expected to germinate per biotype. At the second leaf stage, seedlings were infested using calibrated aphid weight for each biotype i.e. number of plants x 5 aphids x mean aphid weight (g) and weighed with a five-decimal scale. A total of 739, 757, 745 and 664 plants were infested for RWASA1, RWASA2, RWASA3 and RWASA4.

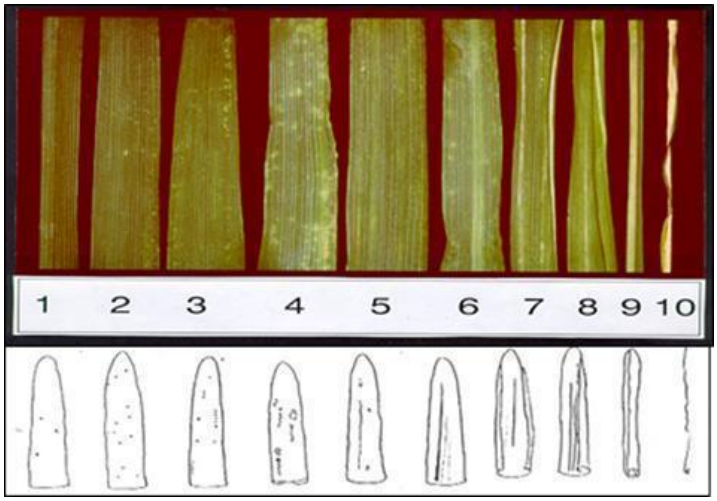

Figure 8: Russian wheat aphid phenotypic damage rating scale: where (1-2) highly resistant; (3-4) resistant; (5-6) moderately resistant; (7) moderately susceptible; susceptible (8-9); plant death (10) highly susceptible (Tolmay *et al.,* 2012).

Each seedling tray was kept in a separate cubicle with natural day/night conditions of 11/13 h (light/dark) and temperatures of 22/12°C for 21 days to avoid cross-contamination of biotypes. The test entries were scored 21 days post-infestation using a ten-point damage rating scale (Tolmay *et al*., 2012) presented in Figure 8, 1 being plants appearing very healthy, 7 being severe chlorotic-streaking and the beginning of leaf rolling and 10 being plant dying or no recovery possible (Table 4). Figure 9 shows a clear example of the difference between resistant and susceptible genotype.

Table 4: Russian wheat aphid damage rating scale, descriptors and resistance categories used for wheat RWA resistance evaluation [Modified from Tolmay *et al.,* 1999; Tolmay *et al.,* 2012; Tolmay and Booyse, 2016].

Scale	Description	Category
1-2	1: Small isolated chlorotic spots 2: Small chlorotic spots	Highly resistant (HR)
3-4	3: Chlorotic spots in rows 4: Chlorotic splotches	Resistant (R)
5-6	5: Mild chlorotic streaks 6: Prominent chlorotic streaks	Moderately resistant (MR)
7	7: Severe streaks, leaves fold conduplicate	Moderately susceptible (MS)
8-9	8: Severe streaks, leaves roll convolute, 9: Severe streaks, leaves roll tightly	Susceptible (S)
10	10: Plant dying or no recovery possible	Plant death (D)

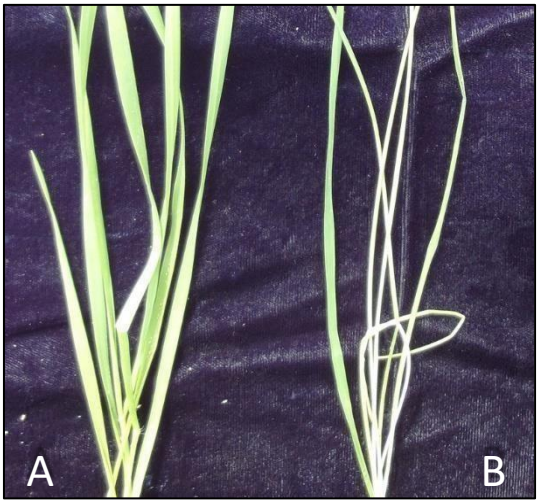

Figure 9: The difference between a resistant and susceptible genotype: (A) a resistant check control CItr 2401 and susceptible check control Hugenoot (Source: Dr Vicki Tolmay).

The resistance level of each accession was evaluated per plant for each of the four biotypes. Once the damage rating was acquired, the best test was performed to determine the significant differences among the genotypes (Tolmay and Booyse, 2016). Each set was analysed separately to confirm that the experiment was done correctly. The combined analysis was done to obtain the list of the most resistant genotypes of all genotypes tested. The resistance pattern of all genotypes either same or different from the known resistance towards all four RWA biotypes was assessed. Resistance to the latest biotypes RWASA3, and RWASA4 was then assessed. The resistance patterns of RRSR, RSRR, RSRS, RSSR, SRSR, SSRR, and SRRS from RWASA1-RWASA4 were considered new and unique for RWA breeding program because of the resistance reaction to either one or both of the latest RWA biotypes RWASA3 and RWASA4. The presence of mixed reaction (HR, R, MR, MS, S, D) was observed within both RWASA3 and RWASA4 tested genotypes. This was noted and the predominant reaction was recorded to represent the overall reaction of that test entry.

3.1.2.4 Data analysis

Data collected was subjected to a multiple *t*-distribution test procedure (Gupta and Panchapakesan, 1979). The genotypes were ranked from the smallest to largest and from the largest to smallest thus giving a resistant category (R), moderate resistance category (MR) and a susceptible category (S).

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Table 5: Wheat genotype name, origin, collection type, USA biotype resistance and reference of the genotypes evaluated in this study for their Russian wheat aphid resistance.

Wheat genotypes	Origin	Collection type	USA biotype resistance		Reference
			RWA1	RWA2	
PI 127097	Afghanistan	Landrace	S	R	USDA-ARS-NPGS
PI 127099	Afghanistan	Landrace	S	R	USDA-ARS-NPGS
PI 127104	Afghanistan	Landrace	S	R	USDA-ARS-NPGS
PI 134117	Afghanistan	Landrace	R	R	USDA-ARS-NPGS
PI 135047	Afghanistan	Landrace	R	R	USDA-ARS-NPGS
PI 135064	Afghanistan	Landrace	R	R	Collins *et al*., 2005; Peng *et al.,* 2009
PI 135076	Afghanistan	Landrace	R	R	USDA-ARS-NPGS
PI 137739''S''	Iran	Landrace	R	-	Schroeder-Teeter *et al.,* 1994
PI 137740	Iran	Landrace	R	R	USDA-ARS-NPGS
PI 137741	Iran	Landrace	R	R	Collins *et al*., 2005
PI 137757	Iran	Landrace	S	R	USDA-ARS-NPGS
PI 140204	Iran	Landrace	R	R	Collins et al., 2005
PI 140213	Iran	Landrace	R	R	Collins *et al*., 2005; Peng *et al.,* 2009
PI 166227	Turkey	Landrace	R	S	USDA-ARS-NPGS
PI 181263	Afghanistan	Landrace	R	R	USDA-ARS-NPGS
PI 189746	Pakistan	Landrace	R	S	USDA-ARS-NPGS
PI 197985	Afghanistan	Landrace	S	R	USDA-ARS-NPGS
PI 220131	Afghanistan	Landrace	R	R	Collins *et al*., 2005; Peng *et al.,* 2009
PI 220133	Afghanistan	Landrace	R	R	Collins *et al*., 2005
PI 243659	Iran	Landrace	R	R	Collins *et al*., 2005; Peng *et al.,* 2009
PI 243679	Iran	Landrace	R	R	Collins *et al*., 2005
PI 243730	Iran	Landrace	R	R	Collins *et al*., 2005
PI 245380	Iran	Landrace	R	R	USDA-ARS-NPGS
PI 245432	Afghanistan	Landrace	S	R	USDA-ARS-NPGS
PI 250791	Afghanistan	Landrace	R	S	USDA-ARS-NPGS
PI 245583	Afghanistan	Landrace	R	R	Collins *et al*., 2005; Peng *et al*., 2009
PI 269408	Afghanistan	Landrace	R	R	Collins *et al*., 2005
PI 321738	Afghanistan	Landrace	-	S	USDA-ARS-NPGS
PI 347003	Afghanistan	Landrace	R	R	Collins *et al*., 2005
PI 347006	Afghanistan	Landrace	R	R	Collins *et al*., 2005; Peng *et al.,* 2009
PI 347017	Afghanistan	Landrace	S	R	USDA-ARS-NPGS
PI 347019	Afghanistan	Landrace	R	R	Collins *et al*., 2005
PI 347030	Afghanistan	Landrace	S	R	USDA-ARS-NPGS
PI 349043	Georgia	Landrace	R	-	USDA-ARS-NPGS
PI 366103	Egypt	Landrace	R	R	Peng *et al.,* 2009
PI 366520	Afghanistan	Landrace	R	-	USDA-ARS-NPGS
PI 366529	Afghanistan	Landrace	S	R	USDA-ARS-NPGS
PI 366533	Afghanistan	Landrace	R	S	USDA-ARS-NPGS
PI 366537	Afghanistan	Landrace	R	R	USDA-ARS-NPGS
PI 366538	Afghanistan	Landrace	R	R	USDA-ARS-NPGS
PI 366545	Afghanistan	Landrace	R	-	USDA-ARS-NPGS
PI 366549	Afghanistan	Landrace	R	-	USDA-ARS-NPGS
PI 366550	Afghanistan	Landrace	R	S	USDA-ARS-NPGS
PI 366565	Afghanistan	Landrace	R	R	USDA-ARS-NPGS
PI 366562	Afghanistan	Landrace	R	R	USDA-ARS-NPGS
PI 366566	Afghanistan	Landrace	R	R	Collins *et al*., 2005
PI 366572	Afghanistan	Landrace	R	R	Collins *et al*., 2005; Peng *et al.,* 2009
PI 366573	Afghanistan	Landrace	R	-	USDA-ARS-NPGS
PI 366985	Afghanistan	Landrace	R	R	Collins *et al*., 2005
PI 367171	Afghanistan	Landrace	R	S	USDA-ARS-NPGS
PI 367172	Afghanistan	Landrace	Mixed	S	USDA-ARS-NPGS
PI 367188	Afghanistan	Landrace	R	S	USDA-ARS-NPGS
PI 478115	Pakistan	Landrace	Mixed	S	USDA-ARS-NPGS
PI 478126	Pakistan	Landrace	Mixed	R	USDA-ARS-NPGS
PI 478127	Pakistan	Landrace	Mixed	S	USDA-ARS-NPGS
PI 478134	Pakistan	Landrace	S	R	USDA-ARS-NPGS
PI 478172	Pakistan	Landrace	Mixed	S	USDA-ARS-NPGS

Table 5: Continued

Wheat genotypes	Origin	Collection type	USA biotype resistance		Reference
			RWA1	RWA2	
PI 478177	Pakistan	Landrace	Mixed	S	USDA-ARS-NPGS
PI 478216	Pakistan	Landrace	Mixed	S	USDA-ARS-NPGS
PI 478257	Pakistan	Landrace	Mixed	S	USDA-ARS-NPGS
PI 478260	Pakistan	Landrace	Mixed	R	USDA-ARS-NPGS
PI 478262	Pakistan	Landrace	Mixed	R	USDA-ARS-NPGS
PI 564249	USA	Breeding material	-	S	USDA-ARS-NPGS
PI 564250	USA	Breeding material	-	S	USDA-ARS-NPGS
PI 564259	USA	Breeding material	-	S	USDA-ARS-NPGS
PI 564260	USA	Breeding material	-	S	USDA-ARS-NPGS
PI 623373	Iran	Landrace	S	R	USDA-ARS-NPGS
PI 623825	Iran	Landrace	-	R	Peng *et al.,* 2009
PI 623836	Iran	Landrace	-	R	Peng *et al.,* 2009
PI 623848	Iran	Landrace	-	R	USDA-ARS-NPGS
PI 623857	Iran	Landrace	-	R	USDA-ARS-NPGS
PI 624023	Iran	Landrace	-	R	USDA-ARS-NPGS
PI 624151	Iran	Landrace	-	R	Peng *et al.,* 2009
PI 624152	Iran	Landrace	-	R	Peng *et al.,* 2009
PI 624188	Iran	Landrace	-	R	USDA-ARS-NPGS
PI 624253	Iran	Landrace	-	R	USDA-ARS-NPGS
PI 623671	Iran	Landrace	-	R	USDA-ARS-NPGS
PI 634770 (*Dn9*)	RSA	Breeding material	-	-	USDA-ARS-NPGS
94M370 (*Dn7*)	RSA	Breeding material	R	R	Puterka *et al.,* 2014
RWA MATRIX 2414	-	*Dn2414* donor	R	R	USDA-ARS-NPGS
Differential checks					
CItr 2401	Tajikistan	*Dn2401* resistant check	R	R	Jankielsohn, 2014
Gariep	RSA	RWASA1 differential check	R	R	Tolmay *et al.,* Unpublished data
Hugenoot (PI 591944)	RSA	Susceptible check	-	-	Tolmay and Booyse, 2016
PAN 3144	RSA	RWASA3 differential check	R	S	Jankielsohn, 2014
Yumar (PI 605388)	USA	RWASA2 differential check	R	R	Puterka *et al.,* 2014

3.2 Yield trait phenotyping and genotyping

3.2.1 Aim

To contribute to the ARC-SG germplasm development program which in turn contributes to all wheat improvement programs in SA, both State and privately owned through the selection of wheat landraces with resistance to RWASA3 and RWASA4 and high yield.

3.2.2 Material and methods

3.2.2.1 Plant material

Five individual plants from each of the 30 selected RWA resistant genotypes were used in this study. The collection was composed of genotypes originating from Afghanistan (14), Iran (11), Pakistan (3) and United States (3). They were chosen based on their resistance to the latest biotypes of RWA i.e. RWASA3 and RWASA4. Eighteen wheat genotypes had resistance to both RWASA3 and RWASA4, four had resistance to RWASA3 while eight had resistance to RWASA4 (Table 6). The selection of RWA scores per genotype would be better understood when referring to appendix three to five.

Table 6: Genotypes, their origin, RWASA3 and RWASA4 resistance scores used in the study.

Wheat genotypes	Origin	Biotype resistance	Scores
PI 127099	Afghanistan	RWASA4	44444
PI 134117	Afghanistan	RWASA4	44446
PI 137739''S''	Iran	RWASA3	44444
		RWASA4	44444
PI 137740	Iran	RWASA3	44655
		RWASA4	44444
PI 137741	Iran	RWASA3	46444
		RWASA4	44444
PI 137757	Iran	RWASA3	44444
PI 140204	Iran	RWASA3	44655
		RWASA4	44556
PI 140213	Iran	RWASA3	65455
		RWASA4	44444
PI 181263	Pakistan	RWASA3	44444
		RWASA4	44444
PI 197985	Afghanistan	RWASA3	43444
		RWASA4	33433
PI 243659	Iran	RWASA3	44444
		RWASA4	33433
PI 243679	Iran	RWASA4	53365
PI 245583	Afghanistan	RWASA3	44444
		RWASA4	44444
PI 250791	Afghanistan	RWASA3	44444
PI 269408	Afghanistan	RWASA3	44444
		RWASA4	44433
PI 347019	Afghanistan	RWASA3	44444
		RWASA4	44444
PI 269408	Afghanistan	RWASA3	44444
		RWASA4	44433

Table 6: Continued

PI 347019	Afghanistan	RWASA3	44444
		RWASA4	44444
PI 269408	Afghanistan	RWASA3	44444
		RWASA4	44433
PI 347019	Afghanistan	RWASA3	44444
		RWASA4	44444
PI 347030	Afghanistan	RWASA3	44444
PI 366529	Afghanistan	RWASA4	44444
PI 366537	Afghanistan	RWASA4	44444
PI 366538	Afghanistan	RWASA4	45454

PI 366550	Afghanistan	RWASA3	44444
PI 366566	Afghanistan	RWASA3	44444
		RWASA4	44444
PI 366573	Afghanistan	RWASA3	44444
		RWASA4	44444
PI 478172	Pakistan	RWASA3	46446
		RWASA4	44344
PI 478216	Pakistan	RWASA4	33333
PI 624848	Iran	RWASA4	44444
PI 624152	Iran	RWASA4	44444
PI 624253	Iran	RWASA3	55554
		RWASA4	44444
Breeding lines			
PI 564250	USA	RWASA3	66466
		RWASA4	44444
PI 564259	USA	RWASA3	44444
		RWASA4	44344
PI 564260	USA	RWASA3	44444
		RWASA4	44443

3.2.2.2 Trial establishment

Following the RWA bioassay, the five most resistant plants to RWASA3 and RWASA4 per genotype used were each transplanted into a 2l pot (size: 17 cm height x 12 cm diameter) containing 2.5 kg of soil. After transplanting, the soil in the pots was brought to field capacity by tap water irrigation. Pots were watered 3 times a week until the plants were ready for harvest. The plants were sprayed with registered fungicides and pesticides (Folicur: 2.5ml/l for mildew control and Aphox: 0.5 g/l for aphid control) for mildew and insect pests when necessary when necessary. Furthermore, weeds were controlled by hand. The pots were kept in a glasshouse with 11/13 h day/night conditions and temperatures of 22/12°C day/night conditions.

The first three heads of each plant were tagged with different colors and the number of days to heading (HD) and the number of days to anthesis (AD) were recorded daily. The HD was recorded at Zadock 59 growth stage when the head is completely out of the flag leaf sheath while the AD were recorded at Zadock 61 growth stage when the anthers begin to release pollen from the heads (Zadocks *et al.,* 1974) which is from heading. When all plants had reached maturity all heads in each pot were harvested and the traits measured are presented in Table 7.

Table 7: Wheat agronomic traits and how they were measured.

Agronomic traits	How the traits were measured
Spike numbers per plant (NSP)	Total number of spikes counted from each plant per pot after harvesting
Spike length (SL)	Measured from the base of the rachis to the top uppermost of the spike excluding the awns
Grain number per spike (GNS)	Total number of grains counted from each spike after threshing
Grain numbers per plant (GNP)	Counted as total number of grains counted per plant
Grain weight per spike (GW)	Total weight of grains after threshing
Total grain weight per plant (TGW)	Grain weight per plant

Table 7: Continued

Thousand kernel weight (TKW)	Grain weight per plant divided by grain numbers per plant 1000 then multiplied by 100

3.2.2.3 Data analysis

The phenotypic data obtained were subjected to analysis of variance (ANOVA) using General Linear Models Procedure (PROC GLM) of SAS software (SAS Institute 2018, Version 9.4). Shapiro-Wilk test was performed on the standardized residuals from the model to verify normality.

3.2.2.4 Genotyping

3.2.2.4.1 Genomic DNA isolation

Five samples of leaf tissues were harvested from each RWASA3 and RWASA4 resistant plant per landrace and genomic DNA was isolated using the CTAB (cetyl trimethyl ammonium bromide) DNA extraction procedure (Cota-Sánchez *et al*., 2006): Two 5 mm stainless steel ball bearings and 750 µl of CTAB buffer (100 mM Tris-HCl, pH 8.0), 20 mM ethylene-diaminetetraacetate (EDTA, pH (8.0)) were added to the 2 ml Eppendorf tubes with leaf tissue. The leaf tissue with a buffer inside the tubes were homogenised with tissue-lyser (Tissue-lyser, Qiagen Retsch®, Germany) for two minutes at 30 revolutions per second. Following homogenisation, the tubes were incubated at 65°C in a water bath (Memmert 854 Schwabach W, Germany) for 1 hour. Five hundred µl of 100% 2-Isopropanol was transferred into an empty 1.5 ml Eppendorf tubes then cooled at 4°C. Chloroform (500 µl): Isoamyl alcohol (ratio of 24:1) was added to the incubated tubes, vortexed and centrifuged (Prism microcentrifuge) at 12 500 g (gravity) for 5 minutes. The supernatant was then transferred to cooled tubes containing the 100% 2Isopropanol, then vortexed and centrifuged at 12 500 *g* for 5 minutes. The DNA pellet was washed with 200 µl of 70% ethanol then centrifuged at 12 500 g for 5 minutes. The ethanol was discarded leaving the DNA pellet to dry at room temperature for 1 hour and then re-suspended with 200 µl of 1X TE buffer (10 mM Tris-HCl, pH 8.0, 1 mM EDTA, pH 8.0). RNase (2 µl) was added to breakdown the ribonucleic acid (RNA) then incubated at 37°C for 1 hour. The DNA concentration was quantified at 260 nm using a NanoDrop 2000 spectrophotometer (ND-2000 V3.5, NanoDrop Technologies, Inc.). The DNA quality was determined using an absorbance ratio of 260/280 nm with an acceptable quantity of 1.8. The DNA was adjusted to a 50 ng/ug final concentration and stored at 4˚C for further use (Figure 10).

3.2.2.4.2 Polymerase chain reaction: DNA bulking, marker analysis and enzyme restriction

Three CAPS markers i.e. *MQ*, *Caps4A-Ags*, and *Caps5D-Ags* and one SSR marker *GS7D*, linked to known and validated TKW, grain length and grain numbers genes were used (Table 8). A representative bulk of each wheat landrace was compiled with 5 µl of DNA of each of the five resistant plants per landrace and transferred into 1.5 ml Eppendorf tubes to create a DNA bulk. The PCR was performed in a final volume of 20 µl containing 4 µl of genomic DNA, 10 µl of One*Taq*® Quick load® 2X buffer (New England Biolabs®, *OneTaq*®, Quick labs) containing 0.2 mM dNTPs, 1.8 mM $MgCl_2$, 5 µl of Nuclease-free water (VWR® International LLC.) and 0.5 µl of each primer. Nucleasefree water (17 µl), 10 µl of PCR product, 2.5 µl of enzyme buffer and 0.5 µl of each restriction enzyme for each marker were added into a new PCR plate. The PCR conditions were an initial denaturation at 94ºC for 5 minutes, denaturation at 94ºC for 45 seconds, primer annealing at 55-62ºC (depending on the primer) followed by extension at 72ºC for 45 seconds and a final extension at 72ºC for 5 minutes before holding at 4ºC. The primer and enzyme names used in this study are listed in Table 8. The PCR products that gave the null allelic reaction were repeated twice to confirm null allele status of the genotypes.

3.2.2.4.3 Agarose and gel electrophoresis for separating the SSR and CAPS markers

The digest products were separated on 2% (w/v) high-resolution Seakem® Le agarose gel (Lonza, Lonza Rockland, Inc) which was prepared by melting 4 g of the agarose gel in 200 ml of 1X TBE buffer. The 1X TBE buffer was prepared by adding 20 ml of 10X TBE buffer in 180 ml of double-distilled water. The mixture was dissolved in a microwave for 2-5 minutes until it was bubble-free and stained with 10 µl of SYBR® Safe DNA gel stain (Fischer scientific Inc.). The mixture was poured into a gel casting tray set with 28-tooth combs. The combs were removed after the gel set and the gel was immersed in the electrophoresis chamber containing 1 X TBE buffer. The digested product was loaded into the gel wells and after gel loading, 10 µl of 100 bp DNA ladder was added, one on the left-hand side and the other on the right-hand side. The gel was run for 2 hours and 30 minutes. Following UV light exposure, gel photographs were taken with a gel documentation system (Bio-imaging systems, Lasec SA) to view the gel bands (Figure 10). The allele sizes of the different fragments were manually determined and scored.

