Emergence and Spread of New Races of Wheat Stem Rust Fungus: Continued Threat to Food Security and Prospects of Genetic Control

Ravi P. Singh, David P. Hodson, Yue Jin, Evans S. Lagudah, Michael A. Ayliffe, Sridhar Bhavani, Matthew N. Rouse, Zacharias A. Pretorius, Les J. Szabo, Julio Huerta-Espino, Bhoja R. Basnet, Caixia Lan, and Mogens S. Hovmøller

First, eleventh, and twelfth authors: International Maize and Wheat Improvement Center (CIMMYT), Apdo. Postal, 6-641, 06600, Mexico, D.F.; second author: CIMMYT, Addis Ababa, Ethiopia; third, seventh, and ninth authors: United States Department of Agriculture–Agricultural Research Service, Cereal Disease Laboratory, University of Minnesota, St. Paul 55108; fourth and fifth authors: Commonwealth Scientific and Industrial Research Organisation (CSIRO) Plant Industry, Agriculture Flagship, GPO Box 1600, Canberra, ACT, 2601, Australia; sixth author: CIMMYT, ICRAF House, United Nations Avenue, Gigiri, Village Market-00621, Nairobi, Kenya; eighth author: University of the Free State, Bloemfontein 9300, South Africa; tenth author: Campo Experimental Valle de México INIFAP, Apdo. Postal 10, 56230, Chapingo, Edo de México, México; and thirteenth author: Department of Agroecology, Aarhus University, Forsøgsvej 1, DK-4200, Slagelse, Denmark.
Accepted for publication 6 March 2015.

ABSTRACT

Singh, R. P., Hodson, D. P., Jin, Y., Lagudah, E. S., Ayliffe, M. A., Bhavani, S., Rouse, M. N., Pretorius, Z. A., Szabo, L. J., Huerta-Espino, J., Basnet, B. R., Lan, C., and Hovmøller, M. S. 2015. Emergence and spread of new races of wheat stem rust fungus: Continued threat to food security and prospects of genetic control. Phytopathology 105:872-884.

Race Ug99 (TTKSK) of *Puccinia graminis* f. sp. *tritici*, detected in Uganda in 1998, has been recognized as a serious threat to food security because it possesses combined virulence to a large number of resistance genes found in current widely grown wheat (*Triticum aestivum*) varieties and germplasm, leading to its potential for rapid spread and evolution. Since its initial detection, variants of the Ug99 lineage of stem rust have been discovered in Eastern and Southern African countries, Yemen, Iran, and Egypt. To date, eight races belonging to the Ug99 lineage are known. Increased pathogen monitoring activities have led to the identification of other races in Africa and Asia with additional virulence to commercially important resistance genes. This has led to localized but severe stem rust epidemics becoming common once again in East Africa due to the breakdown of race-specific resistance gene *SrTmp*, which was deployed recently in the 'Digalu' and 'Robin' varieties in Ethiopia and Kenya, respectively. Enhanced research in the last decade under the umbrella of the Borlaug Global Rust Initiative has identified various race-specific resistance genes that can be utilized, preferably in combinations, to develop resistant varieties. Research and development of improved wheat germplasm with complex adult plant resistance (APR) based on multiple slow-rusting genes has also progressed. Once only the *Sr2* gene was known to confer slow rusting APR; now, four more genes—*Sr55*, *Sr56*, *Sr57*, and *Sr58*—have been characterized and additional quantitative trait loci identified. Cloning of some rust resistance genes opens new perspectives on rust control in the future through the development of multiple resistance gene cassettes. However, at present, disease-surveillance-based chemical control, large-scale deployment of new varieties with multiple race-specific genes or adequate levels of APR, and reducing the cultivation of susceptible varieties in rust hot-spot areas remains the best stem rust management strategy.

Additional keyword: black rust.

Hexaploid common wheat (*Triticum aestivum*) and tetraploid durum wheat (*T. turgidum* var. *durum*) are cultivated on more than 215 million hectares worldwide at a wide range of altitudes and latitudes, with a record estimated production by FAO of 725 million metric tons in 2014, up 7.6 million metric tons (or 1.1%) from the 2013 record level, providing approximately one-fifth of the calories and protein intake in humans (WHEAT 2014). The demand for wheat continues to increase at an annual rate of 1.6% and some estimates indicate that 60% more wheat will be needed by 2050 (WHEAT 2014).

Stem rust, or black rust, caused by *Puccinia graminis* f. sp. *tritici*, can occur wherever wheat is grown (Roelfs et al. 1992). Although not the most widespread or common among the wheat rusts, stem rust disease has the potential to cause the most damage when an epidemic occurs (Dean et al. 2012). Severe wheat stem rust epidemics occurred in the United States in 1919, 1920, 1923, 1927, 1935, 1953, and 1954 (Roelfs 1978). Average statewide wheat yield losses during these epidemics were 25.4% in Minnesota, 28.4% in North Dakota, and 19.3% in South Dakota.

Corresponding author: R. P. Singh; E-mail address: R.Singh@cgiar.org

http://dx.doi.org/10.1094/PHYTO-01-15-0030-FI

In contrast, since 1954, only localized epidemics have occurred in the United States and the rest of North America. The prevention of widespread stem rust attacks is attributed to the deployment of cultivars with combinations of multiple resistance genes and the removal of the alternate host of *P. graminis* f. sp. *tritici*, barberry (*Berberis vulgaris*) near wheat-growing regions (Kolmer et al. 1991).

Worldwide, stem rust is mostly found in regions with a continental climate where summer temperatures regularly exceed 25°C. The disease has caused wheat losses during different historical periods in Canada (Kolmer 2001), the Southern Cone of South America (German et al. 2007), continental Europe, the Indian subcontinent, Australia (Park 2007), South Africa (Pretorius et al. 2007), East Africa (Wanyera et al. 2006), and China (Knott 1989; Roelfs et al. 1992).

EMERGENCE, EVOLUTION, AND SPREAD OF THE UG99 RACE GROUP

In 1998, a new race of stem rust fungus with a notably unique virulence to resistance gene *Sr31* was found in Uganda and subsequently named Ug99 (Pretorius et al. 2000). Race-typing experiments using urediniospore samples identified Ug99 to be the race that caused the 2004 epidemics in Kenya (Wanyera et al. 2006)

and suggested that it was likely the cause of preceding epidemics that occurred after 2002 in the country. Following the North American stem rust nomenclature system (Roelfs and Martens 1988), Ug99 was designated as TTKS (Wanyera et al. 2006). Evaluations of international breeding germplasm and varieties through seedling tests in greenhouse and field screening revealed that the Ug99 race possessed broad virulence to stem rust resistance genes, especially those predominant in varieties and breeding germplasm (Jin and Singh 2006; Jin et al. 2007; Pretorius et al. 2000; Singh et al. 2006). Although the TTKS race code appeared to be unique, this designation did not reflect one of the most unique characteristics of Ug99 and overlooked its virulence to resistance genes *Sr31* and *Sr38*. This necessitated a revision of the North American stem rust nomenclature system and resulted in the addition of resistance genes *Sr24, Sr31, Sr38*, and *SrMcN* into the differential set (Jin et al. 2008). This led to the redesignation of race TTKS to TTKSK, which recognized virulence to *Sr31* and *Sr38*, one of the most significant and unique virulence combinations known in *P. graminis* f. sp. *tritici* populations worldwide.

Recognizing its potential threat to wheat production, 1970 Nobel Laureate Dr. Norman Borlaug raised the alarm and called for global action to fight the disease. The subsequent launch of the Borlaug Global Rust Initiative (BGRI) in 2005 led to a concerted international collaborative effort to combat the emerging threat of wheat rusts. A key component of BGRI is surveillance and monitoring, which has successfully tracked the evolution and spread of important stem rust races (Hodson et al. 2012).

A series of reviews by Singh et al. (2006, 2008, 2011a) have documented the significance, emergence, evolution, and geographical spread of the Ug99 race group at regular intervals. In each review, an update on the latest known status of the Ug99 race group and highlights of all major changes since 2010 are provided. New emerging stem rust threats are also featured, along with an assessment of the current status of global rust monitoring systems, which were established in response to the Ug99 threat. Seven races, phenotypically recognized as a lineage, were identified until 2010 (Singh et al. 2011a), and these were distributed across nine countries in Africa, the Middle East, and Asia. Currently, there are eight confirmed races in the Ug99 lineage and these have been detected in 13 countries (unpublished data), indicating that the pathogen has continued to evolve and expand in geographical range. All known races in the Ug99 lineage and their key characteristics are shown in Table 1.

Based on virulence phenotypes, it became evident that Ug99 should be regarded as a race group. One of the most intriguing races in the Ug99 race group is TTKSF, a member that is avirulent to *Sr31*. TTKSF was first detected in South Africa in 2000 (Boshoff et al. 2002) and was distinctive from local races based on its virulence to *Sr8b* and *Sr38*. Race TTKSF or its theoretical ancestor race PTKSF can be regarded as the most likely candidates as originators of this lineage if the Ug99 race complex resulted from asexual mutations. However, sexual recombination may have contributed to the evolution of the lineage because *B. holstii*, known to be susceptible to *P. graminis* f. sp. *tritici*, is present in proximity to wheat production areas in the highlands of eastern Africa (Y. Jin, unpublished). Isolates of *P. graminis* have been recovered from aecial infections in Ethiopia (G. Woldeab and Y. Jin, unpublished), indicating that *B. holstii* can serve as an alternate host in Ethiopia, and *P. graminis* is able to complete its life cycle.

The latest race to be identified within the Ug99 lineage is TTKSF+ (Pretorius et al. 2012). It was detected in samples collected from South Africa and Zimbabwe in 2010 and identified as the result of the susceptibility of the South African 'Matlabas' wheat. The gene affected by TTKSF+ was characterized as *Sr9h* (Rouse et al. 2014a). Race TTKSF+ could be a single-step mutation of the existing race TTKSF, which was first identified in South Africa in 2000, then Zimbabwe (2009) and Uganda (2012). Although TTKSF+ was first reported in 2010, recent recharacterization of older South African isolates has shown virulence to *Sr9h* in a TTKSF culture collected in 2003, indicating that virulence existed much earlier than reported (Z. A. Pretorius, unpublished). Virulence to resistance gene *Sr9h* brings the total number of stem rust resistance genes known to be ineffective against the Ug99 race lineage to 34, whereas at least 39 genes remain effective, including several newly described stem rust resistance genes (Table 2), which is a positive news for breeding resistant varieties.

The geographical distribution of the Ug99 race group has also expanded and the changing distribution over time is illustrated in Figure 1. The countries where Ug99 race group members have been identified include Egypt, Ethiopia, Eritrea, Iran, Kenya, Mozambique, Rwanda, South Africa, Sudan, Tanzania, Uganda, Yemen, and Zimbabwe. Of these countries, Egypt, Eritrea, Rwanda, and Mozambique have confirmed the presence of Ug99 lineage races since 2010. Egypt is the most recent country to confirm detection. Since 2010, specific races have been identified in an expanded range. Notably, race TTTSK (Ug99 + *Sr36* virulence) was detected in three additional countries, including Ethiopia, Uganda, and Rwanda, and race PTKST (virulent on *Sr24*) was also detected in three additional countries, including Eritrea, Mozambique, and Zimbabwe. Detection of race TTKSF (avirulent on *Sr31*) in Uganda (2012) is also noteworthy because this race was previously identified only in southern Africa, which implies a connection between southern and eastern Africa epidemiological zones. Simple-sequence repeat (SSR) fingerprinting by Visser et al. (2009) showed that race TTKSF occurring in South Africa was significantly different from other existing non-Ug99 races; thus, it is

TABLE 1. *Puccinia graminis tritici* races belonging to Ug99 lineage identified in 2014 in various countries, with avirulence or virulence status on discriminating resistance genes

Race[a]	Common alias	Resistance genes and avirulence (A) or virulence (V) status					Confirmed countries (year detected)
		Sr31	*Sr21*	*Sr24*	*Sr36*	*Sr9h*	
TTKSK	Ug99	V	V	A	A	A	Uganda (1998), Kenya (2001), Ethiopia (2003), Sudan (2006), Yemen (2006), Iran (2007), Tanzania (2009), Eritrea (2012), Rwanda (2014), Egypt (2014)
TTKSF		A	V	A	A	A	South Africa (2000), Zimbabwe (2009), Uganda (2012)
TTKST	Ug99+*Sr24*	V	V	V	A	A	Kenya (2006), Tanzania (2009), Eritrea (2010), Uganda (2012)
TTTSK	Ug99+*Sr36*	V	V	A	V	A	Kenya (2007), Tanzania (2009), Ethiopia (2010), Uganda (2012), Rwanda (2014)
TTKSP		A	V	V	A	A	South Africa (2007)
PTKSK		V	A	A	A	A	Kenya (2009), Ethiopia (2007), Yemen (2009)
PTKST		V	A	V	A	A	Ethiopia (2007), Kenya (2008), South Africa (2009), Eritrea (2010), Mozambique (2010), Zimbabwe (2010)
TTKSF+		A	V	A	A	V	South Africa (2010), Zimbabwe (2010)

[a] Race designation follows the North American nomenclature system described by Jin et al. (2008). Race TTKSF+ is given a temporary name because it exceeds the current North American 20-differential gene set.

highly likely that the Ug99 lineage was a foreign introduction. In fact, the detection of TTKSF in Uganda once again supports the hypothesis of rust inoculum exchange between southern and eastern Africa. Additional samples of stem rust are currently undergoing analysis and it is likely that the pattern of continued evolution within the Ug99 lineage and geographical expansion will continue in the future.

