Review

Current status, likely migration and strategies to mitigate the threat to wheat production from race Ug99 (TTKS) of stem rust pathogen

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Abstract

Stem or black rust, caused by *Puccinia graminis tritici*, has historically caused severe losses to wheat (*Triticum aestivum*) production worldwide. Successful control of the disease for over three decades through the use of genetic resistance has resulted in a sharp decline in research activity in recent years. Detection and spread in East Africa of race TTKS, commonly known as Ug99, is of high significance as most wheat cultivars currently grown in its likely migration path, i.e. to North Africa through Arabian Peninsula and then to Middle East and Asia, are highly susceptible to this race and the environment is conducive to disease epidemics. Identifying/developing adapted resistant cultivars in a relatively short time and replacing the susceptible cultivars before rust migrates out of East Africa is the strategy to mitigate potential losses. Although several alien genes will provide resistance to this race, the long-term strategy should focus on rebuilding the '*Sr2*-complex' (combination of slow rusting gene *Sr2* with other unknown additive genes of similar nature) to achieve long-term durability. A Global Rust Initiative has been launched to monitor the further migration of this race, facilitate field testing in Kenya or Ethiopia of wheat cultivars and germplasm developed by wheat breeding programmes worldwide, understand the genetic basis of resistance-especially the durable type, carry out targeted breeding to incorporate diverse resistance genes into key cultivars and germplasm, and enhance the capacity of national programmes. A few wheat genotypes that combine stem rust resistance with high yield potential and other necessary traits have been identified but need rigorous field testing to determine their adaptation in target areas.

Keywords: *Puccinia graminis*, *Triticum aestivum*, Black rust, Resistance, Breeding, Epidemiology

Introduction

Stem or black rust of wheat, caused by fungus *Puccinia graminis* Pers. f. sp. *tritici* Eriks. & E. Henn., was at one time the most feared disease of wheat worldwide. The first detailed reports of wheat stem rust were given independently by Italian scientists Fontana and Tozzetti in 1767 [1, 2] and the causal organism was named as *Puccinia graminis* in 1797 by Persoon. The fear from stem rust was understandable because an apparently healthy looking crop about 3 weeks prior to harvest could reduce to a black tangle of broken stems and shriveled grain by harvest.

It was not until the beginning of the 20th century and soon after the rediscovery of Mendel's laws, that Biffen [3] demonstrated that inheritance of resistance to wheat yellow rust, caused by *Puccinia striiformis*, followed Mendel's laws. After two devastating stem rust epidemics in North America in 1904 and 1916, another important finding came from the work of Stakman and Piemeisel [4] who showed that stem rust pathogen had various forms

Table 1 Originating genus and species and usefulness of designated *Sr* genes in conferring seedling and/or adult plant resistance to Ug99 race of stem rust pathogen *P. graminis* f. sp. *tritici*

Origin of *Sr* genes	Stem rust resistance (*Sr*) genes	
	Ineffective	Effective
Triticum aestivum	5, 6, 7a, 7b, 8a, 8b, 9a, 9b, 9f, 10, 15, 16, 18, 19, 20, 23, 30, 41, 42, Wld-1	28[1], 29[2], Tmp
Triticum turgidum	9d, 9e, 9g, 11, 12, 17	2[2], 13[1,2], 14[1]
Triticum monococcum	21	22, 35
Triticum timopheevi		36[1], 37
Triticum speltoides		32, 39
Triticum tauschii		33[2], 45
Triticum comosum	34	
Triticum ventricosum	38	
Triticum araraticum		40
Thinopyrum elongatum		24[1], 25, 26, 43
Thinopyrum intermedium		44
Secale cereale	31	27[1], 1A.1R

[1]Virulence for the gene is known to occur in other races.
[2]Level of resistance conferred in the field usually not enough.

or races. These races varied in their ability to infect different wheat varieties which later were found to carry distinct resistance genes or combinations thereof. Strong emphases to identify resistance to stem rust and breed resistant wheat cultivars were given in the USA, Canada, Australia and Europe. A simultaneous effort was also made to understand rust epidemiology and evolution, which led to the barberry eradication programme in North America and Europe and formulation of genetic control strategies. Efforts to find a solution to stem rust also initiated global collaboration among wheat scientists who grew and evaluated wheat germplasm for resistance to stem rust.

The International Spring Wheat Rust Nursery Program, initiated in 1950 by B.B. Bayles and R.A. Rodenhiser of USDA-ARS (United States Department of Agriculture-Agricultural Research Services), Beltsville, operated continuously until the mid-1980s. The objectives of the programme were: (1) to find new genes or combinations of genes in wheat that condition field resistance to rusts throughout the world, and (2) to test new varieties and promising selections of wheat developed by plant breeders and pathologists for resistance to rusts. The germplasm and information generated were made available to the global wheat community. This nursery was the foundation of numerous other international nurseries and led to global cooperation to achieve resistance to diseases and pests of several crops. CIMMYT (International Maize and Wheat Improvement Center) and several other centres continue to use this methodology to not only distribute improved germplasm they develop but also to evaluate their performance for agronomic and disease resistance attributes.

Almost 50 different stem resistance genes are now catalogued [5], several of which are incorporated in wheat from alien relatives of wheat (Table 1). All but one of 50 resistance genes are race-specific, and are expressed in

both seedling and adult plants. Race specificity derives from the gene-for-gene relationship between the host plant resistance gene and corresponding virulence genes in the pathogen. Gene *Sr2*, transferred to wheat from 'Yaroslav emmer' by McFadden [6], is the only catalogued gene that is not race-specific. *Sr2* can confer slow rusting [7] resistance of adult-plant nature. Resistance gene *Sr2*, in addition to other unknown minor genes derived from cultivar Hope and commonly known as '*Sr2*-Complex', provided the foundation for durable resistance to stem rust in germplasm from University of Minnesota in the USA, Sydney University in Australia, and the spring wheat germplasm developed by Dr N.E. Borlaug as part of a programme sponsored by the Mexican Government and the Rockefeller Foundation [8, 9]. Cultivar Yaqui 50, released in Mexico during the 1950s, and other *Sr2*-carrying wheats released since then had stabilized the stem rust situation in Mexico and many other countries where modern semidwarf wheats were adopted. Changes in stem rust races have not been observed in Mexico for almost 40 years and natural infections are non-existent. Another *Sr2*-carrying cultivar Sonalika, released in 1960 in the Indian subcontinent and subsequently grown on millions of hectares, remained resistant to stem rust. When present alone, the *Sr2* gene confers slow rusting that is not adequate under heavy disease pressure, but does provide adequate resistance in combination with other minor genes. Unfortunately, not much is known about the other genes in the *Sr2* complex and their interactions. Knott [10] has shown that adequate levels of multigenic resistance to stem rust can be achieved by accumulating approximately five minor genes.

