Tracking the wheat rust pathogens

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Abstract

Rapid progress has been made towards the goal of establishing a Global Cereal Rust Monitoring System. The system has reached the point where it can now be regarded as a fully operational global disease monitoring system. Creation of a functional surveillance and monitoring network, covering 35 countries and a large proportion of the developing world wheat area is seen as a significant achievement. Through this network it has been possible to track the spread and status of important stem rust races such as those within the "Ug99 race group". New technologies are playing an increasingly important role in rust tracking. These are already having an impact in several different areas from survey data collection to pathogen diagnostics and their role is likely to increase in the future. A robust and functional data management system - the Wheat Rust Toolbox - is now in place. This includes extensive rust surveillance and race databases and a suite of dynamic visualization tools. New web resources are providing access to a wealth of information regarding rust surveillance and monitoring in ways not previously possible. Global collaboration is ensuring that key databases are shared and being integrated into different information platforms. Despite good progress, several areas are seen as priorities for further work. These include: expansion of the data management system, increased information relating to host genotypes and rust resistance genes, early warning systems and disease mitigation planning. Activities are planned in the near future in all these areas.

Introduction

The appearance of stem rust race TTKSK (Ug99) in East Africa (Pretorius et al. 2000) was the catalyst to put in place a global monitoring system for wheat rusts. Identification of a race of *Puccinia graminis* f. sp. *tritici* to which a large proportion of commercial wheat cultivars were susceptible was a clear signal that tracking rust pathogen races and monitoring disease status on a global basis was a high priority. The sheer mobility of rust pathogens coupled to their inherent ability to change through mutation, recombination or somatic hybridization makes continual monitoring across large geographical areas an absolute necessity (Brown and Hovmøller 2002; Park 2007). Significant progress in the development of such a global monitoring system has been made largely under the auspices of the Durable Rust Resistance in Wheat (DRRW) project. Progress in the conceptualization, development and implementation of a Global Cereal Rust Monitoring System (GCRMS) has been previously described in detail by Hodson et al. (2009, 2011) and Park et al. (2011). The series of reviews

relating to Ug99 by Singh et al. (2006; 2008, 2011) have also provided updates and summary information regarding the GCRMS. Through the work of the GCRMS it has become clear that Ug99 cannot be regarded as a single entity, because a number of different races have been identified that are considered to belong to a clonal lineage that includes the original Ug99. For the purposes of this paper the term "Ug99 race group" is used throughout. The working definition of this term is: 'the group of *Pgt* races sharing almost identical molecular fingerprints to the original Ug99 isolate (race TTKSK). Most races in the group have virulence to Sr31, but Sr31-avirulent progenitors/relatives are also included'. The GCRMS is not a static entity and considerable efforts are being undertaken by an international coalition to improve and expand the existing system. Changes, progress, improvements and remaining gaps in the current GCRMS will be the focus of this paper. Technology is playing an increasingly important role in improving the efficiency of rust monitoring and these new interventions will be described. Well managed and accessible information is also critical if informed decisions are to be made to mitigate the threat of rusts. Several advances in this area will also be highlighted. It has long been recognized that any successful surveillance and monitoring system must take a holistic view, covering the pathogen, and equally important, linking this with relevant knowledge of the host as well. Progress towards this goal of a more integrated and holistic approach will be reported. Despite good progress, several areas are seen as priorities for further work to advance the current GCRMS. Future planned activities to enhance the current global monitoring efforts will be described.