RACE TKTTF AND ITS IMPLICATIONS ON UG99-RESISTANT CULTIVARS

The global rust monitoring system established in response to the Ug99 threat is engaged in detecting and monitoring other races virulent to commercially important resistance genes, leading to the detection of various races with a broad geographic footprint. Those efforts led to the detection of RRTTF and TKTTF, two races with wide distribution throughout East Africa, the Middle East, and South Asia (Fig. 2). Race RRTTF was witnessed in Iran in 1997 and was still present in 2007 (Nazari and Mafi 2013). RRTTF is also present in Ethiopia (2007), Yemen (2007), and Pakistan (2009). Admassu et al. (2009) reported a widely distributed race, RRTTR, in Ethiopia from stem rust samples collected during 2006 and 2007 based on a differential set described in Fetch and Dunsmore (2004) in which *Sr24*, *Sr31*, and *Sr38* were not used. It is considered likely that the race reported by Admassu et al. (2009) was actually RRTTF hence this race was likely present in Ethiopia at least since 2006.

Races similar to TKTTF occurred in Turkey in the 1990s and still predominate (Mert et al. 2012). They have also been detected in Iran (2010), Lebanon (2012), Ethiopia (2012), and Egypt (2013). This race group does not belong to the Ug99 lineage based on avirulences to *Sr11* and *Sr31* and molecular fingerprints (Olivera Firpo et al. in press). The presence of stem rust races with identical virulence profiles throughout this vast region implies that there are inoculum exchanges.

In 2013, Ethiopia experienced localized but severe stem rust epidemics in the southern wheat production region and the epidemics continued into the 2014 crop season (Olivera Firpo et al. in press). Race analyses of samples from the epidemic regions detected race TKTTF to be the causal race behind these epidemics. Race TKTTF is highly virulent to the widely grown 'Digalu' wheat, which possesses resistance gene *SrTmp* that is effective against the Ug99 race group and ineffective against TKTTF. Through the global rust-monitoring system established by BGRI, not only was the causal race identified but also its dispersal patterns were elucidated and germplasm screening activities initiated to identify existing or new resistant cultivars.

As discussed, available evidence indicates the strong likelihood of an origin and presence for race TKTTF in the Middle East region (Newcomb et al. 2013). The first confirmed detection of race TKTTF in Ethiopia was in August 2012 but the race remained at a low frequency and escaped further detection until early October 2013. By mid-November 2013, a severe epidemic on the most widely grown variety (Digalu) had started. This cultivar, despite resistance to the Ug99 race group and prevalent races of stripe rust, was highly susceptible to race TKTTF and suffered grain yield losses of up to 100% covered an area exceeding 10,000 ha. Despite an early warning, which led to a major national awareness campaign and extensive fungicide use, the absence of seed of varieties resistant to stem rust race TKTTF made effective control impossible in the 2014–15 growing season. Thousands of hectares were severely affected by the devastating epidemic. The enormous inoculum load poses a threat for future crops in Ethiopia and the surrounding region and also increases the risk of mutation and further evolution.

Reports of stem rust in some fields of 'Robin', a variety released in 2011 and postulated to carry resistance gene *SrTmp*, emerged in the wheat crop sown in the 2014 off-season in Kenya. In the 2014 main crop season, some fields of Robin, especially those of small farmers, suffered significant losses, although losses to stem rust for large-scale farmers were limited due to the use of fungicides. Host reactions on International Maize and Wheat Improvement Center (CIMMYT) wheat breeding materials and checks possessing different resistance genes at Njoro indicate a likely evolution and selection of *SrTmp* virulence in the Ug99 lineage. Analyses are in progress to determine whether the stem rust outbreak on Robin in Kenya was due to race TKTTF or yet-to-be characterized variants of Ug99 with added virulence to *SrTmp*.

Strong messages emerge from the Ethiopian TKTTF experience. The presence of a race in one region and incursion into a new region can have a devastating impact. The speed with which a stem rust epidemic can develop and spread is incredibly fast. Effective control of stem rust, especially under small-holder farming systems, is virtually impossible in the absence of resistant varieties. The current stem rust situation in Ethiopia reinforces the need for

TABLE 2. Origin and usefulness of cataloged and temporarily designated *Sr* genes in conferring seedling or adult plant resistance to all *Puccinia graminis* f. sp. *tritici* races belonging to the Ug99 lineage

	Stem rust resistance (*Sr*) genes	
Origin of *Sr* genes	Ineffective	Effective
Triticum aestivum[a]	5, 6, 7a, 7b, 8a, 8b, 9a, 9b, 9f, 9h, 10, 16, 18, 19, 20, 23, 30, 41, 49, 54, McN, Wld-1	15[a,b,c], 28[b], 29[c,d], 42[b,c], 48, 55[c,e], 56[c,e], 57[c,e], 58[c,e], Tmp (or Sha7)[b,c], Huw234[b,c], ND643[c], Yaye[c]
T. turgidum	9d, 9e, 9g, 11, 12, 17	2[c,e], 13[b,c], 14[b,c]
T. monococcum	21	22, 35[b]
T. timopheevi	36	37[d]
Aegilops speltoides	…	32[d], 39[d], 47[f]
A. tauschii	…	33[c], 45[c,d], 46[b,f], TA10171[f], TA10187[b,f], TA1662[b,f]
A. searsii	…	51
A. geniculata	…	53
Dasypyrum villosum	…	52
T. comosum	34	…
T. ventricosum	38	…
T. araraticum	…	40[d]
Thinopyrum elongatum	24	25[b], 26, 43[b,d]
T. intermedium	…	44[b,d]
Secale cereale	31	27[b], 50[b], 1RS(Amigo)[b,c]

[a] Data from multiple research groups are not consistent. Initial studies determined that *Sr15* was ineffective; however, recent data shows avirulence in Ug99 lineage.
[b] Virulence for the gene is known to occur in other races.
[c] Level of resistance conferred in the field usually inadequate under high disease pressure.
[d] Unsuitable for utilization due to linkage with undesirable traits in the translocation.
[e] Confers slow rusting adult plant resistance.
[f] Not tested for resistance to Ug99 in field trials to determine effectiveness.

effective global rust surveillance and monitoring, the critical need for the continued development and promotion of durable rust resistant varieties, and the diversification of varietal and cropping systems.

RACES JRCQC AND TRTTF AND THEIR VIRULENCE COMBINATIONS TO DURUM WHEAT

A large proportion of the North American and CIMMYT durum wheat lines, selected for Ug99 resistance trials in Kenya in 2005, became susceptible when tested in a field stem rust nursery in Debre Zeit, Ethiopia, suggesting the presence of stem rust races that were virulent to the TTKSK-effective genes present in durum wheat germplasm. Race-typing studies identified two races, JRCQC and TRTTF, the latter also occurring in Yemen (Olivera et al. 2012). Both JRCQC and TRTTF possess virulence on stem rust resistance genes *Sr13* and *Sr9e*, two genes constituting major components of stem rust resistance in North American and CIMMYT durum cultivars and germplasm. In addition to *Sr9e* and *Sr13* virulence, race TRTTF is virulent to at least three stem rust resistance genes

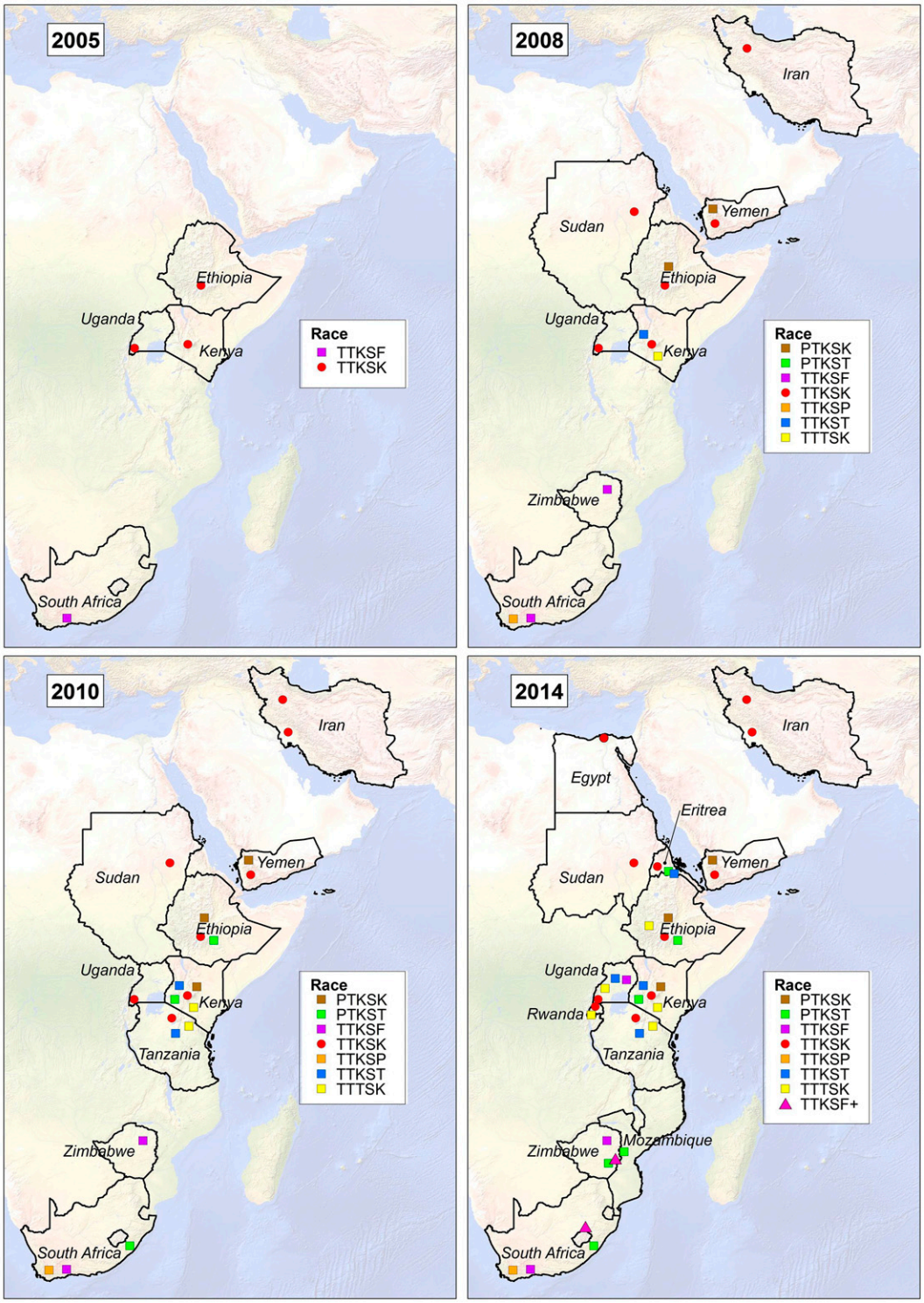

Fig. 1. Detection and distribution of *Puccinia graminis* f. sp. *tritici* races belonging to the Ug99 (TTKSK) lineage in 2005, 2008, 2010, and 2014.

that are effective to race TTKSK, including *Sr36*, *SrTmp*, and a temporarily designated resistance gene *Sr1RS* (Amigo) located on 1AL.1RS rye translocation. Race TRTTF is the first known race with virulence to the stem rust resistance gene *Sr1RS* (Amigo), which represents one of the few genes effective against Ug99 race group in winter wheat cultivars from the United States. The presence of these races in Ethiopia led to the establishment of screening wheat materials, especially durum wheat, at the Debre Zeit field site in Ethiopia under the BGRI to identify resistant sources that can contribute to durum wheat improvement.