The importance of stem rust declined worldwide with the deployment of various other alien resistance genes such as *Sr24*, *Sr26*, *Sr31* and more recently *Sr38*. Translocations carrying these genes, except that with *Sr26*, also carried additional genes that conferred resistance to

some other important diseases such as leaf rust, yellow rust or powdery mildew. The decrease in incidence of stem rust to almost non-significant levels by the mid-1990s throughout most of the world was coincident with a decline in research and breeding emphasis to such a level that in many countries breeding was done in the absence of this disease. CIMMYT scientists continued to select for stem rust resistance in Mexico using artificial inoculation with six *P. graminis tritici* races of historical importance. Emergence of new stem rust races, however, did not occur as the pathogen population stopped evolving since the 'Green Revolution' [11]. Moreover, a majority of wheat lines selected in Mexico remained resistant at international sites as a result of absence of disease, inadequate disease pressure, or presence of races that lacked necessary virulence for the resistance genes contained in CIMMYT wheat germplasm.

Susceptibility of Global Wheat Germplasm to *P. graminis tritici* Race Ug99 Present in East Africa

Race Ug99, first identified in Uganda during 1999 [12], is the only known race of *P. graminis tritici* that has virulence for gene *Sr31* known to be located in the translocation 1BL.1RS from rye (*Secale cereale*). Later this race was designated as TTKS by Wanyera *et al.* [13] using the North American nomenclature system [14]. Although designation TTKS is scientifically more correct, we have preferred to use Ug99 that has become a more commonly acceptable designation for this race. Unfortunately, race Ug99 not only carries virulence to gene *Sr31* but also this unique virulence is present together with virulences for most of the genes of wheat origin and virulence for gene *Sr38* introduced in wheat from *Triticum ventricosum* that is present in several European and Australian cultivars and a small portion of new CIMMYT germplasm (Table 1).

The 1BL.1RS translocation initially present in Russian winter wheat cultivars 'Kavkaz' and 'Aurora' became widespread in winter, facultative and spring growth habit cultivars from Europe, China and the USA and several cultivars in developing countries derived from CIMMYT germplasm during the mid-1980s and later. CIMMYT spring wheat germplasm developed from the crosses of spring wheats with winter wheats during the early 1980s showed significant grain yield advantage and wide adaptation with superior disease resistance attributed to the presence of the 1BL.1RS translocation [15]. The alien chromosome segment from *T. ventricosum* carries stem rust resistance gene *Sr38* together with genes *Yr17* and *Lr37* for resistance to other two rust diseases and resistance to powdery mildew and eye spot. Such alien chromosome segments on the one hand are very useful for controlling multiple diseases but on the other hand could lead to 'vertifolia' or masking effect [16], resulting in decrease in frequency or even loss of other useful genes, especially minor types, in breeding materials.

The frequency of 1BL.1RS translocation went up to approximately 70% at one stage in CIMMYT's spring wheat germplasm but has declined to about 30% in more recent advanced lines. Reaction of current germplasm to the Ug99 race in Kenya shows that the frequency of germplasm with 'Sr2 gene complex' has gone down to negligible levels in new advanced lines even though *Sr2* is still present in relatively high frequency in about 60% lines. All wheat lines of CIMMYT origin evaluated in Kenya, irrespective of the presence or absence of the 1BL.1RS translocation, were highly resistant to stem rust in Mexico and remain highly resistant in other parts of the world, indicating that high frequency of this translocation in 1980s and 1990s cultivars explains only a portion of the current susceptibility of wheat germplasm to race Ug99 in Kenya. The major susceptibility is likely the result of the specific nature of avirulence/virulence combination that Ug99 possesses, which had led to the susceptibility of many wheat materials irrespective of where they were developed. Jin and Singh [17] compared seedling reactions of US wheat cultivars and germplasm with highly virulent races present in USA and race Ug99. Several wheat lines, especially spring wheat that were highly resistant to US races and did not carry the 1BL.1RS translocation, were also found to be susceptible to Ug99. This further supports the fact that race Ug99 carries a unique combination of virulence to known and unknown resistance genes present in wheat germplasm.

Predicting the Migration Path of Race Ug99

Dispersal Mechanisms of Rust Spores

To understand the potential movement and migration that may occur as a result of the emergence of race Ug99 in eastern Africa, it is helpful to consider the main modes of dispersal for rust pathogens.

Wheat rust pathogens are biotrophs and therefore need live wheat plants or other secondary hosts for survival in the absence of alternate hosts. They produce huge numbers of urediniospores during the crop season and subsequent wind dispersion transmits these urediniospores onto the same or new host plants. Physical barriers therefore do not succeed in stopping the urediniospores dispersal. Predicted patterns of movement of airborne pathogens are filled with uncertainty, although advances in air-borne modelling and prediction are offering some interesting new insights [18]. Typically, most spores will be deposited close to the source [19], however long-distance dispersal is well documented, with three principal modes of dispersal known to occur.

The first mode of dispersal is single event, extremely long-distance (typically cross-continent) dispersal that results in pathogen colonization of new regions. Dispersion of this type is rare under natural conditions and by nature inherently unpredictable. It is also difficult to

specifically attribute long-distance dispersal. However, rusts are one pathogenic group with reasonably strong evidence for unassisted, long-distance dispersal under natural airborne conditions. Several examples of long-distance dispersal have been described by Brown and Hovmøller [20], including the introduction of sugarcane rust into the Americas from Cameroon in 1978 and a wheat stem rust introduction into Australia from southern Africa in 1969. Both of these examples provide strong evidence for being unassisted natural long-distance wind-borne dispersals. Deposition in new areas is primarily through rain-scrubbing of airborne spores onto susceptible hosts [21]. More recently, the arrival of Asian soybean rust into the USA in 2004 from northern South America/Caribbean was most likely carried by hurricane Ivan [22]. An enabling factor in this mode of dispersal, particularly for rusts, is the robust nature of spores ensuring protection against environmental damage [23].

Assisted long-distance dispersal, typically on travellers' clothing or infected plant material, is another increasingly important element in the colonization of new areas by pathogens. Despite strict phytosanitary regulations, increasing globalization and air travel both increase the risk of pathogen spread. There is strong evidence to support an accidental introduction of wheat yellow rust into Australia in 1979, probably on travellers' clothing, from Europe [24]. More recently, concerns over non-accidental release of plant pathogens as a form of 'agricultural bio-terrorism' have arisen, with wheat stem rust considered one pathogen of concern [25], primarily due to its known ability to cause devastating production losses to a major food staple [26].