Table 8: Detailed description of molecular markers used in this study.

Gene	Marker name	Annealing temperature (ºC)	Enzyme name	Digest temperature	Product sizes (bp)	Trait associated	References
TaCWI- 4A	*Caps4A-Ags*	60ºC	*Tai I*	37ºC	885 354 and 531	Higher thousand- kernel weight Higher grain numbers	Jiang *et al.,* 2015
TaCWI-5D	*Caps5D-Ags*	58ºC	*Bst YI*	60ºC	405 and 244 409, 143 and 97	Higher thousand- kernel weight Lower thousand- kernel weight	Jiang *et al.,* 2015
TaTGW-7A	*MQ*	55-62ºC	*Bsm AI*	55ºC	250 196	Higher thousand- kernel weight Lower thousand- kernel weight	Hu *et al.,* 2016
TaGS-D1	*GS7D*	52ºC	None	None	562 522	Higher thousand- kernel weight Lower thousand- kernel weight	Zhang *et al.,* 2014

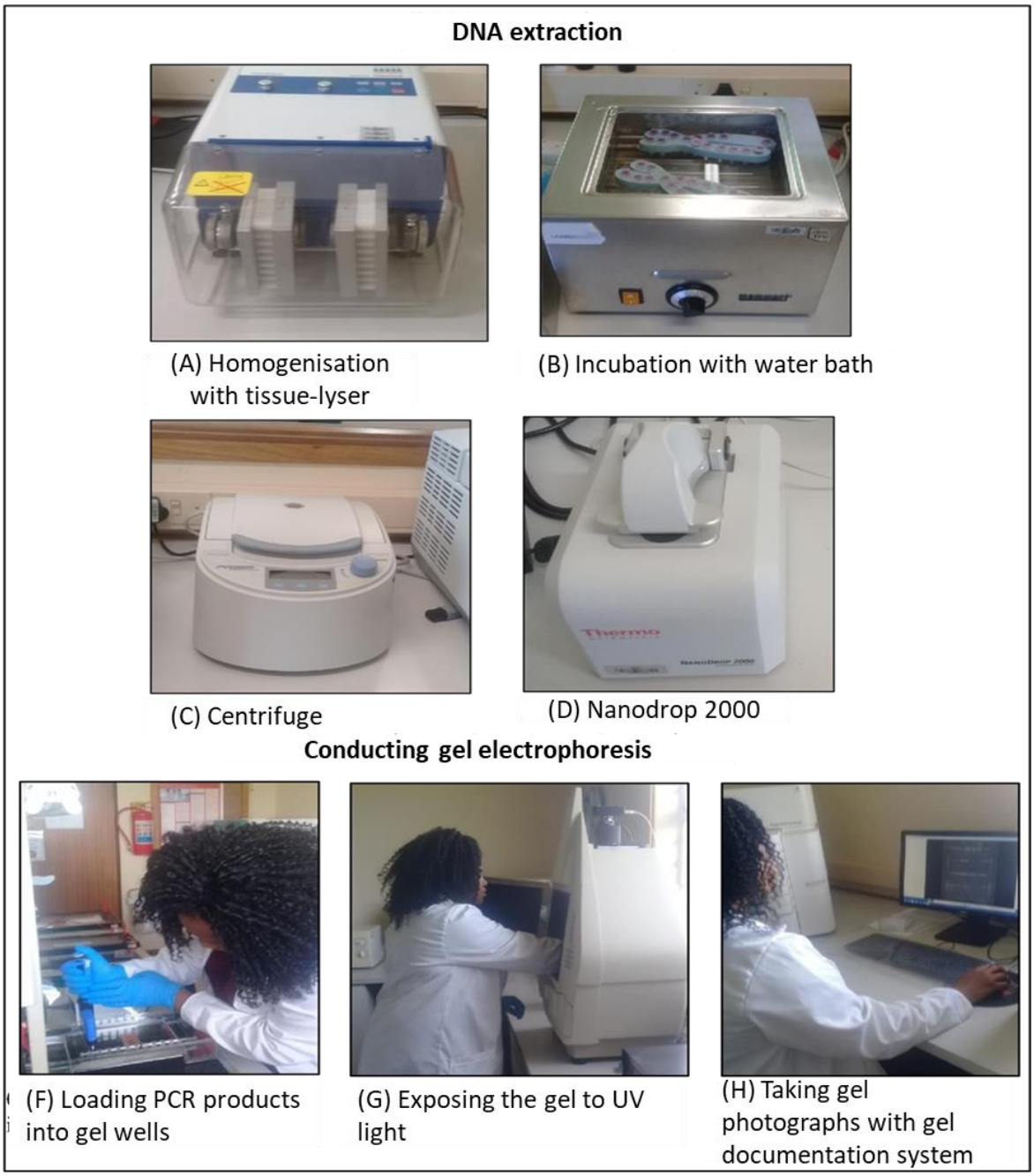

Figure 10: Conducting DNA extraction and gel electrophoresis on selected RWASA3 and RWASA4 resistant genotypes at ARC-SG.

Chapter 4

4. Results

4.1 Russian Wheat Aphid

4.1.1 The mean damage variation of all four Russian wheat aphid (RWA) biotypes

Significant differences (*P*<0.0001) were observed in the means of the four RWA biotypes. The highest mean was obtained from RWASA3 (7) and the lowest mean (5) obtained from RWASA1. However, means of RWASA2 (6) and RWASA4 (6) were not different from one another. Therefore, these findings show that the RWASA3 was the most damaging biotype among the four biotypes on these sets of wheat lines. Moreover, this indicates that the biotype would colonise wheat despite the genes present. Therefore, resistance to this biotype needs extensive screening and selection of resistant plants from wheat landraces.

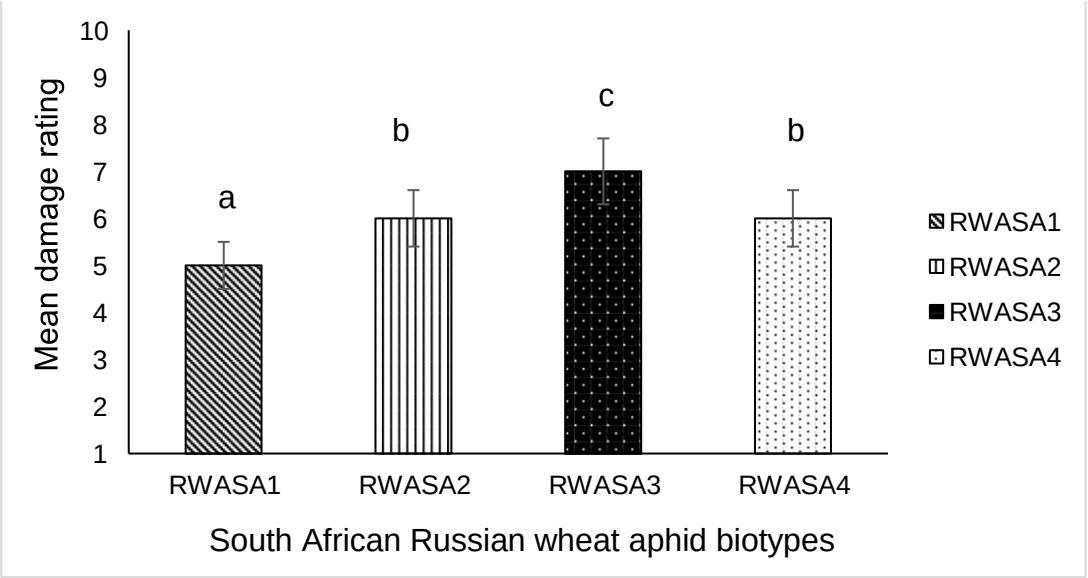

Figure 11: Mean damage rating of each of the four South African Russian wheat aphid biotypes RWASA1, RWASA2, RWASA3 and RWASA4.

The resistance proportion of the 80 genotypes against the four RWA biotypes is presented in Figure 12. This figure supports Figure 11 by showing the extent of resistance proportion and the virulence of the four biotypes on the genotypes. Genotypes with no germination were not included in the graph below. RWASA1 and RWASA2 displayed high levels of resistance by 56% and 65% respectively. Thirty-four percent of the genotypes were resistant to RWASA3 while RWASA4 (45%) displayed low levels of resistance among all RWA biotypes. RWASA1 displayed higher levels of moderate resistant

genotypes at 26% followed by RWASA3 at 15%. On the other hand, RWASA2 (10%) and RWASA4 (11%) slightly differed from one another with the amount of moderate resistant genotypes in their bioassays. Furthermore, RWASA3 (54%) and RWASA4 (44%) displayed high levels of susceptible genotypes. Moreover, RWASA1 (18 %) and RWASA2 (25%) had low levels of susceptible genotypes.

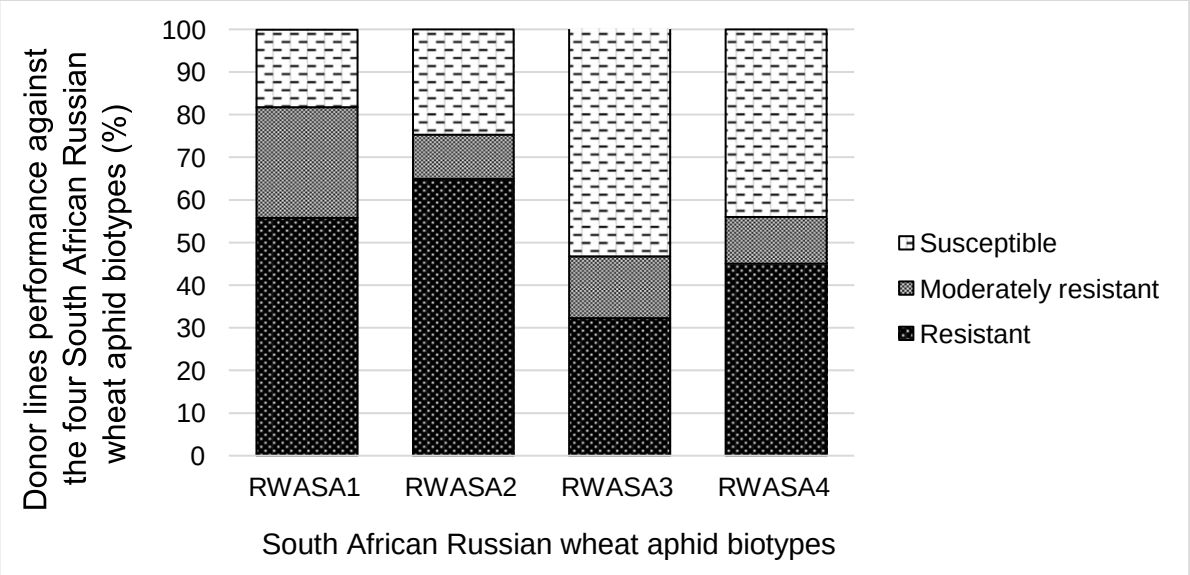

Figure 12: The performance of all 80 genotypes tested against all the four Russian wheat aphid biotypes**.**

4.1.2 Genotypes resistance to all the four RWA biotypes

Table 9, 10 and 11 shows the ranking of the test genotypes, presented by category namely resistant, moderately resistant and susceptible based on the multiple *t*-distribution test (Gupta and Panchapakesan, 1979). The genotypes showed marked variation over biotypes suggesting differential responses of the test genotypes for RWA resistance. Twenty-five genotypes showed comparable resistance to differential check CItr 2401 that is resistant to all four RWA biotypes thus these genotypes were considered best performers based on their resistance to these biotypes. These include PI 137739''S'', PI 137740, PI 137741, PI 140204, PI 181263, PI 197985, PI 243659, PI 245380, PI 245583, PI 269408, PI 347003, PI 347019, PI 366103, PI 366529, PI 366550, PI 366566, PI 366573, PI 366985, PI 367188, PI 478172, PI 564250, PI 564259, PI 564260, PI 624253 and RWA MATRIX 2414 (Table 11). The resistance shown by these genotypes is important and should be included in the RWA breeding programs or commercialized for use in production under areas where RWA occurs. This does not necessarily mean the

genotypes carry the same gene. Therefore, these new resistance sources suggest the presence of unknown resistance genes that still need to be identified except for RWA MATRIX 2414 and PI 137739''S''. Three resistance genes *Dn7*, *Dn2401*, and *Dn2414* are already known to be resistant to all SA biotypes and foreign biotypes. However, only *Dn2401* donors are useful commercially since *Dn7* and *Dn2414* have been introduced from 1RS translocation and this translocation is associated with bad dough traits. For further analysis, highly resistant single plants from RWASA3 and RWASA4 resistant genotypes were selected. This practice provides a good step towards identifying or developing markers linked to RWA resistance from these lines in the future.

Nineteen genotypes were resistant to at least three RWA biotypes. These include PI 127097, PI 127099, PI 140213, PI 135047, PI 220133, PI 245432, PI 366537, PI 366538, PI 623848, PI 624152, and PI 624188 with resistance to RWASA1, RWASA2, and RWASA4 and susceptible to RWASA3. However, PI 135047 showed moderate resistance to RWASA2 while PI 245432 and PI 624188 showed moderate resistance to RWASA4. Genotypes PI 347030 and PI 624151 were both susceptible to RWASA4, however; PI 347030 showed moderate resistance to RWASA1 and RWASA2 and was resistant to RWASA3 while PI 624151 showed resistance to RWASA1 and RWASA2 and was moderately resistant to RWASA3. PI 478127 showed moderate resistance to RWASA1, RWASA3, and RWASA4 and was susceptible to RWASA2 while PI 250791 showed resistance to RWASA1, RWASA3, and RWASA4 and was susceptible to RWASA2. Only PI 134117 was susceptible to RWASA1 and resistant to RWASA2, RWASA3, and RWASA4. From the above genotypes, only five performed comparably to differential check PAN3144. The genotypes include PI 349043, PI 366520 and PI 624151 that showed resistance to RWASA1, RWASA2, and moderate resistance to RWASA3 and were susceptible to RWASA4. PI 366565 was resistant to RWASA1, RWASA2 and RWASA3, and susceptible to RWASA4.

Sixteen genotypes were resistant to two RWA biotypes. These include PI 135076, PI 220131, PI 243730, PI 347006, PI 347017, PI 366533, PI 366572, PI 623373, PI 623825, PI 623857, PI 623671, PI 634770 and 94M370 that showed resistance to RWASA1 and RWASA2 and were susceptible to RWASA3, and RWASA4. However, among the above genotypes, PI 243730 and PI 366533 showed moderate resistance to RWASA1, resistant to RWASA2, susceptible to RWASA3 and RWASA4 except PI 366533 which did not germinate under RWASA3 bioassay. Genotypes PI 347006, PI 347017, PI 366572 and 94M370 showed moderate resistance to RWASA1 and RWASA2, as they were susceptible to RWASA3 and RWASA4 except 94M370 with no data generated for RWASA4. PI 243679 showed moderate resistance to RWASA1, resistant to RWASA4 and

susceptible to RWASA2 and RWASA4. PI 478126 was susceptible to RWASA1 and RWASA3 and resistant to both RWASA2 and RWASA4 while PI 478216 was moderately resistant to both RWASA1 and RWASA3 and was susceptible to RWASA2 and RWASA4. From the 16 genotypes, five performed comparably to a differential check Yumar that was resistant to RWASA1 and RWASA2 and susceptible to RWASA3 and RWASA4. This includes genotypes PI 135076, PI 220131, PI 623825, PI 623857 and PI 623871 respectively. A further five genotypes including PI 347006, PI 347017 and PI 366572 that were moderately resistant to RWASA1 and RWASA2 while PI 243730 and PI 634770 were moderately resistant to RWASA1 and resistant to RWASA2 and susceptible to RWASA3 and RWASA4 may also be compared with Yumar.

Five genotypes namely PI 127104, PI 135064, PI 137757, PI 478127 and PI 623836 were resistant to only one RWA. From these genotypes, three genotypes, PI 127104, PI 137757 and PI 623836 showed a similar resistance profile to differential check Gariep as they were moderately resistant to RWASA1 and susceptible to RWASA2, RWASA3, and RWASA4. However, PI 135064 was susceptible to RWASA1, RWASA3, and RWASA4 and was moderately resistant to RWASA2. On the other hand, PI 478257 was susceptible to RWASA1, RWASA2, and RWASA4 and was moderately resistant to RWASA3. Ten genotypes i.e. PI 166227, PI 189746, PI 367171, PI 367172, PI 478115, PI 478134, PI 478177, PI 478260, PI 478262 and PI 624023 performed comparably to the susceptible check Hugenoot not showing resistance to any of the four biotypes used in this study. The three genotypes PI 366545, PI 366549 and PI 366562 did not germinate in any bioassay.

Table 9: Resistance categories and the ranking set one genotypes based on multiple *t*-distribution test (P<0.0001) of all the three sets and five differential checks, analysed separately against all the four South African Russian wheat aphid biotypes.

Resistance category for set one	**Separate analysis of set 1 accession against the four South African Russian wheat aphid biotypes**			
	RWASA1	**RWASA2**	**RWASA3**	**RWASA4**
Resistant	RWA MATRIX 2414	RWA MATRIX 2414	RWA MATRIX 2414	PI 220133
	PI 245583	PI 366573	PI 245583	PI 366566
	PI 137739	PI 137741	CItr 2401	PI 564249
	PI 366573	PI 140213	PI 366985	RWA MATRIX 2414
	PI 137741	PI 243659	PAN 3144	PI 243659
	PI 269408	PI 245583	PI 269408	PI 269408
	CItr 2401	PI 366566	PI 564260	PI 137741
	PI 243659	CItr 2401	PI 366573	CItr 2401
	PI 220133	PAN 3144	PI 366566	PI 366573
	PI 366985	PI 564250	PI 366103	PI 245583
	PI 140213	PI 220133	PI 564259	PI 140213
	PI 140204	PI 269408	PI 137739	PI 366985
	PI 564259	PI 366985	PI 137741	PI 366103
	PAN 3144	PI 140204	PI 564250	PI 564260
	PI 366566	PI 564259	PI 243659	PI 564259
	PI 564260	PI 634770		PI 564250
		PI 564249		PI 243679
		PI 564260		PI 140204
		PI 137739		
Moderately resistant	PI 366103	Yumar	PI 140204	PI 137739
	PI 347006	PI 347003	PI 347003	PI 347003
	PI 564249	94M370		PAN 3144
	PI 347003	PI 366572		
	PI 564250	PI 347006		
	PI 634770	PI 135064		
	PI 366572			
	94M370			
	Gariep			
	PI 243679			
	Yumar			
Susceptible	PI 135064	PI 243679	PI 564249	PI 347006
	PI 321738	PI 321738	PI 140213	PI 366572
	Hugenoot	Gariep	PI 135064	PI 321738
		Hugenoot	94M370	PI 634770
			PI243679	Gariep
			Yumar	Yumar
			PI347006	PI 135064
			Hugenoot	Hugenoot
			PI 634770	
			PI 321738	
			Gariep	
			PI 366572	
No germination	PI 366562	PI 366562	PI 366562	PI 366562

Table 10: Resistance categories and the ranking set two genotypes based on multiple *t*-distribution test (*P*<0.0001) of all the three sets and five differential checks, analysed separately against all the four South African Russian wheat aphid biotypes.

Resistance category for set one	**Separate analysis of set 1 accession against the four South African Russian wheat aphid biotypes**			
	RWASA1	**RWASA2**	**RWASA3**	**RWASA4**
Resistant	PI 366537	PI 197985	CItr 2401	CItr 2401
	PI 366529	PI 134117	PI 134117	PI 135047
	PI 127097	PI 366537	PI 181263	PI 347019
	PI 127099	PI 137740	PI 367188	PI 366529
	PI 135047	PAN 3144	PAN 3144	PI 366537
	PI 135076	CItr 2401	PI 366529	PI 366538
	PI 197985	PI 135076	PI 366550	PI 134117
	PI 347019	PI 245380	PI 245380	PI 181263
	CItr 2401	PI 366550	PI 137740	PI 366550
	PAN 3144	PI 127099	PI 347030	PI 137740
	PI 367188	PI 347019	PI 366565	PI 197985
	PI 366550	PI 127097	PI 366566	PI 245380
	PI 245380	PI 135047	PI 250791	PI 127299
	PI 366538	PI 366529		PI 250791
	PI 137740	PI 243730		PI 127097
	PI 366565	PI 181263		
	PI 181263	PI 366565		
	PI 245432	PI 367188		
	Yumar	PI 366538		
	PI 243730	PI 245432		
	PI 250791			
	Gariep			
Moderately resistant	PI 137757	Yumar	PI 197985	PI 367188
	PI 127104	PI 347030	PI 347019	PAN 3144
	PI 347017	PI 347017		PI 245432
	PI 347030			
Susceptible	PI 134117	PI 137757	PI 137757	PI 347017
	PI 189746	PI 127104	PI 347017	PI 243730
	PI 166227	PI 250791	PI 127097	PI 367171
	PI 367171	PI 367171	PI 127104	PI 127104
	PI 367172	Gariep	PI 367172	PI 347030
	Hugenoot	PI 367172	PI 243730	Yumar
		Hugenoot	PI 135047	PI 137757
		PI 189746	PI 366537	PI 135076
		PI 166227	PI 366538	PI 367172
			PI 135076	PI 366565
			PI 189746	Gariep
			PI 127099	PI 166227
			PI 367171	PI 189746
			Yumar	PI 127099
			PI 166227	Hugenoot
			PI 245432	
			Gariep	
			Hugenoot	

Table 11: Resistance categories and the ranking set three genotypes based on multiple *t*-distribution test (P<0.0001) of all the three sets and five differential checks, analysed separately against all the four South African Russian wheat aphid biotypes.

