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Wheat Improvement

Food Security in a Changing Climate

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Chapter 8
Wheat Rusts: Current Status, Prospects of Genetic Control and Integrated Approaches to Enhance Resistance Durability

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Abstract The three rusts are the most damaging diseases of wheat worldwide and continue to pose a threat to global food security. In the recent decades, stem rust races belonging to the Ug99 (TTKSK) and Digalu (TKTTF) race group resurfaced as a major threat in Africa, the Middle East and Europe threatening global wheat production. In addition, the evolution and migration of new aggressive races of yellow rust adapted to warmer temperatures into Europe and Asia from Himalayan region are becoming a significant risk in several wheat production environments. Unique and complex virulence patterns, continuous evolution to overcome effective resistance genes in varieties, shifts in population dynamics, transboundary migration have resulted in localized/regional epidemics leading to food insecurity threats. This underscores the need to identify, characterize, and deploy effective rust resistant genes from diverse sources into pre-breeding lines and future wheat varieties. The use of genetic resistance and deployment of multiple race specific and pleiotropic adult plant resistance genes in wheat lines can enhance resistance durability. Recent advances in sequencing annotated wheat reference genome with a detailed analysis of gene content among sub-genomes will not only accelerate our understanding of the genetic basis of rust resistance bread wheat, at the same time wheat breeders can now use this information to identify genes conferring rust resistance.

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Progress in genetic mapping techniques, new cloning techniques and wheat transformation methods over the last two decades have not only resulted in characterizing new genes and loci but also facilitated rapid cloning and stacking multiple genes as gene cassettes which can be future solution for enhancing durable resistance.

Keywords Rust resistance · Race specific genes · Adult plant resistance genes · Breeding technologies

8.1 Learning Objectives

- Geographical distribution of three rust diseases, impact, management strategies and briefly address the new molecular tools in the current era to enhance resistance breeding and opportunities for wheat improvement.

8.2 Economic Importance, Historical Impacts, Status of Rust Diseases

Pests and diseases (P&D) have historically affected food production either directly through losses in crop production or quality. Currently, these losses are exacerbated by the changing climate threatening food security and rural livelihoods across the globe. Nearly 200 wheat pests and diseases in wheat have been documented, of which fifty are considered economically important because of their potential to cause substantial yield losses. Two studies estimated potential grain yield losses due to disease at 18% and 21.5% at global level and (10.1–28.1%) per hotspot for wheat [1], however, losses can be significantly higher in areas where susceptible wheat varieties are still grown. Rust pathogens are present in all wheat growing environments and have constantly hindered global wheat production since domestication and still continue to threaten the global wheat supplies. It is estimated that global annual losses to wheat rust pathogens can be around 15 million tons valued at US$ 2.9 billion [2]. Documented evidence suggesting wheat rusts could be one of the earliest pathogens wherein spores of SR dating back to 1300 BC were detected in Israel, rust was reported as serious disease of cereals in Italy and Greece more than 2000 years ago and festival called “Robigalia” was celebrated to protect the crops from rusts and smuts [3]. The continuous effort to increase genetic gains is not possible without overcoming several of the current barriers such as climate change coupled with a variety of unpredictable abiotic and biotic stresses that pose significant threat to wheat production both locally and globally (see Chap. 7). Genetic uniformity of wheat in the quest of developing high-performing cultivars, has also contributed pathogen resurgence to the point wherein diseases threaten global wheat production. This review considers the three rust diseases affecting wheat

productivity, and the emerging threats considering the geographical distribution, impacts, and management strategies and briefly address the new molecular tools in the current era to enhance resistance breeding and deployment opportunities for wheat improvement.

There are three wheat rust diseases, namely stem (black) rust, stripe (yellow) rust and leaf (brown) rust, all belonging to the members of the Basidiomycete family, genus *Puccinia*, and named *P. graminis* f. sp. *tritici* (*Pgt*), *P. striiformis* f. sp. *tritici* (*Pst*) and *P. triticina* (*Pt*), respectively.

8.2.1 Stem Rust

Stem rust (SR), or black rust is common where wheat plants are exposed to warmer environments at later stages of crop growth. SR has the potential to completely annihilate a healthy looking crop when an epidemic occurs and linear yield losses have been observed, early infections can result in no grain fill and panicles can be reduced to chaff [4]. SR epidemics have been significantly curtailed by eliminating its alternate host (barberry species) between 1918 and 1980 in the USA and in the UK, adoption of semi-dwarf, early maturing rust resistant varieties developed by CIMMYT (International Maize and Wheat Improvement Center), and the use of fungicides.