The second major mode of dispersal for pathogens like rusts is step-wise range expansion. This typically occurs over shorter distances, within country or region, and has a much higher probability than the first described dispersal mode. This probably represents the most common or normal mode of dispersal for rust pathogens. A good example of this type of dispersal mechanism would include the spread of yellow rust by a *Yr9*-virulent race of *P. striiformis* that evolved in eastern Africa and migrated to South Asia through the Middle East and West Asia in a step-wise manner over about 10 years, and caused severe epidemics in its path [27]. The authors of this study consider that the entire wheat area in Asia (except China) may comprise a single epidemiologic zone, hence any new race arising in this region would, given time, spread throughout the epidemiologic region.

The third mode of dispersal, extinction and re-colonization, could perhaps be considered a sub-mechanism of step-wise range expansion. This mechanism occurs in areas that have unsuitable conditions for year-round survival. Typically, these are temperate areas with inhospitable winter conditions or seasonal absence of host plants. Two good examples of this mechanism are the 'Puccinia pathways' of North America–a concept that arose from the pioneering work of Stakman [28] in

which rust pathogens over-winter in the southern USA or Mexico and re-colonize wheat areas further north following the prevailing south–north winds as the wheat season progresses. A similar mechanism is now being observed following the arrival of Asian soybean rust into the USA [29]. The second well-documented extinction-re-colonization example is that of wheat yellow rust in China [20]. Year-round pathogen survival conditions exist in southern Gansu and northern Sichuan provinces. These areas then act as sources for re-colonization dispersal into the main winter wheat growing areas in Shaanxi, Shanxi, Henan, Hebei and Shangdong provinces in autumn. Absence of suitable host plants in the main winter wheat growing areas precludes year-round survival in the wheat belt of the Northern provinces.

With any of the dispersal modes described, establishment of viable pathogen populations depends on the presence of suitable host plants and the coincidence of suitable environmental conditions. For epidemics to occur, susceptible host plants to a specific pathogen type must occur over large areas.

Considering all these factors what are the likely dispersal scenarios for *Sr31*-virulent stem rust race Ug99? At the outset, it should be stated that it is unrealistic to rule out any of previously described dispersal mechanisms. Documented historical evidence indicates that all are possible; however for practical reasons this paper does not focus on rare, single event (natural or assisted) long-distance dispersal. The following sections describe the current known status of Ug99, potential migration paths, and potential impact scenarios if migration leading to subsequent epidemics does occur.

Current Distribution of Race Ug99

Race Ug99 was first detected in Uganda in 1999 [12]. Following its detection, investigations in neighbouring countries in East Africa revealed that the same race may have migrated to sites in the Rift Valley province of central Kenya by 1998/1999, with subsequent advancement to sites in Eastern Kenya by 2001. It must also be noted that reports of stem rust, possibly attributable to race Ug99, arose from the western rift valley of Kenya as early as 1993 (KARI, unpublished). In 2003, race Ug99 was detected in Ethiopia with 2005 reports from at least six dispersed site locations (Figure 1). Available evidence suggests that Ug99 is now established in the eastern African highlands and spreading, although extensive field survey results detailing exact distribution are currently unavailable.

The East African highlands are a known 'hot-spot' for the evolution of new rust races (30). The favourable environmental conditions, plus the presence of host plants year-round all favour the buildup of pathogen populations. Available evidence emerging from the East African countries indicates that Ug99 has exhibited a gradual step-wise
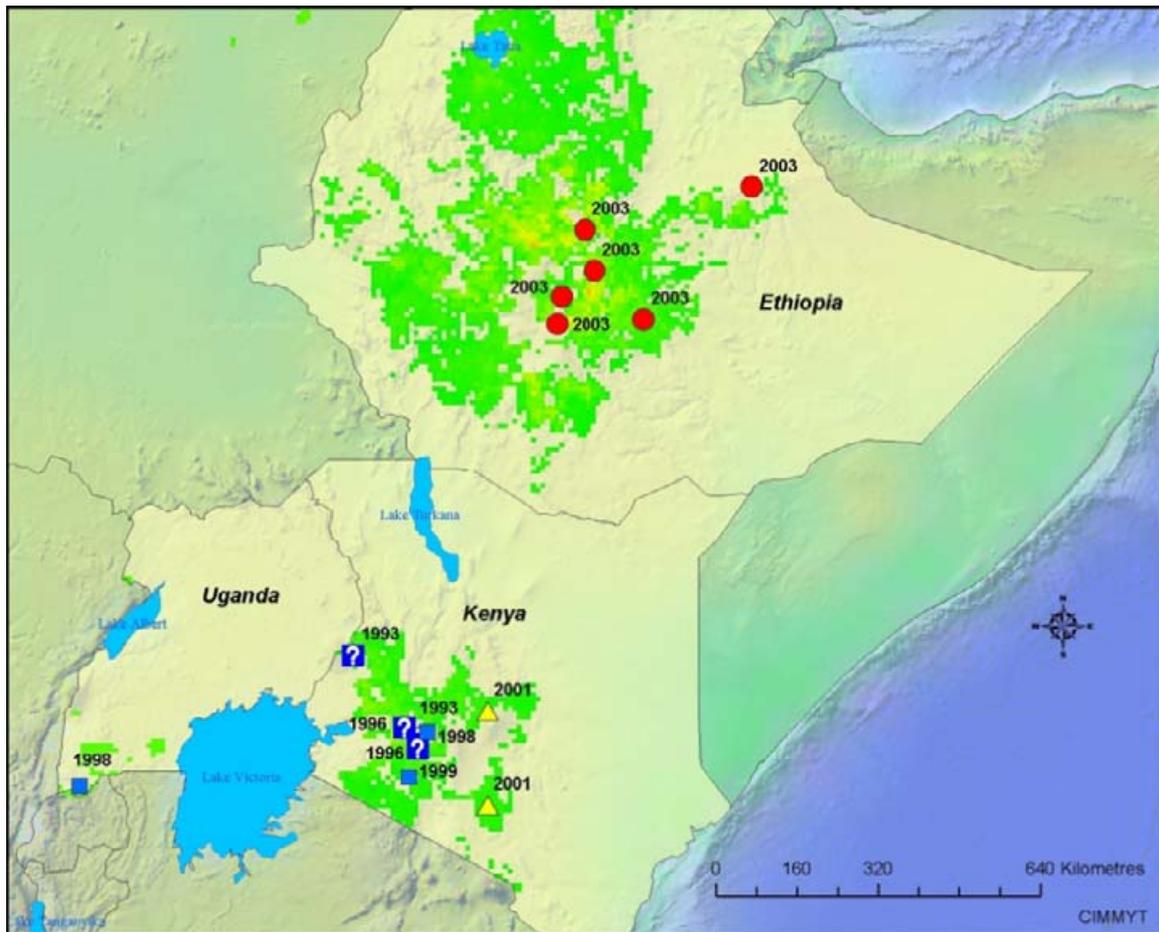
Figure 1   Known status of race Ug99 of the stem rust pathogen and regional wheat production areas. (Dates indicate first year of detection; different symbols and colours indicate chronology: blue squares earliest, yellow triangles intermediate and red circles most recent. Question marks indicate stem rust, but uncertain attribution to race Ug99)

range expansion, following the predominant west–east airflows.

