



**Full Length Article**

## Screening of Resynthesized Hexaploid Wheats for Durable Rust Resistance

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### Abstract

Wheat is one of the most important food crops, and its yield losses range from 20 to 100% due to leaf, stripe and stem rusts caused by *Puccinia triticina*, *P. striiformis* and *P. graminis*, respectively. New sources of resistance are strategically essential to limit the devastating effect of ever-evolving rust pathogens. In this study, we characterized 200 synthetic hexaploid wheats (SHWs) against all three rusts using rust pathotypes at seedling and adult plant stages in Queensland, Australia. We identified 57 accessions resistant to leaf rust, 77 to stripe rust and 69 accessions resistant to stem rust at the seedling stage. Ten SHWs were resistant to all three rusts, while 32 SHWs had dual resistance against leaf and stem rusts, and 28 SHWs had dual resistance to stripe and stem rust. We identified 24 SHWs carrying adult plant resistance (APR) for leaf, stripe and stem rust. The coefficient of correlation between yellow rust scores at seedling and adult plant stages were highest ( $r=0.89$ ), followed by stem rust ( $r=0.77$ ), and leaf rust ( $r=0.72$ ), which indicate the possibility of adult plant resistance in SHWs. The diagnostic competitive allele-specific PCR (KASP) markers for known rust resistance genes revealed that 14 SHW accessions carried *Lr34*, 85 carried *Lr46*, and 3 SHW carried *Lr67*, while none of the SHW carried *Sr2*. This study identified useful SHW lines for breeding resistance against all three types of rusts; some carried known durable resistance genes while others likely carry novel resistance loci. © 2020 Friends Science Publishers

**Keywords:** Wheat rust; Durable resistance genes; Synthetic hexaploid wheat

### Introduction

Bread wheat (*Triticum aestivum* L.) is commonly attacked by three air-borne fungal pathogens, *Puccinia triticina* f. spp. *tritici*, *P. graminis* f. spp. *tritici* and *P. striiformis* f. spp. *tritici* that cause leaf rust, stem rust and stripe rust diseases, respectively. Leaf rust has a comparatively wide range of adaptability to climatic conditions and is the most prevalent among three rust diseases (Riaz *et al.* 2016). Leaf rust damages leaves, florets and consequently shriveled seeds, resulting in yield losses up to 40% (Singh *et al.* 2016). Stem rust has a preference for hot and humid climates. Severe stem rust disease epidemics can cause 50 to 70% yield losses in bread and durum wheat. Similarly, the development of highly virulent stem rust race Ug99 (TTKSK) in Uganda in 1998 threatened global wheat

cultivation due to its widespread virulence on over 80% of varieties cultivated globally (Yu *et al.* 2012; Sharma-Poudyal *et al.* 2014). European countries especially Germany and United Kingdom recorded its first major stem rust outbreak in decades and 80% of UK wheat varieties found susceptible to these new isolates (Lewis *et al.* 2018; Olivera *et al.* 2018). Stripe or yellow rust develops under colder conditions. Chen (2014) have reported that yellow rust susceptible varieties losses are more than 60% of potential yield in the Pacific Northwest. In recent years, highly virulent races of *P. striiformis* with adaptation to high temperatures have emerged from the Himalaya region in Asia, which threaten wheat production in Indo-Gangetic plains (Hovmøller *et al.* 2016). In Mexico, virulence occurred on commercial varieties carrying resistance genes *Yr3*, *Yr27*

and *Yr31*, resulting in an epidemic in 2014 (Singh *et al.* 2016).

The use of chemical fungicides and breeding for genetic resistance are two fundamental approaches to control wheat rust diseases. Genetic resistance has always been environment friendly and is particularly inexpensive for wheat growers in developing countries (Oliver 2014). There are two common types of genetic resistance; race-specific resistance based on major genes, and adult plant resistance based on minor genes. Major genes generally provide resistance at all plant stages, are controlled by single genes, and can be rapidly overcome by the evolving pathogen population. On the contrary, minor genes provide resistance at the adult growth stages, controlled by several minor genes, each with a small effect, thus are more difficult for the pathogen to overcome. Currently, there is more emphasis to discover, characterize and utilize minor genes because these genes are durable and provide rust resistance for a more extended period. Breeding for adult plant resistance to all three wheat rusts is an important task among wheat breeders however is quite challenging because of the minor effect hard to detect in field and complexity of genetic inheritance (Lowe *et al.* 2011; Vazquez *et al.* 2015).

Rust resistance genes can be incorporated from wheat genotypes, landraces and also from relative species of wheat through pre-breeding and breeding. Synthetic hexaploid wheat (SHW) developed by crossing *Triticum turgidum* Desf. Husn. (AABB) and *Aegilops tauschii* Coss. (DD) holds genomic diversity for resistance to many abiotic and biotic stresses as confirmed from evaluation studies of these synthetic wheat germplasm (Ogbonnaya *et al.* 2013; Börner *et al.* 2015; Rasheed *et al.* 2018; Ahmad *et al.* 2019). One of the important stripe rust resistance gene *Yr24/Yr26* was introgressed from SHWs and deployed largely in wheat cultivars in China (McIntosh *et al.* 2018). Recently, Zegeye *et al.* (2014) have reported that SHWs represent rich sources of new stripe rust resistance which is valuable for future wheat breeding. This study was focused on the following aims: (1) to evaluate the genetic diversity for leaf, stem and stripe rust resistance in synthetic hexaploid wheat accessions at the seedling stage; (2) to identify accessions carrying adult plant resistance to all three rusts under the field conditions in Queensland, Australia; (3) to identify sources of potentially durable rust resistance among synthetic hexaploid wheat.

## Materials and Methods

### Rust evaluation

**Plant material and pathotypes:** A panel of 200 synthetic hexaploid wheats was evaluated in this study. Synthetic hexaploid wheat (AABBDD) developed by the artificial crossing of durum wheat (AABB) and *Aegilops tauschii* Coss. (DD). The panel was assessed for disease responses

to triple (leaf, stem and stripe) rust under glasshouse and field conditions available at The University of Queensland, Australia, during the wheat cropping season of 2018. These SHW accessions were provided by the Australian Grains Genebank (AGG) in Australia. At the seedling stage, SHWs were screened using three races of *P. triticina* [76-1,3,5,7,9,10,12+Lr37, 104 1,3,4,6,7,8,10,12 and 104-1,2,3,(6),(7),11,+Lr24], and one for each *P. graminis* (34-1,2,7+Sr38) and *P. striiformis* (134E16A+17+27+) (Table 1). The selected races represented highly virulent and prevalent races throughout the wheat-growing regions of eastern Australia (Wellings 2011; Fetch *et al.* 2016).