Wheat growing environments of East Africa are unique epidemiological regions that favor wheat production all-round the year in different regions providing continuous green bridge for pathogen evolution and survival resulting in frequent localized epidemics. Even though it was under control for over three decades the recent re-emergence of SR race "Ug99" in East Africa posed a serious threat to global wheat production [5].

The stem rust race Ug99 (TTKSK) caused widespread damage in Kenya [6] carrying unique virulence as it was able to overcome over 50% of the known SR resistance genes including widely deployed genes *Sr31* and *Sr38*. Following the spread of race Ug99, resistant cultivar "Kenya Mwamba" was released in 2001 (known to carry gene *Sr24*) which became a popular variety with farmers however; in 2006, race TTKST (Ug99+*Sr24* virulence) was detected in Kenya, resulting in severe localized epidemics in Kenya [7]. Through sustained breeding efforts of CIMMYT, several new varieties with resistance to TTKSK and TTKST were released post 2009, of which "Kenya Robin" became a leading variety combining high yield potential and SR resistance covering 40% of the wheat area in Kenya by 2014. However, in the same year the breakdown of resistance in Robin and two variants of Ug99 race group with virulence to resistance gene *SrTmp* were identified, viz. race TTKTK (Ug99+*SrTmp*), and TTKTT (Ug99+*Sr24*+*SrTmp*) [8]. These two genes (*Sr24* and *SrTmp*) were quite important in conferring effective resistance to SR races in the USA, CIMMYT, South America and Australian wheat germplasm increasing the vulnerability of varieties to Ug99 race group [9] not only for East Africa but predicted migration paths threatening production in other wheat growing

environments [9]. In 2018, another new race with virulence to *Sr8155B* gene was identified in Kenya in 2018 (unpublished data) and currently, seven of the fourteen variants within the Ug99 race group have evolved in Kenya, making it the hot spot for evolution of Ug99 race group.

Stripe rust epidemics in Ethiopia in 2010, prompted release of varieties carrying good levels of stripe rust and SR resistance of which cultivar "Digalu" (carrying high yield potential and rust resistance to both YR and Ug99 race group) became a popular variety with farmers by 2013–2014 occupying approximately 31% of wheat area under production. However, in 2013, devastating localized epidemics of SR were reported on Digalu caused by race TKTTF, a SR race unrelated to the Ug99 race group [10]. This race was able to overcome resistance gene *SrTmp* present in Digalu and was later detected in Kenya, this race was previously reported in Turkey, Lebanon and Iran. Airborne dispersal models also indicated a migration route into East Africa from sources in the Middle East. Race TKTTF has also now been detected in Germany, UK, Sweden, Denmark. In addition to Digalu race group, diverse SR races with rare combination of virulence to *Sr9e* and *Sr13* have been found in the central highlands of Ethiopia [11], which have been quite important for durum wheat as these genes are deployed in both North America and Australia.

Widespread eradication of the alternate host, common barberry, had resulted in effective control of SR in Western Europe until 2013. However, unusual SR infections on winter and spring wheat in 2013 and race analyses identified six SR races, similar to Digalu race with additional virulence to *Sr7a, Sr45,* and *SrTt*-3 [12]. Common barberry is now being implicated as a source of new stem rust race diversity in Georgia and Western Siberia and SR epidemics on oats in Sweden. Since 2014, several large-scale stem rust outbreaks have been reported and virulent races are spreading rapidly. Race TKKTP with virulence combination for *Sr24, Sr36, Sr1A.1R* and *SrTmp* [13], races TRTTF and TKKTF (virulence to *Sr1A.1R*) have also been identified. Race TKKTF is spreading rapidly and now detected in 17 countries across Europe, North Africa, the Middle East and East Africa. Similarly, race TTRTF caused epidemics on durum wheat in Sicily since 2015 and is now detected in 10 countries in Europe, North Africa, the Middle East and East Africa [14].

8.2.2 Stripe Rust

Stripe (yellow) rust (YR) is a common disease in wheat and well adapted to temperate areas with humid and cool weather, aggressive races adapted to warmer temperatures have migrated and spread across geographies since 2000 [15]. Race shifts towards higher rates of mutation for virulence within the *Pst* pathogen has resulted in vulnerability of widely deployed cultivars. Global yield losses to YR is estimated at 5.5 million tons per year [16]. Production losses in North America alone since 2000 exceeded over one million tons, and in China, losses over 1.8–6.0 million tons were observed under epidemic conditions. Similar reports of yield losses to YR in

Europe in the recent decade has been attributed largely to the race shifts derived from the Himlayan region [17]. Historically, impact of newly evolved YR races on wheat productivity have been occasional, however, new incursions have often resulted in widespread damage, e.g. incursion of YR races from Europe into eastern Australia in 1978, western Australia in early 2002. Exotic incursions of YR races replaced the existing populations in the USA since 2000 and race shifts in the European *Pst* populations in 2011 and 2012 by races from the Himalayan region [17] are very good examples of exotic races with different genetic *Pst* lineages causing significant impact on host susceptibility. A recent study linking both virulence and race structure with recent YR epidemics in different geographies [18] suggested different *Pst* races in distinct genetic lineages, where aggressive strains adapted across diverse environments were spreading across continents, including the more recent outbreak of YR in Argentina.