P. GRAMINIS F. SP. *TRITICI* POPULATION GENETICS AND MOLECULAR DIAGNOSTICS

In the last decade, DNA-based tools have begun to be used for population genetics studies of *P. graminis* f. sp. *tritici*. SSR markers were developed (Szabo 2007; Visser et al. 2011; Zhong et al. 2009) and used to examine regional *P. graminis* f. sp. *tritici* populations in Ethiopia (Admassu et al. 2010), South Africa (Visser et al. 2009), and the United States (Stoxen 2012). SSR markers were also used to differentiate the Ug99 race group from other *P. graminis* f. sp. *tritici* lineages (Jin et al. 2008, 2009; Visser et al. 2011). However, these SSR makers have not been very useful in differentiating different members of the Ug99 race group.

Recently, the genome sequencing of a U.S. isolate of *P. graminis* f. sp. *tritici* (Duplessis et al. 2011) and the resequencing of several additional isolates provided a powerful tool for genetic studies and development of molecular diagnostic tools. A preliminary study in which 70 *P. graminis* f. sp. *tritici* isolates were resequenced demonstrated that the Ug99 race group, with 18 isolates representing 7 races, represents a single genetic lineage that has evolved relatively recently (L. J. Szabo and C. A. Cuomo, unpublished data). Using the genomic sequence data from this study, a polymerase chain reaction (PCR)-based diagnostic method was developed for the Ug99 lineage (Szabo 2012). This assay is highly specific for the Ug99 genetic lineage and is able to discriminate between several of the members of the Ug99 race group (TTKST, TTKST, TTTSK, TTKSF, TTKSP, and PTKST/PTKSP). In addition, the development of this assay has identified multiple genotypes per race phenotype (L. J. Szabo, unpublished data). For example, four different genotypes have been identified within the race TTKSK. Currently, this assay is being used to monitor the movement and distribution of members of the Ug99 genetic lineage.

The availability of an extensive single-nucleotide polymorphism (SNP) database for *P. graminis* f. sp. *tritici* has also facilitated the development of a high-throughput SNP assay (*P. graminis* f. sp. *tritici* SNP Chip) (L. J. Szabo and J. L. Johnson, unpublished data). This SNP assay was used to genotype isolates of race TKTTF collected from Ethiopia during the stem rust epidemic of 2013 to 2014 (Olivera Firpo et al. in press). Two distinct genetic types were identified within isolates of the TKTTF race group and it was discovered that these genetic types are part of the same genetic lineage that contains isolates of race RRTTF, a common race found in Ethiopia in recent years. However, the TKTTF/RRTTF lineage is very distinct from the Ug99 genetic lineage. At this point, the origin of the TKTTF genetic group is not clear, and genotyping of additional samples from Africa, the Middle East, and Central Asia is needed for determining it.

RESISTANCE IN WHEAT TO CURRENT *P. GRAMINIS* F. SP. *TRITICI* RACES

Race-specific resistance genes. The broad virulence spectrum present in Ug99 and its derivatives has been implicated with partial to high susceptibility of numerous important wheat varieties sown on over 80 to 95% of total wheat area, as well as breeding materials, regardless of the originating countries (Singh et al. 2006, 2008, 2011b). Virulences for resistance genes *Sr24*, *Sr31*, *Sr36*, and *Sr38* were considered the most significant because these genes were providing resistance to other predominant *P. graminis* f. sp. *tritici* races and were present at a relatively high frequency in adapted wheat backgrounds. In all, 39 resistance genes continue to confer moderate to adequate resistance to the Ug99 race group; however, other races are known with virulence to 18 of these genes (Table 2). Moreover, several of the remaining effective genes were recently transferred to wheat from related genera and species that likely bring negative linkage drag present in the translocation. Development of successful commercial cultivars in the future will demonstrate their commercial utility.

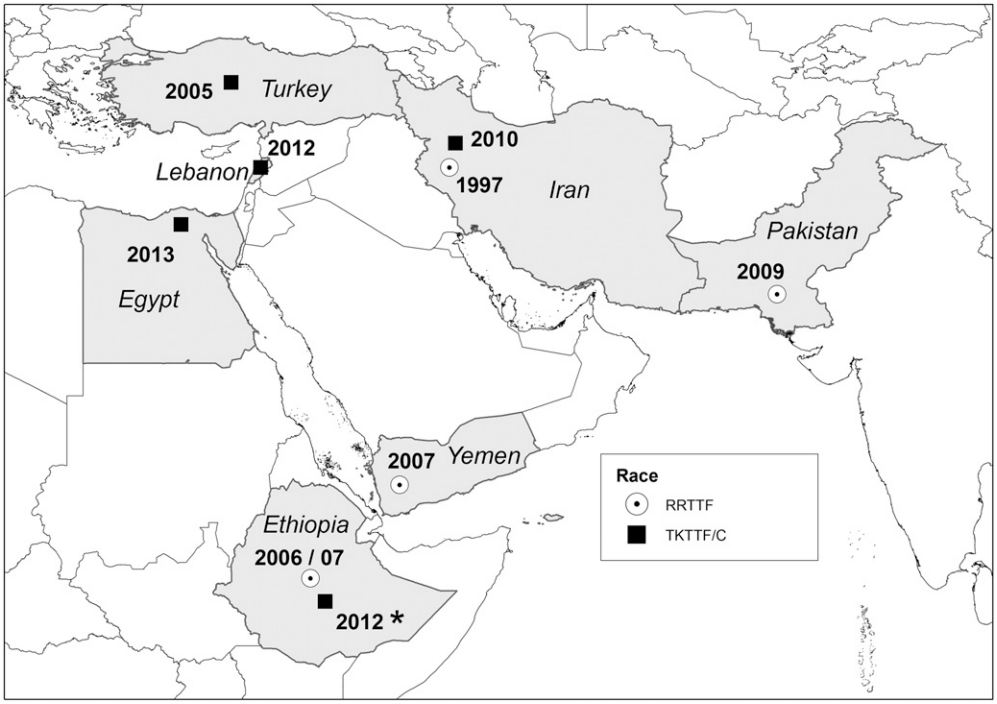

Fig. 2. Detection and distribution of *Puccinia graminis* f. sp. *tritici* races RRTTF and TKTTF (or TKTTC).

Genes *Sr22*, *Sr25*, *Sr26*, *Sr33*, *Sr35*, *Sr45*, and *Sr50* are, at present, possibly the most useful race-specific resistance genes, provided that they are used in combinations. Some other temporarily designated genes (Table 2) that are common in high-yielding wheat germplasm offer additional possibilities of gene combinations. If proper care in their deployment is not taken, then these genes are expected to lose effectiveness in eastern and southern Africa, where high populations of the Ug99 race group and other races exist each year. The "boom-and-bust" phenomenon was once again seen with the utilization of gene *SrTmp* (or *SrSha7*) in Digalu and Robin wheat in Ethiopia and Kenya, respectively. Recent studies (Ghazvini et al. 2012; Hiebert et al. 2011; Lopez-Vera et al. 2014a) have shown that *Sr42*, *SrTmp*, *SrSha7*, *SrNini*, and *SrCad* are colocated on chromosome arm 6DS; however, virulence to resistance gene *SrTmp* in Digalu and Robin shows that two different resistance genes, or alleles, are likely involved because temporarily designated resistance gene *SrCad* carried by Canadian 'AC Cadillac' wheat and breeding lines carrying *SrNini* remained resistant. Relationships between *Sr42*, *SrCad*, and *SrNini* need elucidation because all three map in the same region on chromosome arm 6DS.

Adult plant resistance genes. Although adult plant resistance (APR) to stem rust has been known for a long time, *Sr2* was the only well-studied gene. *Sr2* and a linked or pleiotropic gene, *Pbc*, that conferred pseudo-black chaff (PBC) were transferred to hexaploid wheat 'Hope' and 'H44-24a' from tetraploid emmer wheat 'Yaroslav' by E. S. McFadden in the United States (McFadden 1930, 1939). An independent transfer was also possibly made from 'Khapli' emmer by W. L. Waterhouse in Australia, who developed hexaploid wheat 'Khapstein'. *Sr2* is known to confer modest APR that can be inadequate under high disease pressure; however, both semidwarf and tall wheat varieties with *Sr2* and expressing high levels of resistance are also known (Hare and McIntosh 1979; McIntosh 1988; Njau et al. 2010; Rajaram et al. 1988; Singh and McIntosh 1986). Knott (1982) showed that an adequate level of multigenic resistance to stem rust was achieved by accumulating approximately five minor resistance genes of additive effects. This led to the use of the terminology "*Sr2*-complex" when a variety displays PBC in conjunction with high resistance levels because of the lack of genetic information on other contributing resistance genes. Increased emphasis on identifying and characterizing new sources of resistance to stem rust in the last decade led to various molecular mapping studies using biparental populations and association analyses.

Yu et al. (2014) recently summarized results from various published studies, developed consensus maps, and identified several genomic regions that carry minor APR genes or quantitative trait loci (QTL). The most significant finding has been identifications of three pleiotropic APR genes—*Sr55* (= *Lr67/Yr46/Pm46*), *Sr57* (= *Lr34/Yr18/Pm38/Sb1/Bdv1*), and *Sr58* (= *Lr46/Yr29/Pm39*)—and a fourth gene, *Sr56* (Bansal et al. 2014; Herrera-Foessel et al. 2014; Singh et al. 2012, 2013a). Mapping studies using six CIMMYT semidwarf wheat varieties ('Kingbird', 'Kiritati', 'Pavon 76', 'Muu', 'Juchi', and 'Huiviris') with high levels of stem rust resistance in Kenya showed a continuous variation for stem rust severity in recombinant inbred line (RIL) populations and the presence of three to five QTL in each RIL population (Bhavani et al. 2011; Njau et al. 2013; Singh et al. 2013b). Interestingly, *Sr2* was present in all resistant parents and was the most important APR gene conferring resistance to stem rust. *Sr57* and *Sr58* were also shown to confer APR along with other QTL. A recent study by Rouse et al. (2014b) using a biparental mapping population from the cross of durable APR-carrying but *Sr2*-lacking 'Thatcher' and *Sr57*-carrying 'McNeal' showed the presence of three APR QTL on chromosome arms 3BS, 1AL, and 2BS in addition to *Sr57* on 7DS. The 3BS QTL overlapped to the recessive seedling race-specific gene *Sr12*, a gene also transferred to hexaploid wheat from the tetraploid durum 'Iumillo' and known to be ineffective to the Ug99 race group. The *Sr12* region was also implicated with APR in the Thatcher-derived U.S. 'Chris' wheat to an Australian *P. graminis* f. sp. *tritici* race (Singh and McIntosh 1987). However, whether *Sr12* actually conferred APR, even though defeated, needs to be established, and the presence of a linked APR QTL cannot be ruled out.

It is expected that accumulating multiple slow-rusting APR genes will result in wheat varieties that express high levels of durable stem rust resistance. This kind of resistance is especially important to curtail or reduce the evolution and survival of new virulent races in the East African highlands and other areas where wheat cultivation is continuous year round. High levels of APR to the Ug99 race group, identified in some semidwarf lines soon after the initiation of field screening in Kenya and Ethiopia (Njau et al. 2010; Singh et al. 2008), have allowed targeted breeding efforts to build this complex resistance in new breeding materials at CIMMYT.

RESISTANCE IN WHEAT VARIETIES AND BREEDING GERMPLASM

The emphasis toward phenotyping and breeding to identify and develop stem-rust-resistant wheat varieties increased during the last decade under the BGRI umbrella and supported by the Durable Rust Resistance in Wheat (DRRW) project. Over 350,000 and 87,000 wheat accessions that include released varieties, breeding materials, genetic resources, and mapping populations from up to 32 countries in a single year have been evaluated in Kenya and Ethiopia, respectively, at field sites at Njoro, Kenya (operated by the Kenya Agricultural and Livestock Research Organization, KALRO) and Debre Zeit, Ethiopia (operated by Ethiopian Institute of Agricultural Research, EIAR) (Fig. 3). High emphasis is given to screening durum wheat at Debre Zeit due to the presence of races that overcome commonly present resistance genes in this species.