A major concern is that a significant proportion of global wheat germplasm is potentially at risk from race Ug99. Reynolds and Borlaug [31] estimated that this area might amount to 50 million ha of wheat grown globally i.e., about 25% of the world's wheat area. Germplasm with resistance to Ug99 is available [17], but for many parts of the world, material of this type is not present in varieties grown in farmers' fields. Major questions that now arise are: how likely is it that Ug99 might spread, where Ug99 might spread to, and what the likely consequences of any movement are?

Potential Migration Paths for Race Ug99

Mechanisms of dispersal for Ug99 have been described in the previous section, but information relating to likely migration routes is a vital input into any ex ante impact assessment or mitigation planning processes. Obviously there are considerable uncertainties associated with any pathogen movement prediction studies, but several

factors relevant for spread of wheat stem rust can be included to guide analysis. Nobel Laureate, Dr Norman Borlaug, eloquently identified these key factors in an expert assessment of the stem rust outbreak in Africa [32], namely: area and distribution of susceptible material; degree of susceptibility; optimum temperatures and moisture; amount of initial inoculum; and air movement and complacency. One additional sub-factor that might be added to this list is historical precedent of movement through known regions.

Most evidence, albeit circumstantial, indicates that Ug99 is likely to spread beyond the borders of the three East African countries in which it is currently present. The sheer mobility of rust spores led an international panel of rust experts to conclude that 'it is only a matter of time until Ug99 reaches across the Saudi Arabian peninsula and into the Middle East, South Asia, and eventually, East Asia and the Americas' [32]. In addition, there is documented evidence connecting East Africa with West and South Asia for migration of rust races of East African origin [27].

Given the inherently spatial nature of Ug99 migration, GIS tools are starting to be used as a framework to
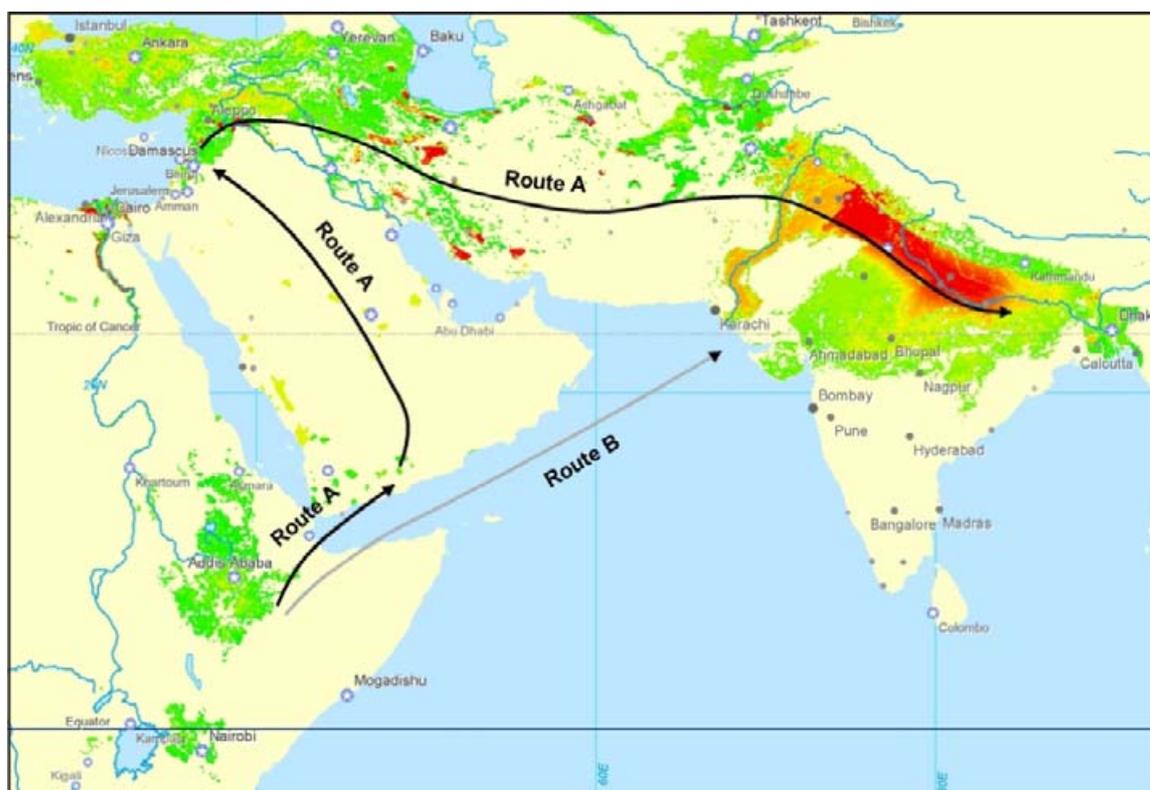
Figure 2   Potential migration routes for race Ug99 of the stem rust pathogen based on prevailing airflows and regional wheat production areas. Route A via Arabian peninsular, Middle-East and South Asia is considered to have a higher probability

integrate relevant factors determining likely movement [33]. Thus far these factors have included: the current status and distribution of Ug99, prevailing winds, climatic factors that favour survival and sporulation, distribution of wheat production zones, human populations within those production zones, historical migration patterns of rust races originating in East Africa, and known susceptibility of existing cultivars. This initial study is perhaps the first to assess likely Ug99 movements using any quantitative data inputs and supports the hypothesis of Singh et al. [27] that East Africa and the wheat area of Asia may comprise a single epidemiologic zone. Key elements of the Hodson et al. [33] study, and supplementary information, are summarized below.