**Evaluation at the seedling stage:** The 200 SHW accessions were screened for all three rusts (leaf rust, stem rust and stripe rust) at the seedling stage under controlled glasshouse conditions at The University of Queensland, St Lucia, Queensland, Australia. The seeds of all accessions were imbibed at room temperature for 24 h and then transferred to 4°C in a refrigerator for 2 days, and then returned to room temperature for one day. Rust-susceptible stander cultivar “Morocco” was also planted as a check. Germinating seeds were then transplanted into ANOVApot® pots (140 mm) filled with potting media comprised of 70% fines compost of pine bark (0 to 5 mm) and 30% of coco peat with 5.5 to 6.5 of pH. At the time of transplanting Osmocote® fertilizer (slow release) was applied at a rate of 2 grams/pot. Each pot contained four seedling locations (*i.e.*, from the pot tag location 1 to 4 clockwise), where every location holds five sprouted seeds of the same SHW clumped together. Five seedlings of each SHW entry were grown in non-replicated augmented design, according to Zegeye *et al.* (2014). Seedlings were grown in the glasshouse with a standard day temperature of 25°C for 12 h and 17°C of the night for 12 h under diurnal-conditions, and temperature fluctuations were  $\pm 2^\circ\text{C}$ . For screening of leaf, stem and stripe rust seedlings, the batches were planted separately using intervals of one week. Each batch of seedlings at the two-leaf growth stage was inoculated discretely with pathotypes mentioned above.

Preserved urediniospores of leaf rust, stem rust and stripe rust were removed from the -80°C freezer, heat-shocked at 45°C for 3 min. These *Puccinia* isolates were purified using the single spore method and increased using the rust susceptible variety “Morocco”. The rust inoculum was made ready by preparing a suspension of urediniospores in Isopar-6 (mineral oil) @ 0.005 g/mL. Rust inoculum was applied on the wheat seedling leaves with a concentration of  $6 \times 10^5$  urediniospores/mL by using an IWATA power jet lite® (air atomizer). After inoculation deionized water was lightly misted over seedlings and kept in a 100% humid chamber (10°C for stripe rust, and 22°C for leaf rust and stem rust) for 18 h and humidity was maintained using an electric fogger. After that, plants were transferred for disease development to a controlled rust-free glasshouse. Light, temperature and other glasshouse

conditions for triple rust seedling assays were kept according to Hickey *et al.* (2012) and Riaz and Hickey (2017).

Rust infection types (ITs) of leaf and stem rusts were recorded twice: 12- and 16-days post-infection using the 0–4 scale (Stakman *et al.* 1962) and stripe rust ITs were recorded using the 0–9 scale (McNeal *et al.* 1971). In the case of leaf rust and stem rust, lower ITs (0–2) were assessed as resistant, and ITs (2+) were considered moderate, while higher ITs (3–4) were considered susceptible. Stripe rust seedlings ITs 0–3 were recorded as resistant, 4–6 as moderate reactions and 7–9 as susceptible, as described by Line and Qayoum (1992). To allow statistical analysis of glasshouse reactions and to compare disease responses to adult data sets, the seedling which contains both numbers and symbols were converted to the 1–9 scale according to Riaz *et al.* (2016) and Ziems *et al.* (2014). For mixed rust ITs, every reading was changed individually to the 1–9 scale, and the means were obtained.

**Rust evaluation in the field:** The same panel of 200 synthetic hexaploid wheats (SHW) was assessed for leaf rust and stem rust under field conditions at the Department of Fisheries and Agriculture, Redlands Research Farm, Queensland, Australia during wheat cropping season 2018. However, stripe rust evaluation carried out at Gatton Research Farm, Queensland, The University of Queensland, Australia. Eight seeds of each SHW accessions were planted as hill plots with two replications. A mixture of rust susceptible genotypes was planted between each bay to spread inoculum and ensure uniform disease infection. A set of wheat rust standards, including Thatcher, Thatcher+*Lr28*, Chinese Spring, Chinese Spring+*Sr2*, Chara, Chara Mutant, Avocet, Avocet+*Yr17*, Avocet+*Yr18*, Avocet+*Yr27*, Avocet+*Yr29*, Wyalkatchem+*Yr29*, Lalbhador, Lalbhador+*Yr18* and Lalbhador+*Yr29*, were planted twice in the experimental fields to observe the rust epidemic development. Each bay consisted of two rows of hill plots. After 35 days of sowing, Morocco plants infected with pathotypes of *P. triticina*, *P. graminis* and *P. striiformis*, same pathotypes used in seedling evaluations, were transplanted into the spreader rows to establish disease in the field experiments. The rust epidemics were escalated at both experimental sites (Redlands and Gatton) by using overhead sprinkler irrigations when temperature and humidity were conducive in the evenings for rust infection. When the triple rust epidemics had adequately established on rust standards to permit a definite difference among resistant and susceptible wheat lines, rust responses were scored using the 1–9 scale, where a genotype reaction score of 1 was recorded very resistant (VR), 2 resistant (R), 3 resistant to moderately resistant (RMR), 4 moderately resistant (MR), 5 moderately resistant to moderately susceptible (MRMS), 6 moderately susceptible (MS), 7 moderately susceptible to susceptible (MSS), 8 susceptible (S) and 9 very susceptible (VS) (Bariana *et al.* 2007; Jighly *et al.* 2016). Multiple triple rust disease responses were recorded from stem elongation to early grain filling with weekly intervals.