Current status of the GCRMS

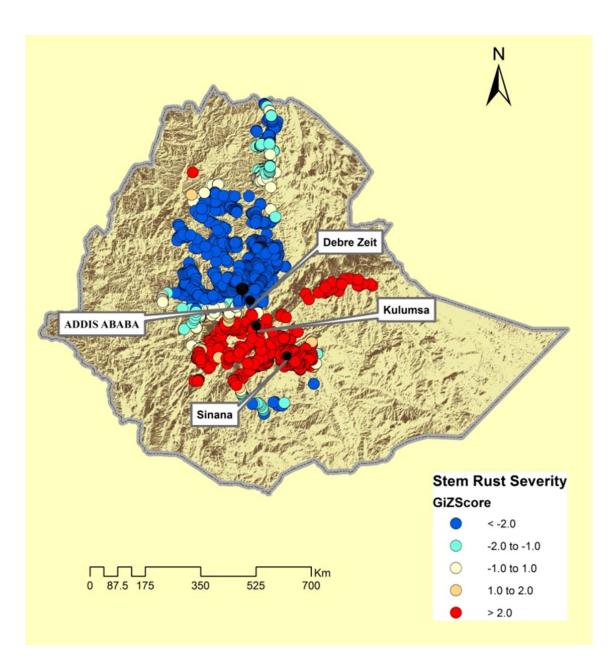
At the inception of the GCRMS in 2007, only two stem rust-affected East African countries (Ethiopia & Kenya) had undertaken rust surveys in a standardized way and made data available. In the subsequent years, the global network contributing to the GCRMS has expanded substantially and now 27 African and Asian wheat-producing countries have contributed surveillance data. Many of these countries are now undertaking surveys and contributing data on an annual basis. These 27 countries account for an estimated 42 million ha of wheat, approximately 20% of the world wheat area. An additional eight countries (both developed and developing) have contributed valuable data on stem rust races. In total, the GCRMS now includes consolidated stem rust race data from 21 countries. This current global network of 35 countries represents the first example of comprehensive developing country rust monitoring. The GCRMS network is now delivering routine information on the rusts on a vast geographical scale rarely seen before, enhancing knowledge not only of stem rust but other wheat rusts as well.

The global surveillance has revealed the predominance of stem rust in East and Southern Africa, with isolated pockets of the disease occurring in other areas. Stripe rust is more widespread, dominating throughout the Central, West Asia, North Africa (CWANA) region and into South Asia. There are some indications that stripe rust may be increasing in the East African highlands. In other parts of the world, e.g. Europe, China, USA and Australia, stripe rust is increasingly the major biotic constraint to wheat production. Leaf rust is the most cosmopolitan of the rusts, being recorded in all areas surveyed. An example illustrating the geographical coverage of on-going surveillance and relative importance of the three rusts in 2011 is given in Fig. 1. An increasingly rich surveillance database (over 9,000 geo-referenced records) is permitting secondary analysis and value addition to the original survey data. Geo-spatial analysis of multiple years of standardized survey data is facilitating the identification of disease hot-spots. Geo-statistical approaches such as hot-spot analysis using the Getis-Ord

Gi* statistic (Mitchell 2005) permit investigation of where statistically significant clusters of high (hot-spot) or low (cold spot) disease severity occur. Spatial interpolation of disease severity data permits visualization of areas of either high or low disease severity. These approaches result in the development of risk maps that can then help guide decision making regarding disease control and mitigation measures. Examples of stem rust risk maps for Ethiopia and Kenya are given in Fig. 2. Initial risk maps have been produced for all countries with survey data, and these are being updated as soon as new data are available. Data mining of the original surveillance data to extract secondary data (e.g. climate, elevation) for possible use in disease forecasting and early warning is the next step to be undertaken.



Fig. 1 Relative importance of the three wheat rusts based on BGRI survey data in 2011 (Black bars = proportion of surveyed fields with stem rust, brown bars = proportion with leaf rust and yellow bars = proportion with stripe rust) *Source*: Wheat Rust Toolbox