A clear trend of increased resistance is evident in wheat germplasm screened at Kenya during the last 5 years (Fig. 4), with approximately 20% of entries showing good levels and another 20% showing intermediate levels of resistance on the average for all countries. To illustrate further, resistance to the Ug99 race group observed during the 2014 main season at Njoro field in wheat varieties and in advanced lines in registration trials from India, Pakistan, and Ethiopia indicated that 38.4, 56.7, and 69.4% of entries, respectively, showed high to moderate resistance (<30% disease severity) (Fig. 5). This level of resistance is expected to be sufficient for wheat-growing areas of India and Pakistan; however, for Ethiopia, 32.8% entries grouped under near-immune and resistant categories should be a better option for deployment because stem rust is present throughout the year in the highlands of East Africa, causing an early build up of diseases in favorable years.

Resistance in wheat varieties and breeding materials from the United States of America. After the emergence of Ug99, a study by Jin and Singh (2006) assessed the level of resistance in U.S. wheat varieties to Ug99 and found that though 93 and 94% of the hard-red spring wheat varieties assessed were resistant to the two most virulent *P. graminis* f. sp. *tritici* races present in the United States, only 16% varieties were considered resistant to the original Ug99 race TTKSK. Resistant hard-red spring wheat varieties possessed stem rust resistance genes *Sr24* or *SrTmp* and included 'Ember', 'Guard', 'Ivan', 'Keene', and 'Stoa', and were released prior to 2000. Since 2006, wheat breeders throughout the United States have assessed their advanced breeding lines for field stem rust response at the field site in Njoro, Kenya and for seedling stem rust responses to a select array of *P. graminis* f. sp. *tritici* races, including the Ug99 race group, at the United States Department of Agriculture–Agricultural Research Service (USDA-ARS) Cereal Disease Laboratory (CDL). As a result of this work, two spring wheat varieties, 'Tom' (Anderson et al. 2012) and 'Linkert', released by the University of Minnesota in 2008 and 2013, respectively, exhibited APR to the Ug99 race group.

Screening of 174 U.S. spring wheat varieties and breeding lines in 2013 indicated that only 4% were resistant as seedlings to race TTKSK (Fig. 6). When the same germplasm was screened with *P. graminis* f. sp. *tritici* races TRTTF and TKTTF, only two breeding lines displayed seedling resistance to all three races. Field screening at Njoro and Debre Zeit combined with the seedling responses indicated that 4.6% of the lines displayed susceptible infection types to race TTKSK and exhibited an average disease severity of less than 30% across the four seasons (Fig. 6).

Stem rust has historically not caused severe yield losses in winter wheat in the United States. In total, 48% of hard red winter wheat and 27% of soft red winter wheat cultivars and breeding lines were demonstrated to be resistant to race TTKSK (Jin and Singh 2006). Genes conferring resistance included *Sr24*, *Sr36*, *SrTmp*, and *Sr1RS* (Amigo). The emergence of races TTKST and TTTSK in the Ug99 lineage with additional virulences to *Sr24* and *Sr36*, in addition to race TRTTF with virulence to *SrTmp* and *Sr1RS* (Amigo), suggests that nearly all U.S. winter wheat is susceptible to at least one of the *P. graminis* f. sp. *tritici* races detected in Africa or Asia in the last decade.

U.S. researchers have made substantial progress in developing molecular markers linked to available Ug99 resistance genes, identification of new Ug99 resistance genes, and facilitation of foreign disease-screening nurseries. However, U.S. wheat breeders appear to have not succeeded thus far in selecting Ug99 resistance, with few exceptions (e.g., Tom and Linkert). Emphasis on improving grain yield, abiotic stress tolerance, protein content, processing quality, and resistance to diseases or races that are already present in the United States are likely to be high priorities for U.S. wheat breeders.

Breeding for stem rust resistance in CIMMYT's international spring wheat germplasm. The improved spring wheat germplasm developed each year by CIMMYT in Mexico is distributed worldwide annually through international trials and nurseries. These trials and nurseries are grown by cooperators in both public and private sectors and are used as sources of new genetic diversity for various traits of interest. National breeding programs in Asia, the Middle East, Africa, Latin America, and southern European countries also select adapted lines for direct release as varieties. This flagship breeding program was initiated during the mid-1940s by Dr. Norman Borlaug, who introduced shuttle breeding to shorten breeding time by growing segregating populations and other breeding materials twice each year at two distinct field sites, Ciudad Obregon and Toluca, in Mexico. This selection scheme also exposed breeding materials to two very distinct climatic conditions and multiple diseases of global importance and gave rise to widely adapted and input-responsive semidwarf varieties that triggered what subsequently became known as the "Green Revolution". In the post-Green Revolution period, new spring wheat germplasm developed in Mexico has resulted in several successful varieties grown in large areas in many countries (Lantican et al. 2005).

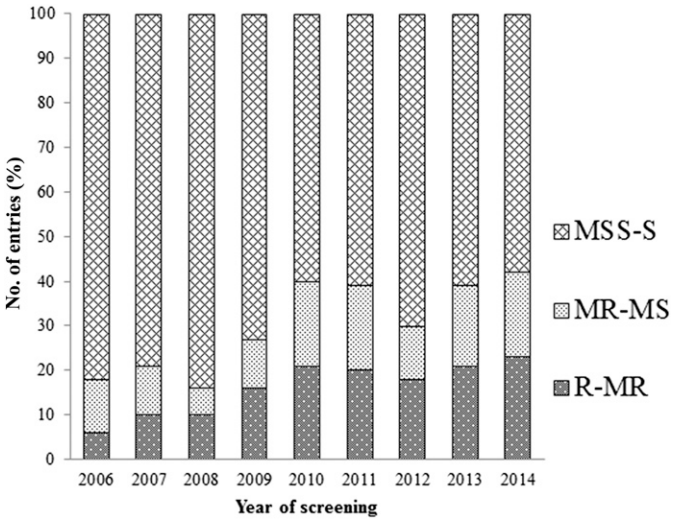

Fig. 4. Resistance to Ug99 race group of stem rust fungus in wheat materials screened at Njoro, Kenya during 2006 to 2014.

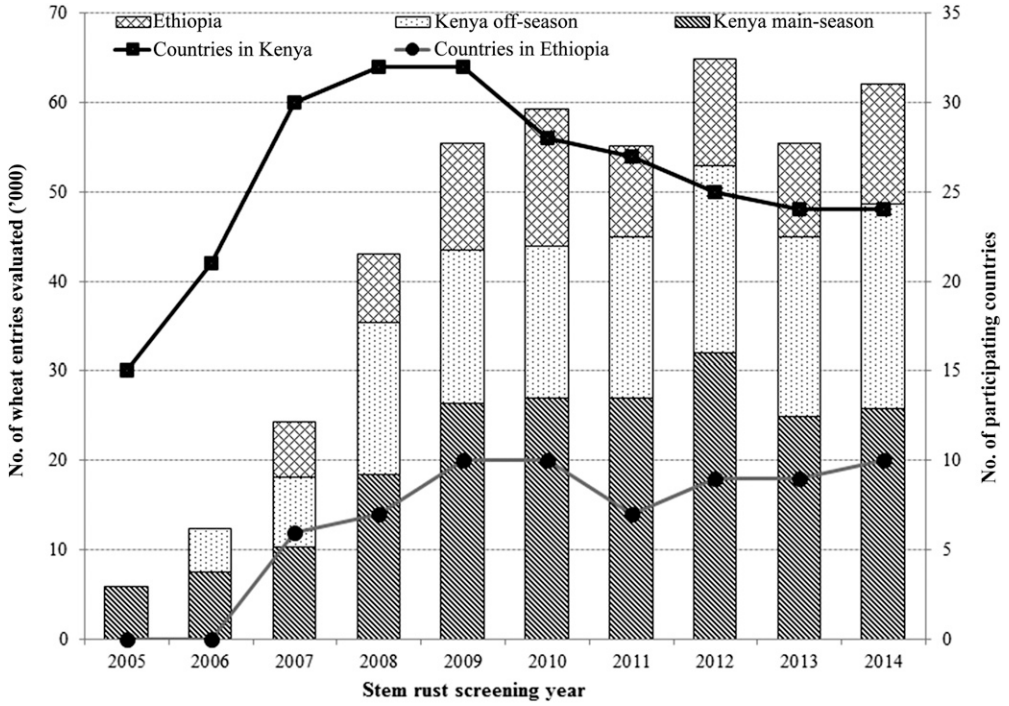

Fig. 3. Wheat varieties, genetic resources, breeding materials, and mapping populations screened in field trials at Njoro, Kenya and Debre-Zeit, Ethiopia for resistance to stem rust from 2005 to 2014.

Wheat lines identified as resistant to Ug99 (Njau et al. 2010) in field screening since 2005 have been distributed worldwide since 2007 as sequentially numbered "Stem Rust Resistance Screening Nurseries" (SRRSN) after verifying the resistance through repeated field testing at Njoro and seedling phenotyping at biosafety greenhouses at the USDA-ARS CDL. Considering the continued importance of CIMMYT wheat germplasm in enhancing the productivity and sustainability of wheat in eastern Africa and other regions where Ug99 race group was identified and predicted to migrate through wind trajectories (Singh et al. 2008, 2011a), a targeted breeding effort was initiated in 2006 to develop high-yielding spring wheat germplasm by utilizing a "Mexico-Kenya shuttle-breeding scheme" that allows growing and selecting segregating materials in Mexico and Njoro, Kenya twice a year (Singh et al. 2011b). Lines identified as Ug99 resistant in 2005 and 2006 and other sources of Ug99-effective race-specific resistance genes were used as donors of resistance and crossed with high-yielding materials. A high emphasis was given to building APR because some wheat lines with high levels of APR could be identified soon after screening was initiated (Njau et al. 2010), and it also became obvious that Ug99 was evolving and continuing to add new virulences.

Approximately 1,800 F_3/F_4 generation populations are currently selected at Njoro, Kenya during the off-season (January to May) under high stem rust pressure. The resulting F_4/F_5 populations are then sown again at Njoro in the main season for another round of selection. The advanced lines derived from these populations are brought back to Njoro from Mexico for phenotyping during three to four seasons. The most resistant lines are also phenotyped with Ug99 and other select arrays of races at the seedling stage by CDL. All data for various traits, generated over 2 years, are used in selecting approximately 600 of the best lines that are then distributed internationally through targeted yield trials and screening nurseries. The highest recorded stem rust severity data during the four seasons of screening is used to give a final resistance rating to wheat lines that carry APR, and we also postulate race-specific resistance genes effective against the Ug99 lineage in case distributed lines carry them. Seedling phenotyping data combined with pedigree and linked molecular marker (if available) information are used in postulating race-specific resistance genes in wheat lines distributed internationally.