**Screening for durable rust resistance genes:** Total genomic DNA was extracted from each accession following previously described protocol by Ain *et al.* (2015). The presence of durable resistance genes *Lr34/Yr18/Sr57/Pm38*, *Lr46/Yr29/Sr58/Pm39*, *Lr67/Yr46/Sr55/Pm46*, and *Sr2* were determined using gene-specific markers by the KASP method using *Lr34\_TCCIND*, *Lr46\_JF2-2A*, *CSTM4\_67G*, and *Sr2\_ger9\_3p* markers, respectively (Rasheed *et al.* 2016). The Master mix included 2  $\mu$ L of 50-100 ng/ $\mu$ L template DNA, 2.5  $\mu$ L of 2X KASP master mix, 0.07  $\mu$ L of KASP assay mix and 2.5  $\mu$ L of water. PCR was performed in a 384-well format (S1000, Thermal Cycler, U.S.A.) by the following procedure: hot start at 95°C for 15 min, followed by 10 touchdown cycles (95°C for 20 s; touchdown at 65°C initially and decreasing at -1°C per cycle for 25 s), followed by 30 additional cycles of annealing (95°C for 10 s; 57°C for 60 s). The allele-specific primers were designed carrying the standard FAM (5' GAAGGTGACCAAGTTCATGCT 3') and HEX (5' GAAGGTCGGAGTCAACGGATT 3') tails and with the targeted SNP at the 3' end.

### Statistical analysis

The basic statistical analysis and frequency distribution were calculated and visualized using Microsoft Excel 2018. The multivariate principal component analysis (PCA) was carried out using R version 3.5.2 and PCA biplot was made using 'ggplot' function in R v. 3.5.2.

## Results

### Rust evaluations at the seedling stage

**Rust responses at the seedling stage:** Of the 200 SHWs, 192 were germinated, and the responses of these to leaf, stem and yellow rust were assessed at the seedling stage under glasshouse conditions (Fig. 1). In the leaf rust seedling assay, 60 (31%) accessions showed resistant, 14 (7%) moderately resistant and 106 (55%) showed moderately susceptible to susceptible responses against the three pathotypes. Among the resistant accessions, 3 accessions scored very resistant, 24 scored resistant and 33 accessions scored resistant to moderately resistant responses. The stem rust seedling assay showed that 131 (68%) SHWs were resistant, 26 (13%) were moderately resistant and 24 (12%) were susceptible to highly susceptible against the stem rust pathotype. Among the 131 stem rust-resistant accessions, 15 were recorded very resistant, 16 were found resistant and 100 accessions were identified as resistant to moderately resistant. For stripe rust, 99 (52%), 50 (26%), 13 (7%) and 30 (15%) SHWs were found resistant, moderately resistant and moderately susceptible to susceptible respectively. Of the 99 stripe rust seedling resistant genotypes, 25 scored highly resistant, 42 recorded resistant and 32 scored resistant to moderately resistant.

**Triple rust resistance at the seedling stage in SHWs:** Of the 200 SHWs, ten accessions (SHW-6, SHW-22, SHW-26,

**Table 1:** Virulence profile of *Puccinia triticina* (Pt), *Puccinia graminis* (Pgt) and *Puccinia striiformis* (Pst) pathotypes used in this study

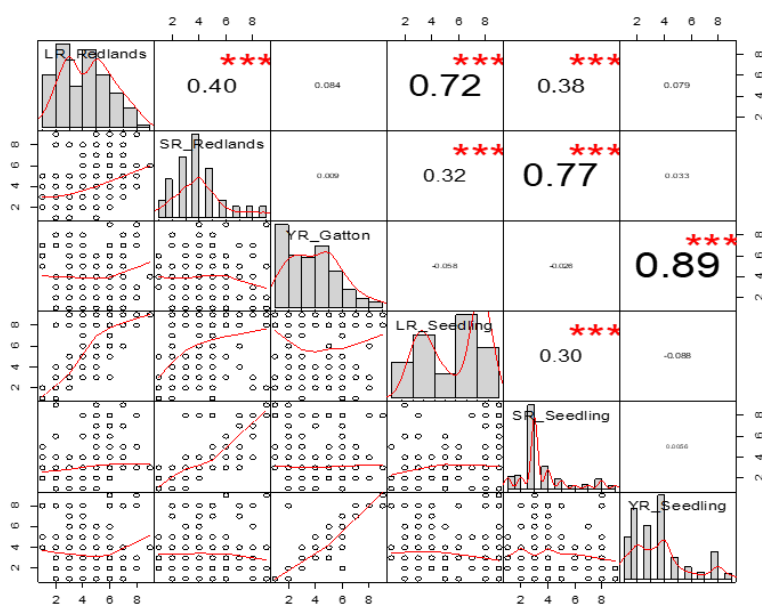
| Pathotypes                     | Virulence on genes   | Avirulence on genes  |
|--------------------------------|--|--|
| Pt 76-1,3,5,7,9,10,12          | Lr13, Lr14a, Lr15, Lr17a, Lr17b, Lr20, Lr27 and Lr31                           | Lr1, Lr2a, Lr3a, Lr3ka, Lr16, Lr23, Lr24, Lr26, Lr28 and Lr37  |
| Pt 104-1,3,4,6,7,8,10,12 +Lr37 | Lr1, Lr3a, Lr12, Lr13, Lr14a, Lr15, Lr17a, Lr17b, Lr20, Lr27+31, Lr28 and Lr37 | Lr2a, Lr3ka, Lr16, Lr23, Lr24 and Lr26                         |
| Pt 104-1,2,3,(6),(7),11,+ Lr24 | Lr14a, Lr16, (Lr17a), Lr20, Lr23, Lr24 and (Lr27, Lr31)                        | Lr1, Lr2a, Lr3a, Lr3ka, Lr13, Lr15, Lr17b, Lr26, Lr28 and Lr37 |
| Pgt 34-1,2,7 +Sr38             | Sr5, Sr6, Sr9g, Sr11, Sr15 and Sr38  | Sr8a, Sr9b, Sr17, Sr22, Sr24, Sr26, Sr27, Sr30, Sr31 and Sr36  |
| Pst 134 E16 A+ 17+ 27+         | Yr2, Yr6, Yr7, Yr8, Yr9, Yr17, Yr25, Yr27 and YrA                              | Yr1, Yr3, Yr4, Yr5, Yr10, Yr15, Yr32, Yr33 YrJ and YrT         |

Cereal Rust Report 2018, Plant Breeding Institute, The University of Sydney, Australia.