The first component of the study investigated the enabling climatic conditions for migration. Analysis of predominant monthly wind vector data [34] during the wheat growing season, supports the notion of two potential airborne migration routes for Ug99 (Figure 2). The first route (A), which matches that described by Singh et al. [27] for the Yr9-virulent race of P. striiformis, is considered the most likely option. The second route (B), connecting East Africa directly with southern Pakistan/ western India, has no known precedent and is highly speculative and of much lower probability (this may be considered to be in the rare, single-event, long-distance dispersal class). Only the more likely major route (East

Africa–Middle East–West Asia–South Asia) is considered in any detail. Wind vector data during May–September indicates the possibility of movement of rust spores from the southern highlands of Ethiopia to Yemen – on the fringes of the southwest monsoon circulation system (Figure 3). Frequency or probability of airborne transmission by this route across the Arabian Gulf is currently unknown. It is noteworthy that the Yr9-virulent race took five years to achieve this crossing. However, any spores successfully crossing to the Arabian Peninsula would encounter suitable over-wintering conditions, as year-round conditions suitable for sporulation, based on a temperature range of 5–40°C [35] in combination with high relative humidity (>60% all year), were found to occur notably in most of East Africa and coastal zones of the Arabian Peninsula (Figure 4). In November–February, coincident with the wheat season, prevailing airflows would carry spores up the Arabian peninsular only to encounter a west-east airflow around the Mediterranean basin, that would move spores through the wheat belt of the Middle East and into the bread baskets of South Asia (Figure 4). This pattern of airflows, combined with wheat distribution, supports the migration route taken by the Yr9-virulent race in the 1990s.

The second component of the study investigated the characteristics and susceptibility of the wheat production areas along the potential migration route. Wheat is the
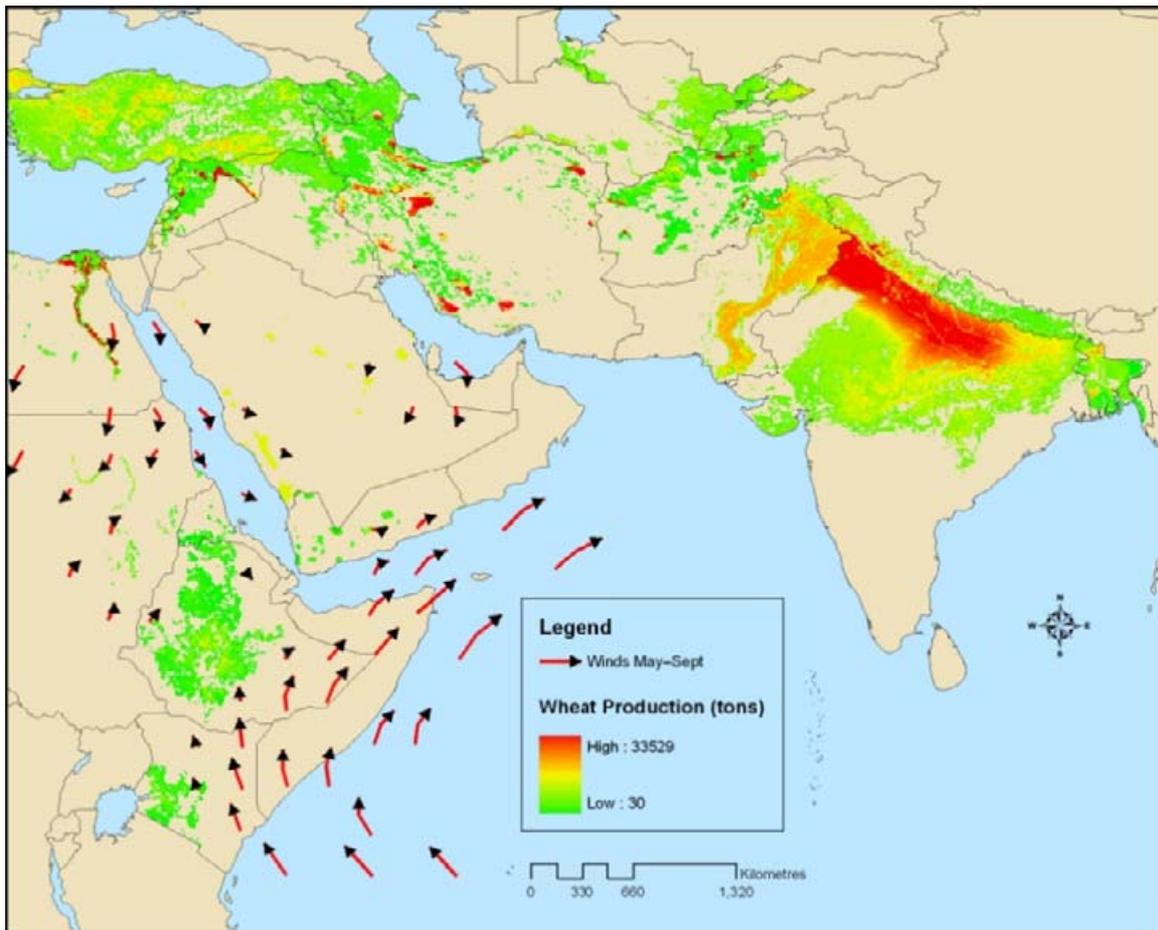
Figure 3 Predominant winds around the Horn of Africa during May–September

predominant crop, with an estimated 19% of global production (~.117 million tons) occurring in the potential Ug99 migration path, and an estimated 1 billion people living within these wheat production areas. Availability of finely disaggregated raster data for crop production [36] and population estimates [37] greatly facilitated the characterization of proposed epidemiologic zone. Susceptibility of wheat germplasm to Ug99 was another key consideration. Using data on area planted to known varieties (CIMMYT, unpublished), known pedigrees and corresponding susceptibility ratings to Ug99 from field-screening in Kenya between 2005 and 2006, the vast majority of current cultivars grown on at least 90% of the area in the potential risk zone were found to be susceptible. The huge areas observed in India and Pakistan result from the predominance of 'mega-cultivars' 'PBW343' and 'Inqualab 91' in the two countries, both of which have proved highly susceptible to Ug99. Only 0.3% of a total reported area of over 44 million ha planted to known cultivars in the epidemiologic zone was rated as being moderately resistant to Ug99.