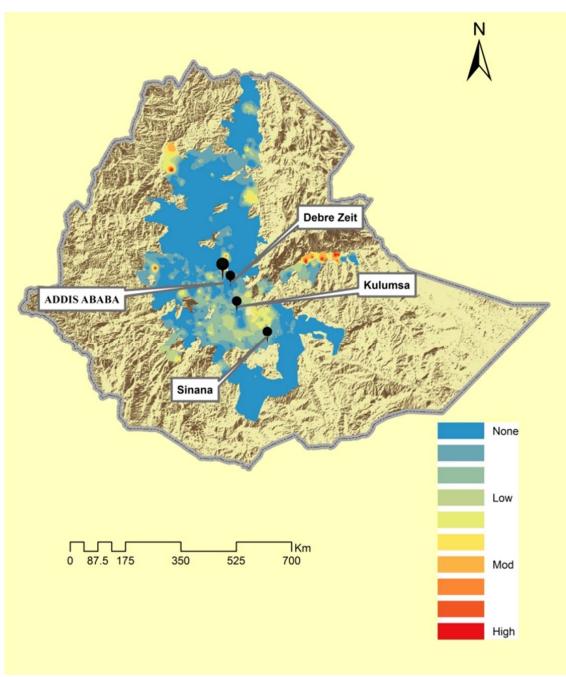


Fig. 2 Examples of stem rust risk maps for Ethiopia and Kenya. **Map A** indicates geostatistical hot-spots, with red dots representing statistically significant hot-spots of high disease severity. **Map B** shows an interpolated surface (Inverse distance Weighting) of disease severity. Surface masked to wheat growing areas

Pathogen monitoring

Stem rust race data has been obtained from 21 countries, with multiple years of data now available for the majority of them. Data from 1,075 isolates are currently included in the GCRMS core database. The "Ug99 race group" has been the focus of much of this work. Through the global sampling and race analysis network, the "Ug99 race group" has been

successfully tracked, both in terms of geographical distribution and race variation. Members of the "Ug99 race group" have been identified in 10 countries (Uganda, Kenya, Ethiopia, Sudan, Yemen, Iran, South Africa, Tanzania, Zimbabwe, Mozambique). A total of 8 races within the "Ug99 race group" have been positively identified. A summary of the known status of the "Ug99 race group" is given in Table 1. The majority of these races are present in Eastern and Southern Africa. Only the original Ug99 variant – race TTKSK – has been confirmed outside of Africa. However, future spread of additional Ug99 variants out of Africa is considered highly likely. An overview of the current geographical status of the "Ug99 race group" is summarized in Fig. 3. Within Africa, "Ug99 race group" variants with virulence to Sr24 are increasingly widespread and are often the predominant races (Pretorius et al. 2010; Mukovi et al. 2011; Wolday et al. 2011). These races include TTKST, PTKST and TTKSP, and one or more have been recorded in seven African countries (Kenya, Ethiopia, Eritrea, Tanzania, Mozambique, Zimbabwe, and South Africa). From current monitoring data the original Ug99 (race TTKSK) now predominates only in Ethiopia (Table 2). In all other countries, Ug99 variants other than TTKSK have been identified more frequently in analysed samples. Recent identification of race TTKSF+ in South Africa and Zimbabwe (Pretorius et al. 2012) represents the eighth race in the "Ug99 race group". No expansion of the geographical range of the "Ug99 race group" has been detected since Mukoyi et al. (2011) reported confirmation of the "Ug99 race group" in Mozambique and Zimbabwe and Wolday et al. (2011) confirmed the presence of the "Ug99 race group" in Eritrea. Recent race analysis data from Eritrea (T. Fetch, unpublished) indicates a predominance of "J" races – virulent for Sr13 and likely originating from durum wheat – among samples collected in 2011. Only 1 isolate (out of 15) collected in 2011 was typed as a member of the "Ug99 race group" (PTKST). This contrasts to sampling in 2010 when "Ug99 race group" Sr24 variants (PTKST, TTKST) were the only races identified in Eritrea (Wolday et al. 2011).

Race ¹	Common alias	Key virulence (+) or avirulence (-)	Country (year of 1 st detection)	
TTKSK	Ug99	+Sr31	Uganda (1998), Kenya (2001), Ethiopia (2003), Sudan (2006), Yemen (2006), Iran (2007), Tanzania (2009)	
TTKSF		-Sr31	South Africa (2000), Zimbabwe (2009)	
TTKST	Ug99 + Sr24	+Sr31, +Sr24	Kenya (2006), Tanzania (2009), Eritrea (2010)	
TTTSK	Ug99 + Sr36	+Sr31, +Sr36	Kenya (2007), Tanzania (2009)	
TTKSP		-Sr31, +Sr24	South Africa (2007)	
PTKSK		+Sr31, -Sr21	Ethiopia (2007), Kenya (2009)	
PTKST	Ug99 + Sr24	+Sr31, +Sr24, -Sr21	Ethiopia (2007), Kenya (2008), South Africa (2009), Eritrea (2010), Mozambique (2010), Zimbabwe (2010)	
TTKSF+		-Sr31	South Africa (2010), Zimbabwe (2010)	