Resistance category for set one	**Separate analysis of set 1 accession against the four South African Russian wheat aphid biotypes**			
	RWASA1	**RWASA2**	**RWASA3**	**RWASA4**
Resistant	CItr 2401 PI 624152 PAN 3144 PI 624188 PI 623825 PI 624151 PI 366520 PI 624253 PI 623836 PI 623848 PI 366533 PI 632671 PI 623373 PI 349043 PI 623857 Yumar Gariep	CItr 2401 PAN 3144 PI 624152 PI 624151 PI 624188 PI 623825 PI 366520 PI 623848 PI 632671 PI 349043 PI 366533 PI 478172 PI 624253 PI 623373 PI 623857 Yumar PI 220131 PI 478126	CItr 2401 PAN 3144	PI 624152 CItr 2401 PI 624253 PI 623848 PI 478172 PI 478126
Moderately resistant	PI 220131 PI 478172 PI 478127 PI 478216		PI 478172 PI 624253 PI 624151 PI 478127 PI 366520 PI 349043	PI 478127 PI 624188
Susceptible	PI 478257 PI 478134 PI 478262 PI 478177 PI 478126 PI 624023 PI 478260 PI 478115 Hugenoot	PI 478127 PI 623836 PI 478177 PI 478115 PI 478262 PI 624023 PI 478257 Gariep PI 478260 Hugenoot PI 478134 PI 478216	PI 623857 PI 478262 PI 478260 PI 624152 PI 624188 PI 623836 PI 623825 Yumar PI 478126 PI 624023 PI 623373 PI 623848 Gariep PI 478115 PI 220131 PI 478134 PI 478177 PI 623671 Hugenoot	PI 366533 PI 366520 PI 632671 PI 623857 PI 623825 PI 624151 PI 220131 PI 478262 PI 349043 Yumar PAN 3144 PI 478260 PI 478257 PI 478115 Gariep PI 478177 PI 624023 PI 623836 PI 478216 PI 478257 PI 478134 Hugenoot
No germination	PI 366545 PI 366549	PI 366565 PI 366549	PI 366533 PI 366565 PI 366549	PI 366565 PI 366549 94M370

4.1.3 Possible unique resistance to the latest Russian wheat aphid biotypes RWASA3 and RWASA4

The study was narrowed down to focus only on RWASA3 and RWASA4. The reaction of all 80 genotypes against RWASA3 and RWASA4 is presented in Table 11 and 12. Twenty-nine genotypes showed varying levels of resistance (resistance and moderate resistance) to either one or both RWASA3 and RWASA4. However, 25 genotypes were susceptible to both biotypes. Some genotypes displayed moderate resistance and resistance to RWASA4 but were susceptible to RWASA3. This include 13 genotypes that were susceptible to RWASA3 and resistant to RWASA4. Some genotypes displayed moderate resistance and resistance to RWASA3 but were susceptible to RWASA4. This include seven genotypes that were resistant to RWASA3 and susceptible to RWASA4. With the currently limited resistance to these biotypes, the high numbers of resistant genotypes to these biotypes are important for transferring useful resistance traits to well-known and adapted wheat cultivars and other genotypes.

4.1.4 Unique resistance pattern of the 80 wheat genotypes towards all four RWA biotypes

The summary of the resistance pattern of all genotypes tested with all the four South African RWA biotypes is presented in Table 12. The resistance patterns from RWASA1 to RWASA4 i.e. RRSR, RSRR, RSRS, RSSR, SRSR, SSRR and SRRS identified in the 80 genotype were considered new for incorporation in the RWA pre-breeding programmes because of resistance reaction to one or both latest RWA biotypes RWASA3 and RWASA4 in addition to RWASA1 or RWASA2. Moreover, these resistance patterns are different from the known 11 *D. noxia* resistance genes i.e. *Dn1*-*Dn9*, *Dnx* and *Dny*. Highly resistant or moderate resistance to any biotype, in this case, were considered resistant since they fall under a resistant category. Of all 80 genotypes screened, 22 gave the above different resistance patterns. These include five genotypes PI 347030, PI 349043, PI 366520, PI 366565 and PI 624151 that which had a resistance pattern of RRRS. However, PI 349043, PI 366520 and PI 624151 showed moderate resistance to RWASA3 while PI 347030 showed moderate resistance to RWASA1 and RWASA2 respectively. Resistance pattern of RSRR was observed in PI 250791 and PI 478127. Only PI 478127 showed moderate resistance to RWASA1, RWASA3, and RWASA4. Moreover, PI 134117 portrayed resistance pattern SRRR whereby the genotypes was resistant to RWASA2, RWASA3, and RWASA4 and susceptible to only RWASA1. A resistance pattern of RRSR was observed in 11 genotypes including PI 127097, PI 127099, PI 135047, PI 140213, PI 220133, PI 245432, PI 366537, PI 366538, PI 623848,

PI 624152 and PI 624188. However, PI 245432 and PI 624188 showed moderate resistance to RWASA4 while PI 135047 showed moderate resistance to RWASA2.

These genotypes expressed high levels of resistance to three RWA biotypes. Therefore, these genotypes can be crossed with each other for gene pyramiding to result in lines that are resistant to all four RWA biotypes. The resistance pattern SRSR in PI 478126 and RSRS in PI 478216 may be beneficial if crossed with each other. PI 478216 showed moderate resistance to RWASA1 and RWASA3 and was susceptible to RWASA2 and RWASA4. Furthermore, PI 243679 is unique RSSR for inclusion in the breeding program although the genotype showed moderate resistance to RWASA1. These genotypes are valuable genetic resources for further breeding. As already mentioned that resistance pattern identified on the above accessions is unique and useful because it contains resistance to RWASA3 or RWASA4. Combination of these genotypes may give better resistance to these biotypes.

Table 12: Summary of the resistance pattern of all 80 genotypes and five differential checks evaluated against RWASA1, RWASA2, RWASA3, and RWASA4.

Wheat accession	South African RWA biotypes				Wheat accession	South African RWA biotypes			
	RWASA1	RWASA2	RWASA3	RWASA4		RWASA1	RWASA2	RWASA3	RWASA4
PI 127097	R	R	S	R	PI 347003	MR	MR	MR	MR
PI 127099	R	R	S	R	PI 347006	MR	MR	S	S
PI 127104	MR	S	S	S	PI 347017	MR	MR	S	S
PI 134117	S	R	R	R	PI 347019	R	R	MR	R
PI 135047	R	MR	S	R	PI 347030	MR	MR	R	S
PI 135064	S	MR	S	S	PI 349043	R	R	MR	S
PI 135076	R	R	S	S	PI 366103	MR	R	R	R
PI 137739''S''	R	R	R	MR	PI 366520	R	R	MR	S
PI 137740	R	R	R	MR	PI 366529	R	R	R	R
PI 137741	R	R	R	R	PI 366533	MR	R	-	S
PI 137757	MR	S	S	S	PI 366537	R	R	S	R
PI 140204	R	R	R	R	PI 366538	R	R	S	R
PI 140213	R	R	S	R	PI 366545	-	-	-	-
PI 166227	S	S	S	S	PI 366549	-	-	-	-
PI 181263	R	R	R	MR	PI 366550	R	R	R	R
PI 189746	S	S	S	S	PI 366565	R	R	R	S
PI 197985	R	R	MR	R	PI 366562	-	-	-	-
PI 220131	R	R	S	S	PI 366566	R	R	R	R
PI 220133	R	R	S	R	PI 366572	MR	MR	S	S
PI 243659	R	R	R	R	PI 366573	R	R	R	R
PI 243679	MR	S	S	R	PI 366985	R	R	R	R
PI 243730	MR	R	S	S	PI 367171	S	S	S	S
PI 245380	R	R	R	R	PI 367172	S	S	S	S
PI 245432	R	R	S	MR	PI 367188	R	R	R	MR
PI 245583	R	R	R	R	PI 478115	S	S	S	S
PI 250791	R	S	R	R	PI 478126	S	R	S	R
PI 269408	R	R	R	R	PI 478127	MR	S	MR	MR
PI 321738	MR	S	S	S	PI 478134	S	S	S	S

Table 12: Continued

Wheat accession	South African RWA biotypes				Wheat accession	South African RWA biotypes			
	RWASA1	RWASA2	RWASA3	RWASA4		RWASA1	RWASA2	RWASA3	RWASA4
PI 478172	MR	R	MR	R	PI 624023	S	S	S	S
PI 478177	S	S	S	S	PI 624151	R	R	MR	S
PI 478216	MR	S	MR	S	PI 624152	R	R	S	R
PI 478257	S	S	MR	S	PI 624188	R	R	S	MR
PI 478260	S	S	S	S	PI 624253	R	R	MR	R
PI 478262	S	S	S	S	PI 623671	R	R	S	S
PI 564249	MR	R	R	R	PI 634770	MR	R	S	S
PI 564250	MR	R	R	R	94M370 (*Dn7*)	MR	MR	S	-
PI 564259	R	R	R	R	RWA MATRIX 2414	R	R	R	R
PI 564260	R	R	R	R	**Differential checks**				
PI 623373	R	R	S	-	Hugenoot	S	S	S	S
PI 623825	R	R	S	S	Gariep (*Dn1*)	R	S	S	S
PI 623836	MR	S	S	S	Yumar (*Dn4*)	R	R	S	S
PI 623848	R	R	S	R	PAN3144 (*Dn5*)	R	R	R	S
PI 623857	R	R	S	S	CItr 2401 (*Dn2401*)	R	R	R	R

R: Resistant MR: Moderately resistant MS: Moderately susceptible S: Susceptible

4.1.5 Uniformity existing within wheat landraces evaluated with RWASA3 and RWASA4.

Genotypes with a uniform reaction to both RWASA3 and RWASA4 were found and the results are presented in Table 13. Six genotypes including PI 134117, PI 181263, PI 245583, PI 366985, PI 367188 and RWA MATRIX 2414 and two differential checks PAN3144 and CItr 2401 displayed stable resistance to RWASA3. With the landraces, the reaction is the same; however, it does not necessarily mean the genetics of the lines is the same since they are not true-breeding lines. Furthermore, five genotypes including PI 366538, PI 366572, PI 321738 and PI 634770 and differential check Gariep displayed uniform susceptibility to RWASA3 respectively. For RWASA4, seven genotypes namely PI 135047, PI 243659, PI 269408, PI 347019, PI 366529, PI 366573 and PI 624152 as well as differential check CItr 2401 were uniformly resistant to RWASA4. Only differential check Hugenoot was uniformly susceptible to RWASA4.

A significant mixed reaction was observed among wheat genotypes when tested against RWASA3 and RWASA4. For example, a genotype may give a composite score that is moderately resistant but still contain mostly susceptible plants in between. From the 80 genotypes, 63 genotypes showed a mixed reaction to RWASA3 as stable resistance was found on six genotypes i.e. PI 137117, PI 181263, PI 245583, PI 366985, PI 367188 and RWA MATRIX 2414 and stable susceptible reaction was found on four other genotypes i.e. PI 321738, PI 366538, PI 366572 and PI 634770 (Table 13). From the 63 genotypes giving a mixed reaction to RWASA3, 16 genotypes were dominated by RWASA3 resistant plants, while six genotypes PI 137741, PI 140204, PI 347019, PI 478172, 564250 and PI 624253 were dominated by moderate resistant plants, one genotype PI 564249 was dominated by moderate susceptible plants and 40 genotypes were dominated by susceptible plants. One genotype PI 624151 had an equal number of moderately resistant and susceptible plants. Therefore, resistant plant may be selected for future used and susceptible plants discarded. Six genotypes PI 349043, PI 366520, PI 366533, PI 366545, PI 366549 and PI 366562 had no data for RWASA4. Two differential checks i.e. Hugenoot and Yumar were dominated by moderately susceptible plants. Of the 80 genotypes screened with RWASA4, 60 genotypes were heterogeneous. From the 60 genotypes, 22 genotypes were dominated by resistant plants, three genotypes PI 197985, PI 243679 and PI 564250 and three differential checks i.e. Gariep, Yumar and PAN3144 were dominated by moderate susceptible plants, followed by 34 genotypes containing susceptible plants. Four genotypes PI 140204, PI 347003, PI 478127 and PI 478134 had equal amounts of resistant and susceptible plats indicating that selection for resistance would be needed before lines could be used. No data was obtained from nine

genotypes i.e. PI 2201.33, PI 349043, PI 366533, PI 366545, PI 366549, PI 366562, PI 564249, PI 623373 and 94M370 (Table 13).

The presence of a mixed reaction in most genotypes made it necessary to select highly RWASA3 and RWASA4 resistant single plants for future research studies. Selection of resistant single plants from mixed germplasm is a pre-requisite for effective pre-breeding. A mixed reaction is expected from landraces because they are genetically diverse, and some genotypes carry useful resistance genes that still need to be investigated further. However, the selection of the unique resistant plants for developing molecular markers for application in molecular breeding will be good for MAS. Moreover, for effective deployment and utilization of these genotypes, there is a need to keep selecting resistance sources from landraces to derive or purify and characterise resistant genotypes with minimum linkage to undesirable traits.

Table 13: Wheat accession number, observed reaction, dominant reaction of each accession number evaluated against RWASA3 and RWASA4.

Wheat accession	Observed reactions					
	RWASA3	Dominant reaction	% of dominant plants	RWASA4	Dominant reaction	% of dominant plants
PI 127097	R,S	S	73.3	R,MR,S,D	R	46.6
PI 127099	R,S,D	S	73.3	R,S,D	R	53.3
PI 127104	R,MS,S	S	86.6	R,MR,S,D	S	73.3
PI 137117	R	R	100	R,MS,S	R	80.0
PI 135047	MS,S	S	86.6	R	R	100
PI 135064	R,MS,S	S	66.6	MS,S	S	60.0
PI 135076	MS,S	S	93.3	R,MS,S,D	S	60.0
PI 137739''S''	R,MR,S	R	80.0	R,MR,S	R	53.3
PI 137740	R,MR,S	R	66.6	R,MR,S	R	46.6
PI 137741	R,MR,MS	MR	46.6	R,MR	R	93.3
PI 137757	R,MR,MS,S, D	S	53.3	MR,MS,S	S	66.6
PI 140204	R,MR,MS,S	MR	53.3	R,MR,MS,S	*	MR=MS
PI 140213	R,MR,S	S	53.3	R,MR	R	93.3
PI 166227	MS,S	S	93.3	S,D	S	73.3
PI 181263	R	R	93.3	R,MR,MS	R	60.0
PI 189746	MS,S	S	86.6	S,D	S	66.6
PI 197985	R,MR,S	S	46.6	R,MR,S	MR	40.0
PI 220131	MS,S,D	S	86.6	MR,MS,S,D	S	40.0
PI 220133	MR,MS,S	S	80.0	-	-	-
PI 243659	E,R,MR,S	R	40.0	R	R	100
PI 243679	MR,MS,S	S	53.3	R,MR,MS	MR	53.3
PI 243730	R,MR,MS,S, D	S	80	R,MS,S,D	S	60.0
PI 245380	R,MR,S	R	53.3	R,MS,S	R	20.0
PI 245432	S,D	S	80.0	R,MR,MS,S	S	40.0
PI 245583	R	R	100	R,MS	R	93.3
PI 250791	R,MR,MS,S	R	40.0	R,MR,MS,S	R	40.0
PI 269408	R,MR	R	93.3	R	R	100
PI 321738	S	S	100	R,MS,S	S	46.6
PI 347003	R,MR,MS,S	R	46.6	R,MS,S	*	R=S
PI 347006	R,MS,S	S	86.6	R,MS,S	S	46.6
PI 347017	R,S,D	S	53.3	R,MS,S	S	60.0

Table 13: Continued

Wheat accession	Observed reactions					
	RWASA3	**Dominant reaction**	**% of dominant plants**	**RWASA4**	**Dominant reaction**	**% of dominant plants**
PI 347019	R,MR,MS,S	MR	46.6	R	R	100
PI 347030	R,MR,S	R	60.0	R,MS,S	S	66.6
PI 349043	-	-	-	-	-	-
PI 366103	R,MR	R	60	R,MR	R	66.6
PI 366520	-	-	-	R,MR,MS,S,D	S	46.6
PI 366529	R,MR	R	73.3	R	R	100
PI 366533	-	-	-	R,MS,S,D	S	33.3
PI 366537	MR,S	S	86.6	R,S	R	80.0
PI 366538	S	S	93.3	R,MS,S	R	86.6
PI 366545	-	-	-	-	-	-
PI 366549	-	-	-	-	-	-
PI 366550	R,MR,S	R	80.0	R,MR,MS,S	R	60.0
PI 366562	-	-	-	-	-	-
PI 366565	R,S	R	66.6	R,MS,S,D	S	46.6
PI 366566	R,MR	R	-	-	-	-
PI 366572	S	S	100	R,MR,MS,S	S	26.6
PI 366573	R,MR	R	73.3	R	R	100
PI 366985	R	R	100	R,MR	R	73.3
PI 367171	S,D	S	86.6	R,MS,S,D	S	40.0
PI 367172	R,S	S	86.6	R,S	S	93.3
PI 367188	R	R	100	R,MR,S,D	S	40.0
PI 478115	MR,MS,S	S	80.0	MS,S,D	S	53.3
PI 478126	R,MS,S,D	S	66.6	R,MS,S,D	S	40.0
PI 478127	R,MR,MS,S	S	53.3	R,MS,S,D	*	MS=S
PI 478134	MS,S,D	S	80.0	S,D	*	S=D
PI 478172	R,MR	MR	80.0	R,MR,MS,S,D	R	60.0
PI 478177	MS,S,D	S	86.6	R,S,D	S	66.6
PI 478216	R,S	S	73.3	MS,S,D	S	46.6
PI 478257	R,MR,MS,S	S	60.0	R,MS,S,D	S	40.0
PI 478260	R,MR,MS,S	S	60.0	MS,S,D	S	80.0
PI 478262	R,MS,S	S	60.0	R,MS,S,D	S	60.0
PI 564249	R,MS,S	MS	46.6	-	-	-
PI 564250	R,MR	MR	80.0	R,MR,MS	MR	66.6
PI 564259	R,MR	R	66.6	R,MR,MS	R	66.6
PI 564260	R,MS	R	93.3	R,MR,MS	R	73.3
PI 623373	MR,MS,S,D	S	60.0	-	-	-
PI 623825	MS,S,D	S	53.3	R,MR,MS,S,D	S	60.0
PI 623836	R,MS,S,D	S	66.6	MR,MS,S,D	D	46.6
PI 623848	MS,S,D	S	66.6	R,MR,MS,S	R	53.3
PI 623857	MR,MS,S	S	46.6	MR,MS,S	S	53.3
PI 624023	MR,MS,S,D	S	53.3	MS,S,D	S	60.0
PI 624151	MR,MS,S	*	MR=S	R,MR,MS,S,D	S	40.0
PI 624152	MS,S	S	46.6	R	R	100
PI 624188	MR,MS,S,D	S	53.3	R,MS,S	S	53.3
PI 624253	R,MR,MS,S, D	MR	46.6	R,MS,S	R	66.6
PI 632671	MS,S,D	S	73.3	R,MR,MS,S,D	S	46.6
PI 634770	S	S	100	R,MR,MS,S	S	40.0
94M370	R,S	S	73.3	-	-	-
RWA MATRIX 2414	R	R	100	HR,R	R	86.6
Differential checks						
Hugenoot	MS,S	S	93.3	S	S	100
Gariep (*Dn1*)	S	S	100	MR,MS,S	MS	53.3
Yumar (*Dn4*)	MS,S	S	73.3	MS,S	MS	53.3
PAN3144 (*Dn5*)	R	R	100	R,MS	MS	80.0

Table 13: Continued

Wheat accessions	Observed reactions					
	RWASA3	**Dominant reaction**	**% of dominant plants**	**RWASA4**	**Dominant reaction**	**% of dominant plants**
CItr 2401 (*Dn2401*)	R	R	100	R	R	100

E: Escape HR: Highly resistant R: Resistant MR: Moderate resistance
MS: Moderately resistant S: Susceptible D: Death

4.1.6 Discussion

In this evaluation, RWASA3 was the most damaging biotype followed by RWASA4 while RWASA1 and RWASA2 were less damaging biotypes. These damage differences could be explained by different resistance genes that still need to be identified, present in the genotypes. Tolmay and Booyse (2016) reported RWASA4 as the most damaging biotype followed by RWASA3 while RWASA1 and RWASA2 were the less damaging biotypes to the genotypes tested. However, these two studies indicates that the RWASA3 and RWASA4 are the most damaging biotypes as compared to other biotypes. Eighteen genotypes resistant to USA biotypes RWA1 and RWA2 including PI 135064, PI 137741, PI 140204, PI 140213, PI 220131, PI 220133, PI 243659, PI 243679, PI 243730, PI 245583, PI 269408, PI 347003, PI 347006, PI 366103, PI 366566, PI 366572, PI 366985 and 94M370 and four landraces reported resistant to RWA2 including PI 623825, PI 623836, PI 624151 and PI 624152 (Collins *et al*., 2005; Peng *et al.,* 2009; Puterka *et al*., 2014) showed varying levels of resistance to the SA biotypes. There are some discrepancies regarding PI 243679 resistance to RWA2. This accession was found to be resistant to USA biotype RWA2 (Collins *et al*., 2005) and later was utilised as an RWA2 susceptible landrace (Peng *et al.,* 2009). In this study, PI 245583, PI 269408, PI 366985, PI 137741, PI 243659 and PI 366566 were resistant to all four SA biotypes necessitating the need for further characterisation for other traits such as RWA resistance mechanisms. Among all 27 genotypes that were evaluated against all SA biotypes, (Tolmay and Booyse, 2016), five genotypes i.e. PI 137741, PI 220131, PI 243730 PI 347019 and PI 366985 were also used in this study. In the present study, the results aligned with the authors results except for PI 347019 and PI 137741. PI 137741. Both studies found PI 137741 resistant to RWASA1, RWASA2 and RWASA3, however, for RWASA4 this study found the genotypes resistant while it was reported to be susceptible in the authors results. However, PI 347019 aligned for RWASA3 with moderate resistance and deviated for RWASA2, and RWASA4 with resistance as the genotype was found susceptible to these biotypes by Tolmay and Booyse (2016). Moreover, PI 347019 slightly deviated for

RWASA1 with resistance (Table 11) while it was found moderately resistant to this biotype in the study of Tolmay and Booyse (2016).

Reports from previous studies stated that RWASA1 is virulent to wheat genotypes containing *Dn2* and *dn3*, RWASA2 is virulent to wheat genotypes containing *Dn1-dn3*, *Dn8*, and *Dn9*, RWASA3 is virulent to wheat genotypes containing *Dn1-Dn4, Dn8,* and *Dn9* while RWASA4 is virulent to wheat genotypes containing *Dn1-Dn5* and *Dn9*, (Jankielsohn, 2011; 2014). RWASA3 was also observed to be virulent to *Dny* resistance gene source (Tolmay *et al.,* 2012). In this study, PI 634770 (*Dn9*) showed resistance to RWASA2 and moderate resistance to RWASA1 and was susceptible to RWASA3 and RWASA4. Sikhakhane (2017) also found PI 634770 (*Dn9*) susceptible to RWASA2 while PI 137739''S'' (*Dn1*) was resistant to RWASA2. Tolmay *et al.,* (2012) reported 14 *Dn4*-containing resistance sources moderately resistant to RWASA3, which is known to be virulent to the cultivar Yumar, a *Dn4* containing genotype. RWA MATRIX 2414 (*Dn2414*) was found resistant to all four biotypes while 94M370 was susceptible to RWASA3. The same difference was observed in other studies. 94M370 was evaluated with USA biotypes RWA1-RWA5 and was tested susceptible to RWA3 and RWA4 (Burd *et al.,* 2006) while later it was tested resistant to RWA3 and RWA4 (Randolph *et al.,* 2009; Puterka *et al.,* 2014). These genotypes come from different pedigrees, created by different people therefore; it is not known what was inherited along the way. Human error is also inevitable and could have resulted in Type I or II error. Type I error may be reporting the presence of resistance/susceptibility when it's not actually there while Type II error may be reporting the absence of resistance/susceptibility when its actually present. Aphid feeding duration and density could be the reason across different results in these studies. Identical phenotypic and marker profiles conferred by 94M370 (*Dn7*) and RWA MATRIX 2414 (*Dn2414*) to most RWA biotypes (local/foreign) are useful for RWA resistance breeding i.e. science (Anderson *et al.,* 2003; Haley *et al.,* 2004: Lapitan *et al.,* 2007; Weiland *et al.,* 2008; Randolph *et al.,* 2009; Mornhinweg 2012; Puterka, 2017) and not useful for wheat industry. These resistance sources in some studies are used as resistant controls or resistant parental lines for developing crosses (Collins *et al*., 2005; Lapitan *et al.,* 2006; Peng *et al.,* 2007; Sotelo *et al.,* 2009; Jankielsohn, 2014; Puterka *et al.,* 2012 and 2014). This new resistance can be bred with other cultivars or breeding lines to introduce new traits that confer resistance to biotypes that were previously virulent.