In conclusion, the seemingly favourable environmental conditions, coupled with the extensive coverage of susceptible wheat varieties, are a grave cause of concern if Ug99 does spread unchecked. Conditions favourable for outbreaks of epidemics currently exist in the migration path of highest probability. If an epidemic from Ug99 does occur, extremely large numbers of wheat farming families would be seriously affected–especially those who have few alternative livelihoods. In these circumstances, landless labourers dependent on agricultural jobs would also be seriously affected, and one could anticipate an increase in the rural–urban migration of landless labourers and small farmers. If large production losses occur there would be significant implications for rural and national economic growth rates in seriously affected countries, and could even affect global wheat markets. Given the serious implications, there is a clear need for more detailed spatial modelling and improved prediction of the likely spread and impacts of Ug99. This should be combined with the establishment of systems for accurate monitoring and early warning to directly support the efforts of wheat researchers and national policy-makers working to prevent the spread of the disease and to alleviate its impacts.
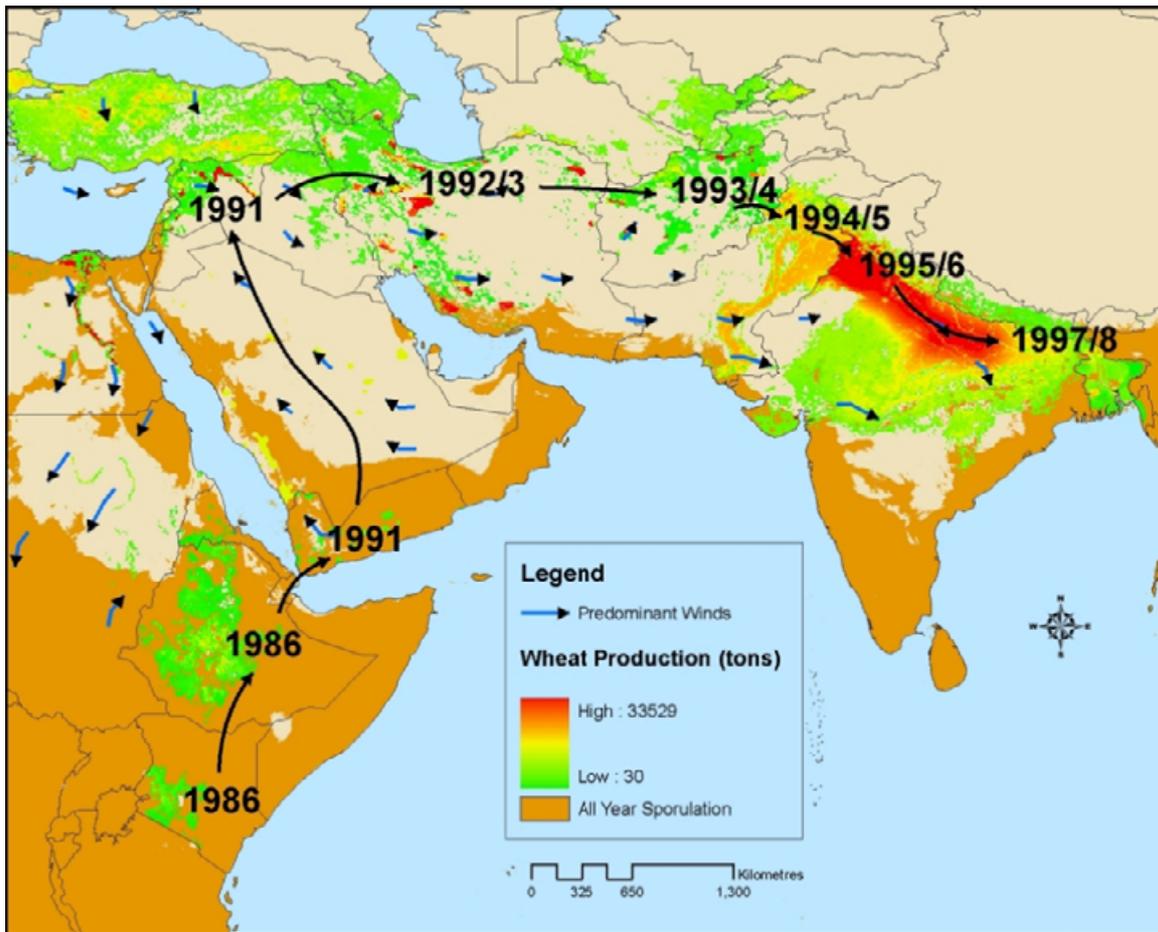
Figure 4 Year-round favourable climatic conditions, prevailing winds and historical, year-by-year movement of a *Yr9*-virulent race of the yellow rust pathogen that evolved in East Africa and migrated to Asia

Strategies to Mitigate the Risks of Losses From Epidemics Caused by Race Ug99

As described in the above section, race Ug99 is at present localized in the highlands of East Africa and poses a major risk to wheat production in Kenya, Ethiopia, Eritrea, Uganda and Tanzania. The best control strategy is to identify resistant wheat genotypes that can adapt to the prevalent environments in these countries, and release them after proper testing while simultaneously producing the seed. An aggressive strategy to promote these resistant cultivars in farmers' fields is the only viable option as resource-poor farmers in most of East Africa, except some commercial farmers in Kenya, cannot afford to use chemical control. A reduction in disease pressure in East Africa will also reduce chances of migration beyond the region. Nine and six new high-yielding wheat lines that have shown adequate resistance to race Ug99 during 2005 evaluation are being yield tested and simultaneously multiplied in Ethiopia and Kenya, respectively, during the 2006 crop season. An older Ethiopian cultivar 'Pavon 76' and two new cultivars, 'HAR3116' and 'FH6-1-7', have shown adequate resistance but it may be difficult to promote them successfully as their yield potentials are significantly lower than the current popular cultivars.

Reducing the area planted to susceptible cultivars in the Arabian Peninsula, North Africa, Middle East and West and South Asia is also the best strategy if major losses are to be avoided when race Ug99 migrates to these areas. The 'Global Rust Initiative', launched during 2005 and led by CIMMYT in partnership with ICARDA and various National and Advanced Research Institutions, is using the following strategies to reduce the possibilities of major epidemics: (1) monitoring the spread of race Ug99 beyond eastern Africa, (2) screening of released cultivars and germplasm for resistance, (3) distributing sources of resistance worldwide for either direct use as cultivars or for breeding, and (4) targeted breeding to incorporate diverse resistance genes and adult plant resistance into high-yielding adapted cultivars and germplasm (www. globalrust.org). Awareness of the potential losses was also raised through a summit that was held in Nairobi on 9 September 2005 with follow up meetings with different National Programs.