The first group of high-yielding wheat lines derived from targeted breeding was distributed internationally in 2011, and subsequent distribution has continued since then (Singh et al. 2011a,b). A summary of resistance to the Ug99 race group in 656 wheat lines to be distributed in 2015 as international yield trials and screening nurseries is given in Table 3. The ninth SRRSN, distributed for sowing in the 2014–15 crop season in various countries, included 235 of the most resistant lines based on three seasons of screening. Approximately half of the 656 lines possessed high to moderate levels of APR. Lines with "near-immune resistant" (NIR), "resistant" (R), and "resistant to moderately resistant" (R-MR) resistance categories (i.e., maximum recorded stem rust severities of 1, 5 to 10, and 15 to 20%, respectively) are better candidates for

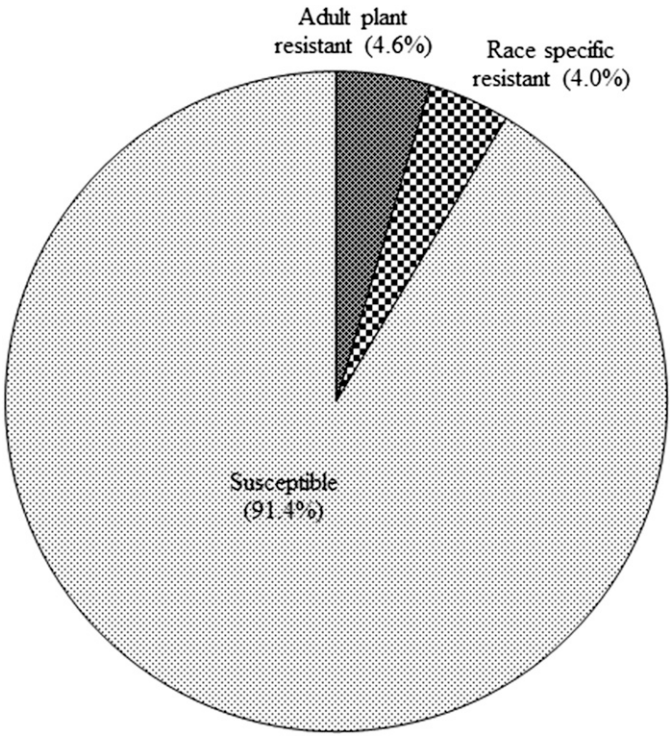

Fig. 6. Proportion of United States spring wheat germplasm susceptible, seedling (all-stage) resistant, and adult plant resistant to the Ug99 race group of *Puccinia graminis* f. sp. *tritici*. Field response was measured in disease nurseries dominated by races TTKST in Njoro, Kenya and TTKSK in Debre Zeit, Ethiopia. Germplasm included 174 varieties and breeding lines from the University of Minnesota, South Dakota State University, Montana State University, University of Idaho, Washington State University, and University of California at Davis. Lines classified as adult plant resistant displayed average disease severity less than 30% in four environments or seasons in Africa.

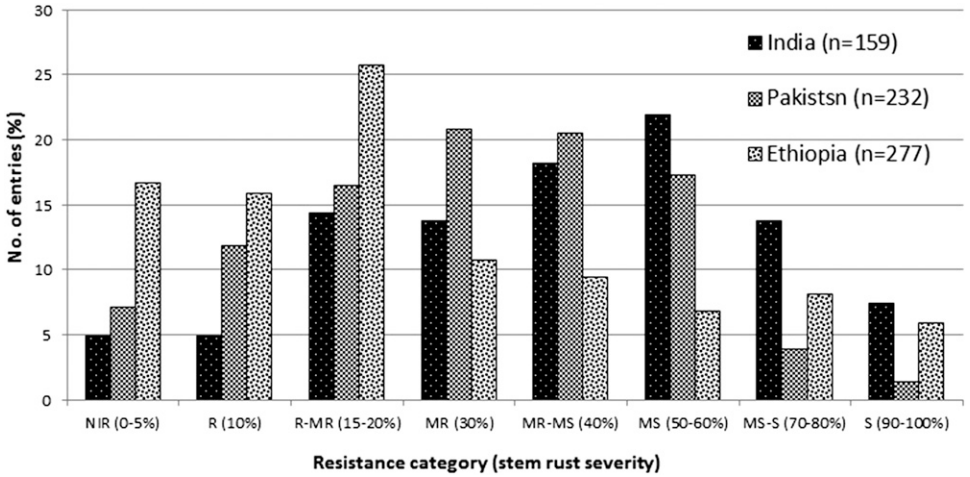

Fig. 5. Resistance to *Puccinia graminis* f. sp. *tritici* race TTKST belonging to Ug99 lineage in wheat varieties and varietal candidates from India, Pakistan, and Ethiopia when tested in field trials at Njoro, Kenya during the 2014 main season.

grain-yield performance evaluation and further stem rust testing at field sites in the eastern African highlands for identifying varieties that combine high yields with high and stable APR. Some lines are already in advanced stages of national varietal performance testing (Fig. 5). In wheat-growing regions where stem rust is not endemic, wheat lines with resistance categories "moderately resistant" (MR) and "moderately resistant to moderately susceptible" (MR-MS), with highest stem rust severities of 30 and 40%, respectively, are likely to confer adequate resistance. Using these lines enhances the possibility of identifying varieties with increased yield potential, resistance to other important diseases, climate resilience, and preferred processing quality.

The presence of various race-specific resistance genes was also postulated and found to be present in 21.5% of the lines (Table 3). Temporarily designated resistance genes *SrND643*, *SrHuw234*, *SrYaye*, and *SrNini* were mapped on chromosome arms 4AL, 2BL, 2BS, and 6DS (Basnet et al. 2015; Lopez-Vera et al. 2014b; Yu et al. 2014) but others need further studies to determine if they are different. These genes are associated with inconsistent seedling reactions and disease severities ranging from low to intermediate on wheat lines carrying them, indicating that their expression is enhanced in the presence of APR genes. This complicates their precise phenotyping in mapping populations; however, their combinations with APR genes could be developed by selecting plants with lower disease severities and reduced infection types. Resistance gene *SrTmp* was effective until 2014 off season to *P. graminis* f. sp. *tritici* races used for screening at Njoro. However, in the 2014 main season, virulence to *SrTmp* was well distributed in field nurseries and the reaction of several *SrTmp*-carrying wheat lines, including popular variety Robin, changed. In all, 61 lines, previously postulated to carry *SrTmp*, showed varying levels of APR in resistance categories R (2 lines), R-MR (4 lines), MR (7 lines), MR-MS (8 lines), MS (21 lines), MS-S (13 lines), and S (6 lines), supporting the previously reported results by Singh et al. (2011a) that APR genes enhance the expression of moderately effective race-specific resistance genes such as *SrTmp* (= *SrSha7*). Hiebert et al. (2011) reported the enhancement of field resistance conferred by *SrCad* in the presence of pleiotropic APR gene *Sr57/Lr34* in Canadian AC Cadillac wheat. Therefore it is recommended that molecular-marker-assisted incorporation of race-specific resistance genes should be done in conjunction with field-based phenotyping.

TABLE 3. Resistance to the Ug99 race group of stem rust fungus in 656 spring wheat lines that will be distributed internationally through various CIMMYT trials and nurseries

Resistance category[a]	Highest disease severity (%)[b]	Entries	
		N	%
High-adequate APR (50.2%)			
NIR (near-immune resistant)	1	1	0.2
R (resistant)	5–10	28	4.3
R-MR (resistant to moderately resistant)	15–20	86	13.1
MR (moderately resistant)	30	84	12.8
MR-MS (moderately resistant to moderately susceptible)	40	130	19.8
Race-specific (21.5%)			
Sr13	5–40	21	3.2
Sr25	1–20	10	1.5
Sr26	30	1	0.2
Sr1R(Amigo)	30–50	2	0.3
SrBau	1–30	47	7.2
SrHuw234	5–40	24	3.7
SrYaye	1–30	15	2.3
SrND643	1–40	10	1.5
SrCbrd	5–10	3	0.5
SrNini	1–5	2	0.3
SrTnmu	20–40	2	0.3
SrUnknown	1–30	4	0.6
Inadequate-susceptible (28.4%)			
MS (moderately susceptible)	50	90	13.7
MS-S (moderately susceptible to susceptible)	60–70	72	11.0
S (susceptible)	80–100	24	3.7

[a] Stem rust resistance category is given to simplify communication regarding the adult-plant resistance (APR) behavior of a line and is based on the highest disease severity recorded regardless of the season.

[b] Disease severities follow modified Cobb Scale, as described by Roelfs et al. (1992). The final disease severity was recorded when the susceptible check 'Cacuke' became necrotic approximately a week after 100% stem rust severity during the off and main seasons of 2013 and 2014 at Njoro, Kenya.

ADVANCES IN CLONING OF RUST RESISTANCE GENES

A relatively small but increasing number of rust resistance genes have been cloned from wheat and other plant species (Table 4). All race-specific rust resistance genes cloned to date encode proteins containing an N-terminal nucleotide-binding site (NBS) domain coupled to a C-terminal domain consisting of a series of degenerate leucine-rich repeat (LRR) motifs. An overly simplistic but nevertheless appropriate model of resistance protein function is that the LRR motif is primarily involved in pathogen recognition while the N-terminal domain is involved in activating a plant defense response. These NBS-LRR proteins are common to all land plant species and provide resistance to a diverse array of fungal, bacterial, viral, and insect pathogens (Yue et al. 2012).

The six race-specific, NBS-LRR wheat rust resistance genes cloned (*Lr1*, *Lr10*, *Lr21*, *Sr33*, *Sr35*, and *Yr10*) confer a spectrum of resistance specificities even though they encode the same class of protein. Currently, there is insufficient information to predict whether an NBS-LRR protein will confer a broad or narrow spectrum of rust resistance or which rust pathogens species it will recognize based upon protein sequence. It is noteworthy that the *Sr33* stem rust resistance gene is a homolog of the *Mla* powdery mildew resistance gene family first described in barley and later in the diploid A genome of wheat (Jordan et al. 2011; Seeholzer et al. 2010; Wei et al. 2002). It has been suggested that other members of the *Mla/Sr33* gene family are also likely to encode *Sr31* and *Sr50* based upon genetic colocalization of *Mla/Sr33* homologs and these two resistance specificities (Periyannan et al. 2013). Thus, it appears that diversification of a single NBS-LRR gene family in wheat and its relatives can provide resistance to at least two pathogen species, one of which is a rust pathogen. All six cloned, race-specific wheat rust resistance genes have related gene members elsewhere in the wheat genome or in at least one of the diploid progenitors of bread wheat. Whether some of these other NBS-LRR gene homologs also confer additional resistances like the *Mla/Sr33* family is unknown.

Lr1, *Lr21*, *Sr33*, *Sr35*, and *Yr10* resistance specificities are each encoded by a single NBS-LRR gene; however, *Lr10* resistance is conferred by a pair of adjacent NBS-LRR genes, each required for function (Loutre et al. 2009). Several disease resistances in other plant species are also encoded by two tandemly inverted NBS-LRR genes. The products of these gene pairs often interact by forming dimers, with one member involved in pathogen recognition whereas the other is involved in plant defense signaling (Cesari et al. 2014). With more than 150 rust resistance genes cataloged in wheat as race specific, it is likely that the majority of these genes will also encode NBS-LRR proteins. Sophisticated methodologies have been developed that enable all NBS-LRR genes present in a plant genome to be sequenced. When coupled with mutagenesis, these strategies are likely to greatly accelerate the discovery of additional wheat rust resistances that are encoded by NBS-LRR genes and, therefore, bypass tedious, conventional map-based cloning approaches (Jupe et al. 2013; Wulff and Moscou 2014).

Unlike race-specific seedling resistances, various APR genes confer partial or slow-rusting phenotypes at the adult plant stage of

development. Some APR genes are race specific while others recognize all rust pathogen races (i.e., race nonspecific). Two race-nonspecific APR genes have been cloned from wheat, and the obvious difference in APR resistance phenotypes, compared with seedling resistance phenotypes, is paralleled by APR genes encoding entirely different proteins. The first APR gene to be cloned, *Lr34/Yr18/Sr57/Pm38/Sb1/Bdv1* (hereafter referred to as *Lr34*), confers adult-plant race-nonspecific resistance to six pathogen species. The predicted *Lr34* protein is a 12-transmembrane domain ABC transporter that transports an unknown substrate that presumably confers disease resistance (Krattinger et al. 2009). The protein encoded by the resistance gene differs from that of the susceptibility allele by just two amino acids; however, such apparently minor differences have been shown to result in significant changes in substrate transport of other ABC-transporter proteins.

The second wheat APR gene to be cloned is the *Yr36* gene. This gene, derived from *T. turgidum* subsp. *dicoccides*, provides partial APR to all wheat stripe rust pathogen races tested and, unlike *Lr34*, is associated with plant cell death at rust infection sites (Fu et al. 2009). The *Yr36* protein contains a functional N-terminal serine-threonine kinase domain fused to a predicted steroidogenic acute regulatory protein-related lipid transfer (START) domain (Fu et al. 2009). This latter domain has been implicated in lipid trafficking, metabolism, and sensing, while binding of START domain proteins to sterols and ceramides causes protein conformational changes. The fusion of a kinase to a possible lipid receptor suggests that this protein may be involved in a lipid-based signaling response, although there is currently no evidence to support this model. Unlike *Lr34*, the *Yr36* gene has not been used extensively in agriculture; therefore, its long-term durability is unknown.