Deviation within each category e.g. highly resistant (1-2) resistance (3-4), moderate resistance (4-5) and susceptible (8-9) is explainable; however, deviation from one category to another e.g. resistance (3-4) to susceptible (8-9), is difficult to explain across studies if he biotypes have been used in the evaluation. Possible explanation for

this deviation may be the presence of heterogeneity. In the USA, wheat cultivars containing the *Dn4* resistance gene are resistant to RWA1 and RWA8 and susceptible to RWA2-RWA7 while wheat genotype containing the *Dn9* resistance gene is resistant to RWA8 and susceptible to RWA1-RWA7 (Jankielsohn, 2014; Puterka *et al.,* 2014). Betta*Dn9* also showed resistance to one USA biotype RWA8 but was susceptible to seven biotypes (Puterka *et al.,* 2014). The deviation of resistance across all these studies shows how RWA studies are complex.

Very few studies have been conducted to investigate different reactions wheat landraces express when evaluated with RWA biotypes (Xu *et al.,* 2015). In their study, they proved the existence of mixed landrace germplasm necessitating extensive identification, selection, and purification of plants with resistant phenotype before deployment and utilization in breeding programs. Therefore, the results from this study adds to the few among others that have reported different reactions a wheat landrace gives in RWA evaluations. The results from this study were compared with the results of Xu *et al.* (2015). PI 135054 and PI 624152 showed stable resistance to USA biotype RWA2 (Xu *et al.,* 2015), however, when tested against SA biotypes RWASA3 and RWASA4, PI 135064 displayed mixed reaction dominated by susceptible plants to both biotypes. Moreover, PI 624152 showed mixed reaction, dominated by susceptible plants to RWASA3 and uniform resistance to RWASA4. USA biotype RWA2 and SA biotype RWASA3 and RWASA4 are the most damaging biotypes with a limited number of resistant lines reported to these biotypes. The presence of diversity or mixed reaction may be due to the genetic nature of wheat landraces, collected either as mixed germplasm because the same germplasm may or may not contain the same genotypes (seeds genetically different), therefore, resulting in inconsistent results across different evaluations (Xu *et al.,* 2015). RWA MATRIX 2414 homogeneous resistance to RWASA3 and RWASA4 is expected as this genotype is a selection from CItr 2401 and exhibits high levels of resistance to SA and foreign biotypes (Weiland *et al.,* 2008; Puterka *et al.,* 2012).

Four of the mixed resistant and susceptible genotypes identified here have been purified before, designated a resistance gene, and mapped on chromosome 7D and 1D respectively. The genotypes include PI 137739 containing *Dn1* resistance gene located on chromosome 7D (Du Toit, 1987; Schroeder-Teeter *et al.,* 1994) and later proved to be on the short arm of chromosome 7D: - 7DS (Liu *et al.,* 2001). In this study, PI 137739 showed to be mixed in reaction and dominated by resistant plants to RWASA3 and RWASA4. PI 634770 contains the *Dn9* resistance gene (Tolmay *et al.,* 2006) and *Dn9* resistance sources are supposed to be resistant to RWASA1 and susceptible to RWASA2-RWASA3, similar findings were found in PI 634770 with uniform susceptibility to

RWASA3 and mixed reaction dominated by susceptible plants to RWASA4 as expected. PI 372129 was also purified and designated to carry the *Dn4* resistance gene and mapped on chromosome 1DL (Nkongolo *et al.,* 1991b; Liu *et al.,* 2002). These lines do not possess the 1RS/1BL location. These results reveal the complexity of RWA host plant resistance suggesting that selection from wheat landraces is crucial for novel resistance.

As mentioned before, the test entries used in this study have been evaluated with either one or both of the USA RWA biotypes RWA1 and RWA2. Assessing resistance patterns of i.e. RRSR, RSRR, RSRS, RSSR, SRSR, SSRR and SRRS from RWASA1 to RWASA4 enabled comparison with the USA resistance patterns of RR, RS and SR from RWA1 and RWA2. Five genotypes PI 347030, PI 349043, PI 366520, PI 366565 and PI 624151 had a resistance pattern of RRRS. From these genotypes, PI 366565 had a resistance pattern of RR for USA RWA biotypes RWA1 and RWA2 (USDA-ARS-NPGS). PI 347030 had a resistance pattern of SR while PI 366550 had a resistance pattern of RS for USA RWA biotypes RWA1 and RWA2 (USDA-ARS-NPGS). PI 349043 only reported resistant to USA RWA biotype RWA1 (USDA-ARS-NPGS) while PI 624151 was only reported resistant to USA RWA biotype RWA2 (Peng *et al*., 2009). PI 250791 and PI 478127 had a resistance pattern of RSRR. Both genotypes reacted differently to the USA RWA biotypes RWA1 and RWA2. PI 250791 had a resistance pattern of RS while PI 478127 had a resistance pattern of MS (M for mixed) for USA RWA biotypes RWA1 and RWA2 (USDA-ARS-NPGS). PI 134117 portrayed resistance pattern SRRR. To USA RWA biotypes RWA1 and RWA2, this genotype had a resistance pattern of RR.

Eleven genotypes had a resistance pattern of RRSR including PI 127097, PI 127099, PI 135047, PI 140213, PI 220133, PI 245432, PI 366537, PI 366538, PI 623848, PI 624152 and PI 624188. From these genotypes, PI 135047, PI 140213, PI 220133 and PI 366538 had a resistance pattern of RR for USA RWA biotypes RWA1 and RWA2 (USDA-ARS-NPGS; Collins *et al*., 2005; Peng *et al*., 2009). Furthermore, PI 127097, PI 127099 and PI 245432 had a resistance pattern of SR for USA RWA biotypes RWA1 and RWA2 (USDA-ARS-NPGS). Only genotypes PI 623848, PI 624152 and PI 624188 were evaluated and reported resistant to RWA2 while PI 366573 was only evaluated and reported resistant to RWA1 (USDA-ARS-NPGS; Peng *et al*., 2009). PI 478126 had a resistance pattern of SRSR while PI 478216 had a resistance pattern of RSRS. Both genotypes reacted the same for USA RWA biotype RWA1 and opposite for RWA2. PI 478126 had a resistance pattern of MR while PI 478216 had a resistance pattern of MS where M for mixed (USDA-ARS-NPGS). PI 243679 had the resistance pattern RSSR while for USA RWA biotypes RWA1 and RWA2; the genotype showed a resistance pattern of RR (Collins *et al*., 2005). Different biotypes in different locations raise concerns

about whether the resistance sources to RWA biotypes in one location may/may not combat RWA biotypes in other locations. Therefore, there is a possibility that these genotypes will continue to be effective in combating RWA biotypes in other countries.

4.1.7 Conclusions

The RWA occurrence in SA once again threatens the wheat industry following resistance breaking biotype development after deployment of resistant cultivars. The RWA complex is dynamic and continues to change with the plant material used. The study reveals that new resistance sources are needed to combat the latest biotypes RWASA3 and RWASA4 as they are the most damaging among to the lines tested in this study. RWASA3 and RWASA4 were reported in 2009 and 2011 respectively. Since then, RWA breeders across SA have devoted their research into developing resistance to these latest biotypes, however, not much progress have been made or reported to these biotypes. This study further reports new germplasm with effective resistance to the latest RWA biotypes and this will be helpful in identifying RWASA5 resistance in the future. New resistance sources to all four RWA biotypes were also found thus strengthening the RWA host plant resistance. Therefore, landraces proved to be valuable sources for novel germplasm. New resistance patterns different from known genes were found based on RWASA3 and RWASA4 suggesting that these genotypes carry useful genes that need to be explored further. Stewardship of valuable resistance genes needs deployment in RWA pre-breeding to maximise resistance to this pest. This is crucial for gene pyramiding as opposed to relying on one gene. Wheat landraces are naturally collected as mixed genotypes; however, exploring uniformity within the genotypes has shown that uniform and natural resistance does exist naturally within the donor lines. Since one cannot breed with a large number of donor lines at once, selections have been made within resistant plants for transferring useful traits. These germplasms will be useful to the ARC-SG RWA pre-breeding.

Genotypes PI 137741, PI 220131, PI 243730, PI 347019, PI 366985 and PI 372129 have been used in pre-breeding programs but not deployed in the field. 94M370 has been studied academically but not used in pre-breeding programs nor deployed in the field. PI 634770 has been deployed in combination with the *Dn5* but not used on its own in the pre-breeding programs nor deployed in the field. The majority of the genotypes used have no pedigree information available; therefore, characterization of these novel resistance sources is imperative for their efficient use in wheat breeding. The characterisation may involve studying the mechanisms underlying resistance, genomic studies whereby molecular markers are developed for use in MAS.

4.2 Yield potential of wheat genotypes with resistance to RWASA3 and RWASA4

4.2.1 The growth period and spike traits of the selected RWASA3 and RWASA4 resistant genotypes

The analysis of variance, *R*-square and the coefficient of variance for the RWASA3 and RWASA4 resistant genotypes evaluated for their number of days to heading (HD), the number of days to anthesis (AD), the number of spikes per plant (NSP), spike length (SL), the grain numbers per spike (GNS), the grain weight per spike (GWS), the grain numbers per plant (GNP), grain weight per plant (GWP) and thousand-kernel weight (TKW) showed significant differences ($P<0.0001$). Table 14 presents the data of the agronomic and the spike traits. The breeding lines PI 564250, PI 564259 and PI 564260 displayed twice the potential as compared to the wheat landraces thus representing the true meaning of improved genotypes. Data was omitted from one RWASA3 and nine RWASA4 resistant genotypes due to plant inability to grow after transplanting. For genotypes with RWASA3 resistance, the HD varied from 70 days to 140 with an average of 109 days. The genotypes were short to medium growing suggesting that they would adapt greatly to the eastern Free State region should they be evaluated under field conditions. Only 21.1% of the genotypes: plants in PI 137739''S'', PI 137741, PI 140204, PI 140213, PI 243659, PI 245583, PI 269408, PI 366566 and PI 478172 have shown to be medium growing (123 to 140 days) thus the remaining 78.9% being the short growing genotypes. Flowering and pollination of wheat may take three to five days (Grain SA, 2012). The AD varied from one day to 21 days with an average of 6 days. However, 56.88% of the plants across all RWASA3 resistant genotypes displayed a flowering period of one to five days respectively. All the plants in genotypes PI 137741, PI 243659 and PI 269408 showed to be within the same range (one to five) of the flowering period while the plants in PI 137740 expressed flowering period of beyond 6 days. Approximately 30 days before anthesis and 10 days' post-anthesis, the number of kernels that will form in the spike are determined (Grain SA, 2012).

The NSP can vary depending on the genotype and the conditions under which the genotype is grown. In this study, the NSP varied from one to 16 with an average of eight. Approximately 51.8% of the plants across all genotypes displayed a considerable variation of eight to 16 spikes. PI 137739''S'', PI 197985, PI 269408, PI 366550 and PI 366566 have shown to be a bad choice for selection as fewer spikes were obtained. Crop management practices such as inadequate water supply or uptake by the plant may have caused the plants to not reach their full development potential. The SL varied from 4.3 cm to 12.0 cm with an average of 8.13 cm. Only (0.02%) of the wheat genotypes (a plant in PI

250791) indicated SL of below 5 cm making the plant undesirable for selection. The GNS varied from one to 60 with an average of 29 grains. In this case, 32.1% displayed the GNS of below 25. This includes one to three plants in each genotype thus contributing the fluctuation in the number of grain within each genotype. The GWP spike varied from 0.3 g to 2.6 g with an average of 1.1 g. Only 16.5% of the wheat genotypes showed grain weight per spike of 1.5 g to 2.6 g. This includes plants in genotypes PI 243659, PI 250791, PI 347019, PI 478172, PI 366573, PI 564250, PI 564259 and PI 564260. These genotypes are valuable sources for crop improvement. The GNP varied from one to 402 with an average of 159 grains per plant. Plants in genotypes PI 137740, PI 137757, PI 197985, PI 347030, PI 366566, PI 366537 and PI 366550 need further improvement for more grains in the future. The TKW varied from 1.0g to 15.5 g with an average of 5.4 g. Precisely 60% of the wheat landraces had GWP of below 5 g (Table 14).

For genotypes with RWASA4 resistance, the HD varied from 74 days to 138 days with an average of 108 days. One to three plants in genotypes PI 127097, PI 134117, PI 137739''S'', PI 243659, PI 245583, PI 347019, PI 366529, PI 366573, PI 478216 and PI 623848 were mid growing genotypes with HD of 123 to 138 days. Only 11.1% of the genotypes had HD of up to 90 days (73 to 90 days). The AD varied from one to 17 days with an average of 6 days. Approximately 65.8% of the wheat donor lines displayed a flowering period of one to 5 days. One to three plants in genotypes PI 366529, PI 366537, PI 366538 and PI 478172 have shown that it would be a bad choice if selected. As mentioned earlier that the number of spikes per plant can differ, the variation in this study was from one to 13 with an average of seven. This is completely different compared to the RWASA3 resistant genotypes that showed the ability to produce up to 16 spikes per plant. A selection for crop improvement could be made on 45% of the wheat donor lines with the spike numbers of eight to 13 respectively. This is accounted by plants in genotypes PI 134117, PI 137739''S'', PI 137741, PI 140204, PI 140213, PI 181263, PI 243659, PI 243679, PI 269408, PI 366573, PI 564250, PI 564259, PI 564260, PI 623848, PI 624152 and PI 624253. The SL varied from 3 cm to 12.7 cm with an average of 8.09 cm. Ninety six percent of the wheat donor lines displayed a spike length of 5 cm to 12.7 cm. Up to three plants in genotypes PI 127097, PI 197985, PI 366529, PI 366550, PI 478172 and PI 478216 have shown the need for improvement.

The GNS varied from one to 62 with an average of 29 grains. However, 62.69% accounted for grain variation of 25 to 62 respectively. From this, it was noted that the SL was not a positive influence on the grain numbers but rather the spikelets per spike.

Genotypes PI 137739''S'', PI 137741, PI 140204, PI 140213, PI 181263, PI 243659, PI 243679, PI 245583, PI 269408, PI 347019, PI 366573, PI 478172, PI 478216, PI 564259, PI 564250, PI 564260, PI 623848, PI 624152 and PI 624153 also shown that they would be good sources for higher yield in the future. The GWS varied from 0.4 g to 3.7 g with an average of 1.14 g. Only 24.6% of the donor lines showed a grain weight of 1.5 g to 3.7 g. This means that the genotypes were composed of thick seeds that are ideal for crop improvement. The GNP varied from one to 381 with an average of 148 grains. However, 61.9% of the wheat donor lines showed potential for selection with grain numbers varying from 100 to 381 grains per plant respectively. These criteria of selection were on the basis that they are high yielding compared to other genotypes. Genotypes PI 127097, PI 137740, PI 197985, PI 366537, PI 366538, PI 366550, PI 478172, PI 478216, PI 624152 and PI 624253 have shown that there is a huge need for improvement in these lines as this would lead to low yield in utilised in the future. The GWP varied from 0.3 g to 16.1 g with an average of 5.4 g. Only 42.5% of the wheat landraces displayed grain weight per plant of below 5 g. However, TKW varied from 13.8 g to 74.6 g with an average of 36.14 g (Table 15). The subjection of plants to RWA screening and transplanting was two major stresses that plants could not have equal ability to recover from thus leading to major variation among the traits measured.

This form of significance indicates greater diversity among the genotypes studied suggesting a considerable response for selection. Moreover, the variation of the traits indicates the direct and indirect relationship of the traits with each other.

Table 14: Genotype, number of days to heading, number of days to anthesis, spike numbers per plant, spike length, grain numbers per spike, grain weight per spike, grain numbers per plant, grain weight per plant and thousand-kernel weight of the RWASA3 resistant genotypes.

Genotype	Number of days to heading	Number of days post heading	Spike numbers	Spike length (cm)	Grain numbers per spike	Grain weight per spike(g)	Grain numbers per plant	Grain weight per plant (g)	Thousand kernel weight (g)
PI 137739''S''	128	11	7	8,2	17	0,5	95	2,1	22,10
	120	5	10	10,0	32	1,4	168	6,2	36,9
	122	4	9	9,8	35	1,4	184	6,7	36,41
	121	7	8	10,5	39	1,3	168	5,5	32,7
	128	4	6	9,3	30	1,0	95	3,1	32,6
PI 137740	98	13	12	7,3	27	0,6	157	3,4	21,7
	106	14	13	6,0	18	0,6	168	5,0	29,8
	102	16	1	7,0	23	0,9	23	0,9	39,1
	105	21	10	7,3	36	0,8	105	3,7	35,2
	96	8	5	5,5	17	0,6	64	1,9	29,7
PI 137741	122	2	9	7,8	35	0,9	152	4,3	28,3
	123	4	7	8,7	29	0,9	150	3,2	21,3
	125	5	7	8,8	37	0,9	170	3,9	22,9
	123	3	8	8,8	32	1,1	174	5,0	28,7
	123	3	9	8,2	34	1,2	197	6.4	32,5
PI 137757	79	3	4	4,5	8	0,3	8	0,3	37,5
	76	4	3	6,3	15	0,7	30	1,3	43,3
	81	7	3	5,5	15	0,7	44	2,0	45,5
	84	17	3	5,6	16	0,7	48	2,2	45,8
	78	2	3	6,2	20	0,9	60	2,8	46,7
PI 140204	130	7	3	7,2	13	1,5	40	1,1	27,5
	121	3	11	10	32	1,1	224	7,5	33,5
	114	4	11	9,8	35	1,1	260	7,5	28,8
	117	4	10	9,7	35	0,9	207	6	29
	116	3	5	9,5	34	1,2	179	6,1	30,1
PI 140213	120	2	5	7,3	20	0,6	72	3,3	45,8
	119	3	9	8,5	25	1,2	124	6,0	48,4
	124	6	5	8,8	32	1,3	161	5,8	36,02
	120	6	6	8,0	25	1,1	118	4,5	38,14
	117	5	8	8,0	28	1,3	136	5,7	41,9

Table 14: Continued

Genotype	Number of days to heading	Number of days to anthesis	Spike numbers	Spike length (cm)	Grain numbers per spike	Grain weight per Spike (g)	Grain numbers per plant	Grain weight per plant (g)	Thousand kernel weight (g)
PI 181263	98	6	13	7,8	32	0,9	196	4,2	21,42
	94	9	3	6,2	20	0,7	60	1,9	31,7
	111	16	13	7,0	32	0,5	242	4,2	17,35
	109	7	16	7,2	27	0,8	231	5,2	16,2
	104	7	8	7,0	31	0,8	142	4,0	28,2
PI 197985	103	13	4	7,0	23	0,9	86	2,9	33,7
	92	4	2	6,5	21	1,0	47	2,0	42,5
	107	5	3	8,3	29	1,2	42	1,6	38,1
	115	2	3	7,5	23	0,9	36	1,8	50
	70	7	1	8,0	24	0,9	24	0,9	37,5
PI 243659	111	4	13	11,7	36	1,1	297	8,1	27.23
	116	1	11	12,0	34	1,2	222	7,2	32,43
	126	3	10	11,7	39	1,5	244	8,1	33,2
	131	3	6	8,5	25	0,6	159	2,9	18,23
	127	5	9	8,5	23	1,1	116	1,6	40,5
PI 245583	122	5	9	9,5	45	1,9	214	9,2	43.0
	122	3	11	10,2	42	1,2	332	9,0	27,11
	126	3	7	8,3	20	0,9	132	4,5	34,1
	125	2	8	8,8	25	1,0	150	4,2	28.0
	125	7	9	9,0	35	1,0	180	4,3	23,9
PI 250791	91	5	6	7,0	29	0,8	155	3,7	23,9
	109	3	10	7,2	27	1,0	241	8,8	36,5
	108	8	10	7,8	32	1,5	235	9,1	38,7
	78	11	9	6,0	24	0,8	137	3,7	29,1
	83	4	5	4,3	14	0,6	70	2,6	37,1
PI 269408	120	3	7	8,2	23	0,5	97	3,4	24,3
	134	4	5	7,3	27	0,5	116	3,9	34,0
	128	3	6	7.3	27	0.5	124	4,2	38,9
	129	2	3	7,7	8	0,4	107	2,6	24,3
	140	2	3	7,3	16	0,4	114	2,9	25,4

Table 14: Continued

Genotype	Number of days to heading	Number of days post heading	Spike numbers	Spike length (cm)	Grain numbers per spike	Grain weight per Spike (g)	Grain numbers per plant	Grain weight per plant (g)	Thousand kernel weight (g)
PI 347019	104	11	7	8,5	33	1,2	153	5,5	35,9
	109	5	7	9,2	32	1,6	123	5,7	46,3
	115	6	15	7,3	28	1,2	246	10,8	43,9
	108	9	11	8,8	34	1,4	212	7,9	37,3
	114	5	8	9,0	33	1,3	166	6,8	41,0
PI 347030	93	5	11	7,1	21	0,8	187	8,4	44,9
	110	14	8	8,0	29	1,0	152	4,9	32,2
	118	4	4	5,8	3	0,1	11	0,3	27,3
	113	4	10	7,4	31	0,8	162	5,6	34,6
	107	7	10	7,5	26	0,6	179	6,7	37,4
PI 366529	80	1	1	6	22	0,9	22	0,9	40.8
	79	4	1	5	23	0,8	23	0,8	34.8
	76	6	2	6,5	26	0,9	26	0,9	34,6
PI 366550	80	1	3	7,0	27	1,0	81	3,2	39,5
	79	4	8	6,0	20	0,8	147	4,5	30,6
	76	6	5	6,2	17	0,7	72	3,3	45,8
	81	5	2	6,8	20	1,0	40	2,0	50,0
	81	8	1	7,0	23	1,2	23	1,2	52,2
PI 366566	-	-	-	-	-	-	-	-	-
	136	1	6	9,2	22	0,5	122	2,6	21,31
	131	5	5	10,0	27	0,8	95	2,6	27,4
	128	7	8	8,7	25	1,0	62	2,1	16,12
	132	2	5	9,2	26	0,7	89	2,5	28,8
PI 366573	108	10	12	9,2	41	1,6	271	9,8	26,16
	103	7	9	8,5	46	1,8	300	10,0	33,33
	110	7	13	8,2	39	1,4	308	10,5	34,1
	102	8	7	8,8	51	2,2	275	11,0	40,0
	111	9	9	7,7	25	1,2	157	7,2	45,9
PI 478172	129	5	9	8,4	29	1,3	185	6,5	35,1
	100	6	7	5,8	24	0,9	168	5,5	32,7
	100	6	9	8,5	28	1,2	181	7,7	42,5
	115	3	8	5,8	20	0,7	107	3,4	31,8
	102	5	6	7,9	30	1,2	144	5,1	35,4

Table 14: Continued

Genotype	Number of days to heading	Number of days post heading	Spike numbers	Spike length (cm)	Grain numbers per spike	Grain weight per Spike (g)	Grain numbers per plant	Grain weight per plant (g)	Thousand kernel weight (g)
PI 564250	117	5	7	10,0	36	1,3	211	7,4	35,07
	111	12	5	10,2	48	1,5	224	6,3	28,13
	104	8	7	9,0	27	1,3	185	7,0	37,83
	101	3	9	9,5	31	1,0	207	6,4	30,91
	97	8	10	9,3	28	1,2	227	9,0	39,64
PI 564259	98	7	7	8,2	46	2,0	291	12,1	41,58
	100	7	8	8,7	38	1,7	189	7,7	40,74
	99	2	11	9,5	34	1.7	360	14.0	38,9
	102	6	11	8,5	42	1,7	300	13,2	44,0
	100	3	11	8,8	45	2,6	402	15,5	38,6
PI 564260	99	5	7	9,5	48	1,8	374	11,5	30,7
	110	3	6	8,8	56	2,0	209	7,7	36,84
	109	1	10	10,0	50	1,8	328	11,5	35,1
	104	8	10	9,3	45	1,8	297	10,1	34,0
	104	2	7	10,2	60	2,3	296	11,9	40,2
PI 624253	104	13	10	7,8	21	0,6	161	3,6	22,4
	107	2	12	7,5	23	0,6	170	4,0	23,5
	113	13	2	6,5	12	0,5	24	0,9	37,5
	108	6	16	9,2	27	0,8	226	7,1	31,4
	105	5	13	8,8	26	0,8	143	3,8	26,6
Mean	109	6	7,54	8,06	29	1,04	159	5,27	33,92
$LSD_{0.0001}$	8,9	3,7	3,3	1,1	8,42	0,33	68,8	2,52	7,24
R^2/CV	0,83/6,35	0,51/49,43	0,57/33,99	0,73/10,9	0,68/22,48	0,73/25,03	0,7/33,67	0,72/37,37	0,60/16,6

Table 15: Genotype, number of days to heading, number of days to anthesis, spike numbers per plant, spike length, grain numbers per spike, grain weight per spike, grain numbers per plant, grain weight per plant and thousand kernel weight of the RWASA4 resistant genotypes.