8.2.3 Leaf Rust

Leaf (or brown) rust (LR), is the most common rust disease in both winter wheat and spring wheat growing areas, as well as on durum wheat. Yield losses due to LR can be substantial if susceptible varieties are infected at early stages coupled with favourable temperatures and moisture conditions resulting in rapid progress in short time span. Yield losses are largely due to the reduction of kernels per spike and lower kernel weights.

Populations of *Pt*, are specifically adapted to either tetraploid durum wheat or hexaploid common wheat and races conferring virulence to several of the *LR* genes are prevalent throughout the world [19]. Since the early 2000s, races of *Pt* that are highly virulent on durum wheat cultivars have spread across South America, Mexico, Europe, the Mediterranean basin, and the Middle East. These races confer virulence to *Lr71* gene, widely present in durum wheat, however, are avirulent to many of the LR genes that are found in common wheat. In Ethiopia another group of *Pt* races have been found that are highly virulent on durum wheat yet avirulent to the highly susceptible common wheat cultivars such as “Thatcher” and “Little Club” [20] and these isolates are unique to Ethiopia.

On a global scale, most populations of *Pt* are unique in their virulence and molecular genotypes. Even though the most common mode of evolution is through mutation and selection in a given environment, there is evidence for recent migration of *Pt* races between different continental regions. Since the mid 1990s isolates of *Pt* with virulence to *Lr1, Lr3a*, and *Lr17a,* and avirulence to *Lr28,* have increased and spread across the U.S. and Canada. These isolates also had a unique molecular genotype, which indicated that these were likely recently introduced to North America. Since the early 2000s these isolates with identical or highly similar virulence and molecular genotypes have been found in Europe, South America, Ethiopia, Turkey and Pakistan [21]. Similarly isolates of *P. triticina* with virulence to durum wheat that also have identical or highly related molecular genotypes have been

found in the Middle East, South America, Europe, Ethiopia, Tunisia, Mexico and the U.S [22].

8.3 Global Rust Phenotyping Network – Critical Tool to Understand Host Resistance and Pathogenic Diversity on a Global Scale

A global network of precision field-based wheat disease phenotyping platforms of the CGIAR Program WHEAT (see http://wheat.org), were developed with the support of national agricultural research institutes. The objective is to generate multi-location disease phenotypic data, under defined management practices, and fostering germplasm exchange. The selected locations also represent hotspots for specific diseases and future-climate analogue sites. This model opens opportunities to increase coordination in wheat phenotyping, avoiding duplications, and building on efficiency and capacity for research. The global wheat phenotyping network (Fig. 8.1) has eight regional hubs/hotspot sites that facilitate screening and selection for diseases, viz. SR and YR (Kenya, Ethiopia, Turkey, India), Fusarium (China, Uruguay), wheat blast (Bolivia and Bangladesh), leaf blight (Nepal and India), soil borne diseases (Turkey), Septoria (Kenya, Ethiopia, Uruguay and Tunisia) alongside CIMMYT, HQ stations of Toluca (YR, Septoria), El Batan (leaf rust), Agua Fria (leaf blight) and Obregon (leaf rust) (Mexico).

In the last decade, effective partnership between CIMMYT, KALRO (Kenya Agriculture and Research Organization), EIAR (Ethiopian Institute of Agriculture Research) and BGRI (Borlaug Global Rust Initiative) through DRRW (Durable rust