Greenhouse studies on avirulence/virulence, and the evaluation of wheat germplasm in greenhouse and

Table 2 Frequency of wheat cultivars and advanced breeding lines of different origins for their field response to Ug99 race of the stem rust pathogen at Njoro, Kenya during 2006

Country/institution	Response and frequency			
	Resistant[1]	Moderately resistant[2]	Moderately susceptible and susceptible[3]	Total
Bangladesh	0	3	81	84
China	1	1	116	118
Egypt	3	0	146	149
India	16	7	79	102
Iran	1	1	98	100
Khazakstan	2	1	83	86
Nepal	1	1	103	105
Pakistan	0	6	99	105
Russia	0	1	34	35
Turkey	14	2	69	85
CIMMYT-Irrigated[4]	94	56	400	550
CIMMYT-Semiarid[4]	50	9	161	220
CIMMYT-High Rainfall[4]	11	6	99	116

[1]Genotypes grouped under resistant category had up to 20% disease severity based on modified Cobb Scale [49] and small to intermediate sized uredinia with necrosis or chlorosis [35].
[2]Genotypes grouped under moderately resistant category had 15–30% disease severities and medium to large uredinia with or without chlorosis and necrosis.
[3]Genotypes grouped under moderately susceptible and susceptible category displayed 40–100% disease severity and medium to large uredinia without chlorosis and necrosis.
[4]Most recent CIMMYT germplasm that will be distributed worldwide through International Yield and Screening Nurseries targeted for irrigated, semiarid and high-rainfall spring wheat regions.

field have identified germplasm with known and some unknown race-specific resistance genes that can be used in wheat improvement. Some lines with adequate levels of adult plant resistance could also be identified. Frequency distribution of stem rust responses of wheat genotypes from various countries and CIMMYT observed during 2006 testing in Kenya is given in Table 2. Resistance of a large portion of the resistant lines is suspected to be based on gene *Sr24*, which in itself is a cause of concern. A majority of resistant wheat cultivars from Australia, Argentina, Uruguay, Brazil and the USA also carry *Sr24* gene for resistance (data not presented).

Race-Specific Resistance Genes

A large portion of the highly resistant germplasm from South America, Australia and CIMMYT possess *Sr24*. This gene is located on the *Thinopyrum elongatum* translocation on chromosome 3DL where the leaf rust resistance gene *Lr24* is present. There are three distinct *Sr24*-carrying translocations: the original one linked to a gene for red grain colour, the shorter segment with white grain, and a third segment where a very small segment has been retranslocated onto chromosome 1BS. In all three segments both *Sr24* and *Lr24* are present together. Therefore, selection for *Lr24* with avirulent leaf rust isolates can be used as an indirect selection strategy. This gene would look like an attractive candidate for future breeding efforts; however it must be used in combination with

other effective resistance genes because virulence to *Sr24* is already known in South Africa and India.

The *Sr25* gene is also located on a *Th. elongatum* translocation together with leaf rust resistance gene *Lr19* on chromosome 7DL. Despite the fact that this translocation is known to enhance the yield potential (38, 39), it was not used widely because it is linked to a gene associated with accumulation of undesirable levels of yellow pigment. A white floured mutant of the translocation, developed by Knott [40], was recently transferred into some Australian and CIMMYT wheat backgrounds. *Sr25* conferred a high level of resistance only in some genetic backgrounds, especially when the slow rusting adult plant resistance gene *Sr2* was also present, e.g. lines 'Super Seri#1' (yellow flour) and 'Wheatear' (white flour). Virulence to *Sr25* is not known yet.

Gene *Sr26*, also of *Th. elongatum* origin, translocated to chromosome 6AL, has been used successfully in Australia and remains effective despite its large-scale deployment in the 1980s. It is not known to be present in cultivars from other countries and the translocation used initially may confer a yield penalty [41].

Gene *Sr27*, of rye origin, has not been used in wheat improvement. Its deployment in triticale in Australia resulted in a rapid evolution of virulence [42]. This gene has also become ineffective in South Africa. Strategically, this gene should be left for triticale improvement in areas where virulence is not known.

Gene *Sr36*, derived from *Triticum timopheevi*, exhibits an immunity (no symptoms) to race Ug99 at both seedling

and adult plant stages [17]. This gene occurs in a high frequency in US soft winter wheat [17]. Although races with virulence for *Sr36* are common, it could be used effectively as a component for Ug99 resistance breeding.

Although race Ug99 is avirulent on genes *Sr28* and *Sr36*, numerous races virulent to these genes are known to occur worldwide. Genes *Sr29, 32, 33, 35, 37, 39, 40* and *44* have not been tested widely for their effectiveness to other races and also not used in breeding. Gene *SrTmp* from 'Triumph' is present in some US wheat cultivars [17] and can be used in breeding. An additional unnamed resistance gene located in rye chromosome translocation 1A.1R, is present in some US winter wheats such as 'Amigo', 'TAM107' and 'TAM200' [17], and can also be used [17]. The relationship between the stem rust resistance gene on 1A.1R translocation and *Sr31* is unknown. This translocation is present in a CIMMYT spring wheat line 'TAM200/Tui'. Both 'TAM200' and 'TAM200/Tui' also possess the *Sr24* gene, and may therefore be good parents to transfer two effective resistance genes simultaneously. Certain hexaploid synthetic (*Triticum turgidum* × *Aegilops tauschii*) wheat-derived advanced lines and some lines where certain Chinese cultivars such as 'Shanghai#7' are parents have also shown adequate level of resistance. However, the genetic basis of resistance is not known.

Durable Resistance and Hope Derived Sr2-Complex'

Durable stem rust resistance of some older US, Australian and CIMMYT spring wheats is believed to be due to the deployment of *Sr2* in conjunction with other unknown minor, additive genes. McFadden [6] transferred Gene *Sr2* to hexaploid wheat in the 1920s from tetraploid emmer wheat cultivar 'Yaroslav'. The slow rusting gene *Sr2* confers only moderate levels of resistance when present alone [43]. Its presence can be detected through its complete linkage with pseudo-black chaff phenotype, which can be prominently expressed under certain environments leading to its elimination in some breeding programmes. On wheat lines that displayed pseudo-black chaff, we observed varying degrees of disease in Kenya with a maximum severity reaching about 60% compared to 100% severity for highly susceptible materials. Reaction types varying from MR to S (moderately resistant to susceptible) on the same internodes of *Sr2*-bearing plants clearly indicated that *Sr2* did confer at least some resistance. However, the level of resistance conferred by *Sr2* alone under high disease pressure in Kenya was not sufficient. *Sr2* was detected in several highly resistant old, tall Kenyan cultivars, including 'Kenya Plume' [44], and CIMMYT-derived wheats 'Pavon 76', 'Juchi 2000' and 'Kritati'. Pavon 76 and Kiritati were resistant during 2004, 2005 and 2006 with maximum disease score of 20MR-MS. Because all three of these wheat cutlivars are susceptible as seedlings with race TTKS, their resistance as speculated

earlier [9] is based on multiple additive genes where *Sr2* is an important component.