 Table 1 Summary of the known status of the "Ug99 race group"

¹Some uncertainty exists over the reaction of the Sr21 gene (this influences the initial code letter being "T" (+Sr21) or "P" (-Sr21). Current table presents most plausible races. All names are using the North American 20 differential set nomenclature

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Table 2 Predominant Pgt races in African countries based on BGRI sampling studies 2008-2011 (Predominant race in year is highlighted in bold. Number in parentheses = number of isolates)

					Current predominant
Country	2008	2009	2010	2011	race
Eritrea			PTKST (5)	JRHSF (8)	JRHSF
			TTKST (4)	JRHSC (4)	
				JRCSC (2)	
				PTKST (1)	
Ethiopia		TTKSK (41)	TTKSK (56)		TTKSK (Ug99)
		JRCQC (12)	TTKST (3)		
		TRTTF (4)	TRTTF (1)		
		PTKST (1)	JRCQC (2)		
		PTKSK (1)			
Kenya	TTKSK (38)	TTKSK (15)	TTKST (81)	TTKST (13)	TTKST (Ug99 +
	TTKST (15)	TTKST (13)	PTKST (10)	TTKSK (3)	Sr24)
	TTTSK (2)	PTKST (5)	TTKSK (1)	PTKST (2)	
	PTKST (2)	PTKSK (2)			
		RRTTF (1)			
		RTRJP (2)			
J		RTGDK (1)			
Tanzania		TTTSK (21)			TTTSK (Ug99 +
		TTKST (12)			Sr36)
		TTKSK (6)			
Zimbabwe		TTKSF (3)	PTKST (3)		PTKST (Ug99 +
			TTKSF (3)		Sr24) or TTKSF
			TTKSF+ (1)		
South Africa	TTKSF (23)	TTKSF (49)	TTKSF (82)	TTKSF (35)	TTKSF (Ug99
	TTKSP (10)	TTKSP (18)	TTKSP (2)	2SA105 (4)	progenitor?)
	2SA105 (12)	PTKST (18)	PTKST (2)	2SA102 (1)	
	2SA104 (5)	2SA105 (26)	2SA105 (23)		
	2SA102 (5)	2SA104 (10)	2SA104 (3)		
	2SA55 (3)	2SA103 (3)	TTKSF+ (1)		
		2SA102 (16)			

Table 2 Predominant *Pgt* races in African countries based on BGRI sampling studies 2008-2011 (Predominant race in year is highlighted in bold. Number in parentheses = number of isolates)

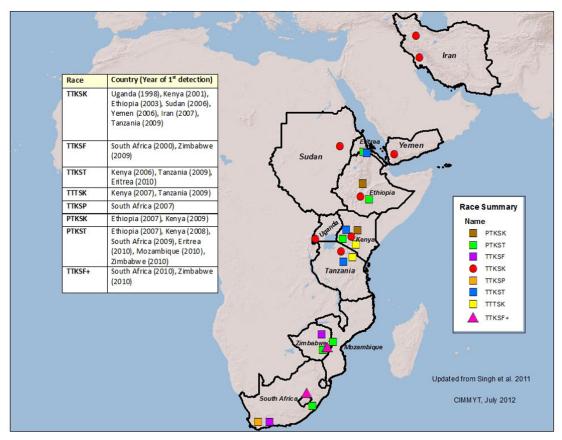


Fig. 3. An overview of the current status and geographical distribution of the "Ug99 race group"

New tools for rust monitoring

Modern information and communication technologies offer increasing opportunities to enhance and improve the efficiency of disease monitoring efforts. Technological interventions are now being implemented throughout the entire rust monitoring chain from field survey data collection, through analysis, to information dissemination. Hand-held GPS provide a quick and reliable way to geo-reference survey locations and sampling sites. These have been used in parallel with standardized survey forms since global wheat rust monitoring was initiated. Whilst basic handheld GPS devices provide accurate location data, they do not permit the entry and capture of survey observation data. This limitation requires the subsequent manual integration of both data sets, which is achieved practically using traditional pen and paper format. Subsequent conversion to electronic format can be both time consuming and a source of errors. The advent of an increasing array of affordable handheld devices offers new opportunities for field data capture and transfer.