Genotype	Number of days to heading	Number of days to anthesis	Spike numbers	Spike length (cm)	Grain numbers per spike	Grain weight per Spike (g)	Grain numbers per plant	Grain weight per plant (g)	Thousand kernel weight (g)
PI 127097	93	3	3	6,8	15	0,5	7	1,2	28,6
	74	17	1	3,0	7	0,2	44	1,5	34,1
	97	6	3	5,5	17	1,0	52	3.0	57,7
	133	3	5	7,0	23	1,1	141	6,1	43,3
	114	3	4	6,8	29	1,4	105	4,8	45,7
PI 134117	100	20	9	8,3	16	0,7	72	3,3	45,8
	127	3	10	10,5	31	1,3	217	8,6	39,6
	110	3	9	9,8	27	0,9	127	4,3	33,9
	123	3	5	7,5	20	0,7	59	2,2	37,3
	83	8	6	7,3	19	0,6	85	3,1	36,5
PI 137739''S''	130	5	6	9,7	33	1,2	152	5,3	34,9
	120	2	7	10,0	35	1,0	143	3,7	25,9
	125	3	4	10,8	32	1,3	103	4,1	39,8
	116	6	12	11,0	46	1,8	226	7,0	31.0
	117	7	9	11,2	45	2,0	232	9,0	38,8
PI 137740	98	12	4	7,7	29	0,7	111	2,0	18,0
	110	7	5	6,3	17	0,7	52	1,0	19,2
	106	4	4	7,0	29	1,1	88	3,7	42,0
	111	3	7	7,0	37	1,4	203	9,1	44,8
	120	5	4	9,2	36	1,8	134	6,4	47,8
PI 137741	110	5	8	8,2	32	1,3	157	6.8	43,3
	109	6	10	8,3	37	1,5	189	7,0	37,0
	119	2	8	7,0	17	0,4	55	1,4	25,5
	115	4	12	8,3	32	1,3	190	7,7	40,5
	115	8	10	8,2	27	1,2	165	7,5	45,5

Table 15: Continued

Genotype	Number of days to heading	Number of days to anthesis	Spike numbers	Spike length (cm)	Grain numbers per spike	Grain weight per Spike (g)	Grain numbers per plant	Grain weight per plant (g)	Thousand kernel weight (g)
PI 140204	96	9	8	8,2	32	1,3	221	7,2	35,6
	97	3	10	8,3	37	1,5	231	8,0	34,5
	116	3	5	8,3	29	1,6	149	8,5	32,2
	116	5	9	5,2	28	1,2	264	7,0	24,6
	105	5	5	8,5	29	1,5	153	5,3	34,6
PI 140213	99	4	13	10,3	28	1,3	319	11,4	35,7
	100	3	9	10,2	32	1,4	217	8,8	40,6
	115	4	5	10,5	48	2,5	200	10,4	52
	107	7	7	8,5	41	1,6	301	12,0	39,9
	112	3	5	10,7	62	3,1	234	11,6	49,6
PI 181263	107	5	7	7,2	40	1,3	218	7,1	32,6
	110	8	5	7,3	34	1,1	71	5,3	74,6
	108	4	8	7,2	28	1,1	196	8,3	45,4
	104	3	9	6,3	26	0,9	183	3,2	17,5
	121	8	5	7,7	38	1,3	135	4,1	30,4
PI 197985	83	2	2	6,3	22	1,2	44	2,6	59,1
	86	6	3	5	12	0,7	44	1,9	54,3
	88	5	5	5,7	13	0,8	35	3,5	57,4
	94	4	2	4	5	0,3	61	0,3	60
	88	2	3	4,5	1	*	1	*	*
PI 243659	119	1	13	9,7	28	1,0	270	6,1	22,6
	120	5	7	10,5	23	0,7	72	3,4	47,2
	109	3	9	12.7	40	1,5	270	9,1	33,7
	30	2	13	12.5	38	1,5	306	9,6	31,4
	123	3	8	12.2	41	1,3	374	13,3	35,6
PI 243679	112	4	9	10,2	23	0,9	198	7.6	38.4
	112	3	7	10,8	45	2.1	230	10.8	50
	99	3	10	10,2	42	1.8	381	16.1	42.3
	105	5	9	9,2	42	2.2	272	12.9	47.4
	120	5	4	9,2	36	1.8	134	6.4	47.8

Table 15: Continued

Genotype	Number of days to heading	Number of days post heading	Spike numbers	Spike length (cm)	Grain numbers per spike	Grain weight per Spike (g)	Grain numbers per plant	Grain weight per plant (g)	Thousand kernel weight (g)
PI 245583	130	2	3	8.0	21	0.6	48	1.4	29.2
	123	3	7	9.3	38	1.5	210	6.7	31.9
	122	2	8	11.0	39	1.1	173	4.4	25.4
	121	11	8	9.8	30	1.1	129	4.1	31.8
	123	2	11	10,5	40	1,2	309	7,5	24,3
PI 366529	114	4	6	6.3	35	1.2	179	5.5	30.7
	100	119	4	3.7	13	0.4	65	1.3	20
	89	17	3	7	24	0.8	72	2.5	34.7
	-	-	-	-	-	-	-	-	-
	102	5	5	6	20	0.9	78	2.9	37.2
PI 366537	90	15	3	5,5	14	0,4	27	1,0	37,0
	96	14	1	5,0	16	0,7	16	0,7	43,8
	84	5	5	6,5	27	1,1	53	2,1	39,6
	73	22	3	6,5	21	0,9	53	2,1	39,6
PI 366538	110	5	2	7,8	33	1,1	33	2,2	66,6
	105	7	3	6,7	26	1,0	102	4,0	39,2
	97	19	5	7,0	24	0,8	97	3,5	36,1
	97	19	5	7,0	24	0,8	97	3,5	36,1
	100	3	4	6,7	25	1,1	92	3,8	41,3
	103	10	4	6,2	19	0,8	82	1,9	42,1
PI 366550	105	4	3	6,5	19	0,8	56	2,5	44,6
	104	17	3	7,5	20	1,8	90	3,7	41,1
	95	7	6	6,7	26	1,2	113	4,7	41,6
	117	7	4	4,0	7	0,4	7	0,4	57,1
PI 366573	99	4	8	7,7	28	1,3	136	4,2	30,9
	113	2	9	8,0	27	1,3	230	8,0	34,8
	99	2	10	9,5	46	1,6	262	10,0	38,2
	115	3	6	7,3	25	1,1	130	4,0	30,8
	112	4	9	8,7	39	1,5	185	6,0	32,4
PI 478172	124	9	3	8,2	32	1,4	97	4,2	43,3
	118	3	4	5,8	24	1,1	47	2,0	42,6
	83	12	5	5,0	24	1,0	24	1,0	41,7
	-	-	-	-	-	-	-	-	-
	84	15	5	4,7	14	0,5	63	1,7	27,0

Table 15: Continued

Genotype	Number of days to heading	Number of days to anthesis	Spike numbers	Spike length (cm)	Grain numbers per spike	Grain weight per Spike (g)	Grain numbers per plant	Grain weight per plant (g)	Thousand kernel weight (g)
PI 478216	137	5	3	8,2	32	1,4	97	4,2	43,3
	128	3	2	5,8	24	1,1	47	2,0	42,6
	126	8	1	5,0	24	1,0	24	1,0	41,7
	-	-	-	-	-	-	-	-	-
	123	13	5	4,7	14	0,5	63	1,7	27,0
PI 564250	97	5	6	8,7	31	1,5	117	4,5	38,5
	100	12	10	10,7	37	1,5	234	10,3	44,0
	99	8	9	9,3	24	1,0	172	6,1	35,5
	91	3	10	10,5	41	1,5	220	8,6	39,1
	96	8	9	9,5	36	1,4	223	7,0	31,4
PI 564259	96	7	9	7,5	28	1,2	185	7,0	37,8
	106	7	10	8,2	39	1,7	256	10,3	40,2
	99	2	9	8,7	39	1,4	249	9,7	39,0
	99	6	8	8,2	32	1,5	283	8,0	28,3
	100	3	11	7,7	34	1,3	295	10,6	35,9
PI 564260	101	5	10	9,5	47	1,6	374	11,4	30,5
	104	3	8	9,7	42	1,7	199	8,6	43,2
	105	1	9	9,2	27	0,8	288	7,2	25,0
	120	8	10	9,8	44	1,7	341	12,0	35,2
	99	2	10	10,2	60	2,3	251	10,0	39,8
PI 623848	126	4	5	11,3	35	1,4	132	2,2	16,7
	138	6	1	6,0	26	1,1	26	1,1	39,6
	128	4	13	9,8	26	0,7	158	3,8	24,1
	-	-	-	-	-	-	-	-	-
	128	4	8	8,2	25	0,7	150	3,9	26,0
PI 624152	112	5	10	9,3	20	0,5	87	1,2	13,8
	112	5	9	7,8	19	0,6	142	4,1	28,9
	-	-	-	-	-	-	-	-	-
	111	5	12	8,5	30	0,9	158	3,6	22,8
	107	5	5	8,3	16	0,5	72	1,0	13,9
PI 624253	-	-	-	-	-	-	-	-	-
	104	3	8	7,3	20	2,5	62	0,8	12,9
	116	8	9	9,2	19	0,4	73	0,9	15,2
	119	8	5	9,0	21	0,5	79	1,3	16,5
	87	17	5	8,0	30	0,4	51	1,3	25,5

Mean	108	6	7	8,15	29	1,2	151	5,37	36,14
$LSD_{0,0001}$	12,1	4,4	2,9	1,3	10,25	0,52	78,81	2,85	9,4
R^2/CV	0,62/8,4	0,44/59,28	0,56/33,96	0,78/12,29	0,56/27,3	0,65/33,54	0,66/39,96	0,69/40,70	0,69/40,70

4.2.2 Pearson's correlation coefficients among the agronomic traits

Correlation coefficients were estimated between HD, AD, NSP, SL, GNS, GWS, GNP, GWP and TKW at the phenotypic level to know the inter-relationship, nature, magnitude, and direction of selection pressure to be applied for practical consideration. Table 15 presents the results of the correlation coefficients of the spike traits measured. A significant correlation of the wheat spike traits at P < 0,000 was observed among the traits. Crop management i.e. water supply to the plant, water uptake by the plant, insect control with insecticides, mildew control with fungicides could have contributed to the sources of variation among the traits.

The NSP showed positive and significant association with SL (0,49), GNS (0,45), GNP (0,73) and GWP (0,63). The trait was positive and not significantly associated with HD (0,18) but negatively correlated with TKW (-0,28) and AD (-0,07). The SL strongly and positively correlated with GNS (0,66), GWS (0,45), GNP (0,62) and GWP (0,61), and was not significantly correlated with HD (0,33), negatively correlated with TKW (-0,06) and AD (-0,2). The GNS showed positive and significant correlation with the GWS (0,61), GNP (0,72) and GWP (0,75) and was not significantly correlated with TKW (0,008) and HD (0,12). A negative correlation was observed between the trait and the AD (-0,16). The GWS showed positive and significant correlation with GNP (0,52) and GWP (0,64) and was not significantly correlated with TKW (0,25) and AD (0,01). Only AD showed a negative correlation to this trait (-0,08). The GNP showed strong and positive correlation with GWP (0,92) and was not significantly correlated with HD (0,12) and negatively correlated with TKW (-0,20) and AD (-0,13). The GWP was not significantly correlated with TKW (0,12) and HD (0,04) but negatively correlated with AD (-0,21). The TKW showed a negative correlation with HD (-0,24) and AD (-0,08). The HD showed negative correlation with AD (-0,22) (Table 16).

Table 16: Pearson's correlation coefficients among the agronomic traits.

Traits	NSP	SL	GNS	GWS	GNP	GWP	TKW	HD	AD
NSP	1								
SL	0,49***	1							
GNS	0,45***	0,66***	1						
GWS	$0,22^{NS}$	0,45***	0,61***	1					
GNP	0,73***	0,62***	0,72***	0,52***	1				
GWP	0,63***	0,61***	0,75***	0,64***	0,92***	1			
TKW	-0,28	-0,06	$0,008^{NS}$	$0,25^{NS}$	-0,20	$0,12^{NS}$	1		
HD	$0,18^{NS}$	$0,33^{NS}$	$0,12^{NS}$	-0,08	$0,12^{NS}$	$0,04^{NS}$	-0,24	1	
AD	-0,07	-0,2	-0,16	$0,01^{NS}$	-0,13	-0,21	-0,08	-0,22	1

NSP: Spike numbers per plant SL: Spike length GNS: Grain numbers per spike
GWS: Grain weight per spike GNP: Grain numbers per plant GWP: Grain weight per plant
TKW: Thousand kernel weight HD: Number of days to heading AD: Number of days to anthesis
NS: Not significant Significance level at < 0,0001

4.2.3 Analysis of the SSR and CAPS markers on RWASA3 and RWASA4 resistant landraces for identifying high TKW, grain numbers and grain length genotypes

Four markers *MQ*, *Caps4A-Ags* and *Caps5D-Ags* (CAPS) and *GS7D* (SSR) linked to high TKW, grain numbers and longer grain length were used for identifying the presence or absence of these traits in wheat landraces that have RWASA3 and RWASA4 resistance. Table 10 presents the marker names, linked genes and PCR amplified fragment sizes while Table 17 gives the results from this study for the same markers. The landraces used in this study have not been used in yield studies to enable comparison of results, However, the markers used have been used on different genetic backgrounds i.e. RILs (Hu *et al*., 2015, Zhang *et al*,, 2016). In this case, these markers serve as the reference for indicating which genes are present in these genotypes through band sizes acquired, Using genotypes containing or assumed to contain *TaTGW-7A* gene, one would expect the genotype to contain marker $MQ_{250\ bp}$ for higher TKW while marker $MQ_{196\ bp}$ signals the absence of the gene (Hu *et al,,* 2016). Marker $Caps4A\text{-}Ags_{885\ bp}$ is linked to the *TaCWI-4A* gene for higher TKW expressed under irrigation regions while $Caps4A\text{-}Ags_{531,354\ bp}$ is linked to the *TaCWI-4A* gene for higher grain numbers expressed under rainfed production regions (Jiang *et al.,* 2015). Furthermore, marker $Caps5D\text{-}Ags_{244,405\ bp}$ is linked to *TaCWI-5D* gene for higher TKW while marker $Caps5D\text{-}Ags_{97,143,409\ bp}$ is linked to lower TKW (Jiang *et al.,* 2015). Moreover, marker $GS7D_{562\ bp}$ is linked to *TaGS-D1* for higher TKW and longer grain length while marker $GS7D_{522\ bp}$ is linked to lower TKW (Zhang *et al.,* 2014). These markers were significantly polymorphic, informative and useful, although there was segregation for the presence or the absence of some genes. All reported bands were recorded across genotypes.

4.2.3.1 Analysis of SSR and CAPS markers on RWASA3 resistant genotypes

Of all genotypes screened, only one RWASA3 resistant genotype PI 197985 showed to be linked to four genes: *TaTGW-7A* and *TaCWI-5D* for higher TKW, *TaGS-D1* for higher TKW and longer grain length and *TaCWI-4A* for higher grain numbers shown by allele $MQ_{250\ bp}$, $Caps4A\text{-}Ags_{354,531\ bp}$, $Caps5D\text{-}Ags_{405}$, and $GS7D_{562}$ respectively. Genotypes PI 137740, PI 181263, PI 243659, PI 366566 and PI 366573 have shown linkage to three genes *TaGS-D1* for higher TKW and longer grain length, *TaCWI-5D* for higher TKW and *TaCWI-4A* for higher grain numbers shown by alleles $GS7D_{562\ bp}$, $Caps4A\text{-}Ags_{354,531\ bp}$, and $Caps5D\text{-}Ags_{405\ bp}$. The above genotypes also showed no linkage to gene *TaTGW-7A* by marker $MQ_{196\ bp}$. Genotype PI 269408 contained marker $GS7D_{562\ bp}$ and $Caps5D\text{-}Ags_{405\ bp}$ for gene *TaGS-D1* and *TaCWI-5D* (higher TKW). Genotype PI 137757 also contained genes *TaGS-D1* for higher TKW and longer grain length, *TaCWI-4A* and *TaCWI-5D* for high TKW shown by alleles $GS7D_{562\ bp}$, $Caps4A\text{-}Ags_{885\ bp}$ and $Caps5DAgs_{244,405,649\ bp}$. Allele $Caps5D\text{-}Ags_{649\ bp}$ in these or any other genotype indicates the presence or the absence of the gene of interest in some plants from the genotype.

Linkage to two genes was observed in genotypes PI 137741, PI 140204, PI 250791, PI 269408, PI 347019, PI 478172, PI 564250, 564260 and PI 624253. In particular, PI 137741, PI 269408, PI 347019 and PI 564250 showed linkage to gene *TaGS-D1* for higher TKW and longer grain length by marker $G7SD_{562\ bp}$ and *TaCWI-5D* for higher TKW by marker $Caps5D\text{-}Ags_{405\ bp}$. No genotypes showed linkage to *TaTGW-7A* by marker $MQ_{196\ bp}$. Genotypes PI 564260 and PI 624253 showed linkage to genes *TaGS-D1* for higher TKW and *TaCWI-4A* for higher grain numbers by marker *Caps4A-Ags*, However, marker $GS7D_{562\ bp}$ and $Caps5D\text{-}Ags_{405\ bp}$ linked to high TKW gene *TaGS-D1* and *TaCWI-5D* were identified in PI 250791 and PI 478172 and both genotypes had no linkage to *TaTGW-7A* gene by marker $MQ_{196\ bp,}$ Moreover, both genotypes showed that not all five representative plants per genotype were linked to *TaGS-D1* by marker $GS7D_{522\ bp}$. Genotypes PI 564260 and PI 624253 showed linkage to genes *TaGS-D1* for higher TKW and longer grain length by marker $GS7D_{562\ bp}$ and *TaCWI-4A* for high grain numbers by marker $Caps4A\text{-}Ags_{531\ bp}$ although PI 564260 also contained $Caps4A\text{-}Ags_{354\ bp}$ for high grain numbers. Only PI 140204 showed linkage to *TaGS-D1* and *TaCWI-5D* by marker $GS7D_{562\ bp}$ and $Caps5D\text{-}Ags_{244,405\ bp}$ linked to high TKW and marker $Caps4A\text{-}Ags_{649\ bp}$. PI 347030 contained marker $Caps4AAgs_{354,531\ bp}$ linked to gene *TaCWI-4A* for higher grain numbers and $Caps5D\text{-}Ags_{405\ bp}$ linked to *TaCWI-5D* for higher grain numbers respectively. No genotypes showed linkage to gene *TaTGW-7A* by marker $MQ_{250\ bp}$. No information was obtained for PI 140204 to marker *Caps4A-Ags* while PI 347030 showed no linkage to gene *TaGS-D1* by marker $GS7D_{522\ bp}$. Genotypes PI 137739''S'' and PI 140213 contained

marker *Caps5D-Ags*$_{405\ bp}$ for gene *TaCWI-5D* linked to high TKW, *MQ*$_{196\ bp}$ for no linkage to gene *TaTGW-7A*. There was no information obtained in PI 137739''S'' to marker *Caps4A-Ags* and PI 140213 for marker *GS7D* and *Caps4A-Ags.* Genotype PI 245583 container marker *GS7D*$_{562\ bp}$ for gene *TaGS-D1*, marker *MQ*$_{196\ bp}$ which indicates no linkage to *TaTGW-7A* and no information was obtained for marker *Caps4A-Ags* and *Caps5D-Ags*. The abundance of high yield potential genes in these genotypes and their good RWASA3 resistance could be used in crop improvement and RWA resistance breeding (Table 17).