**Table 2:** Triple rust-resistant synthetic hexaploid wheats, their pedigree and disease scores for all three rusts at seedling and adult plant stages

| S. No.  | Pedigree   | LR-Adult | LR-Seedling | SR- Adult | SR-Seedling | YR-Adult | YR- Seedling |
|---------|--|----------|-------------|-----------|-------------|----------|--------------|
| SHW-6   | CPI/GEDIZ/3/GOO//JO69/CRA/4/AE.SQUARROSA (409)       | 3        | 3           | 3         | 3           | 1        | 1            |
| SHW-22  | CETA/AE.SQUARROSA (1030)                             | 2        | 2           | 2         | 3           | 3        | 3            |
| SHW-26  | GAN/AE.SQUARROSA (180)                               | 3        | 3           | 3         | 3           | 3        | 2            |
| SHW-43  | SCA/AE.SQUARROSA (518)                               | 2        | 3           | 2         | 3           | 1        | 1            |
| SHW-82  | DOY1/AE.SQUARROSA (415)                              | 2        | 2           | 2         | 3           | 3        | 2            |
| SHW-93  | GARZA/BOY//AE.SQUARROSA (520)                        | 3        | 2           | 1         | 2           | 3        | 2            |
| SHW-126 | ALTAR 84/AE.SQUARROSA (191)                          | 3        | 3           | 3         | 3           | 2        | 2            |
| SHW-162 | GARZA/BOY//AE.SQUARROSA (307)                        | 2        | 2           | 2         | 1           | 2        | 2            |
| SHW-164 | 68.111/RGB-U//WARD/3/AE.SQUARROSA (322)              | 2        | 1           | 1         | 1           | 3        | 3            |
| SHW-190 | 68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE.SQUARROSA (629) | 2        | 2           | 3         | 3           | 3        | 3            |

LR= Leaf rust SR= Stem rust YR= Stripe rust



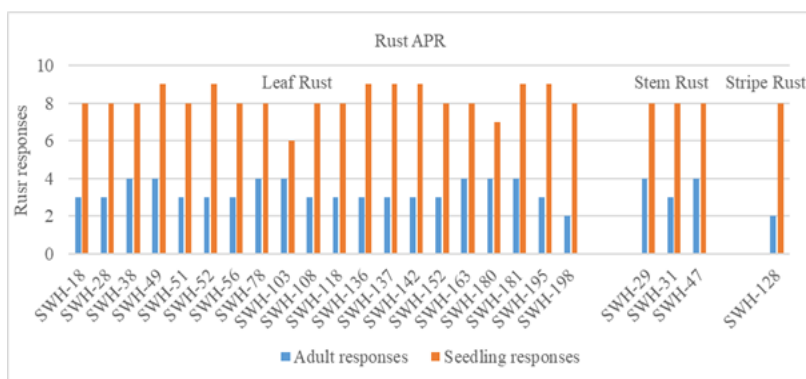
**Fig. 1:** Co-efficient of correlation, histogram and scatterplot of synthetic hexaploid wheats evaluated against all three rusts at seedling and adult plant stages

SHW-43, SHW-82, SHW-93, SHW-126, SHW-162, SHW-164 and SHW-190) showed resistance to all three rusts at the seedling stage based on the responses recorded with varying degrees of resistant infection types (Table 2). The accession, SHW-164, showed immunity against leaf rust and stem rust pathotypes and showed high resistance symptoms against stripe rust pathotype. Similarly, SHW-6 and SHW-43 showed highly resistant reactions against yellow rust and displayed very resistant reactions for leaf and stem rust. According to our seedling screening results SHW-26, SHW-82, SHW-93, SHW-126 and SHW-162 showed nearly-immune reactions against stripe rust race.

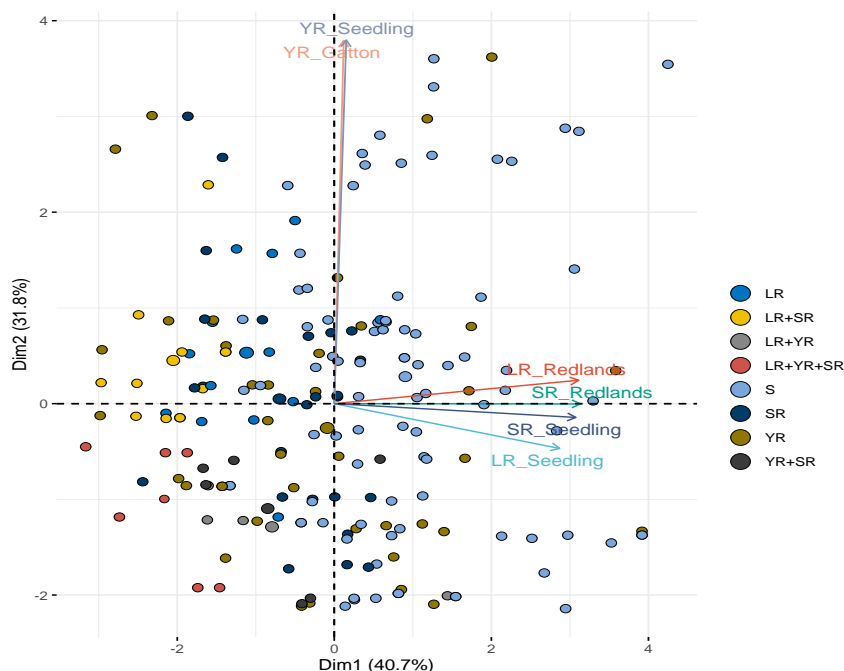
Notably, SHW-93 exhibited nearly-immune reactions for all three rust pathotypes. Moreover, genotypes SHW-82 and SHW-162 showed nearly-immune reactions against *P. triticina* and *P. striiformis* pathotypes.