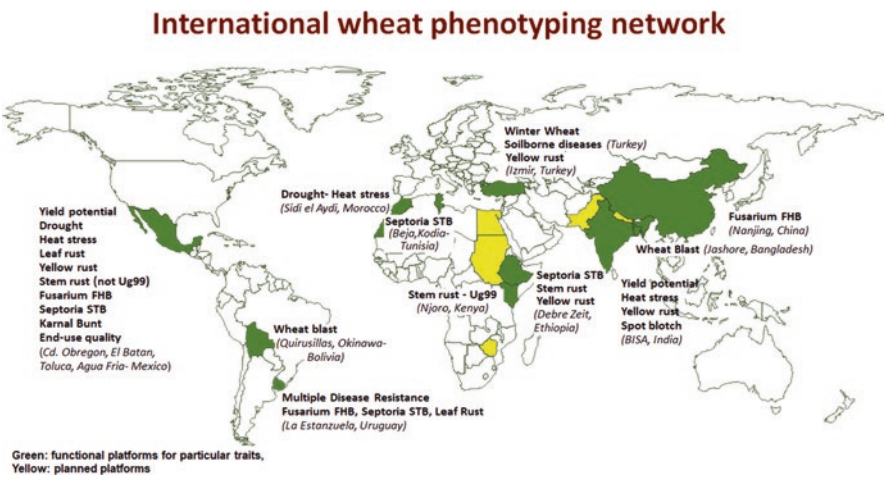

Fig. 8.1 International wheat phenotyping hubs spread across several countries led by NARS in collaboration with CIMMYT/ICARDA

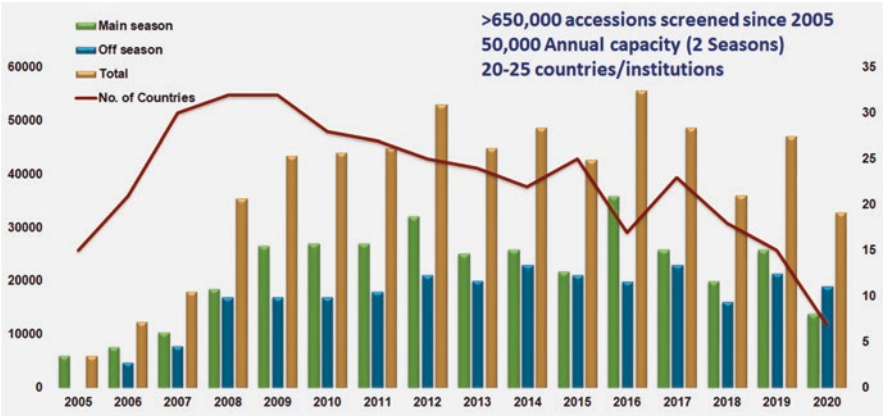

Fig. 8.2 Wheat accessions phenotyped during 2005–2020 for Ug99 resistance at Njoro (Kenya) and participating countries, in partnership with Kenya Agriculture Livestock Research Organization, Kenya

resistance in wheat) and DGGW (Delivering Genetic Gains in Wheat) projects have established functional SR phenotyping platforms which have made a significant impact to the global wheat research in addressing the threat of SR. International SR phenotyping platforms established at Njoro (KALRO, Kenya) and Debrezeit (EIAR, Ethiopia) play key roles in evaluating global wheat germplasm from several countries and institutions. Over 650,000 wheat accessions have been screened against Ug99 and derived races since 2005, and the screening capacity at KALRO, Njoro has increased to 50,000 lines from over 20–25 countries and research institutions each year [4] (Fig. 8.2). The results from the international nurseries show a shift to higher frequencies of lines with resistance to SR races, since the screening activities were initiated in 2008. Similarly, close to 150,000 wheat land races and advanced durum wheat breeding lines and varieties have been evaluated in Debrezeit.

Reliable phenotypic data generated from these phenotyping platforms led to the characterization of over 35 SR genes/loci in collaboration with global partners (Matt Rouse CDL, unpublished data), Genomic prediction models for APR (Adult-Plant Resistance) showed promising results [23]. Release of over 17 varieties in Kenya and Ethiopia and more than 200 varieties released in several countries globally over the years is testament to the success of the impacts from the phenotyping platforms. CIMMYT-Kenya shuttle breeding has resulted in rapid cycling of over 2000 populations each year between Mexico and Kenya to evaluate and select lines in early generations against virulent SR. Candidates of the stage I (10,000 lines) and stage II (1500 lines) yield trials are also evaluated and the selected lines are included in international nurseries and trials and distributed to NARS partners.

8.4 International Research Networks in Mitigating the Threats of Emerging New Races-Early Detection, Forecasting and Prediction