EXPLORING EFFECTOR BIOLOGY AND GENE CASSETTES FOR BUILDING DURABLE RESISTANCE

Colonization of plant cells by rust infection involves the insertion of haustoria into mesophyll cells. Many, possibly hundreds, of small secreted proteins called effectors are released from the fungal haustorium and enter the plant cytoplasm and are believed to suppress plant basal defense mechanisms and alter plant cell homeostasis for the pathogen's benefit (Giraldo and Valent 2013; Rafiqi et al. 2010). NBS-LRR proteins recognize, either directly or indirectly, the invasive action of a single, specific fungal effector protein (a recognized effector is known as an avirulence protein), whereupon a defense response is activated, often leading to a hypersensitive cell death response. The flax rust pathogen *Melampsora lini* is the only rust species for which effectors with demonstrated avirulence activity have been characterized and interaction with a cognate NBS-LRR protein demonstrated (Dodds et al. 2006; Ravensdale et al. 2011).

A single pathogen effector protein is dispensable, presumably due to redundancy, which enables rapid changes in pathogen avirulence by mutation or loss of recognized effectors. Consistent with this molecular model of rapid pathogen avirulence loss is the inherent lack of durability of NBS-LRR resistance genes, particularly when deployed singularly. To counter rapid pathogen change, plant NBS-LRR proteins also evolve rapidly by sequence diversification and intergenic recombination (Michelmore and Meyers 1998). Therefore, a constant counter evolution of new pathogen races and new plant resistance gene specificities occurs in a molecular coevolutionary "arms race" (Maor and Shirasu 2005).

The increasing number of cloned wheat rust resistance genes now makes stacking of multiple resistance genes together on a small chromosomal region in wheat a real possibility using plant transgenesis. These *cis*-gene stacks would greatly simplify future breeding efforts and prevent the single gene deployment of rust-resistance genes. A second major advance that has potentially enabled production of *cis*-gene stacks in wheat is the development of a highly efficient *Agrobacterium*-mediated wheat transformation system (Ishida et al. 2014; Richardson et al. 2014). However, wheat rust resistance genes are generally large (8 to 16 kb), making the production of very large multigene constructs and subsequent transgenic plants technically challenging. Nonetheless, these experiments are being attempted and it is imperative that more wheat rust resistance genes are cloned to increase potential gene combinations available for *cis*-gene stacks.

To maintain the durability of a *cis*-gene stack, it is essential that individual resistance genes within the stack are not deployed singularly, resulting in the erosion of the resistances present at the multigene locus. This caveat is probably more applicable to race-specific genes rather than APR genes. It is difficult to prevent deployment of single genes that are already present in wheat or which can be easily introgressed by conventional breeding. However, intellectual property protection can provide restrictions on the use of cloned genes in transgenic material. Consequently, future *cis*-gene stacks are likely to consist of *trans*-genes cloned from other plant species that are not sexually compatible with wheat and the only means of introducing these genes is via transgenesis. Potentially, these resistance genes may be derived from both hosts and nonhosts of cereal rust fungi (Bettgenhaeuser et al. 2014). The challenge of *cis*-gene stacks is not restricted to resistance genes and the increasing number of agronomically beneficial transgenes makes the development of gene-stacking technologies a biotechnological imperative (Que et al. 2010).

TABLE 4. Cloned rust resistance genes in wheat, barley, maize, and flax

Species	Gene	Product[a]	Rust pathogen	Reference
Wheat	*Lr1*	NBS-LRR protein	*Puccinia triticina*	Cloutier et al. 2007
	Lr10	NBS-LRR protein	*P. triticina*	Feuillet et al. 2003
	Lr21	NBS-LRR protein	*P. triticina*	Huang et al. 2003
	Lr34/Yr18/Sr57/Pm38	ABC transporter	Multiple pathogens	Krattinger et al. 2009
	Sr33	NBS-LRR protein	*P. graminis tritici*	Periyannan et al. 2013
	Sr35	NBS-LRR protein	*P. graminis tritici*	Saintenac et al. 2013
	Yr10	NBS-LRR protein	*P. striiformis*	Liu et al. 2014
	Yr36	START domain kinase	*P. striiformis*	Fu et al. 2009
Barley	*Rpg1*	Protein kinase	*P. graminis tritici*	Brueggeman et al. 2002
	Rpg5	NBS-LRR protein	*P. graminis tritici*	Brueggeman et al. 2008
Maize	*Rp1*	NBS-LRR protein	*P. sorghi*	Collins et al. 1999
	Rp3	NBS-LRR protein	*P. sorghi*	Webb et al. 2002
Flax	*L*	NBS-LRR protein	*Melampsora lini*	Lawrence et al. 1995
	M	NBS-LRR protein	*M. lini*	Anderson et al. 1997
	N	NBS-LRR protein	*M. lini*	Dodds et al. 2001a
	P	NBS-LRR protein	*M. lini*	Dodds et al. 2001b

[a] NBS-LRR = nucleotide-binding site leucine-rich repeat and START = steroidogenic acute regulatory protein-related lipid transfer.

Continuing the understanding of the molecular basis of rust disease is also likely to lead to new disease resistance strategies. Very little is known about the host cell targets of cereal rust effector proteins. However, in other pathosystems, modifications of the plant components targeted by pathogen effector molecules can generate novel resistance. For example, the bacterial pathogen of rice *Xanthomonas oryzae* produces an effector that acts as a transcription factor that specifically targets and upregulates the endogenous rice sugar transporter gene *OsSweet14*. Modification of this endogenous rice gene with site-specific nucleases prevents effector binding to the *OsSweet14* promoter and results in enhanced disease resistance (Li et al. 2012). However, this type of novel resistance strategy will require a much greater understanding of wheat rust effectors and their cognate plant targets for future development in wheat.

CONCLUDING REMARKS

The spread of the Ug99 race group of stem rust in Eastern and Southern Africa and beyond has brought stem rust research and development activities back onto the international wheat improvement agenda under the BGRI. Significant progress was made in pathogen surveillance, field screening of global wheat germplasm, identifying diverse race-specific and APR resistance genes, cloning of rust resistance genes, breeding rust resistant varieties, and training wheat scientists. Existing varieties with resistance were identified and new varieties with race-specific or APRs were released in various countries. A decade of breeding at CIMMYT has started to deliver new, high-yielding wheat germplasm resistant to races belonging to the Ug99 lineage and other important races identified recently in Asia, the Middle East, and Africa. Chemicals remain an option for emergency control and are being used in eastern Africa; however, their large-scale use by small farmers is neither feasible nor economical. Therefore, success in controlling stem rust is likely to be achieved in Africa when new high-yielding varieties with a high level of durable resistance are widely grown and susceptible varieties are simultaneously removed from farmers' fields. This will require a concerted effort by researchers working in different disciplines and agencies responsible for varietal releases, seed multiplication, and their distribution and promotion. International developmental agencies interested in food security and the income of small farmers in Africa can play a crucial role in achieving sustainable stem rust control by supporting the abovementioned activities, which should also help mitigate the stem rust threat in other wheat-growing regions.

ACKNOWLEDGMENTS

We salute the late Dr. Norman Borlaug for his vision and efforts to mobilize the global wheat community and donors to support international efforts to mitigate the threat from Ug99 and other *P. graminis* f. sp. *tritici* races. We thank our own institutions (CIMMYT, USDA-ARS, University of the Free State, CSIRO, and INIFAP) for their support, particularly the DRRW Project managed by Cornell University (supported by the Bill and Melinda Gates Foundation and DFID), USAID, ICAR-India, USDA-ARS, and GRDC-Australia, among others, for their financial support; the Kenyan Agricultural and Livestock Research Organization (KALRO), and Ethiopian Agricultural Research Institute (EIAR) for the provision of field and other facilities that have been crucial in generating some of the information presented in the article; and J. Mollins for technical editing of the article.

LITERATURE CITED

Admassu, B., Friedt, W., and Ordon, F. 2010. Genetic characterization of *Puccinia graminis* f. sp. *tritici* populations from Ethiopia by SSRs. J. Phytopathol. 158:806-812.

Admassu, B., Lind, V., Friedt, W., and Ordon, F. 2009. Virulence analysis of *Puccinia graminis* f. sp. *tritici* populations in Ethiopia with special consideration of Ug99. Plant Pathol. 58:362-369.

Anderson, J. A., Wiersma, J. J., Linkert, G. L., Kolmer, J. A., Jin, Y., Dill-Macky, R., Wiersma, J. V., Hareland, G. A., and Busch, R. H. 2012. Registration of 'Tom' wheat. J. Plant Regist. 6:180-185.

Anderson, P. A., Lawrence, G. J., Morrish, B. C., Ayliffe, M. A., Finnegan, E. J., and Ellis, J. G. 1997. Inactivation of the flax rust resistance gene *M* associated with loss of a repeated unit within the leucine-rich repeat coding region. Plant Cell 9:641-651.

Bansal, U., Bariana, H., Wong, D., Randhawa, M., Wicker, T., Hayden, M., and Keller, B. 2014. Molecular mapping of an adult plant resistance gene *Sr56* in winter wheat cultivar Arina. Theor. Appl. Genet. 127:1441-1448.

Basnet, B. R., Sukhwinder, S., Lopez-Vera, E. E., Huerta-Espino, J., Bhavani, S., Jin, Y., Rouse, M. N., and Singh, R. P. 2015. Molecular mapping and validation of *SrND643*: A new wheat gene for resistance to the stem rust pathogen Ug99 race group. Phytopathology 105:470-476.

Bettgenhaeuser, J., Gilbert, B., Ayliffe, M., and Moscou, M. J. 2014. Nonhost resistance to rust pathogens – a continuation of continua. Front. Plant Sci. 5:664.

Bhavani, S., Singh, R. P., Argillier, O., Huerta-Espino, J., Singh, S., Njau, P., Brun, S., Lacam, S., and Desmouceaux, N. 2011. Mapping durable adult plant stem rust resistance to the race Ug99 group in six CIMMYT wheats. Pages 43-53 in: Proc. Borlaug Global Rust Initiative 2011 Tech. Workshop, St. Paul, MN. R. McIntosh, ed. Borlaug Global Rust Initiative Online publication. www.globalrust.org

Boshoff, W. H. P., Pretorius, Z. A., Van Niekerk, B. D., and Komen, J. S. 2002. First report of virulence in *Puccinia graminis* f. sp. *tritici* to wheat stem rust resistance genes *Sr8b* and *Sr38* in South Africa. Plant Dis. 86:922.

Brueggeman, R., Druka, A., Nirmala, J., Cavileer, T., Drader, T., Rostoks, N., Mirlohi, A., Bennypaul, H., Gil, U., Kudrna, D., Whitelaw, C., Kilian, A., Han, F., Sun, Y., Gil, K., Steffenson, B., and Kleinhofs, A. 2008. The stem rust resistance gene *Rpg5* encodes a protein with nucleotide-binding-site, leucine-rich, and protein kinase domains. Proc. Natl. Acad. Sci. USA 105: 14970-14975.

Brueggeman, R., Rostoks, N., Kudrna, D., Kilian, A., Han, F., Chen, J., Druka, A., Steffenson, B., and Kleinhofs, A. 2002. The barley stem rust-resistance gene *Rpg1* is a novel disease-resistance gene with homology to receptor kinases. Proc. Natl. Acad. Sci. USA 99:9328-9333.

Cesari, S., Bernoux, M., Moncuquet, P., Kroj, T., and Dodds, P. 2014. A novel conserved mechanism for plant NLR protein pairs: The 'integrated decoy' hypothesis. Front. Plant Sci. 5:606.

Cloutier, S., McCallum, B. D., Loutre, C., Banks, T. W., Wicker, T., Feuillet, C., Keller, B., and Jordan, M. C. 2007. Leaf rust resistance gene *Lr1*, isolated from bread wheat (*Triticum aestivum* L.) is a member of the large *psr567* gene family. Plant Mol. Biol. 65:93-106.

Collins, N., Drake, J., Ayliffe, M., Sun, Q., Ellis, J., Hulbert, S., and Pryor, T. 1999. Molecular characterization of the maize *Rp1-D* rust resistance haplotype and its mutants. Plant Cell 11:1365-1376.

Dean, R., Van Kan, J. A. L., Pretorius, Z. A., Hammond-Kosack, K., Di Pietro, A., Spanu, P., Rudd, J. J., Dickman, M., Kahmann, R., Ellis, J., and Foster, G. D. 2012. The top 10 fungal pathogens in molecular plant pathology. Mol. Plant Pathol. 13:414-430.