**Rust evaluations in the field**

**Rust responses at the adult plant stage:** Of the 200 SHWs, 187 were assessed against leaf rust, 188 against stem rust and 192 evaluated for stripe rust and rest were not germinated in the field. In the field evaluations, the SHW accessions exhibited a wide range of leaf rust, stem



**Fig. 2:** Synthetic hexaploid wheats with adult plant resistance (APR) for leaf rust, stem rust and stripe rust



**Fig. 3:** Biplot displaying results from principal component analysis (PCA) of rust responses obtained by the synthetic hexaploid wheats (Dim 1 vs. Dim 2 displayed). The color legend describes the accessions resistant to relevant rust disease (LR for leaf rust, YR for stripe rust and SR for stem rust) and ‘S’ represents susceptible accessions

rust and stripe rust response types at the adult plant stage. The responses ranged from highly resistant to highly susceptible when screened under high disease pressure of three rust pathotypes (Fig. 1). Leaf rust adult plant screening showed that 70 (37%) accessions were resistant, 21 (11%) were moderately resistant and 55 (29%) were susceptible against three-leaf rust pathotypes in the Redlands field experiment. Among the 70 leaf rust field resistant SHW accessions, 5 recorded highly resistant, 22 scored resistant and 43 found resistant to moderately resistant. Stem rust field assay showed that 73 (38%) SHWs were resistant, 52 (28%) were moderately resistant and 32 (17%) were found moderately susceptible to highly susceptible against the stem rust at the adult stage at

Redlands. Among 73 stem rust-resistant accessions at the adult stage, 11 were highly resistant, 24 were resistant and 38 found resistant to moderately resistant. Stripe rust adult plant evaluation depicted that 82 (42%), 30 (16%) and 40 (21%) SHWs were resistant, moderately resistant and susceptible respectively in the Gatton field experiment. Of the 82 yellow rust adult stage resistant accessions, 18 scored highly resistant, 32 found resistant and also 32 were resistant to moderately resistant. Stripe rust adult plant evaluation results exhibited the highest number (82) of resistant SHWs in the field when compared to leaf rust and stem rust. Adult and seedling responses of all the rust-resistant SHWs against leaf rust, stem rust, and stripe rust are given in Table 3.

**Table 3:** Adult and seedling responses of rust-resistant SHWs against leaf rust (LR), stem rust (SR) and stripe rust (YR)