US wheat cultivar 'Chris', which is not known to carry *Sr2* but possesses several seedling resistance genes including *Sr7a* [45], also displayed adequate level of resistance in the field in Kenya. Preliminary studies of inheritance of seedling resistance to TTKS in Chris indicated that Ug99 resistance in 'Chris' is controlled by two complementary recessive genes (Y. Jin, unpublished), and the same seedling resistance is present in 'AC Barrie' (a Canadian spring wheat cultivar) and 'Bonza 65' (a CIMMYT-derived cultivar). Singh and McIntosh [45] indicated the possibility that the adult plant resistance to *Sr7a*-avirulent Australian races may involve interaction of moderately effective gene *Sr7a* and other unknown adult plant resistance genes. Seedling tests conducted at St. Paul, MN, USA indicated that Ug99 is virulent on *Sr7a*-tester line although Chris did show seedling resistance. Singh and McIntosh [45] indicated that resistance conferred by *Sr7a* is difficult to evaluate both in seedlings and adult plants when the gene is present alone. Therefore, at this stage we cannot determine the role *Sr7a* may have played in resistance of 'Chris' observed in Kenya. Even though seedling tests indicate that *Sr23*, another gene whose expression is difficult to evaluate in seedlings and adult plants when present alone, may be ineffective against Ug99, adequate resistance in 'Selkirk' might involve interactions of moderately effective genes *Sr2* and *Sr23* (linked to leaf rust resistance gene *Lr16*) and perhaps additional unknown adult plant resistance genes. Although these observations need validation through genetic analyses, they indicate that complex resistance to stem rust present in some tall as well as some semidwarf cultivars developed in the 1960s and 1970s continues to remain effective.

Breeding Strategies for Resistance to P. graminis tritici Race Ug99

The fastest way to reduce the susceptibility of important wheat cultivars and the best new germplasm is to systematically incorporate diverse sources of resistance into them through limited or repeated backcrossing. Race-specific resistance genes that will confer resistance on Ug99 and other important races were described earlier. Because most of these genes are of alien origin, co-segregating molecular markers for several of them are already available [46, 47] and can aid selection. Where the alien stem rust resistance genes are linked to leaf rust resistance genes, screening for leaf rust in seedlings or adult plants can also be practised.

The best strategy to use race-specific resistance genes is to use them in combinations. Molecular markers provide a powerful tool to identify plants that carry combinations of resistance genes. However, it may be difficult to combine more than two translocations in a single genotype

as their negative impact on yield and quality can be quite large. To transfer two or more effective resistance genes into an adapted cultivar the better crossing strategy would be to first cross the resistance sources and then cross the F_1 plants with the adapted cultivar. Molecular markers can then be used to select top-cross plants that have desirable agronomic features and carry the targeted resistance genes. Because such plants are expected to be in low frequency, it is desirable to maintain a large family size of approximately 400, which can be obtained by emasculating and pollinating 20 spikes. Further backcross on selected plants will help restore the characteristics of the recurrent parent.

A better strategy is to restore the durable resistance in current cultivars and new wheat germplasm. At present little is known about the genes involved in durable resistance; however, some earlier work done by Knott [48] and knowledge on durable resistance to leaf and yellow rusts [27] indicate that such resistance will involve multiple minor genes with additive effects. Accumulating such complex resistance in the absence of disease pressure caused by Ug99 race and a lack of molecular markers will not be an easy task. Molecular markers linked to the slow rusting resistance gene $Sr2$ are known and can be used in selection; however, this gene can also be identified in the field under certain environments from its linkage with pseudo-black chaff phenotype. $Sr2$ is present in over half of the current CIMMYT's spring wheat germplasm including some of the most high-yielding recent genotypes that have high level of resistance to leaf and yellow rusts and good industrial quality.

Our strategy is to transfer the adult-plant resistance from Pavon 76, and a few other wheats identified so far, to a range of important CIMMYT wheat germplasm by using the 'single-backcross selected-bulk' breeding approach [27]. In this strategy the resistance sources are crossed with the adapted high-yielding wheats and then a single backcross is made with the recurrent parent to obtain about 400 BC_1 seeds. BC_1 plants were then selected for desired agronomic features and resistance to leaf and yellow rusts, and harvested as bulk. Large F_2 populations of about 2500 plants will be grown and plants will be selected in Mexico for agronomic traits and resistance to other diseases and harvested as bulk. A similar selection will be practised in the F_3 generation to obtain F_4 populations. At this stage we will try to select for adult-plant resistance by growing densely sown F4-bulk populations in Kenya or Ethiopia, under high stem rust pressure created by inoculating with Ug99 race. Populations will be bulk harvested and plumper grains selected to grow F_5 generation in Mexico. Because stem rust affects grain filling, we expect that plants with insufficient resistance will have shrivelled grains. Moreover, by F_4 generation enough homozygosity is achieved for the selection of additive resistance genes. Individual plants with desired agronomic features and resistance to other diseases will be selected in the F_5 generation and those

with good grain characteristics will be grown in F_6 as hill plots or short rows in Kenya or Ethiopia as well as small plots in Mexico for final selection. Finally, the resistant F_6 plots will be harvested for conducting yield trials in the following crop season. The same methodology is also proposed to transfer resistance from old, tall Kenyan cultivars into adapted semidwarf wheats. The proposed approach is expected to rebuild the durable resistance in modern wheat germplasm. Genetic analyses will be necessary to understand the number and type of resistance genes involved in sources contributing the adult plant resistance. Genomic locations of minor, additive resistance genes will be determined through molecular mapping. Such information will be useful to establish and enhance genetic diversity for minor genes.

Conclusion

Detection and spread in East Africa of the highly virulent *P. graminis tritici* race TTKS, commonly known as Ug99, once again reminds us to shake off the complacency from past successes. Stem rust disease of wheat, which has been controlled for several decades worldwide through the use of genetic resistance, could once again become the cause of food shortages and famines in Africa, the Middle East and Asia if the spread of race Ug99 remains unchecked. Replacement of currently popular susceptible cultivars in these areas with high-yielding resistant cultivars is the best strategy to protect wheat production. This would require a concerted effort involving scientists, governmental and non-governmental agencies and interventions from progressive farmers.

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