4.2.3.2 Analysis of SSR and CAPS markers on RWASA4 resistant genotypes

The markers that were used on RWASA3 genotypes were also used on the genotypes that had RWASA4 resistance. Two genotypes PI 137741 and PI 140204 had the presence of three genes *TaGS-D1*, *TaCWI-4A* and *TaCWI-5D* with varying number of bands per genotype. Both genotypes contained marker *Caps5D-Ags*$_{405\ bp}$. Both genotypes contained marker *Caps5D-Ags*$_{405\ bp}$ (*TaCWI-5D*) for higher TKW and *Caps5DAgs*$_{143\ bp}$ for low TKW in their bulks. However, the presence of gene *TaGS-D1* was shown by marker *GS7D*$_{562\ bp}$ in both genotypes with marker *GS7D*$_{522\ bp}$ only in PI 140204 for low TKW genotypes. Both genotypes shared common band for marker *Caps4A-Ags*$_{531\ bp}$ for high grain numbers (*TaCWI-4A*) whereas PI 140204 contained marker *Caps4A-Ags*$_{354\ bp}$ for the same trait and marker *Caps4A-Ags*$_{885\ bp}$ for high TKW (*TaCWI-4A*). Some genotypes showed linkage to two genes with different band sizes acquired per genotype. Both PI 137740 and PI 140213 contained plants linked and some not linked to genes *TaGS-D1* and *TaCWI-5D* by markers *GS7D*$_{522,562\ bp}$ and *Caps5D-Ags*$_{244,409\ bp}$ in PI 137740 and *Caps5D-Ags*$_{143,244,405\ bp}$. PI 181263 and PI 245583 contained markers *GS7D*$_{562\ bp}$ (*TaGS-D1*) and *Caps5D-Ags*$_{405\ bp}$ (*TaCWI-5D*) for high TKW and no linkage to gene *TaTGW-7A* by marker *MQ*$_{196\ bp}$ and both genotypes showed that some plants were not linked to *TaCWI-5D* by *Caps5D-Ags*$_{143\ bp}$. Genotypes PI 366573 and PI 478172 contained markers *Caps4A-Ags*$_{885\ bp}$ (*TaCWI4A*) and *Caps5D-Ags*$_{244,405\ bp}$ (*TaCWI-5D*) for higher grain numbers. Both genotypes showed no linkage to *TaTGW-7A* by marker *MQ*$_{196\ bp}$ and no information was obtained for marker *GS7D*. PI 137739 showed linkage to gene *TaCWI-4A* by marker *Caps4A-Ags*$_{531\ bp}$ was composed of plants linked and some not linked to *TaCWI-5D* by marker *Caps5D-Ags*$_{244,409\ bp}$. PI 624152 and PI 624253 showed linkage to gene *TaGS-D1* and *TaCWI-4A* by marker *GS7D*$_{562\ bp}$ and *Caps4A-Ags*$_{531\ bp}$. No genotypes were linked to *TaTGW-7A* by marker *MQ*$_{196\ bp}$ nor information obtained for marker *TaCWI-5D*. Gene *TaGS-D1* seemed to be the only prevalent gene present in six genotypes as there was no linkage to the other three genes. The genotypes PI 127097, PI 134117, PI

197985, PI 243659, PI 366529 and PI 366537 contained marker $GS7D_{562\ bp}$ for high TKW (*TaGS-D1*) and marker $MQ_{196\ bp}$ which indicates no linkage to gene *TaTGW-7A*.Genotype PI 269408 contained plants linked and some not linked to gene *TaCWI-5D* by marker $Caps5D\text{-}Ags_{143,405\ bp}$ (Table 17).

From these results, it can be noted that breeding for higher TKW, longer grain length, and higher grain numbers is attainable as one gene if not all four genes assessed was found in almost all genotypes. Some genotypes showed the absence of genes for higher TKW and grain number and longer grain length thus making their RWA resistance still important for RWA breeding. Therefore, genotypes with useful genes are important sources for crop improvement. This allows effective selections for further characterization and utilization in yield breeding for the development of elite germplasm. These genotypes can be tested further for their adaptation to the environment or their yield assessed under varying environmental conditions.

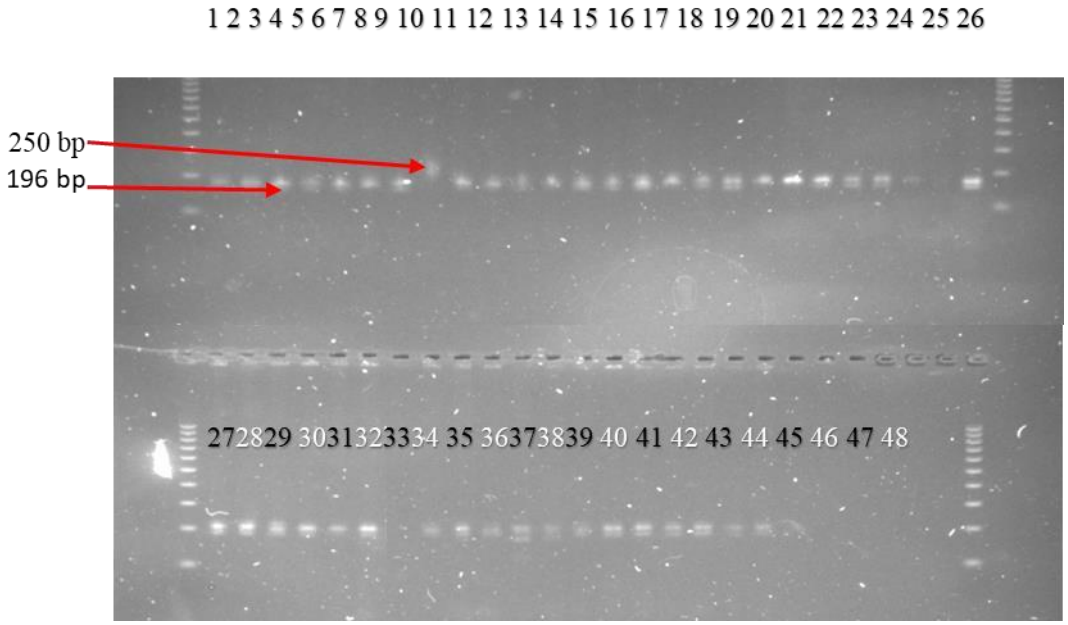

Figure 13: PCR amplification products of marker *MQ* on the RWASA3 and RWASA4 resistant genotypes. Lane 1 to 22 carries entries with RWASA3 resistance while lane 23 to 48 carries RWASA4 resistance.

1: PI 137739''S''	2: PI 137740	3: PI 137741	4: PI 137757	5: PI 140204	6: PI 140213
7: PI 181263	8: PI 197985	9: PI 243659	10: PI 245583	11: PI 250791	12: PI 269408
13: PI 347019	14: PI 347030	15: PI 366550	16: PI 366566	17: PI 366573	18: PI 478172
19: PI 564250	20: PI 564259	21: PI 564260	22: PI 624253	23: PI 127097	24: PI 134117
25: PI 137739''S''	26: PI 137740	27: PI 137741	28: PI 140204	29: PI 140213	30: PI 181263
31: PI 197985	32: PI 243659	33: PI 245583	34: PI 269408	35: PI 347019	36: PI 366529
37: PI 366537	38: PI 366538	39: PI 366550	40: PI 366573	41: PI 478172	42: PI 478216
43: PI 623848	44: PI 624152	45: PI 624253	46: PI 564250	47: PI 564259	48: PI 564260

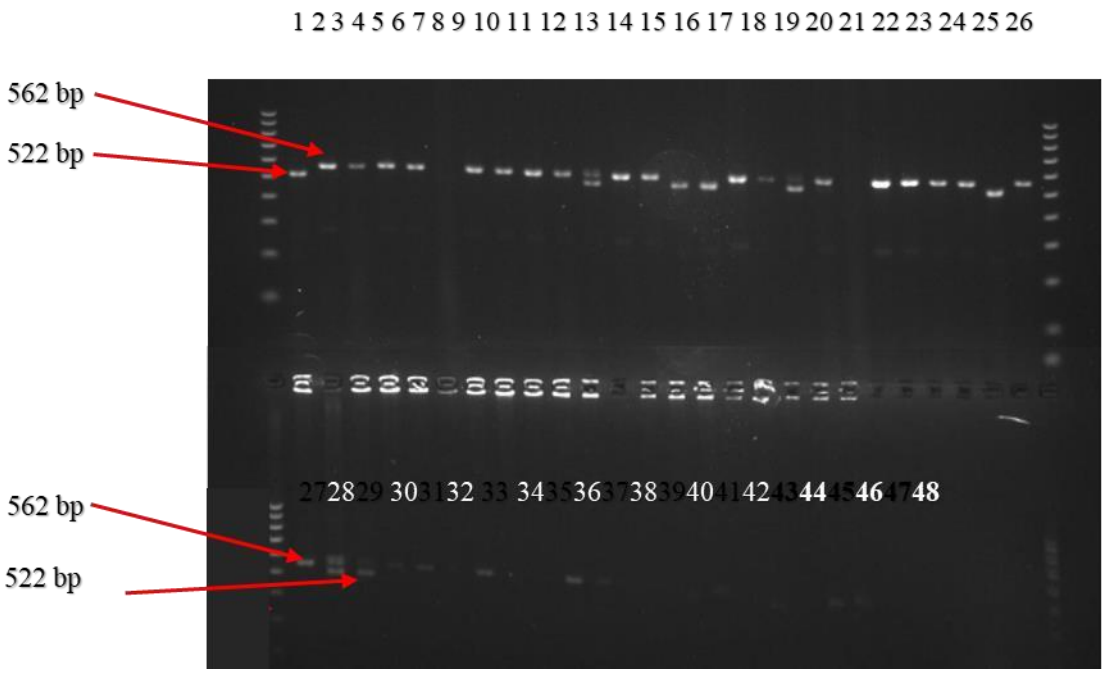

Figure 14: PCR amplification products of marker *GS7D* on the RWASA3 and RWASA4 resistant genotypes. Lane 1 to 22 carries entries with RWASA3 resistance while lane 23 to 48 carries RWASA4 resistance.

1: PI 137739''S''	2: PI 137740	3: PI 137741	4: PI 137757	5: PI 140204	6: PI 140213
7: PI 181263	8: PI 197985	9: PI 243659	10: PI 245583	11: PI 250791	12: PI 269408
13: PI 347019	14: PI 347030	15: PI 366550	16: PI 366566	17: PI 366573	18: PI 478172
19: PI 564250	20: PI 564259	21: PI 564260	22: PI 624253	23: PI 127097	24: PI 134117
25: PI 137739''S''	26: PI 137740	27: PI 137741	28: PI 140204	29: PI 140213	30: PI 181263
31: PI 197985	32: PI 243659	33: PI 245583	34: PI 269408	35: PI 347019	36: PI 366529
37: PI 366537	38: PI 366538	39: PI 366550	40: PI 366573	41: PI 478172	42: PI 478216
43: PI 623848	44: PI 624152	45: PI 624253	46: PI 564250	47: PI 564259	48: PI 564260

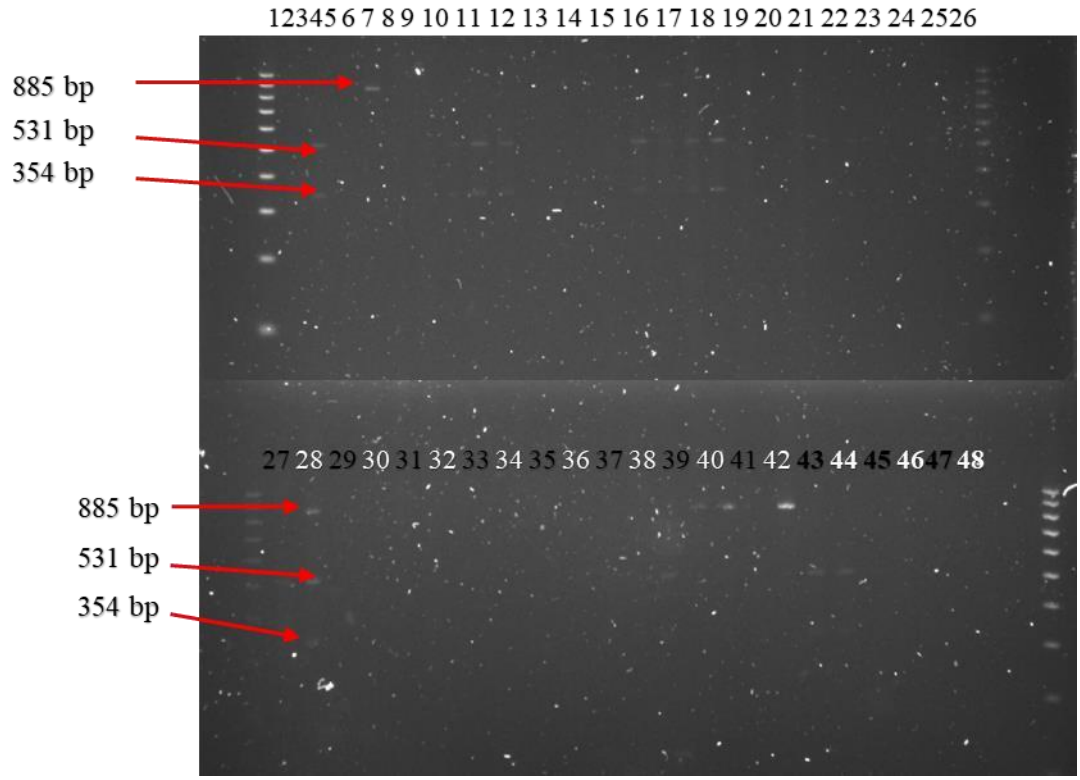

Figure 15: PCR amplification products of marker *Caps4A-Ags* on the RWASA3 and RWASA4 resistant genotypes. Lane 1 to 22 carries entries with RWASA3 resistance while lane 23 to 48 carries RWASA4 resistance.

1: PI 137739''S''	2: PI 137740	3: PI 137741	4: PI 137757	5: PI 140204	6: PI 140213
7: PI 181263	8: PI 197985	9: PI 243659	10: PI 245583	11: PI 250791	12: PI 269408
13: PI 347019	14: PI 347030	15: PI 366550	16: PI 366566	17: PI 366573	18: PI 478172
19: PI 564250	20: PI 564259	21: PI 564260	22: PI 624253	23: PI 127097	24: PI 134117
25: PI 137739''S''	26: PI 137740	27: PI 137741	28: PI 140204	29: PI 140213	30: PI 181263
31: PI 197985	32: PI 243659	33: PI 245583	34: PI 269408	35: PI 347019	36: PI 366529
37: PI 366537	38: PI 366538	39: PI 366550	40: PI 366573	41: PI 478172	42: PI 478216
43: PI 623848	44: PI 624152	45: PI 624253	46: PI 564250	47: PI 564259	48: PI 564260

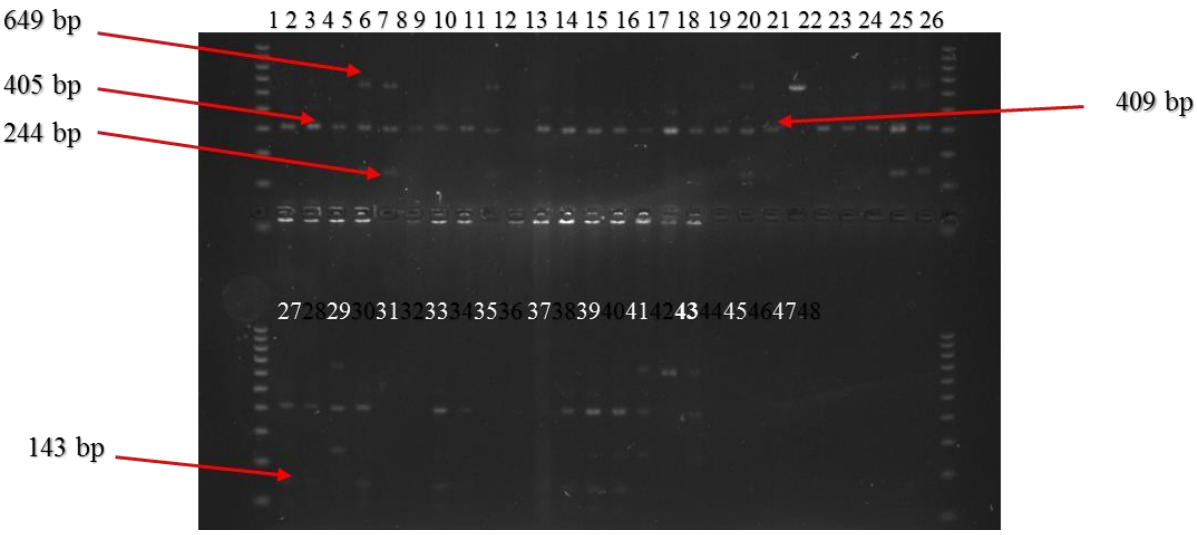

Figure 16: PCR amplification products of marker *Caps5D-Ags* on the RWASA3 and RWASA4 resistant genotypes. Lane 1 to 22 carries entries with RWASA3 resistance while lane 23 to 48 carries RWASA4 resistance.

1: PI 137739''S''	2: PI 137740	3: PI 137741	4: PI 137757	5: PI 140204	6: PI 140213
7: PI 181263	8: PI 197985	9: PI 243659	10: PI 245583	11: PI 250791	12: PI 269408
13: PI 347019	14: PI 347030	15: PI 366550	16: PI 366566	17: PI 366573	18: PI 478172
19: PI 564250	20: PI 564259	21: PI 564260	22: PI 624253	23: PI 127097	24: PI 134117
25: PI 137739''S''	26: PI 137740	27: PI 137741	28: PI 140204	29: PI 140213	30: PI 181263
31: PI 197985	32: PI 243659	33: PI 245583	34: PI 269408	35: PI 347019	36: PI 366529
37: PI 366537	38: PI 366538	39: PI 366550	40: PI 366573	41: PI 478172	42: PI 478216
43: PI 623848	44: PI 624152	45: PI 624253	46: PI 564250	47: PI 564259	48: PI 564260

Table 17: Genotype DNA bulk, biotype resistance and bands acquired per marker.

Genotype	Biotype resistance	Bands of DNA bulks acquired per marker			
		MQ	***GS7D***	***Caps4A-Ags***	***Caps5D-Ags***
PI 127097	RWASA4	196 bp	562 bp	Null	409 bp
PI 134117	RWASA4	196 bp	562 bp	Null	409 bp
PI 137739''S''	RWASA3	196 bp	522 bp	Null	405 bp
	RWASA4	Null	522 bp	531 bp	244, 409,649 bp
PI 137740	RWASA3	196 bp	562 bp	531,354 bp	405 bp
	RWASA4	196 bp	562 bp	Null	244,409,649 bp
PI 137741	RWASA3	196 bp	562 bp	Null	405 bp
	RWASA4	196 bp	562 bp	531 bp	143,405 bp
PI 137757	RWASA3	196 bp	562 bp	885 bp	244,405,649 bp
PI 140204	RWASA3	196 bp	562 bp	Null	244,405,649 bp
	RWASA4	196 bp	522,562 bp	354,531,885 bp	143,405 bp
PI 140213	RWASA3	196 bp	Null	Null	405 bp
	RWASA4	196 bp	522,562 bp	Null	244,405,649 bp
PI 181263	RWASA3	196 bp	562 bp	531,354 bp	405 bp
	RWASA4	196 bp	562 bp	Null	143,405 bp
PI 197985	RWASA3	250 bp	562 bp	531, 354 bp	405 bp
	RWASA4	196 bp	562 bp	Null	Null
PI 243659	RWASA3	196 bp	562 bp	531, 354 bp	244,405,649 bp
	RWASA4	196 bp	562 bp	Null	Null
PI 245583	RWASA3	196 bp	562 bp	Null	Null
	RWASA4	Null	562 bp	Null	143,405 bp
PI 250791	RWASA3	196 bp	522,562 bp	Null	405 bp
PI 269408	RWASA3	196 bp	562 bp	Null	405 bp
	RWASA4	196 bp	Null	Null	143,405 bp
PI 347019	RWASA3	196 bp	562 bp	Null	405 bp
	RWASA4	196 bp	Null	Null	Null
PI 347030	RWASA3	196 bp	522 bp	531,354 bp	405 bp
PI 366529	RWASA4	196 bp	562 bp	Null	Null
PI 366537	RWASA4	196 bp	562 bp	Null	Null
PI 366538	RWASA4	196 bp	Null	Null	143, 405 bp
PI 366550	RWASA3	196 bp	522 bp	531,885 bp	405 bp
	RWASA4	196 bp	Null	531 bp	143,244,405 bp
PI 366566	RWASA3	196 bp	562 bp	531, 354 bp	405 bp
PI 366573	RWASA3	196 bp	562 bp	531,354 bp	405 bp
	RWASA4	196 bp	Null	885 bp	143,244,405 bp
PI 478172	RWASA3	196 bp	522,562 bp	Null	405 bp
	RWASA4	196 bp	Null	885 bp	143,244,405 bp
PI 478216	RWASA4	196 bp	562 bp	Null	649 bp
PI 564250	RWASA3	196 bp	562 bp	Null	244, 405, 649 bp
	RWASA4	Null	Null	Null	Null
PI 564259	RWASA3	196 bp	Null	885 bp	409 bp
	RWASA4	Null	Null	Null	Null
PI 564260	RWASA3	196 bp	562 bp	354,531 bp	649 bp
	RWASA4	Null	Null	Null	Null
PI 623848	RWASA4	196 bp	562 bp	885 bp	649 bp
PI 624152	RWASA4	196 bp	562 bp	531 bp	Null
PI 624253	RWASA3	196 bp	562 bp	531 bp	409 bp
	RWASA4	196 bp	562 bp	531 bp	Null

196 bp: lower TKW
250 bp: higher TKW
Null: no information

522 bp: lower TKW
562 bp: higher TKW

531 and 354 bp: higher grain numbers
885 bp: higher TKW

143 and 409 bp: lower TKW
244 and 405 bp: higher TKW
649 bp: results inconclusive

4.2.4 Discussion

To speed up the breeding process, plant breeders now use molecular markers in MAS to aid in identifying and selecting for genes of interest. Close linkage of a marker to a target gene allows indirect selection for the trait of interest without the need to phenotype first. For this reason, molecular markers are effective, breeder friendly and reliable for identifying and selecting targeted genes in different genetic backgrounds. *TaGS-D1* gene is associated with thousand kernel weight and longer grain length (Fan *et al.,* 2006) while candidate gene *TaTGW-7A* is associated with thousand kernel weight. The *TaCWI-4A* gene is linked to higher grain numbers while gene *TaCWI-5D* is associated with higher TKW respectively (Jiang *et al.,* 2015). Linkage to the above genes will be summarised across all genotypes. The presence of markers showing the absence of the genes investigated will be left out of the discussion as already explained in the results and detailed in Table 17.