Dodds, P. N., Lawrence, G. J., Catanzariti, A. M., Teh, T., Wang, C. I., Ayliffe, M. A., Kobe, B., and Ellis, J. G. 2006. Direct protein interaction underlies gene-for-gene specificity and coevolution of the flax resistance genes and flax rust avirulence genes. Proc. Natl. Acad. Sci. USA 103:8888-8893.

Dodds, P. N., Lawrence, G. J., and Ellis, J. G. 2001a. Contrasting modes of evolution acting on the complex *N* locus for rust resistance in flax. Plant J. 27:439-453.

Dodds, P. N., Lawrence, G. J., and Ellis, J. G. 2001b. Six amino acid changes confined to the leucine-rich repeat β-strand/β-turn motif determine the difference between the *P* and *P2* rust resistance specificities in flax. Plant Cell 13:163-178.

Duplessis, S., Cuomo, C. A., Lin, Y.-C., Aerts, A., Tisserant, E., Veneault-Fourrey, C., Joly, D. L., Hacquard, S., Amselem, J., Cantarel, B. L., Chiu, R., Coutinho, P. M., Feau, N., Field, M., Frey, P., Gelhaye, E., Goldberg, J., Grabherr, M. G., Kodira, C. D., Kohler, A., Kües, U., Lindquist, E. A., Lucas, S. A., Mago, R., Mauceli, E., Morin, E., Murat, C., Pangilinan, J. L., Park, R., Pearson, M., Quesneville, H., Rouhier, N., Sakthikumar, S., Salamov, A. A., Schmutz, J., Selles, B., Shapiro, H., Tanguay, P., Tuskand, G. A., Henrissat, B., Van de Peer, Y., Rouzé, P., Ellis, J. G., Dodds, P. N., Schein, J. E., Zhong, S., Hamelin, R. C., Grigoriev, I. V., Szabo, L. J., and Martin, F. 2011. Obligate biotrophy features unraveled by the genomic analysis of rust fungi. Proc. Natl. Acad. Sci. USA 108: 9166-9171.

Fetch, T. G., Jr., and Dunsmore, K. M. 2004. Physiologic specialization of *Puccinia graminis* on wheat, barley, and oat in Canada in 2001. Can. J. Plant Pathol. 26:148-155.

Feuillet, C., Travella, S., Stein, N., Albar, L., Nublat, A., and Keller, B. 2003. Map-based isolation of the leaf rust disease resistance gene *Lr10* from the hexaploid wheat (*Triticum aestivum* L.) genome. Proc. Natl. Acad. Sci. USA 100:15253-15258.

Fu, D., Uauy, C., Distelfeld, A., Blechl, A., Epstein, L., Chen, X., Sela, H., Fahima, T., and Dubcovsky, J. 2009. A kinase-START gene confers temperature-dependent resistance to wheat stripe rust. Science 323:1357-1360.

German, S. E., Barcellos, A., Chaves, M., Kohli, M., Campos, P., and de Viedma, L. 2007. The situation of common wheat rusts in the Southern Cone of America and perspectives for control. Aust. J. Agric. Res. 58:620-630.

Ghazvini, H., Hiebert, C. W., Zegeye, T., Liu, S., Dilawari, M., Tsilo, T., Anderson, J. A., Rouse, M. N., Jin, Y., and Fetch, T. 2012. Inheritance of resistance to Ug99 stem rust in wheat cultivar Norin 40 and genetic mapping of *Sr42*. Theor. Appl. Genet. 125:817-824.

Giraldo, M. C., and Valent, B. 2013. Filamentous plant pathogen effectors in action. Nat. Rev. Microbiol. 11:800-814.

Hare, R. A., and McIntosh, R. A. 1979. Genetic and cytogenetic studies of durable adult-plant resistance in Hope and related cultivars to wheat rusts. Z. Pflanzenzuchtg. 83:350-367.

Herrera-Foessel, S. A., Singh, R. P., Lillemo, M., Huerta-Espino, J., Bhavani, S., Singh, S., Lan, C., Calvo-Salazar, V., and Lagudah, E. S. 2014. *Lr67/Yr46* confers adult plant resistance to stem rust and powdery mildew in wheat. Theor. Appl. Genet. 127:781-789.

Hiebert, C. W., Fetch, T. G., Zegeye, T., Thomas, J. B., Somers, D. J., Humphreys, D. G., McCallum, B. D., Cloutier, S., Singh, D., and Knott, D. R. 2011. Genetics and mapping of seedling resistance to Ug99 stem rust in Canadian wheat cultivars 'Peace' and 'AC Cadillac'. Theor. Appl. Genet. 122:143-149.

Hodson, D. P., Grønbech-Hansen, J., Lassen, P., Alemayehu, Y., Arista, J., Sonder, K., Kosina, P., Moncada, P., Nazari, K., Park, R. F., Pretorius, Z. A., Szabo, L. J., Fetch, T., and Jin, Y. 2012. Tracking the wheat rust pathogens. Pages 11-22 in: Proc. BGRI Tech. Workshop, Beijing. R. A. McIntosh, ed. Online publication. http://www.globalrust.org/sites/default/files/BGRI-2012-plenary.pdf

Huang, L., Brooks, S. A., Li, W., Fellers, J. P., Trick, H. N., and Gill, B. S. 2003. Map-based cloning of leaf rust resistance gene *Lr21* from the large and polyploid genome of bread wheat. Genetics 164:655-664.

Ishida, Y., Tsunashima, M., Hiei, Y., and Komari, T. 2014. Wheat (*Triticum aestivum* L.) transformation using immature wheat embryos. Pages 189-198 in: *Agrobacterium* Protocols: Volume 1223, Methods in Molecular Biology. K. Wang, ed. Springer, New York.

Jin, Y., and Singh, R. P. 2006. Resistance in U.S. wheat to recent eastern African isolates of *Puccinia graminis* f. sp. *tritici* with virulence to resistance gene *Sr31*. Plant Dis. 90:476-480.

Jin, Y., Singh, R. P., Ward, R. W., Wanyera, R., Kinyua, M., Njau, P., Fetch, T., Pretorius, Z. A., and Yahyaoui, A. 2007. Characterization of seedling infection types and adult plant infection responses of monogenic *Sr* gene lines to race TTKS of *Puccinia graminis* f. sp. *tritici*. Plant Dis. 91:1096-1099.

Jin, Y., Szabo, L. J., Pretorius, Z. A., Singh, R. P., Ward, R., and Fetch, T., Jr. 2008. Detection of virulence to resistance gene *Sr24* within race TTKS of *Puccinia graminis* f. sp. *tritici*. Plant Dis. 92:923-926.

Jin, Y., Szabo, L. J., Rouse, M., Fetch, T., Pretorius, Z. A., Singh, R. P., Wanyera, R., and Njau, P. 2009. Detection of virulence to resistance gene *Sr36* within race TTKS lineage of *Puccinia graminis* f. sp. *tritici*. Plant Dis. 93:367-370.

Jordan, T., Seeholzer, S., Schwizer, S., Töller, A., Somssich, I. E., and Keller, B. 2011. The wheat *Mla* homologue *TmMla1* exhibits an evolutionarily conserved function against powdery mildew in both wheat and barley. Plant J. 65:610-621.

Jupe, F., Witek, K., Verweij, W., Sliwka, J., Pritchard, L., Etherington, G. J., Maclean, D., Cock, P. J., Leggett, R. M., Bryan, G. J., Cardle, L., Hein, I., and Jones, J. D. G. 2013. Resistance gene enrichment sequencing (RenSeq) enables reannotation of the NB-LRR gene family from sequenced plant genomes and rapid mapping of resistance loci in segregating populations. Plant J. 76:530-544.

Knott, D. R. 1982. Multigenic inheritance of stem rust resistance in wheat. Crop Sci. 22:393-399.

Knott, D. R. 1989. The Wheat Rusts—Breeding for Resistance. Springer-Verlag, Berlin.

Kolmer, J. A. 2001. Early research on the genetics of *Puccinia graminis* stem rust resistance in wheat in Canada and the United States. Pages 51-82 in: Stem Rust of Wheat: From Ancient Enemy to Modern Foe. P. D. Peterson, ed. American Phytopathological Society Press, St. Paul, MN.

Kolmer, J. A., Dyck, P. L., and Roelfs, A. P. 1991. An appraisal of stem and leaf rust resistance in North American hard red spring wheats and the probability of multiple mutations to virulence in populations of cereal rust fungi. Phytopathology 81:237-239.

Krattinger, S. G., Lagudah, E. S., Spielmeyer, W., Singh, R. P., Huerta-Espino, J., McFadden, H., Bossolini, E., Selter, L. L., and Keller, B. 2009. A putative ABC transporter confers durable resistance to multiple fungal pathogens in wheat. Science 323:1360-1363.

Lantican, M. A., Dubin, H. J., and Morris, M. L. 2005. Impacts of International Wheat Breeding Research in the Developing World, 1988–2002. International Maize and Wheat Improvement Center, CIMMYT, D.F., Mexico.

Lawrence, G. J., Finnegan, E. J., Ayliffe, M. A., and Ellis, J. G. 1995. The *L6* gene for flax rust resistance is related to the Arabidopsis bacterial resistance gene *RPS2* and the tobacco viral resistance gene *N*. Plant Cell 7:1195-1206.

Li, T., Liu, B., Spalding, M. H., Weeks, D. P., and Yang, B. 2012. High-efficiency TALEN-based gene editing produces disease resistant rice. Nat. Biotechnol. 30:390-392.

Liu, W., Frick, M., Huel, R., Nykiforuk, C. L., Wang, X., Gaudet, D. A., Eudes, F., Conner, R. L., Kuzyk, A., Chen, Q., Kang, Z., and Laroche, A. 2014. The stripe rust resistance gene *Yr10* encodes an evolutionary-conserved and unique CC-NBS-LRR sequence in wheat. Mol. Plant 7: 1740-1755.

Lopez-Vera, E. E., Nelson, S., Singh, R. P., Basnet, B. R., Haley, S. D., Bhavani, S., Huerta-Espino, J., Xoconostle-Cazares, B. G., Ruiz-Medrano, R., Rouse, M. N., and Singh, S. 2014a. Resistance to stem rust Ug99 in six bread wheat cultivars maps to chromosome 6DS. Theor. Appl. Genet. 127: 231-239.

Lopez-Vera, E. E., Singh, S., Bhavani, S., Basnet, B. R., Huerta-Espino, J., Rouse, M., Xoconostle-Cázares, B., and Medran, R. R. 2014b. Identification of genes on wheat chromosomes 2BS and 2BL conferring resistance to stem rust fungus Race Ug99. In: Plant Anim. Genome XXII Conf. San Diego, CA.

Loutre, C., Wicker, T., Travella, S., Galli, P., Scofield, S., Fahima, T., Feuillet, C., and Keller, B. 2009. Two different CC-NBS-LRR genes are required for *Lr10*-mediated leaf rust resistance in tetraploid and hexaploid wheat. Plant J. 60:1043-1054.

Maor, R., and Shirasu, K. 2005. The arms race continues: Battle strategies between plants and fungal pathogens. Curr. Opin. Microbiol. 8:399-404.

McFadden, E. S. 1930. A successful transfer of emmer characters to *vulgare* wheat. J. Am. Soc. Agron. 22:1020-1034.

McFadden, E. S. 1939. Brown necrosis, a discoloration associated with rust infection in certain rust resistant wheats. J. Agric. Res. 58:805-819.

McIntosh, R. A. 1988. The role of specific genes in breeding for durable stem rust resistance in wheat and triticale. Pages 1-9 in: Breeding Strategies for Resistance to the Rusts of Wheat. N. W. Simmonds and S. Rajaram, eds. CIMMYT, D.F., Mexico.

Mert, Z., Karakaya, A., Dusunceli, F., Akan, K., and Cetin, L. 2012. Determination of *Puccinia graminis* f. sp. *tritici* races of wheat in Turkey. Turk. J. Agric. For. 36:107-120.

Michelmore, R. W., and Meyers, B. C. 1998. Clusters of resistance genes in plants evolve by divergent selection and a birth-and-death process. Genome Res. 8:1113-1130.

Nazari, K., and Mafi, M. 2013. Physiological races of *Puccinia graminis* f. sp. *tritici* in Iran and evaluation of seedling resistance to stem rust in Iranian wheat cultivars. Phytopathol. Mediterr. 52:110-122.