In response to the resurgence of SR in eastern Africa and the threat of Ug99, and "sounding the alarm" by Dr. N. E. Borlaug in 2005, the international wheat research community led by Cornell university, established the BGRI (Borlaug Global Rust Initiative) to significantly reduce the vulnerability of wheat crop worldwide to three rusts diseases. Improved pathogen monitoring and surveillance activities greatly enhanced the tracking and spread of new and virulent variants of SR, YR and LR. Global cereal rust monitoring system (GCRMS) is an information platform that includes standardized protocols and methods for surveys, preliminary virulence testing, data, sample transmission and management at the field; national and global levels. Collected rust samples are sent under permit to several international specialist rust laboratories for pathotype analysis (GRRC-Denmark [YR+SR], CDL-Minnesota [SR+LR], AAFC- Canada [SR], and ICARDA-Turkey RCRRC- Izmir [SR+YR]). The GCRMS expanded substantially over the years and as of 2019, scientists from 40 countries are participating in wheat rust surveillance and over 44,000 geo-referenced survey records and 9000+ rust isolate records have been collected (see https://rusttracker.cimmyt.org/). For the first time, important *Pgt* race groups, e.g. the Ug99 group, have been successfully tracked in space and time. Other important new *Pgt* and *Pst* race groups that are spreading in Europe, the Middle East and Africa are also being monitored. Integrated data management is achieved through a centralized database (Wheat Rust Toolbox) managed by Aarhus University, Denmark and the tools and database are updated on a routine basis, hence delivering the most recent information in a timely manner. The Wheat Rust Toolbox includes a comprehensive user management system that permits controlled access to specific tools and functionality. Registered users have country-specific access to an on-line data entry system and a suite of country-specific data visualization options for their own data.

A series of reviews on current status of key SR races [4, 24, 25] have provided a recent and comprehensive overview of the status of the Ug99 race group, describing the rapid evolution of new races and its geographical expansion. Technology innovations are now enhancing the global rust monitoring system. Very recently Mobile And Real-time PLant disEase (MARPLE) diagnostics [26] has been developed by John Innes Center, UK and successfully deployed as a portable, genomics-based tool to identify individual strains of complex fungal plant pathogens. Advanced spore dispersal and meteorologically driven epidemiological models, developed by Cambridge University and UK Met Office, are now providing valuable new information on pathogen movements and the basis for near-real time, in-season rust early warning systems. An operational rust early warning system is now operational in Ethiopia and similar systems are being developed in Nepal and Bangladesh.

8.5 Types of Resistance, Strategies to Deploy Different Resistance Mechanisms to Attain Resistance Durability

Currently, over 220 rust resistance genes viz. 79 LR resistance genes, 82 YR resistance genes and 60 SR genes have been formally cataloged and designated of which majority of them confer race specific resistance and only a few genes confer slow rusting /partial adult plant resistance to the three rust diseases.

8.5.1 Race-Specific/Seedling Resistance

Race specific, or seedling resistance, also referred as qualitative resistance or all stage resistance is effective at all growth stages and belongs to the "R gene" class conferring NBS-LRR (Nucleotide Binding Site- Leucine Rich Repeat) domain. Some exceptions are known where R-genes are effective only in post-seedling or adult plant stages. R-genes may confer a major resistance effect/complete resistance expressed as varying degrees of hypersensitive response and are effective one or against few races of the pathogen. However, majority of the R-genes are intermediate and do not confer clean phenotype or adequate levels of resistance and some are influenced by temperature and light regimes. The ease of selecting these genes at both seedling and field stages has made it easier to incorporate such resistance in wheat breeding programs resulting in increased productivity (boom). However, deployment of single R-genes has often resulted in pathogen acquiring virulence post deployment as varieties in a short period leading to breakdown of resistance causing epidemics and severe yield losses (bust) cycles e.g. widespread virulence for *Yr9* and *Yr27*, virulence for SR gene *Sr31* and other important SR genes *Sr24*, *Srtmp* to the Ug99 race group and ineffectiveness of LR resistance genes in the United States. However, deployment of multiple R gene combinations often refereed as "pyramiding" can effectively enhance durability of resistance in an event of when one of the R gene breaks down other genes will continue to protect the variety and keep pathogen populations under check.

8.5.2 APR Genes Conferring Pleiotropic Effects

Race-nonspecific resistance often referred as adult plant resistance or partial resistance is effective against wider races of a pathogen species. APR is generally quantitative, exhibiting incomplete resistance that is usually expressed at later stages of plant development unlike race-specific resistance that is expressed at both seedling and adult plant stage. These genes help slow the disease progress through increased latency period, reduced infection frequency, reduced pustule size resulting in lower spore production. Several of the APR genes confer seedling susceptibility and

usually produce medium to large compatible pustules at low frequency without hypersensitive response and expression of resistance is observed when the plants reach flag leaf or boot leaf stage. The phenotypic effect of such genes is relatively minor to moderate, however, additive effects of multiple APR genes (4–5) in combinations can result in very high levels of resistance [24]. The problem of "boom and bust" cycles prompted wheat breeders to embrace an alternate approach combining slow rusting or partial resistance to enhance resistance durability. Johnson and Law [27] defined durable resistance as "resistance that remained effective after widespread deployment over a considerable period of time". A general concept of a durable resistance source for cereal rusts is that it is minor in effect, polygenic, usually expressed at post-flowering/adult plant stage, non-race-specific and produce non-hypersensitive response to infection.