| S. No. | SHW     | LR adult | LR seedling | SHW     | SR adult | SR seedling | SHW     | YR adult | YR seedling |
|--------|---------|----------|-------------|---------|----------|-------------|---------|----------|-------------|
| 1      | SWH-4   | 2        | 2           | SHW-5   | 3        | 3           | SHW-3   | 2        | 2           |
| 2      | SWH-6   | 3        | 3           | SHW-6   | 3        | 3           | SHW-4   | 1        | 1           |
| 3      | SWH-9   | 3        | 3           | SHW-8   | 3        | 3           | SHW-6   | 1        | 1           |
| 4      | SWH-11  | 2        | 2           | SHW-9   | 3        | 3           | SHW-7   | 2        | 2           |
| 5      | SWH-20  | 1        | 2           | SHW-13  | 3        | 3           | SHW-8   | 3        | 3           |
| 6      | SWH-22  | 2        | 2           | SHW-19  | 3        | 3           | SHW-11  | 2        | 2           |
| 7      | SWH-26  | 3        | 3           | SHW-22  | 2        | 3           | SHW-17  | 1        | 1           |
| 8      | SWH-32  | 3        | 3           | SHW-26  | 3        | 3           | SHW-18  | 1        | 1           |
| 9      | SWH-33  | 2        | 2           | SHW-28  | 3        | 3           | SHW-21  | 2        | 2           |
| 10     | SWH-34  | 3        | 2           | SHW-32  | 1        | 1           | SHW-22  | 3        | 3           |
| 11     | SWH-37  | 3        | 3           | SHW-36  | 2        | 3           | SHW-26  | 3        | 2           |
| 12     | SWH-40  | 2        | 2           | SHW-40  | 1        | 1           | SHW-27  | 1        | 1           |
| 13     | SWH-43  | 3        | 3           | SHW-42  | 3        | 3           | SHW-33  | 3        | 2           |
| 14     | SWH-44  | 4        | 3           | SHW-43  | 2        | 3           | SHW-36  | 1        | 1           |
| 15     | SWH-54  | 3        | 3           | SHW-52  | 3        | 3           | SHW-43  | 1        | 1           |
| 16     | SWH-57  | 3        | 3           | SHW-57  | 1        | 1           | SHW-45  | 1        | 1           |
| 17     | SWH-59  | 2        | 2           | SHW-65  | 3        | 3           | SHW-63  | 2        | 2           |
| 18     | SWH-60  | 2        | 3           | SHW-67  | 2        | 2           | SHW-64  | 3        | 3           |
| 19     | SWH-62  | 3        | 2           | SHW-76  | 3        | 3           | SHW-65  | 2        | 2           |
| 20     | SWH-63  | 3        | 3           | SHW-78  | 3        | 3           | SHW-66  | 2        | 2           |
| 21     | SWH-82  | 2        | 2           | SHW-79  | 1        | 2           | SHW-68  | 2        | 2           |
| 22     | SWH-88  | 2        | 2           | SHW-82  | 3        | 3           | SHW-70  | 2        | 1           |
| 23     | SWH-93  | 3        | 2           | SHW-83  | 1        | 1           | SHW-71  | 3        | 2           |
| 24     | SWH-94  | 3        | 3           | SHW-93  | 1        | 2           | SHW-76  | 2        | 2           |
| 25     | SWH-104 | 1        | 1           | SHW-94  | 2        | 2           | SHW-77  | 1        | 1           |
| 26     | SWH-105 | 2        | 2           | SHW-95  | 1        | 2           | SHW-78  | 3        | 2           |
| 27     | SWH-111 | 3        | 3           | SHW-98  | 3        | 2           | SHW-82  | 3        | 2           |
| 28     | SWH-113 | 1        | 1           | SHW-101 | 2        | 3           | SHW-83  | 2        | 2           |
| 29     | SWH-116 | 2        | 2           | SHW-103 | 2        | 2           | SHW-84  | 2        | 2           |
| 30     | SWH-117 | 3        | 3           | SHW-105 | 1        | 1           | SHW-87  | 2        | 2           |
| 31     | SWH-120 | 3        | 3           | SHW-106 | 2        | 3           | SHW-88  | 3        | 2           |
| 32     | SWH-121 | 2        | 2           | SHW-107 | 2        | 3           | SHW-89  | 2        | 2           |
| 33     | SWH-126 | 3        | 3           | SHW-108 | 3        | 3           | SHW-90  | 2        | 2           |
| 34     | SWH-127 | 3        | 3           | SHW-113 | 2        | 2           | SHW-93  | 3        | 2           |
| 35     | SWH-128 | 3        | 2           | SHW-120 | 3        | 2           | SHW-96  | 3        | 3           |
| 36     | SWH-138 | 3        | 3           | SHW-121 | 1        | 1           | SHW-98  | 2        | 1           |
| 37     | SWH-140 | 2        | 2           | SHW-124 | 3        | 3           | SHW-101 | 2        | 1           |
| 38     | SWH-141 | 2        | 2           | SHW-126 | 3        | 3           | SHW-102 | 3        | 2           |
| 39     | SWH-144 | 3        | 3           | SHW-128 | 3        | 3           | SHW-106 | 2        | 2           |
| 40     | SWH-145 | 1        | 2           | SHW-129 | 2        | 3           | SHW-108 | 2        | 1           |
| 41     | SWH-146 | 3        | 3           | SHW-132 | 3        | 3           | SHW-110 | 2        | 1           |
| 42     | SWH-147 | 3        | 3           | SHW-136 | 3        | 3           | SHW-115 | 1        | 1           |
| 43     | SWH-151 | 2        | 2           | SHW-137 | 2        | 3           | SHW-116 | 3        | 2           |
| 44     | SWH-159 | 3        | 3           | SHW-138 | 1        | 2           | SHW-122 | 3        | 3           |
| 45     | SWH-161 | 1        | 2           | SHW-141 | 1        | 2           | SHW-124 | 2        | 2           |
| 46     | SWH-162 | 2        | 2           | SHW-143 | 3        | 3           | SHW-126 | 2        | 2           |
| 47     | SWH-164 | 2        | 1           | SHW-144 | 2        | 3           | SHW-129 | 3        | 3           |
| 48     | SWH-165 | 2        | 3           | SHW-145 | 3        | 3           | SHW-130 | 2        | 2           |
| 49     | SWH-174 | 3        | 3           | SHW-146 | 2        | 3           | SHW-131 | 2        | 2           |
| 50     | SWH-177 | 3        | 3           | SHW-147 | 2        | 3           | SHW-134 | 2        | 2           |
| 51     | SWH-184 | 2        | 3           | SHW-148 | 2        | 3           | SHW-137 | 1        | 1           |
| 52     | SWH-186 | 2        | 2           | SHW-151 | 3        | 2           | SHW-139 | 3        | 3           |
| 53     | SWH-190 | 2        | 2           | SHW-159 | 3        | 3           | SHW-140 | 2        | 2           |
| 54     | SWH-196 | 3        | 3           | SHW-162 | 2        | 1           | SHW-142 | 1        | 1           |
| 55     | SWH-197 | 3        | 3           | SHW-164 | 1        | 1           | SHW-143 | 2        | 2           |
| 56     |         |          |             | SHW-170 | 2        | 1           | SHW-152 | 3        | 2           |
| 57     |         |          |             | SHW-174 | 1        | 1           | SHW-157 | 2        | 2           |
| 58     |         |          |             | SHW-175 | 4        | 3           | SHW-158 | 2        | 2           |
| 59     |         |          |             | SHW-180 | 2        | 1           | SHW-162 | 2        | 2           |
| 60     |         |          |             | SHW-181 | 2        | 3           | SHW-164 | 3        | 3           |
| 61     |         |          |             | SHW-184 | 3        | 3           | SHW-167 | 1        | 1           |
| 62     |         |          |             | SHW-187 | 2        | 2           | SHW-168 | 2        | 2           |
| 63     |         |          |             | SHW-190 | 2        | 3           | SHW-169 | 1        | 1           |
| 64     |         |          |             | SHW-196 | 1        | 3           | SHW-173 | 2        | 3           |
| 65     |         |          |             | SHW-197 | 2        | 2           | SHW-175 | 1        | 1           |
| 66     |         |          |             | SHW-198 | 2        | 1           | SHW-176 | 1        | 1           |
| 67     |         |          |             | SHW-199 | 2        | 3           | SHW-178 | 2        | 2           |
| 68     |         |          |             | SHW-200 | 1        | 1           | SHW-180 | 3        | 3           |
| 69     |         |          |             |         |          |             | SHW-181 | 1        | 1           |
| 70     |         |          |             |         |          |             | SHW-182 | 1        | 1           |
| 71     |         |          |             |         |          |             | SHW-185 | 1        | 1           |
| 72     |         |          |             |         |          |             | SHW-188 | 1        | 1           |
| 73     |         |          |             |         |          |             | SHW-190 | 3        | 3           |
| 74     |         |          |             |         |          |             | SHW-193 | 2        | 2           |
| 75     |         |          |             |         |          |             | SHW-195 | 2        | 2           |
| 76     |         |          |             |         |          |             | SHW-198 | 3        | 3           |
| 77     |         |          |             |         |          |             | SHW-200 | 3        | 2           |

**Identification for adult plant resistance:** Leaf, stem and stripe rust adult plant resistance was detected in 24 seedling-susceptible SHW genotypes. Among these genotypes, 20 SHWs carried resistance against leaf rust, 3 for stem rust and 1 for stripe rust (Fig. 2). Of these adult plant resistant accessions, 15 were scored susceptible, 7 were highly susceptible, and 2 scored moderately susceptible to the susceptible reaction at the seedling stage. Among the adult plant resistant accessions, 2 were considered highly resistant, 13 were resistant to moderately resistant, and 9 SHWs were moderately resistant with very low rust percent severity in the field evaluations.