Newcomb, M., Mert, Z., Akin, B., Morgounov, A., Carter, M., Johnson, J., Luster, D., Szabo, L. J., and Jin, Y. 2013. Phenotypic and genotypic analyses of Turkish *Pgt* samples collected in 2012. Page 96 in: Proc. BGRI 2013 Tech. Workshop, New Delhi, India. R. A. McIntosh, ed.

Njau, P. N., Bhavani, S., Huerta-Espino, J., Keller, B., and Singh, R. P. 2013. Identification of QTL associated with durable adult plant resistance to stem rust race Ug99 in wheat cultivar 'Pavon 76'. Euphytica 190:33-44.

Njau, P. N., Jin, Y., Huerta-Espino, J., Keller, B., and Singh, R. P. 2010. Identification and evaluation of sources of resistance to stem rust race Ug99 in wheat. Plant Dis. 94:413-419.

Olivera, P. D., Jin, Y., Rouse, M., Badebo, A., Fetch, T., Jr., Singh, R. P., and Yahyaoui, A. 2012. Races of *Puccinia graminis* f. sp. *tritici* with combined virulence to *Sr13* and *Sr9e* in a field stem rust screening nursery in Ethiopia. Plant Dis. 96:623-628.

Olivera Firpo, P., Newcomb, M., Szabo, L.J., Rouse, M., Johnson, J., Gale, S., Luster, D. G., Hodson, D., Cox, J. A., Burgin, L., Gilligan, C. A., Patpour, M., Justesen, A. F., Hovmøller, M. S., Woldeab, G., Hailu, E., Hundie, B., Tadesse, K., Pumphrey, M., Singh, R. P., and Jin, Y. Phenotypic and genotypic characterization of race TKTTF of *Puccinia graminis* f. sp. *tritici* that caused a wheat stem rust epidemic in southern Ethiopia in 2013/14. Phytopathology. In press.

Park, R. L. 2007. Stem rust of wheat in Australia. Aust. J. Agric. Res. 58: 558-566.

Periyannan, S., Moore, J., Ayliffe, M., Bansal, U., Wang, X., Huang, L., Deal, K., Luo, M., Kong, X., Bariana, H., Mago, R., McIntosh, R., Dodds, P., Dvorak, J., and Lagudah, E. 2013. The gene *Sr33*, an ortholog of barley *Mla* genes, encodes resistance to wheat stem rust race Ug99. Science 341:786-788.

Pretorius, Z. A., Pakendorf, K. W., Marais, G. F., Prins, R., and Komen, J. S. 2007. Challenges for sustainable control of cereal rust diseases in South Africa. Aust. J. Agric. Res. 58:593-601.

Pretorius, Z. A., Singh, R. P., Wagoire, W. W., and Payne, T. S. 2000. Detection of virulence to wheat stem rust resistance gene *Sr31* in *Puccinia graminis* f. sp. *tritici* in Uganda. Plant Dis. 84:203.

Pretorius, Z. A., Szabo, L. J., Boshoff, W. H. P., Herselman, L., and Visser, B. 2012. First report of a new TTKSF race of wheat stem rust (*Puccinia graminis* f. sp. *tritici*) in South Africa and Zimbabwe. Plant Dis. 96:590.

Que, Q., Chilton, M.-D., de Fontes, C. M., He, C., Nuccio, M., Zhu, T., Wu, Y., Chen, J. S., and Shi, L. 2010. Trait stacking in transgenic crops. GM Crops 1:220-229.

Rafiqi, M., Gan, P. H., Ravensdale, M., Lawrence, G. J., Ellis, J. G., Jones, D. A., Hardham, A. R., and Dodds, P. N. 2010. Internalization of flax rust avirulence proteins into flax and tobacco cells can occur in the absence of the pathogen. Plant Cell 22:2017-2032.

Rajaram, S., Singh, R. P., and Torres, E. 1988. Current CIMMYT approaches in breeding wheat for rust resistance. Pages 101-118 in: Breeding Strategies for Resistance to the Rust of Wheat. N. W. Simmonds and S. Rajaram, eds. CIMMYT, D.F., Mexico.

Ravensdale, M., Nemri, A., Thrall, P. H., Ellis, J. G., and Dodds, P. N. 2011. Co-evolutionary interactions between host resistance and pathogen effector genes in flax rust disease. Mol. Plant Pathol. 12:93-102.

Richardson, T., Thistleton, J., Higgins, T. J., Howitt, C., and Ayliffe, M. 2014. Efficient *Agrobacterium* transformation of elite wheat germplasm without selection. Plant Cell Tiss. Org. 119:647-659.

Roelfs, A. P. 1978. Estimated losses caused by rust in small grain cereals in the United States-1918-76. U.S. Dep. Agric. Misc. Publ. No. 1363, St. Paul, MN. pages 1-85.

Roelfs, A. P., and Martens, J. W. 1988. An international system of nomenclature for *Puccinia graminis* f. sp. *tritici*. Phytopathology 78:526-533.

Roelfs, A. P., Singh, R. P., and Saari, E. E. 1992. Rust Diseases of Wheat: Concepts and Methods of Disease Management. CIMMYT, D.F., Mexico.

Rouse, M. N., Nirmala, J., Jin, Y., Chao, S., Fetch, T. G., Jr., Pretorius, Z. A., and Hiebert, C. W. 2014a. Characterization of *Sr9h*, a wheat stem rust resistance allele effective to Ug99. Theor. Appl. Genet. 127:1681-1688.

Rouse, M. N., Talbert, L. E., Singh, D., and Sherman, J. D. 2014b. Complementary epistasis involving *Sr12* explains adult resistance to stem rust in Thatcher wheat (*Triticum aestivum* L.). Theor. Appl. Genet. 127:1549-1559.

Saintenac, C., Zhang, W., Salcedo, A., Rouse, M. N., Trick, H. N., Akhunov, E., and Dubcovsky, J. 2013. Identification of wheat gene *Sr35* that confers resistance to Ug99 stem rust race group. Science 341:783-786.

Seeholzer, S., Tsuchimatsu, T., Jordan, T., Bieri, S., Pajonk, S., Yang, W., Jahoor, A., Shimizu, K. K., Keller, B., and Schulze-Lefert, P. 2010. Diversity at the *Mla* powdery mildew resistance locus from cultivated barley reveals sites of positive selection. Mol. Plant-Microbe Interact. 23:497-509.

Singh, R. P., Herrera-Foessel, S. A., Huerta-Espino, J., Bariana, H. S., Bansal, U., McCallum, B. D., Hiebert, C. W., Bhavani, S., Singh, S., Lan, C., and Lagudah, E. S. 2012. *Lr34/Yr18/Sr57/Pm38/Bdv1/Ltn1* confers slow rusting, adult plant resistance to *Puccinia graminis tritici*. Page 173 in: 13th Cereal Rust Powdery Mildew Conf. Friendship Hotel, Beijing.

Singh, R. P., Herrera-Foessel, S. A., Huerta-Espino, J., Lan, C. X., Basnet, B. R., Bhavani, S., and Lagudah, E. S. 2013a. Pleiotropic gene *Lr46/Yr29/Pm39/Ltn2* confers slow rusting, adult plant resistance to wheat stem rust fungus. Page 17 in: Proc. Borlaug Global Rust Initiative, 2013 Tech. Workshop, New Delhi, India.

Singh, R. P., Hodson, D. P., Huerta-Espino, J., Jin, Y., Bhavani, S., Njau, P., Herrera-Foessel, S. A., Singh, P. K., Singh, S., and Govindan, V. 2011a. The emergence of Ug99 races of the stem rust fungus is a threat to world wheat production. Annu. Rev. Phytopathol. 49:465-481.

Singh, R. P., Hodson, D. P., Huerta-Espino, J., Jin, Y., Njau, P., Wanyera, R., Herrera-Foessel, S. A., and Ward, R. W. 2008. Will stem rust destroy the world's wheat crop? Adv. Agron. 98:271-309.

Singh, R. P., Hodson, D. P., Jin, Y., Huerta-Espino, J., Kinyua, M., Wanyera, R., Njau, P., and Ward, R. W. 2006. Current status, likely migration and strategies to mitigate the threat to wheat production from race Ug99 (TTKS) of stem rust pathogen. CAB Rev. Perspect. Agric. Vet. Sci. Nutr. Nat. Resour. 1:1-13. DOI: 10.1079/PAVSNNR20061054.

Singh, R. P., Huerta-Espino, J., Bhavani, S., Herrera-Foessel, S. A., Singh, D., Singh, P. K., Velu, G., Mason, R. E., Jin, Y., Njau, P., and Crossa, J. 2011b. Race non-specific resistance to rust diseases in CIMMYT spring wheats. Euphytica 179:175-186.

Singh, R. P., and McIntosh, R.A. 1986. Genetics of resistance to *Puccinia graminis tritici* and *Puccinia recondita tritici* in Kenya Plume wheat. Euphytica 35:245-256.

Singh, R. P., and McIntosh, R. A. 1987. Genetics of resistance to *Puccinia graminis tritici* in 'Chris' and 'W3746' wheats. Theor. Appl. Genet. 73:846-855.

Singh, S., Singh, R. P., Bhavani, S., Huerta-Espino, J., and Lopez-Vera, E. E. 2013b. QTL mapping of slow-rusting, adult plant resistance to race Ug99 of stem rust fungus in PBW343/Muu RIL population. Theor. Appl. Genet. 126:1367-1375.

Stoxen, S. 2012. Population structure of *Puccinia graminis* f. sp. *tritici* in the United States. M.S. thesis, University of Minnesota. Online publication. http://purl.umn.edu/131092

Szabo, L. J. 2007. Development of simple sequence repeat markers for the plant pathogenic rust fungus *Puccinia graminis*. Mol. Ecol. Notes 7: 92-94.

Szabo, L. J. 2012. Development of a rapid molecular assay for the Ug99 race group of *Puccinia graminis*. (Abstr.) Phytopathology 102:S4.117.

Visser, B., Herselman, L., Park, R. F., Karaoglu, H., Bender, C. M., and Pretorius, Z. A. 2011. Characterization of two new *Puccinia graminis* f. sp. *tritici* races within the Ug99 lineage in South Africa. Euphytica 179:119-127.

Visser, B., Herselman, L., and Pretorius, Z. A. 2009. Genetic comparison of Ug99 with selected South African races of *Puccinia graminis* f. sp. *tritici*. Mol. Plant Pathol. 10:213-222.

Wanyera, R., Kinyua, M. G., Jin, Y., and Singh, R. P. 2006. The spread of *Puccinia graminis* f. sp. *tritici* with virulence on *Sr31* in Eastern Africa. Plant Dis. 90:113.

Webb, C. A., Richter, T. E., Collins, N. C., Nicolas, M., Trick, H. N., Pryor, T., and Hulbert, S. H. 2002. Genetic and molecular characterization of the maize *rp3* rust resistance locus. Genetics 162:381-394.

Wei, F., Wing, R. A., and Wise, R. P. 2002. Genome dynamics and evolution of the *Mla* (powdery mildew) resistance locus in barley. Plant Cell 14: 1903-1917.

WHEAT. 2014. Wheat: Vital grain of civilization and food security. In: 2013 Annual Report, CGIAR Research Program on Wheat. CGIAR Research Program on Wheat (WHEAT), Mexico, D.F.

Wulff, B. H., and Moscou, M. J. 2014. Strategies for transferring resistance into wheat: From wide crosses to GM cassettes. Front. Plant Sci. 5:692.

Yu, L.-X., Barbier, H., Rouse, M. N., Singh, S., Singh, R. P., Bhavani, S., Huerta-Espino, J., and Sorrells, M. E. 2014. A consensus map for Ug99 stem rust resistance loci in wheat. Theor. Appl. Genet. 127:1561-1581.

Yue, J. X., Meyers, B. C., Chen, J. Q., Tian, D., and Yang, S. 2012. Tracing the origin and evolutionary history of plant nucleotide-binding site-leucine-rich repeat (NBS-LRR) genes. New Phytol. 193:1049-1063.

Zhong, S., Leng, Y., Friesen, T. I., Faris, J. D., and Szabo, L. J. 2009. Development and characterization of expressed sequence tag-derived microsatellite markers for the wheat stem rust fungus *Puccinia graminis* f. sp. *tritici*. Phytopathology 99:282-289.