Noteworthy examples of durable resistance is the resistance to SR transferred from tetraploid emmer to North American bread wheat cultivars "Hope" and "H-44", and LR resistance in the South American wheat cultivar "Frontana". Since the early 1980s, significant progress has been achieved in understanding the genes involved with slow rusting and their efficient use in breeding [7]. Currently at CIMMYT, key slow rusting pleotropic genes such as *Lr34*. *Lr46*, *Lr67* and *Lr68* in combination with other minor effect genes continue to enhance durable resistance to the three rust diseases [4, 24].

Lr34 was first reported in cultivar "Frontana", and wheat cultivars containing *Lr34* are widely present and occupy more than 25 million ha in developing countries and is effective in reducing yield losses in epidemic years, and has been mapped on chromosome 7DS. This gene confers pleiotropic resistance effect (Table 8.1) on multiple diseases such as YR, SR, powdery mildew, barley-yellow dwarf virus and spot blotch (*Lr34*/*Yr18*, *Sr57*, *Pm38*, *Bdv1* and *Sb1*), respectively and is associated with a morphological marker leaf tip necrosis (LTN). *Lr34* was cloned and the gene

Table 8.1 Pleotropic APR genes used in CIMMYT wheat breeding program and linked markers

Genes	Reported linked markers	Marker type	Reference stock	Chromosomal location	Reference
Lr34/ Yr18/ Pm38/ Sr57	*wMAS000003, wMAS000004*	STS, SNP	Parula, Thatcher, Glenlea, Jupateco R, Opata, Bezostaya, Chinese Spring.	7DS	[29]
Lr46/ Yr29/ Pm39/ Sr58	*csLV46, csLV46G22*	CAPS	Pavon 76, Parula,	1BL	[30]
Lr67/ Yr46/ Pm46/ Sr55	*csSNP856*	SNP	RL6077	4DL	[31]
Sr2/Yr30	*csSr2, wMAS000005*	CAPS	Pavon76	3BS	[32]
Lr68	*cs7BLNLRR*	CAPS	Parula	7BL	[33]

encodes a full-size ATP-binding cassette (ABC) transporter [28] and gene-specific markers were developed which are widely used in marker assisted selection.

Lr46 was first described in 1998 in cultivar "Pavon 76", located on chromosome 1BL characterized by lower latency period [34], confers partial resistance to other diseases with corresponding designations *Yr29*, *Sr58* and *Pm39*, respectively. *Lr46* is also associated with LTN and is very common in both old and new wheat varieties including durum wheat.

The *Lr67* gene was identified in the common wheat accession "PI250413" and transferred into "Thatcher" to produce the isoline "RL6077" (Thatcher*6/PI250413). *Lr67* also shows pleotropic effect to SR and YR however, with *lower effect of* LR resistance than *Lr34*. Mapping studies mapped *Lr67/Yr46/Pm46* on chromosome arm 4DL. Cloning elucidated that *Lr67* gene encodes a hexose transporter [35].

Lr68 is another APR gene located on chromosome arm 7BL, conferring APR to LR identified in CIMMYT's wheat "Parula", known to carry *Lr34* and *Lr46 and* likely to have originated from "Frontana" [33]. *Lr68* showed a weaker effect than *Lr34, Lr46 and Lr67* but combined effect of *Lr34*, *Lr46* and *Lr68* in Parula resulted in near immunity [33].

Stem rust gene *Sr2* is one of the most important and widely used genes, conferring modest levels of resistance, and has been effective until date (over 100 years) even to the Ug99 and Digalu race groups of SR in East Africa. This gene was transferred from "Hope" and "H-44" into common cultivars and is derived from a tetraploid "Yaroslav" emmer and is located on chromosome 3BS. This gene was widely used by Dr. N. E. Borlaug when he initiated wheat breeding in 1944 in Mexico, which resulted in varieties such as "Yaqui 50" and several high yielding semi dwarf varieties that were deployed in different wheat programs [24]. The *Sr2* gene shows pleiotropic effects with YR gene *Yr30* that also confers moderate resistance. *Sr2* gene is also associated with a morphological marker called pseudo-black chaff (PBC). Efforts to combine *Sr2* with other minor effect genes to enhance SR resistance in breeding materials at CIMMYT has resulted in several resistant or moderately resistant varieties and recently lines combining *Sr2* and Fhb1 have been developed [36]. Several new uncharacterized slow rusting genes, some potentially pleiotropic, have been identified in the recent years suggesting diversity for APR QTL and their potential in breeding.