**Triple rust resistance at the adult plant stage:** Of these evaluated SHWs, ten (SHW-6, SHW-22, SHW-26, SHW-43, SHW-82, SHW-93, SHW-126, SHW-162, SHW-164 and SHW-190) exhibited adult plant resistance to all three rusts scaled between highly resistant to moderately resistant (Table 2). Genotypes SHW-6 and SHW-43 showed nearly-immune to stripe rust and SHW-93, and SHW-164 also displayed nearly-immune to stem rust pathotypes. According to our adult plant screening results, SHW-26 showed resistant reactions against all triple rust pathotypes. Accession SHW-162 exhibited nearly-immune reactions for triple rust pathotypes used in this study at the adult plant stage in the field. Genotypes SHW-43 and SHW-82 both showed nearly-immune infection types against leaf and stem rust pathotypes in the field at the adult stage.

#### Coefficient of correlation and principal component analysis

The response of the SHWs panel to three rusts (leaf, stem and stripe rust) was assessed at the seedling stage under glasshouse conditions and at the adult stage at two experimental field sites in Queensland, Australia. The rust responses at seedling and adult plant stages correlated well with one another (Fig. 1). The strongest correlations were found between YR-Gatton and YR-Seedling responses ( $r=0.89$ ), followed by SR-Redlands SR-Seedling responses ( $r=0.77$ ). LR-Redlands rust responses exhibited significant and positive correlations with LR-Seedling ( $r=0.72$ ), SR-Redlands ( $r=0.40$ ) and SR-Seedling ( $r=0.79$ ). Moreover, LR-Seedling responses were significantly and positively correlated with SR-Redlands ( $r=0.32$ ) and SR-Seedling ( $r=0.30$ ).

The diversity among SHWs was visualized with principal component analysis (PCA) based on the rust scores. The first two principal components explained 72.5% of the total variability (Fig. 3). The vector position and direction of leaf rust and stem rust was similar and quite different from the vector position and direction of yellow rust scores. This indicated the high correlation and similar diversity pattern between leaf rust and stem rust evaluations.

#### Durable rust resistance genes in SHWs

The results revealed that none of the SHW accessions

carried the *Sr2* gene. Among the other durable gene complexes, 14 SHWs carried *Lr34/Yr18/Sr57/Pm38*, and only 3 SHW carried the *Lr67/Yr46/Sr55/Pm46* gene. For the *Lr46/Yr29/Sr58/Pm39* gene complex, 85 SHWs were found positive and because this gene is yet to be cloned, the maker is not entirely diagnostic and could have false-positives.

#### Discussion

Development of new wheat cultivars incorporating genetic resistance to rapidly evolving rust pathogens is essential to sustain food security of wheat growing countries. The importance of SHW has been repeatedly emphasized for genetic improvement of wheat for both biotic and abiotic stresses (Ogbonnaya *et al.* 2013; Börner *et al.* 2015; Rasheed *et al.* 2018). We identified SHW accessions carrying several types of resistance against rust diseases. The SHW carrying triple rust resistance could be exploited in breeding programs to transfer resistance against all three rusts into susceptible high yielding wheat varieties. Furthermore, we identified adult plant resistance to leaf rust, stem rust and stripe rust by efficient assessment of synthetic wheat germplasm.

The presence of complete and moderate resistance at the seedling stage in 74 (38%), 157 (81%) and 149 (78%) for leaf rust, stem rust and stripe rust was quite large in number which indicated that several of these SHWs have the possibility of the presence of uncharacterized resistance genes. So far, more than 100 leaf rust resistance genes (*Lr*) have been designated in bread wheat and its immediate progenitors (McIntosh *et al.* 2016). Among these genes, *Lr21* (1DL), *Lr22* (2DS), *Lr32* (3DS), *Lr72* (2BS), *Lr40* (1DS), *Lr23* (2DS), *Lr39 syn.* and *Lr41* (2DS) have been identified previously in SHWs or derived from *Ae. tauschii*. The leaf rust 104-1,2,3, (6), (7),11, +*Lr24* isolate used in this study had virulence to *Lr23*, which indicated that resistant accessions carry an effective alternative gene. The leaf rust isolates used in this study were virulent to *Lr37*, which is derived from *Ae. ventricosa* through 2NS translocation, and none of the durum parents of SHWs carried this translocation which ruled out the possibility of the presence of *Lr37* in SHWs (Helguera *et al.* 2003). *Lr1* is a member of a multigene family (PSR567) and encodes a CC-NBS-LRR domain. The gene is identified in several *Ae. tauschii* accessions and because leaf rust isolates are avirulent to this gene, it is likely that SHWs accessions carry this gene inherited from *Ae. tauschii* parents (Ling *et al.* 2004). The leaf rust isolates were also avirulent to *Lr26/Yr9/Sr31* which is present in all wheat cultivars with the 1BL.1RS translocation. This translocation is absent in a wide array of SHWs; however, virulence to this is gene is known among other *Puccinia* isolates (Kolmer 2019; Zhang *et al.* 2019).

Until now, more than 83 stripe rust and 86 stem rust resistance gene have been designated in wheat and its wild relatives (McIntosh *et al.* 2016). The stem rust resistance

genes *Sr10171* (7DS), *Sr10187* (6DS), *Sr33* (1DL) and *SrTA1662* have been reported in *Ae. tauschii* accessions and these could be present in the resistant SHWs. The stem rust isolate (34-1,2,7+*Sr38*) used in this study was avirulent to some genes derived from wild relatives of bread wheat; hence their presence can be ruled out in SHWs. For example, *Sr22* is derived from *T. monococcum*, *Sr23* and *Sr26* are derived from *Thinopyrum elongatum*, *Sr27* and *Sr31* are derived from rye by TRS.3AS and 1BL.IRS translocations, respectively; and *Sr36* is derived from *T. timopheevii*. However, it is the possibility that resistant SHWs could carry the *Sr30* gene known to be present in several Australian and Mexican cultivars (McIntosh *et al.* 2016). Another important finding was that none of the SHW carries adult plant resistance gene *Sr2* associated with pseudo-black chaff, and seedling chlorosis and occurs very frequently in commercial cultivars produced and distributed by CIMMYT. Among the stripe rust resistance genes, *Yr28* is derived from SHWs and widely deployed in synthetic-wheat based commercial cultivars in China (Zeng *et al.* 2014). Previously, it was identified by GWAS that several stripe rust resistance loci co-localized with known *Yr* genes including *Yr24/Yr26/Yr28* on chromosome 4DS, *Yr48* on chromosome 5AL, *Yr32* on chromosome 2AS, and *Yr19* on chromosome 5BL (Zegeye *et al.* 2014). It is expected that several of the SHWs could carry *Yr32* because stripe rust isolate is avirulent to this gene. Overall, the resistant SHW accessions represent important sources of new, potentially uncharacterized resistance genes.