Since all genotypes were bulked and screened with different markers, the presence of a single band for the target gene from any marker is important. This could mean that the plants from which the bulks were compiled from may be genetically the same and that all plants are linked to the same gene. In this study, only PI 197985 had a single band of $MQ_{250\ bp}$ (*TaTGW7A*). Genotypes PI 137740, PI 137741, PI 181263, PI 197985, PI 243659, PI 245583, PI 624253 with resistance to RWASA3 and RWASA4 had a single band of $GS7D_{562\ bp}$ (*TaGS-D1*). Genotypes PI 137757, PI 366550 and PI 564259 with RWASA3 resistance were the only genotypes with $Caps4A\text{-}Ags_{885\ bp}$ linked to gene *TaCWI-4A* for higher TKW and an additional band of $Caps4A\text{-}Ags_{531\ bp}$ linked to gene *TaCWI-4A* for higher grain numbers was acquired in PI 366550. From RWASA4 resistant genotypes, PI 366573, PI 478172 and PI 623848 contained marker $Caps4A\text{-}Ags_{885\ bp}$. Thirteen genotypes with resistance to RWASA3 had a single band of $Caps5D\text{-}Ags_{405\ bp}$ (*TaCWI-5D*) in their bulks. This includes PI 137739''S'', PI 137740, PI 137741, PI 140213, PI 181263, PI 197985, PI 250791, PI 269408, PI 347019, PI 347030, PI 366550, PI 366573 and PI 564250, However, genotypes PI 137739''S'' and PI 137741 with resistance to RWASA4 had a single band of $Caps4A\text{-}Ags_{531\ bp}$ (*TaCWI-4A*). The above genotypes did not segregate for each of the markers; therefore, there is a need for further genetic studies in these lines such as developing RILs or double haploid lines for haplotype analysis.

From the RWASA3 resistant genotypes, PI 197985 showed to be the only genotype with linkage to all four genes *TaGW-7A*, *TaGS-D1*, *TaCWI-4A*, and *TaCWI-5D* by markers $MQ_{250\ bp}$, $GS7D_{562\ bp}$, $Caps4A\text{-}Ags_{885\ bp}$ and $Caps5D\text{-}Ags_{405\ bp}$ respectively. This line is a good source for gene pyramiding and could be explored further for other

traits. A breeding population could be made from this line for future genetic studies. Linkage to genes *TaGS-D1*, *TaCWI-4A* and *TaCWI-5D* was also identified in the bulks of genotypes PI 137740, PI 137757, PI 181263, PI 243659, PI 245583, PI 366566 and PI 366573 by markers *GS7D*$_{562\ bp}$, *Caps4A-Ags*$_{531\ bp}$ and *Caps5D-Ags*$_{405\ bp}$. The DNA markers GS*7D*$_{562\ bp}$ and *Caps5D-Ags*$_{405\ bp}$ were found on genotypes PI 137741, PI 250791, PI 269408, PI 347030, PI 347019 and PI 564250 suggesting that these genotypes contain genes *TaGS-D1* and *TaCWI-5D*. Only genotypes PI 366550 and PI 564259 contained markers *Caps4A-Ags*$_{885\ bp}$ with an additional band of *Caps4A-Ags*$_{531\ bp}$ in PI 366550 for higher grain numbers and *Caps5D-Ags*$_{405\ bp}$ for higher TKW thus suggesting linkage to genes *TaCWI-4A* and *TaCWI-5D*. A few lines showed linkage to a single gene thus also proving to be important for yield breeding. These include PI 137739''S'', PI 140213 showing linkage to gene *TaCWI-5D* by marker *Caps5D-Ags*$_{405\ bp}$.

From the RWASA4 resistant genotypes, linkage to genes *TaGS-D1*, *TaCWI-4A*, and *TaCWI-5D* were identified in the bulks of genotypes PI 137741 and PI 140204, by marker GS*7D*$_{562\ bp}$, *Caps4A-Ags*$_{531\ bp}$, and *Caps5D-Ags*$_{405\ bp}$ respectively. None of the genotypes with RWASA4 resistance displayed linkage to gene *TaTGW-7A* respectively, Genotypes PI 137740, PI 140213, PI 181263 only showed linkage to genes *TaGS-D1* by marker *GS7D*$_{562\ bp}$ and *TaCWI-5D* by marker *Caps5D-Ags*$_{405\ bp}$. However, PI 478172, PI 623848, PI 624152 and PI 624253 were linked to genes *TaGS-D1* by marker *GS7D*$_{562\ bp}$ and *TaCWI-4A* by *Caps4A-Ags*$_{531\ bp}$ while PI 137739''S'' was linked to *TaCWI-4A* and *TaCWI-5D*, PI 366573 was linked to gene *TaCWI-4A* by marker *Caps4A-Ags*$_{885\ bp}$ and gene *TaCWI-5D* by marker *Caps5D-AgsA*$_{244,405\ bp}$. Genotypes PI 127097, PI 134117, PI 197985, PI 243659, PI 366529, PI 366537 and showed linkage to gene *TaGS-D1* by marker *GS7D*$_{562\ bp}$ while genotypes PI 269408, PI 366538, PI 366550 had markers *Caps5D-Ags*$_{405\ bp}$ with an additional band of 244 bp in genotype PI 366550, all linked to gene *TaCWI-5D*.

The markers used in this study were useful and polymorphic in distinguishing between high and low TKW and grain numbers and longer grain length genotypes. The magnitude of the genes identified in each genotype makes them valuable genetic sources for crop improvement. Their presence will ensure prolonged yield potential as opposed to one gene in a genotype. From these results, it can be noted that these genotypes can be used for gene combination. The presence of these genes in these genotypes provides a step closer to effective crop improvement. A selection has been made from these genotypes in cases of further analysis. Genetic linkage studies within and between these genotypes need to be conducted to confirm their relatedness.

4.2.5 Conclusions

For a faster breeding process, plant breeders for MAS to identify and select different genes for different purposes use polymorphic molecular markers. This enables indirect selection of traits without the need to phenotype because conventional breeding is time-consuming, costly, labor-intensive and dependent upon the changing environment. However, a very few polymorphic markers are available. Screening the wheat landraces with resistance to RWASA3 and RWASA4 with markers linked high TKW, high grain numbers and longer grain length genes (*TaTGW-7A*, *TaGS-D1*, *TaCWI-4A*, and *TaCWI-5D*) has shown that crop improvement is possible in these genotypes. Almost each genotype had one gene if not all four thus proving that future genetic studies are possible with these lines. Though gene *TaGW-7A* was rare in almost all genotypes, its presence in one-line means that the line can be used to transfer the potential gene into various genetic backgrounds. The breeders now prefer high yielding genotypes with optimum growth period; therefore, these genotypes are vital for crop improvement. All the agronomic traits measured showed a strong relationship with one another. These genotypes would perform better should they be explored further. Use of the higher yielding genotypes identified in this study is recommended for exploration with markers linked to other genes for gene combination. Furthermore, the use of these markers in future yield studies and MAS is also recommended on various genetic backgrounds for both faster breeding progress.

Chapter 5

5.1 Summary

Screening the donor lines with the four South African RWA biotypes has proved effective in identifying resistant lines against potential biotypes thus strengthening the RWA host plant resistance breeding program. Twenty-five resistance sources were found comparable to the differential check CItr 2401 with resistance to all four RWA biotypes. The phenotypic resistance in these lines may be the same, however, this does not mean the genetic base is the same. Seven new resistance patterns from RWASA1 to RWASA4 i,e, RRSR, RSRR, RSRS, RSSR, SRSR, SSRR and SRRS different from *Dn1-Dn9*, *Dnx* and *Dny* sources were found in 22 genotypes. The genotypes with these resistance patterns could be used in combination with genotypes containing known resistance patterns for effective RWA breeding. South African breeders have developed high levels of resistant genotypes to RWASA1 and RWASA2. This is because RWASA2 was reported nearly three decades later after RWASA1. The latest biotypes RWASA3 and RWASA4 were reported nearly two years apart thus up to this far not much resistance has been developed. This study has found new germplasm sources that showed resistance to RWASA3 and RWASA4. These germplasms will be useful to identify resistance to RWASA5 in the future. Exploring uniformity in the germplasm used has shown that natural and uniform resistance exists within landraces.Genotypes with mixed reaction need an intense selection of plants with useful traits. Moreover, the genotypes with uniform resistance need further characterisation including studying resistance mechanisms involved, genes underlying resistance developing molecular markers and molecular mapping of resistance genes. Therefore, wheat landraces are valuable resistance sources when searching for new resistant germplasm. Gene pyramiding approach has been implemented successfully whereby breeders have incorporated multiple resistances against multiple biotypes and high yielding genes in various genotypes. High yielding genotypes were found in the selected RWASA3 and RWASA4 resistant plants suggesting that these germplasms will perform well should they be explored further. Using molecular markers linked to TKW, high grain numbers and longer grain length genes *TaGS-D1*, *TaTGW-7A*, *TaCWI-4A* and *TaCWI-5D* on highly resistant RWASA3 and RWASA4 plants have proved that these landraces carry other useful genes that need to be explored further, Almost all genotypes showed the presence of each gene except for *TaTGW-7A* since only one genotype showed linkage to this gene. This include RWASA3 resistant genotype PI 197985, which was the only genotype potentially linked to *TaTGW-7A* gene. Other RWASA3 resistant genotypes include 17 genotypes that were potentially linked to *TaGS-D1*, 12 potentially linked to *TaCWI-4A* and 19 potentially linked to *TaCWI-5D* gene. From the RWASA4 resistant genotypes, none of the genotypes screened were linked to

TaTGW-7A, however, 16 genotypes that were potentially linked to *TaGS-D1*, nine potentially linked to *TaCWI-4A* and 12 potentially linked to *TaCWI-5D* gene. These means gene pyramiding and crop improvement in these lines is achievable. The results from this study will contribute greatly to RWA breeders and molecular breeders of ARC-SG of SA.

5.2 Study limitations and suggestions for future work

South African RWA resistance breeders have not bred or developed many RWASA3 and RWASA4 resistant lines for use in the breeding programs and commercially. This means resistant lines to these biotypes need to be identified and selected in wheat landraces. There are few reports about intense exploration and evaluation of wheat landraces for uniformity meaning that these types of studies are needed to change or expand the scope of RWA host plant resistance breeding. There have not been many reports about the markers used in this study especially for TKW, grain numbers, and grain length. The genotypes selected genotypes showed very limited linkage to gene *TaTGW-7A*. A breeding population could be developed from the genotype that showed linkage to the gene for haplotype analysis.

5.3 Study contributions

The RWA resistance study conducted will contribute greatly to the ARC-SG pre-breeding for host plant resistance, The genotypes with linkage to high yielding genes will contribute greatly to the wheat crop improvement program.

5.4 Final conclusions and recommendations

Host plant resistance to RWA is a complex study due to resistance breaking biotypes. Linkage to undesirable traits is also another challenge in host plant resistance as some resistance sources carry some undesirable traits. Therefore, the selection of resistance sources with minimum linkage to undesirable traits is important in RWA breeding programs. Moreover, with the current decrease in wheat production, high yielding genotypes with optimum growth period are needed. These selected lines need further phenotyping for other important agronomic traits and resistance to pathogens such as rust, Fusarium head blight, pre-harvest sprouting, and powdery mildew. Pyramiding of resistance genes controlling various biotypes and genes controlling yield components in these selected wheat lines is crucial for the development of elite lines that are beneficial for the wheat industry or necessary for commercial deployment. Molecular markers used in this study showed to be reliable and should be used in other studies and on different genetic backgrounds for the faster breeding process.

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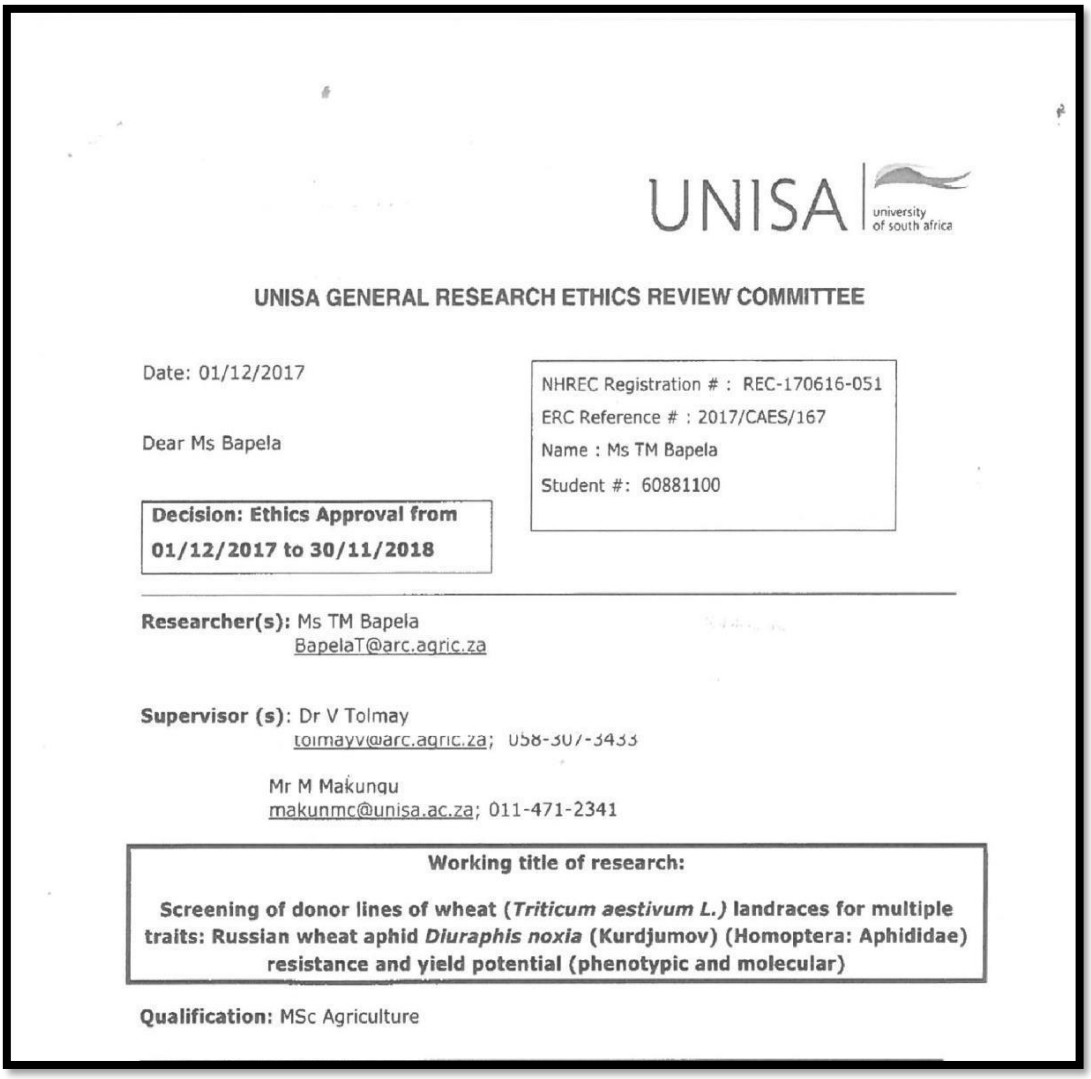
Appendix 1: UNISA ethics clearance approval letter



UNISA university of south africa

UNISA GENERAL RESEARCH ETHICS REVIEW COMMITTEE

Date: 01/12/2017

NHREC Registration # : REC-170616-051
ERC Reference # : 2017/CAES/167
Name : Ms TM Bapela
Student #: 60881100

Dear Ms Bapela

Decision: Ethics Approval from 01/12/2017 to 30/11/2018

Researcher(s): Ms TM Bapela
BapelaT@arc.agric.za

Supervisor (s): Dr V Tolmay
tolmayv@arc.agric.za; 058-307-3433

Mr M Makunqu
makunmc@unisa.ac.za; 011-471-2341

Working title of research:

Screening of donor lines of wheat (*Triticum aestivum L.)* landraces for multiple traits: Russian wheat aphid *Diuraphis noxia* (Kurdjumov) (Homoptera: Aphididae) resistance and yield potential (phenotypic and molecular)

Qualification: MSc Agriculture

Appendix 2: Summary statistics of RWASA1, RWASA2, RWASA3 and RWASA4 phenotypic data.

	RWASA1	**RWASA2**	**RWASA3**	**RWASA4**
Number of observations read	864	864	864	864
Number of observations used	739	757	745	664
Number of missing values	125	107	119	200
Mean	5	6	7	6
Standard deviation	1,74	1,8	1,77	1,81
LSD±	1,39	1,38	1,37	1,41
Error mean square	1,00	1,00	1,00	1,12
Sum of squares	4010,0	3080,8	1967,4	2944,0
R-square	0,89	0,86	0,86	0,79
Co-efficient of variation (%)	19,11	18,21	14,36	17,399

Appendix 3: Set 1 accessions

Accession	RWASA1																RWASA2														
	Rep 1					Rep 2					Rep 3					Av	Rep 1					Rep 2					Rep 3					Av
PI 135064	6	6	6	5	6	9	6	8	6	6	5	7	7	8	9	7	6	7	7	6	7	6	6	7	9	7	6	6	7	4	6	6
PI 137739"S"	2	2	3	3	3	4	4	4	4	4	4	4	4	4	5	4	4	4	4	9	4	4	4	4	4	9	4	4	4	4	9	5
PI 137741	4	4	4	4	4	3	4	3	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
PI 140204	4	4	4	4	4	4	4	4	4	4	4	6	3	4	4	4	4	4	4	4	5	4	4	7	4	4	4	4	4	4	4	4
PI 140213	4	4	4	4	4	4	4	4	4	4	*	*	*	*	*	4	4	4	4	4	4	4	4	4	*	4	4	4	4	4	4	4
PI 220133	5	5	5	5	5	2	5	2	3	3	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	5	4	4	4	4
PI 243659	4	4	3	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
PI 243679	5	7	6	6	9	5	6	6	6	5	7	7	7	*	*	6	7	7	7	4	7	8	9	7	7	7	9	7	7	4	7	7
PI 245583	3	4	3	4	3	3	3	3	3	3	3	4	4	4	4	3	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
PI 269408	4	4	4	4	4	*	4	4	*	3	4	4	4	4	4	4	4	4	4	4	4	4	5	4	4	*	4	4	4	4	4	4
PI 321738	9	7	9	8	6	7	7	8	9	9	9	4	7	9	8	8	7	9	9	8	7	4	4	9	9	4	9	9	9	9	9	8
PI 347003	4	4	7	6	4	4	4	6	4	5	5	4	*	5	*	5	9	9	5	9	8	9	5	4	4	*	4	4	4	4	4	6
PI 347006	4	4	7	4	4	4	5	4	4	5	4	4	9	4	4	5	4	9	9	9	*	4	*	5	4	4	*	*	*	*	*	6
PI 349043	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
PI 366103	4	4	5	4	4	6	4	4	5	4	7	6	4	4	4	5	6	4	5	4	6	6	6	4	4	4	5	5	5	6	5	5
PI 366562	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
PI 366566	5	4	4	4	4	4	*	4	4	4	*	4	5	5	4	4	4	4	4	4	4	4	*	4	4	4	4	4	4	4	*	4
PI 366572	7	6	4	6	4	6	5	7	7	7	3	*	9	4	4	6	4	4	4	9	6	4	4	8	8	5	6	5	7	8	7	6
PI 366573	4	3	3	3	4	3	5	4	4	3	4	4	4	4	4	4	4	4	4	4	4	4	4	4	3	3	4	4	4	4	4	4
PI 366985	5	4	4	3	4	3	4	4	5	3	4	4	5	4	4	4	4	5	4	4	*	4	4	4	*	4	4	4	4	4	4	4
PI 564249	4	7	7	4	4	*	*	*	*	*	3	4	4	6	4	5	7	4	4	6	7	4	4	4	5	4	3	3	4	5	4	5
PI 564250	2	3	6	6	6	6	6	5	4	6	5	5	6	6	5	5	4	4	4	5	4	4	4	4	4	4	4	4	4	4	4	4
PI 564259	7	4	4	5	4	4	3	4	3	4	4	4	4	4	4	4	7	4	4	4	4	4	4	4	5	5	4	4	4	4	4	4
PI 564260	5	4	5	5	*	4	4	6	4	4	4	*	4	4	4	4	9	4	4	8	9	4	4	4	4	4	4	4	4	4	4	4
PI 634770	6	5	5	6	6	6	6	6	*	*	3	*	6	*	*	6	5	5	5	5	5	4	5	5	4	5	4	4	4	4	4	5
94M370	4	7	5	4	5	9	9	4	9	4	4	4	4	9	4	6	9	4	3	4	4	7	7	4	4	4	4	9	7	9	9	6
RWA MATRIX 2414	3	2	4	3	3	4	4	4	3	3	4	3	3	4	3	3	3	4	3	3	*	4	4	4	3	3	3	4	4	4	4	4
Cltr 2401	4	4	3	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
Gariep	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	7	9	8	8	9	7	6	7	6	7	4	4	4	4	4	6
Hugenoot	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	8	9	9	9	9	9	9	9	9	9	9	9	9	9
PAN 3144	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
Yumar	6	6	6	7	7	6	6	7	7	6	4	7	7	7	7	6	8	7	9	9	8	8	7	9	9	8	7	7	7	7	7	8

Accession	RWASA3																RWASA4															
	Rep 1					Rep 2					Rep 3					AV	Rep 1					Rep 2					Rep 3					AV
PI 135064	7	8	9	8	7	8	3	3	9	7	9	9	9	9	9	8	9	9	9	9	7	7	7	7	7	8	7	9	9	9	8	8
PI 137739''S''	4	4	4	9	9	5	4	4	4	4	4	4	4	4	4	5	9	9	4	4	4	4	4	4	4	4	9	9	9	9	9	6
PI 137741	5	6	6	5	6	4	6	4	4	4	*	7	6	4	4	5	4	4	3	3	4	4	4	4	5	4	4	4	4	4	4	4
PI 140204	6	6	6	6	6	4	4	6	5	5	9	7	7	9	7	6	4	4	6	5	6	4	7	7	7	8	*	*	*	*	*	6
PI 140213	6	5	4	6	6	9	9	9	9	9	4	9	9	6	9	7	4	4	4	4	4	4	6	4	4	4	4	4	4	4	4	4
PI 220133	9	9	6	9	6	8	8	9	9	7	9	9	9	9	9	8	4	5	3	*	*	*	*	*	*	*	*	*	*	*	*	4
PI 243659	7	E	6	6	3	4	4	4	4	4	9	8	8	9	9	6	3	3	4	4	3	4	4	4	4	4	4	4	4	4	4	4
PI 243679	9	7	5	5	5	7	9	7	6	9	9	9	9	9	9	8	5	3	3	6	5	6	6	6	6	5	7	7	7	7	7	6
PI 245583	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	7	3	4	4	4	4	4	4	4	4	4	4	4	4	4	4
PI 269408	4	4	4	4	4	4	4	4	4	4	6	4	4	4	4	4	4	4	3	4	4	4	4	4	4	4	4	4	4	4	4	4
PI 321738	9	9	9	9	9	9	9	9	9	9	9	9	8	9	9	9	4	4	4	7	7	9	9	9	9	9	8	7	9	4	4	7
PI 347003	9	4	5	9	*	4	7	4	7	*	9	9	9	9	9	7	4	*	9	8	8	4	*	7	7	*	7	4	*	8	4	6
PI 347006	9	9	9	9	9	9	9	9	4	9	7	9	9	9	9	9	4	3	4	3	*	9	9	9	9	9	7	*	8	8	*	7
PI 349043	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
PI 366103	6	4	5	4	6	4	6	4	4	5	4	5	4	4	4	5	5	4	5	5	5	4	4	4	4	4	5	4	4	4	4	4
PI 366562	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
PI 366566	4	4	4	4	4	4	4	4	4	4	6	5	6	6	4	4	E	5	2	3	*	*	*	*	*	*	*	*	*	*	*	3
PI 366572	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	4	9	*	*	8	9	6	*	9	7	4	*	7	7	4	7
PI 366573	5	6	4	5	4	4	4	5	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
PI 366985	4	4	4	4	5	4	4	4	4	4	4	4	4	4	4	4	4	5	5	4	5	4	4	4	4	4	4	4	4	4	5	4
PI 564249	4	7	9	8	8	7	7	7	4	7	9	8	7	7	4	7	*	4	*	*	*	*	*	*	3	*	*	*	*	*	*	4
PI 564250	6	5	4	6	6	6	6	4	4	6	6	6	6	6	6	6	5	4	7	7	6	6	6	6	6	6	4	6	4	5	6	6
PI 564259	6	6	5	6	6	4	4	4	4	4	4	4	4	4	4	5	4	4	3	4	4	4	7	4	4	4	4	7	6	6	6	5
PI 564260	7	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	3	4	3	6	4	4	7	4	4	7	7	5
PI 634770	9	9	9	9	9	9	9	9	9	9	8	8	9	9	9	9	7	9	8	8	6	8	8	9	4	*	4	4	7	7	7	7
94M370	4	9	4	9	4	9	4	9	9	9	9	8	9	9	9	8	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
RWA MATRIX 2414	4	4	4	4	4	4	4	4	4	4	3	4	4	4	4	4	2	2	4	3	4	4	3	4	4	4	4	4	4	4	4	4
CItr 2401	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	*	4	4	*	*	*	*	*	*	4	*	4	4	4	4
Gariep	9	9	9	9	9	9	9	9	9	9	8	9	9	9	9	9	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
Hugenoot	9	9	9	9	9	9	9	9	9	8	9	9	7	9	8	9	7	6	7	6	6	7	7	7	9	8	7	6	7	7	*	7
PAN 3144	4	4	4	4	4	4	4	4	4	5	4	4	4	4	4	4	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9
Yumar	9	9	9	9	9	9	9	8	7	8	7	7	8	9	9	8	7	7	7	7	7	7	7	7	7	7	4	4	4	7	7	8