Other adult plant resistance genes reported to confer partial or slow rusting include *Lr74 Lr75*, *Lr77,* and *Lr78* for leaf rust, *Yr11, Yr12, Yr13, Yr14, Yr16, Yr36, Yr39, Yr52, Yr59, Yr62, Yr68, Yr71, Yr75, Yr77, Yr78, Yr79, Yr80* and *Yr82* [37] for yellow rust and more recently *Sr56* identified in cultivar 'Arina' for stem rust [38].

8.6 Enhancing Resistance Durability Through Breeding Success, Setbacks and Lessons Learnt

Breeding for rust resistance has been a rigorous exercise owing to the continued evolution and selection of pathogen for new virulence to previously effective resistance genes largely through mutation or sexual recombination, or transboundary

migration of races to new wheat production environments. In most developing countries, varieties with genetic resistance are preferred by farmers; therefore resistance is a required trait for release. Even though several race-specific resistance genes have been identified only a handful of genes are used actively in breeding as several genes are only effective in certain environments and majority are easily overcome in few years of deployment. Linkage drag associated with undesired genes transferred from secondary and tertiary gene pools or originating from unadapted genetic backgrounds remains a major constraint even with modern techniques to shorten e.g. translocations. One of the best approaches to utilize these race-specific resistance genes is through pyramiding, combinations of multiple effective genes in varieties. Molecular markers linked to some of the effective resistance genes have facilitated the selection for multiple resistance genes and releases of varieties that carry them. However, the lack of diagnostic markers to select genes in different genetic backgrounds leaves no option but use field-based selections under artificial epidemics, which continues to be the most common practice in several breeding programs.

Other approach is to utilize quantitative APR in breeding, although the individual effects of pleiotropic APR genes and other QTLs are small or moderate in their effect when present alone; near-immune levels of resistance have been achieved by combining 4–5 of these genes that often have additive effects. Incorporating such type of resistance has been found to enhance durability and significant progress was made for LR resistance, and more recently for resistance to Ug99 race group and stripe rust resistance in CIMMYT germplasm using a single back cross selected bulk scheme. Although breeding for APR resistance is cumbersome initially, additive effect of multiple minor APR genes enables combinations of high disease resistance, which can be simultaneously selected together with high yields with appropriate agronomic traits and the frequency of these genes can be increased within the breeding germplasm. Comparison of grain yield performance of 697 EYT lines (Stage II) 2018–2019 derived from Mexico Shuttle and Mexico Kenya Shuttle breeding schemes identified similar frequency of lines that combine high yield potential and stem rust resistance (Fig. 8.3) and significant progress has been achieved in combining yield potential and rust resistance in CIMMYT breeding lines.

One of the prerequisites for enhancing APR is the absence of epistatic race-specific resistance gene interactions in breeding materials, which enables selection of transgressive segregants with high levels of resistance under high disease pressure. The progress in breeding APR to the Ug99 race group was facilitated by extending shuttle breeding scheme to Kenya and were able to demonstrate success in achieving high levels of complex APR to rusts at CIMMYT.

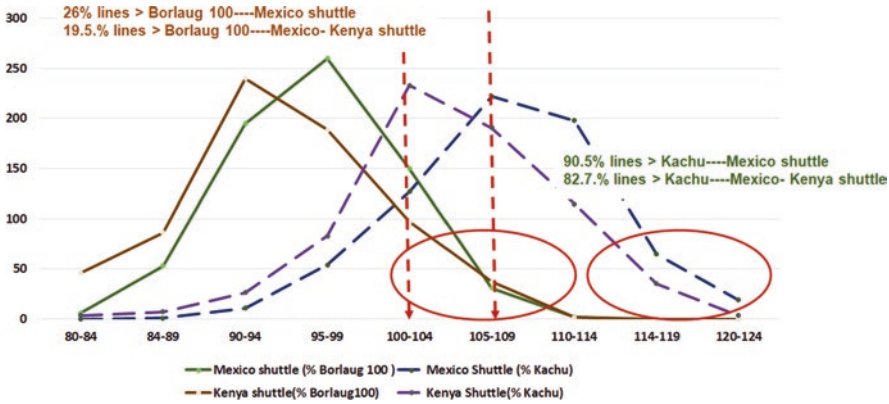

Fig. 8.3 Performance of grain yield of 697 EYT lines (Stage II) 2018–2019 derived from Mexico Shuttle and Mexico Kenya Shuttle breeding schemes

8.7 Integrating New Tools for Resistance Breeding Presents Opportunities for Wheat Improvement

The proven approach to enhance durability of genetic resistance is the deployment of combinations of multiple effective resistance genes. However, limitation to stack multiple genes is their segregation when parents possessing different genes are crossed and the need to grow large populations to identify multiple gene combinations and the need to have complementing diagnostic markers tagging the R-genes to ensure desired gene combinations are achieved. However, incomplete/moderate effect R-genes, race-nonspecific APR genes, or their combinations confers enhanced resistance levels due to additive effects, hence have been shown to be effectively selected in the field under high disease pressures [4, 24].