The presence of multiple disease resistance in SHWs is an exciting strategy which is desirable attribute enhancing the breeding value of SHWs. Das *et al.* (2016) have evaluated 32 SHWs against leaf rust, fusarium head blight, spot blotch and *Septoria tritici* blotch; and identified 7 out of 32 SHWs resistant to leaf rust. However, the phenotyping was only conducted at the adult plant stage in the field at CIMMYT. Therefore, it was not possible to identify the adult plant resistance to leaf rust. Recently, SHWs were characterized for multiple disease resistance (MDR) by evaluating resistance against all three rusts, yellow leaf spot, crown rot and *Septoria nodorum*, and only 9 out of 322 SHWs were resistant to all six fungal diseases (Jighly *et al.* 2016). This was quite similar to our results where we identified 10 out of 200 SHWs resistant to all three rusts. Further, it was identified by genome-wide association studies (GWAS) that one QTL on chromosome 1BL was associated with resistance to all six fungal pathogens and this QTL was associated with well-documented gene *Lr46/Yr29/Sr58* present in 22% SHWs (Jighly *et al.* 2016). We identified *Lr46/Yr29/Sr58* in 42.5% SHWs which is significantly higher frequency than previous studies, and likely a result of using the KASP marker for *Lr46/Yr29/Sr58* diagnosis, which is known to give false-positive results. Similarly, 125 SHWs were evaluated for resistance against six biotic stresses including three rusts, cereal cyst nematodes, crown rot and Hessian fly, and only

6 accessions were resistant to combined five stresses (Bhatta *et al.* 2019). APR gene *Lr67* was only identified in two SHWs, however the source this gene in SHWs is not clear because previously none of the *Ae. tauschii* known to carry *Lr67/Yr46/Sr55/Pm46* and gene was evolved after bread wheat polyploidization (Moore *et al.* 2015). The *Lr67/Yr46/Sr55/Pm46* gene originated from South Asia, and its frequency was pre-dominant in landraces from South Asia (Riaz *et al.* 2016).

A high correlation coefficient was observed for leaf rust, stem rust and stripe rust reactions recorded at the respective seedling and adult plant growth stages. Thus the glasshouse and field data produced in this assay represents highly reliable and repeatable data for genetic studies. This also indicated the presence of both R-genes and adult plant resistance among SHWs. Zegeye *et al.* (2014) have reported that 22% of SHWs susceptible at the seedling stage were resistance to stripe rust at the adult plant stage. In our study, almost 42% of the SHW accessions exhibited resistant to moderately resistant reactions against stripe rust in the field at the adult plant stage. Of these SHW resistant accessions, 40% (77) were resistant at both seedling stage indicated the presence of major resistance gene. The remaining adult plant resistant accessions could carry adult plant resistance based on minor genes which are desirable because they provide long-lasting resistance against newly evolving virulent pathogen races. Similar findings could be implied to the stem rust-resistant SHW accessions, where three accessions (SHW-29, SHW-31 and SHW-47) showed seedling susceptibility and resistance at the adult plant stage, thus were considered to exhibit adult plant resistance.

Based on field results, 24 accessions appeared to carry adult plant resistance for all three rusts, of which 15 were susceptible, 7 were highly susceptible, and 2 were recorded moderately susceptible to susceptible at the seedling stage. The accession SHW-26 SHW-162 displayed near-immune reactions for all three rust pathotypes at the adult plant stage in Redlands and Gatton fields. Entries SHW-43 and SHW-82 both exhibited near-immune disease responses to leaf rust and stem rust races in Redlands field at the adult growth stage. These SHWs are extremely important to discover minor genes or loci, providing durable resistance against all three rusts.

Similarly, ten accessions (SHW-6, SHW-22, SHW-26, SHW-43, SHW-82, SHW-93, SHW-126, SHW-162, SHW-164 and SHW-190) showed triple rust seedling and adult plant stage resistances simultaneously with varying resistance levels. These accessions are important sources of major rust resistance genes for all-stage resistance against all three rusts. The following categories could be established for multiple rust resistance: a) accession SHW-164 showed immunity against leaf and stem rust races and highly resistant to stripe race, b) the accessions SHW-6 and SHW-43 exhibited immunity against stripe rust and showed very resistant reactions for leaf and stem rust at the seedling stage, c) the accessions SHW-26, SHW-82, SHW-93, SHW-126



and SHW-162 showed nearly immune responses against stripe rust and SHW-93 also exhibited same reactions for leaf, stem and stripe rust pathotypes used in this study, and d) the accessions SHW-82 and SHW-162 showed nearly immune responses against leaf and stripe rust.

This study reconfirms the importance of resynthesized hexaploid wheat germplasm to deliver valuable genetic diversity to develop disease-resistant wheat. As the panel of SHW accessions displays a spring growth habit, the resistance can be quickly deployed in elite bread wheat cultivars through repeated cycles of backcrossing under speed-breeding (Watson *et al.* 2017; Li *et al.* 2018). We have started a rust gene discovery initiative by developing mapping populations derived from all 10 SHWs with triple rust resistance and progenies are being advanced following speed breeding protocols. Our findings are also useful for practical breeding because these SHW accessions with multiple rust resistances can be used to pyramid multiple rust resistance genes for durable resistance to most devastating diseases of wheat. This strategy will help not only fast-track isolation of new rust resistance genes and deploy new resistance genes, but will also broaden the genetic diversity in breeding germplasm.

## Conclusion

Breeding for rust resistance is crucial for any wheat breeding program. This involves dynamic rust evaluation and ongoing distribution of new rust-resistant wheat cultivars. The KASP markers for known rust resistance genes revealed that SHWs carried leaf rust resistant genes *Lr34*, *Lr46* and *Lr67*. This study also revealed that these genotypes carried leaf, stem and stripe rust resistance. Synthetic hexaploid wheats with substantial genetic diversity for resistance can be effectively utilized to develop rust-resistant wheat varieties.

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