Appendix 4: Set 2 accessions

Accession	RWASA1																RWASA2															
	Rep 1					Rep 2					Rep 3					Av	Rep 1					Rep 2					Rep 3					AV
PI 127097	4	*	4	4	4	4	4	4	*	4	4	4	4	4	*	4	4	6	4	4	*	4	4	4	4	6	6	4	3	4	4	4
PI 127099	4	4	4	4	*	4	4	4	*	4	4	*	*	*	*	4	4	5	4	4	*	4	*	4	4	4	4	4	4	8	*	4
PI 127104	*	4	*	4	4	4	10	8	8	4	10	9	8	8	8	7	8	4	9	4	*	8	8	6	8	4	8	9	8	8	9	7
PI 134117	4	*	4	4	4	9	10	10	9	0	10	10	9	10	*	4	4	4	3	3	*	4	4	*	4	4	3	4	3	3	*	4
PI 135047	4	4	*	4	4	4	*	4	4	4	4	4	4	*	*	4	4	4	6	*	6	6	4	4	4	4	4	4	4	4	*	4
PI 135076	4	4	4	*	4	4	4	4	4	*	4	4	4	4	*	4	4	4	4	4	*	4	*	4	4	4	4	*	4	4	4	4
PI 137740	*	4	4	6	4	4	4	4	4	4	4	4	4	5	*	4	4	*	4	4	5	4	*	4	4	4	4	3	4	3	3	4
PI 137757	4	4	4	4	*	8	10	8	8	8	4	8	8	*	*	7	8	8	9	8	*	4	6	6	4	4	8	*	*	*	*	7
PI 166227	10	9	*	10	10	9	9	8	4	*	10	8	10	9	*	9	10	9	10	10	*	10	8	9	8	9	8	8	8	9	8	9
PI 181263	*	4	4	4	4	4	4	4	4	4	4	10	8	*	*	4	5	5	6	4	*	4	4	4	4	4	6	3	6	6	3	5
PI 189746	10	10	8	9	*	8	10	10	8	10	4	10	8	*	*	9	9	*	9	8	9	9	8	9	9	9	9	9	9	9	10	9
PI 197985	4	4	4	*	4	*	*	*	*	*	4	3	4	4	*	4	4	4	*	*	*	3	3	4	3	3	3	3	4	3	*	3
PI 243730	4	*	4	*	4	4	*	5	4	4	8	9	7	10	*	6	4	4	8	4	*	6	10	4	4	*	4	4	10	6	*	6
PI 245380	4	4	4	4	*	4	4	4	*	*	4	4	4	*	*	4	4	*	4	4	4	4	4	4	4	4	6	4	4	4	*	4
PI 245432	6	6	4	4	*	6	6	6	6	6	4	4	*	*	*	5	6	5	5	5	*	6	4	4	6	6	4	6	6	4	6	5
PI 250791	8	8	*	8	8	3	3	4	4	4	8	*	8	4	*	6	4	4	8	8	*	8	8	7	*	8	4	10	8	8	*	6
PI 347017	4	4	4	*	4	4	10	*	10	9	*	*	*	*	*	6	*	*	*	*	*	10	8	*	*	*	4	*	4	4	4	6
PI 347019	4	4	4	*	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	*	6	6	6	4	4	4	4	4	4	4	4
PI 347030	7	8	*	8	8	4	8	8	8	4	4	8	9	4	8	7	4	4	4	4	*	8	8	4	9	10	4	8	4	8	9	6
PI 366529	4	4	4	4	*	4	4	4	4	4	3	3	3	*	*	4	4	6	4	4	*	4	6	5	4	4	6	4	4	4	4	5
PI 366537	4	4	*	4	4	3	3	3	3	4	3	3	3	3	3	3	3	*	3	3	4	3	3	3	4	3	6	5	4	4	*	4
PI 366538	4	4	*	4	*	4	4	8	4	*	4	4	*	*	*	4	6	4	6	6	*	6	6	4	6	*	4	4	4	*	4	5
PI 366550	4	*	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	*	4	4	4	4	4	7	4	4	4	*	4
PI 366565	4	4	4	6	*	4	4	4	*	6	*	*	4	*	*	4	4	6	5	6	4	4	4	4	*	4	*	*	*	*	*	5
PI 367171	10	10	9	10	*	10	10	4	8	4	9	10	10	*	9	9	9	8	9	*	10	*	4	8	8	9	8	9	7	8	8	8
PI 367172	*	8	8	*	8	10	9	10	10	9	8	8	8	9	8	9	9	8	9	*	9	9	8	8	9	9	9	8	9	9	8	9
PI 367188	4	4	*	4	4	4	4	4	4	*	4	4	4	4	*	4	4	4	4	4	4	*	*	*	*	*	*	7	8	8	7	6
CItr 2401	4	4	4	4	4	4	4	4	4	*	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	*	4
Gariep	6	6	5	6	5	6	6	6	5	*	6	6	6	6	6	6	10	10	8	8	8	8	8	9	7	8	8	7	9	9	8	8
Hugenoot	9	9	10	*	10	10	10	10	10	10	10	10	9	10	10	10	9	9	9	10	9	9	9	9	10	9	8	8	8	9	8	9
PAN 3144	4	4	*	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	*	4	4	4	4	4	4	4	*	4	4	4	4
Yumar	4	5	5	5	5	6	4	6	5	5	5	6	5	5	6	5	5	6	6	6	*	6	4	5	5	6	6	5	6	6	6	6

Accession	RWASA3																RWASA4															
	Rep 1					Rep 2					Rep 3					AV	Rep 1					Rep 2					Rep 3					AV
PI 127097	9	9	8	8	*	4	4	8	8	*	8	8	8	9	8	8	4	4	4	4	4	8	9	10	8	*	4	6	*	4	8	6
PI 127099	4	8	9	8	*	8	8	10	9	10	8	9	9	8	*	8	4	4	4	4	*	4	4	4	*	4	8	9	10	10	9	6
PI 127104	9	8	8	9	8	8	8	8	9	9	8	7	4	8	8	8	6	6	8	8	8	8	8	9	9	8	6	4	8	8	8	7
PI 134117	4	4	4	4	*	4	*	4	4	4	4	4	4	4	*	4	7	4	7	4	8	4	*	4	4	4	4	4	*	4	4	5
PI 135047	8	8	8	*	7	8	8	9	8	*	8	8	8	9	8	6	4	4	4	4	4	4	4	4	4	4	4	4	4	4	*	4
PI 135076	8	9	8	8	8	9	9	9	8	8	8	8	9	8	*	8	7	4	4	4	*	8	*	8	9	8	8	10	9	9	10	8
PI 137740	3	3	4	4	3	8	9	9	8	6	3	3	4	3	*	8	6	6	6	6	*	4	8	4	4	*	4	4	4	*	4	5
PI 137757	8	8	8	10	*	4	6	6	9	*	4	8	8	8	4	7	6	6	6	6	*	8	8	7	8	*	8	8	4	8	*	7
PI 166227	9	9	9	9	8	8	8	9	9	8	8	9	9	9	*	9	8	8	8	10	9	9	8	9	9	*	8	10	8	*	8	9
PI 181263	4	*	4	4	4	4	4	4	4	4	4	4	4	4	4	4	6	7	6	6	*	4	4	4	4	4	4	*	4	4	4	5
PI 189746	8	8	*	8	7	9	8	9	9	9	8	8	9	8	*	8	9	10	10	8	9	9	9	10	9	8	9	*	10	9	9	9
PI 197985	4	6	8	8	4	9	9	9	8	8	6	4	4	4	4	6	9		6	6	6	6	6	6	4	4	*	4	4	4	*	5
PI 243730	5	4	9	9	*	8	8	8	*	8	10	9	8	9	9	8	4	8	9	7	4	8	8	7	8	8	4	8	9	8	10	7
PI 245380	4	4	4	*	4	4	4	4	6	6	6	6	6	8	4	5	6	7	8	7	*	4	*	*	4	4	*	*	*	*	*	6
PI 245432	10	8	8	9	8	8	8	8	10	9	8	8	8	10	9	9	7	7	8	8	*	*	*	*	*	*	4	6	4	6	*	6
PI 250791	4	4	4	4	*	6	4	5	8	*	8	8	8	4	*	4	4	6	8	7	*	8	7	4	4	*	9	4	4	4	*	6
PI 347017	4	*	4	4	4	8	9	8	8	10	8	8	9	8	10	7	8	7	8	8	*	9	8	4	4	4	*	*	*	*	*	6
PI 347019	8	4	8	9	8	8	4	5	5	6	7	6	6	6	6	6	4	4	4	4	4	4	4	4	4	*	4	8	4	4	4	4
PI 347030	6	9	6	8	4	8	6	4	4	4	4	4	4	4	4	5	7	8	7	7	9	4	8	8	9	4	9	8	8	8	8	7
PI 366529	4	4	6	6	3	4	4	4	4	4	4	6	6	4	4	4	4	4	4	4	4	4	4	4	4	*	4	*	4	4	4	4
PI 366537	6	8	6	9	9	8	8	9	8	8	9	8	9	8	8	8	4	4	4	4	*	4	4	4	4	4	4	4	8	4	*	4
PI 366538	8	8	8	8	9	8	8	9	8	8	8	8	8	9	*	8	4	4	4	4	4	4	4	7	4	8	4	*	4	4	4	5
PI 366550	4	*	4	4	4	6	4	9	4	6	4	4	4	4	4	5	9	4	*	4	4	7	4	4	4	7	4	5	4	5	4	5
PI 366565	4	4	5	*	4	4	4	4	*	4	8	8	4	4	*	4	4	4	9	9	*	9	10	9	9	*	10	9	9	10	*	8
PI 367171	9	10	8	9	8	8	8	8	8	9	*	9	8	8	8	8	7	7	10	9	7	4	9	4	8	*	4	9	8	*	10	7
PI 367172	8	8	8	9	8	8	9	8	8	8	9	8	4	9	*	8	8	8	8	9	8	4	9	8	8	8	8	8	8	9	9	8
PI 367188	4	4	4	4	*	4	*	4	4	4	4	4	4	4	*	4	4	6	4	4	*	8	*	4	4	9	10	9	8	8	*	7
CItr 2401	4	4	3	4	3	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	*	4	4	4	4	4	4
Gariep	8	8	8	8	9	9	9	9	8	8	9	9	8	9	10	9	9	9	9	9	*	8	8	7	6	8	9	8	10	*	9	8
Hugenoot	9	9	9	9	*	9	9	8	8	*	9	9	10	9	9	9	10	10	9	9	10	9	9	9	9	9	9	9	9	9	9	9
PAN 3144	4	4	4	4	*	4	4	4	4	4	4	*	4	4	4	4	4	4	4	4	*	8	8	7	9	8	7	8	9	8	8	7
Yumar	9	*	9	9	8	8	9	8	9	8	8	8	9	9	8	9	7	7	6	7	7	9	7	7	7	8	*	9	8	8	9	8

Appendix 5: Set 3 accessions

Accession	RWASA1																RWASA2															
	Rep 1					Rep 2					Rep 3					AV	Rep 1					Rep 2					Rep 3					AV
PI 220131	6	9	9	8	*	5	*	*	*	*	*	*	*	*	*	7	9	*	7	8	8	5	4	6	5	5	5	5	5	*	*	6
PI 349043	4	4	4	4	*	7	7	4	8		4	4	7	7	*	5	5	5	4	5	*	5	5	4	5	*	4	5	4	4	*	5
PI 366520	*	*	*	*	*	*	5	*	*	*	*	*	*	*	*	5	5	5		5	*	5	5	4	*	*	5	5		4	5	5
PI 366533	5	4	4	5	*	*	*	*	*	*	*	*	*	*	*	5	*	*	*	*	*	*	*	*	*	*	*	*	5	5	4	5
PI 366545	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
PI 366549	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
PI 478115	8	10	8	9	*	*	8	10	8	9	*	*	*	*	*	9	4	5	9	8	*	7	8	8	9	8	9	8	9	9	9	8
PI 478126	8	8	10	8	*	4	*	8	7	8	8	8	10	9	*	8	5	5	4	*	*	*	4	4	4	*	10	10	8	9	10	7
PI 478127	*	5	6	7	*	4	8	7	8	8	10	8	5	4	5	7	9	7	8	7	*	*	4	4	*	4	9	8	8	4	9	7
PI 478134	7	8	8	9	*	8	5	8	6	9	*	*	*	*	*	8	8	9	8	9	10	9	10	10	9	8	8	8	9	8	10	9
PI 478172	9	9	8	8	*	7	4	5	8	4	5	*	5	4	4	6	5	4	4	5	*	4	4	4	4	4	4	7	8	4	4	5
PI 478177	*	8	*	*	*	9	4	8	8	10	10	9	4	8	10	8	6	5	8	8	*	8	8	8	8	7	9	8	9	8	9	8
PI 478216	4	4	4	*	*	8	8	8	8	8	9	10	8	8	*	7	7	9	9	9	8	9	9	9	8	9	10	10	8	9	10	9
PI 478257	9	8	8	9	*	7	4	5	7	*	8	7	9	8	8	7	9	8	8	9	*	9	9	9	7	9	8	7	9	9	*	8
PI 478260	8	9	9	8	*	8	9	8	8	*	*	*	*	*	*	8	9	9	8	9	*	10	9	7	7	9	9	8	9	8	10	9
PI 478262	7	9	8	*	*	9	9	8	7	7	8	8	7	7	*	8	9	8	8	4	8	9	9	8	8	8	8	8	9	8	*	8
PI 623373	*	*	*	*	*	5	*	*	*	*	*		4	8	*	6	4	*	*	*	*	6	4	4	5	*	*	*	4	5	4	5
PI 632671	*	*	*	5	*	*	6	5	5	5	*	*	*	*	*	5	*	*	*	*	*	4	4	5	4	*	5	5	4	5	4	4
PI 623825	4	5	5	4	*	4	4	4	4	5	5	4	4	4	4	4	4	4	4	4	*	4	4	4	4	4	5	5	6	5	4	4
PI 623836	8	10	10	*	*	*	5	*	*	5	*	*	5	*	*	7	4	4	4	*	*	10	10	8	9	10	7	10	10	10	8	8
PI 623848	4	4	5	4	*	5	5	4	5	*	5	5	5	6	5	5		5	5	4	5	4	5	5	4	7	4	4	4	4	4	5
PI 623857	8	4	4	7	*	*	5	5	4	*	*	5	5	4	5	5	8	4	4	6	*	5	5	5	5	4	4	5	4	4	4	5
PI 624023	*	*	*	*	*	8	8	8	8	8	*	*	4	8	*	7	8	7	8	8	*	*	*	5	*	*	8	9	9	*	7	8
PI 624151	5	4	4	4	*	5	4	5	5	4	5	4	5	5	4	5	*	5	5	4	4	5	4	4	4	4	4	5	4	4	4	4
PI 624152	4	5	4	*	*	*	*	*	*	*	5	5	4	4	*	4	5	4	4	5	4	4	4	4	4	4	5	4	4	4	4	4
PI 624188	4	4	5	4	*	4	4	4	4	5	*	4	4	4	4	4	*	*	*	*	*	4	4	5	4	*	5	5	4	4	4	4
PI 624253	5	4	4	4	*	*	5	4	5	*	5	*	4	4	5	4	5	5	4	5	*	*	*	*	*	*	4	5	4	*	*	5
Cltr 2401	4	4	4	4	4	4	*	4	4	4	3	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
Gariep	5	6	*	6	6	6	5	5	5	6	5	6	5	5	5	5	7	10	10	9	8	8	8	8	8	9	9	8	10	8	8	9
Hugeoont	9	9	10	10	10	8	8	8	8	9	10		10	10	10	9	9		9	9	9	9	9	9	8	9	8	9	9	9	9	9
PAN 3144	4	4	4	4	4	4	4	4	4	4	5	4	4	4	4	4	4	4	4	4	4	4	5	4	4	4	5	4	4	4	4	4
Yumar	5	5	6	5	6	6	6	5	6	6	5	6	4	5	5	7	5	6	5	5	5	5	5	5	5	6	5	5	6	5	4	5

Accession	RWASA3																RWASA4															
	Rep 1					Rep 2					Rep 3					AV	Rep 1					Rep 2					Rep 3					AV
PI 220131	8	8	7	8	9	8	8	9	8	*	*	8	8	9	8	8	7	7	9	8	10	9	8	8	7	10	*	7	8	5	5	6
PI 349043	5	5	5	8	4	7	8	4	8	9	8	8	7	9	8	7	7	9	7	8	10	8	7	9	10	9	9	4	4	8	8	8
PI 366520	*	*	*	*	*	9	*	5	*	*	8	5	*	7	*	7	5		5	*	*	*	10	*	10	*	*	*	*	*	*	5
PI 366533	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*		10	8	*	*	*	4	*	*	*	*	7	5	*	*	8
PI 366545	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
PI 366549	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
PI 478115	9	5	8	7	9	9	9	9	8	*	8	8	9	8	9	8	8	8	9	10	8	8	7	7	7	9	8	10	9	10	10	9
PI 478126	8	9	9	3	*	9	9	8	*	10	7	8	9	8	8	8	8	4	8	*	9	3	3	3	3	*	7	8	10	9	9	9
PI 478127	5	4	*	8	8	8	8	8	8	*	4	3	8	8	7	7	7	8	7	8	*	4	4	4	9	8	7	8	7	7	10	9
PI 478134	9	7	9	8	8	8	9	7	9	8	9	8	8	8	10	8	10	9	8	10	10	10	8	8	10	*	10	8	9	10	9	10
PI 478172	4	6	4	4	6	5	6	6	6	5	5	5	6	5	5	5	8	9	10	5	5	4	4	3	4	9	7	4	8	4	9	9
PI 478177	8	7	9	9	9	8	9	9	8	9	8	9	10	7	8	8	10	8	10	9	4	8	9	8	8	9	8	9	10	9	10	10
PI 478216	4	*	8	9	4	8	8	9	4	8	9	9	9	8	8	8	10	9	10	8	7	10	7	8	10	9	10	9	9	10	9	10
PI 478257	9	5	8	9	9	9	5	7	8	*	3	*	8	8	7	7	9	10	10	8	7	9	10	7	8	9	7	4	10	10	9	9
PI 478260	7	9	8	7	8	9	8	5	4	8	9	9	8	7	8	8	8	10	8	7	8	9	8	8	8	9	10	8	8	8	8	8
PI 478262	9	9	7	*	8	8	7	8	8	4	3	9	9	9	*	8	7	8	9	9	10	8	9	4	10	8	8	4	4	8	9	8
PI 623373	9	6	8	7	9	9	8	8	7	10	8	7	8	8	10	8	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
PI 632671	10	9	9	8	9	8	*	9	7	8	9	9	8	9	10	9	*	9	*	*	*	4	4	*	*	*	9	*	*	*	*	*
PI 623825	7	*	8	8	7	7	9	7	8	*	7	*	7	*	*	8	9	8	8	9	5	10	5	7	8	10	4	4	8	8	9	9
PI 623836	4	9	7	*	8	8	8	8	*	7	*	7	*	*	*	7	5	9	10	*	10	9	10	10	8	9	10	10	7	10	8	7
PI 623848	*	8	9	7	8	8	8	8	8	*	7	*	7	8	8	8	5	5	5	4	7	9	4	4	4	4	4	4	4	5	5	5
PI 623857	*	5	7	7	7	*	8	8	*	*	8	8	8	8	7	7	5	5	5		8	*	5		7	*	5	5	*	*	*	5
PI 624023	9	7	8	8	10	8	8	7	9	6	8	6	9	9	10	8	*	*	*	5	*	*	7	10	*	*	*	*	*	10	*	*
PI 624151	*	*	8	5	*	6	6	8	7	*	5	*	8	8	*	7	7	5	5	8	7	9	4	9	8	10	10	9	4	10	8	8
PI 624152	7	8	7	7	8	7	7	7	7	8	8	8	8	8	*	8	4	4	4	4	4	4	4	4	4	4	4	4	3	3	4	4
PI 624188	8	7	5	8	*	7	8	7	9	8	8	*	8	7	*	8	8	7	7	9	8	7	4	4	4	8	9	9	8	7	8	8
PI 624253	5	*	5	5	5	4	4	*	6	6	7	*	7	8	7	6	4	4	4	4	4	*	4	4	4	4	*	7	4	8	*	4
CItr 2401	4	4	4	4	4	4	4	6	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
Gariep	9	8	9	9	9	8	4	8	9	9	8	8	8	8	9	8	8	10	10	8	10	7	8	8	9	*	8	8	10	10	7	8
Hugeoont	9	8	9	9	9	9	9	9	8	9	10	9	9	9	10	9	10	10	9	10	10	9	10	10	8	9	10	10	10	10	10	10
PAN 3144	4	4	4	4	4	4	5	5	4	4	4	4	4	4	4	4	8	8	10	8	9	8	7	9	8	8	8	7	9	9	8	8
Yumar	8	8	7	9	8	8	8	7	8	8	8	9	8	9	8	8	7	9	8	7	8	7	7	8	*	9	7	8	8	10	9	8