In the last two decades several rust resistance genes have been cloned viz. eleven SR resistance genes: *Sr13*, *Sr21*, *Sr22, Sr33, Sr35, Sr45, Sr46 Sr50, Sr55* (pleiotropic with *Lr67*), and *Sr57* (pleiotropic with *Lr34*) and more recently *Sr60* four LR resistance genes *Lr1, Lr10 Lr21*and *Lr22a* and six YR resistance genes *Yr5*, *Yr7*, *Yr10*, *Yr15*, *YrAS2388R* and *Yr36* (see Chap. 19). Also, in the last decade R gene enrichment sequencing (Ren-Seq), approaches have been widely used to clone resistance genes. Resistance genes from wild relatives can be introgressed to engineer broad-spectrum resistance in domesticated crop species using a combination of association genetics with R-gene enrichment sequencing (AgRenSeq) and a relatively new approach called MutRenSeq that combines chemical mutagenesis with exome capture and sequencing has been developed for rapid R-gene cloning [39]. Despite these advances, limited number of widely effective cloned genes and rapid evolution of new races with complex virulences and transcontinental migration reinforces a responsible strategy for their deployment.

The availability of multiple cloned resistance genes has opened the possibility of transforming wheat lines with a stack or cassette of multiple cloned effective resistance genes. This transgenic approach can help combine multiple resistance genes in

a linkage block with one another on a single translocation thereby reducing the chances of segregation upon further breeding processes and up to five cloned genes can be stacked currently transgene cassette of four R-genes (*Sr22, Sr35, Sr45* and *Sr50*), combined with the APR gene *Sr55* [40] (https://2blades.org/). However, the current regulatory framework in most countries does not allow the cultivation of transgenic and cisgenic, wheat. If future policy decisions allow use of transgenic-cassettes this approach has great potential to develop wheat varieties with durable resistance. Genome editing technology in the recent years has shown great potential to surpass the bottlenecks of conventional resistance breeding (see Chap. 29). This technology offers the modification of specific target genes in elite varieties, thus bypassing the whole process of crossing. Recent advances in gene-editing technology can also offer avenues to building resistance durability. Genome editing was found to be effective in improving powdery mildew resistance by editing *Mlo* homologs in wheat to produce a triple knockout in hexaploid wheat [41]. As gene-editing technology develops, site-specific editing of alleles may become practical in the future.

8.8 Key Concepts

Geographical distribution of three rust diseases, impact, management strategies Rust resistance, Race specific genes, Adult plant resistance genes, breeding technologies and new molecular tools in the current era to enhance resistance durability.

8.9 Conclusions

The three rust diseases continue to be a significant challenge in several wheat production environments. Major threat is due to the extreme damage these diseases can cause to susceptible varieties. Severe localized epidemics have been reported in the last two decades largely due to the lack of resistance diversity in host and constantly evolving and migrating rust races that can pose a significant risk wherein breeding for these new incursions or newly evolved races could be a recent undertaking. Genetic resistance through deployment of both race specific genes and APR though quite widely used in breeding programs, effective combinations of partially effective pleotropic race- nonspecific genes such as *Sr2*, *Lr34*, *Lr46*, *Lr67*, and *Lr68* have been found to confer durable resistance in CIMMYT germplasm. Deployment of both APR genes with combinations of multiple race specific genes can be a better strategy to enhance resistance durability. Cloning of rust genes in the last decade and development of gene-specific DNA markers can facilitate pyramiding strategies into desired wheat backgrounds with a possibility to possible to transform wheat lines with a cassette of multiple cloned resistance genes. Significant progress in the area of global rust research including monitoring and surveillance, establishment of phenotyping platforms to facilitate the testing of global wheat germplasm and the

identification and characterization of new sources of race-specific and APR genes, all of this has led to the development and rapid deployment of rust resistant cultivars target countries. CIMMYT breeding will continue to provide improved high-yielding wheat germplasm carrying high to adequate rust resistance to global wheat partners, mitigating the potential threat of these transboundary rust diseases. Even though fungicides are effective in controlling rusts and are widely used in developed countries, lack of availability at the right time and resources are still a limitation for small holder farmers and emerging concerns of new race groups requiring multiple applications (reduced fungicide efficacy) to avert losses highlights the importance of breeding and deployment of resistant germplasm to curtail epidemics.

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