Modern Smartphones incorporate an ever increasing array of technology options, linked to increasingly lower costs. Devices that can transfer data, run a range of custom applications, receive email, take high definition photographs or videos, and are GPS-enabled are now common-place. Tablet devices offer a similar range of functionality to Smartphones, but have the additional advantage of incorporating larger screens. Prices for both Smartphones and

Tablets are now well below US\$200, making them affordable options for use on field surveys.

The GCRMS has started to utilize mobile technology in field surveys. A customized version of the EpiCollect application (Aanensen et al. 2009) has been developed and deployed for Android devices and i-Phones. This customized application has four main features; (i) capture of GPS data, (ii) capture of a geo-referenced photographs, (iii) data entry using a standardized BGRI rust survey data form, and (iv) automated data transfer and synchronization with core, centralized databases. A similar application is currently under development for Tablet devices.

Whilst new mobile technologies offer many advantages for field surveys, it must be borne in mind that several limitations still exist. Use of any electronic devices under field conditions does require careful consideration and evaluation of issues such as screen visibility, battery life, field robustness/ ruggedness and reliability, training of users, ease of use and technology limitations, e.g. text input on small-screen devices, lack of internet access and additional costs of 3G data connections. Ultimately, it is unlikely that a single solution will fit all circumstances and user-groups, hence a range of options will be needed. The GCRMS is taking a multiple solution approach to field survey data collection, aiming to provide a range of data collection options to participating field surveyors.

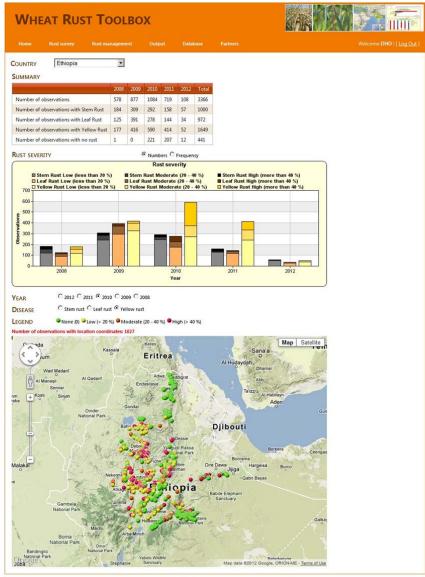
In addition to field survey data collection, new technologies are also providing opportunities in other areas. Molecular diagnostics are playing an increasingly important role in supporting global pathogen monitoring activities. Crouch et al. (2010) reported the development of diagnostic SNP-based markers capable of distinguishing the "Ug99 race group" with a high degree of reliability. The advantages of such techniques include: fewer restrictions on sending dead DNA samples internationally for analysis, it avoids any problems with lack of sample viability, and permits rapid diagnosis (48 hour assay) of the possible presence of the "Ug99 race group". The major limitation is that it lacks the discriminating power for specific race identification afforded by conventional, differential-based analyses. However, this work is expanding and improved diagnostic abilities on specific, important "Ug99 race group" races is likely in the near future.

Information systems

Data management and information dissemination are critical issues for the GCRMS if it is to be successful. Using Google Earth as a platform, the CIMMYT Rust Mapper tool (http://rusttracker.cimmyt.org/?page_id=256) was the first global rust information tool and remains an integral part of the GCRMS. Several additional major advances in data management and information dissemination have been achieved recently. The Wheat Rust Toolbox data management system has been developed in collaboration with the Global Rust Reference Center (GRRC) at Aarhus University, Denmark, and this system is now fully operational. The entire Wheat Rust Toolbox has now been converted to a .NET development framework, permitting more flexibility, improved performance and enhanced functionality. Although the Wheat Rust Toolbox is entirely web-based, it is not a public-domain website. Its function is restricted to i) a data management platform regarding data entry, quality control, management and exchange of data, and ii) a test and development platform for web based data analysis and display tools. The Wheat Rust Toolbox currently has several key components and a brief description of each follows:

Core databases: Two major core databases are currently implemented and populated within the Wheat Rust Toolbox – the rust survey database and the stem rust race database. The rust survey database currently holds over 9,000 geo-referenced, standardized, field survey data records from 27 countries. Survey database records span the period 2007 to 2012 and include information on all three rusts. The stem rust race database includes data from 1,075 isolates and 21 countries. Expansion beyond the current core databases is currently in development with planned inclusion of a Barberry Database (the alternate host for *Pgt* and *Pst*), Trap Nursery Database and a Molecular Diagnostics Database. These additional databases should be available by mid 2013.

Restricted access, user data entry and visualization tools: The Wheat Rust Toolbox includes a comprehensive User Management system that permits controlled access to specific tools and functionality. Registered users have country-specific access to an on-line data entry system and a suite of country-specific data visualization options for their own data. The current system permits secure on-line survey data entry, storage in a structured database, data editing, data visualization and data export (via XML/Excel) of country-specific data. Data visualization options include: a tabular data summary, an interactive graphical display of all rust data by year, a raw data analysis tool, and an interactive map of the country survey data. An example of registered user outputs is given in Fig. 4. These options combined with the advantage of having the data analysed in a global or regional context will be a major motivation for using the system in the future. Only when country data has been checked and approved for publication by the data owner does it enter into the public domain, global data dissemination tools.



Web site provided by <u>Aarhus University</u>, <u>Faculty of Science and Technology</u>, <u>Department of Agroecology</u> Report technical problems to webmaster: Poul Lassen. Optimized for screen size 1024x768

Fig. 4 An example of the Wheat Rust Toolbox registered user outputs for Ethiopia

Public domain, global data dissemination tools: A comprehensive range of visualization tools now provides access to quality-controlled survey and race data. All tools are directly linked to the under-lying databases and all provide interactive rather than static visualizations. Any updates to approved and published data are automatically displayed in the visualization tools. The current set of interactive tools includes: a survey mapping tool for all three rusts with both a country-specific and global version, an overview of the importance of all three rusts across countries (see Fig. 1), a stem rust race frequency graph and map, a stem rust race by country graph, a first detection of stem rust virulence tool, a stem rust virulence by country graph, and a stem rust virulence by year mapping tool. All of the Wheat Rust Toolbox Data Dissemination tools are accessible and implemented within public domain rust surveillance websites. A similar set of interactive race tools are likely to be made available for stripe rust in the near future.

A set of new websites is now available that provide access to the GCRMS data sets. The principal web site for all the rust surveillance and monitoring data is RustTracker.org (http://rusttracker.cimmyt.org/). This site was developed by CIMMYT and partners as a component of the Durable Rust Resistance in Wheat (DRRW) project and is an integral part of the Borlaug Global Rust Initiative (BGRI). The aim of RustTracker.org is to provide a comprehensive set of information about global rust surveillance and monitoring. The site is directly integrated with the Wheat Rust Toolbox and includes implementations of all the data dissemination tools generated by the data management platform. Other key features include a comprehensive set of situation updates regarding current rust status and an extensive set of country-specific pages and tools covering approximately 40 countries. A limited amount of host information is also integrated directly into RustTracker.org and the plan is that this content will increase considerably in the near future. Stem rust, and the "Ug99 race group" in particular, has been an initial focus of RustTracker.org, but information and content is being expanded to include both stripe rust and leaf rust. Ultimately, RustTracker.org aims to be the most comprehensive source of information for all rust surveillance and monitoring related content.

The new GRRC website, <u>www.wheatrust.org</u>, is also directly linked to the outputs of the Wheat Rust Toolbox. All of the outputs from the Data Dissemination Tools are also available at this location. It is envisaged that over time, additional country-based websites hosted by national partners will provide outlets for a selected set of Wheat Rust Toolbox data outputs. The overall vision is that the data held within the GCRMS can be disseminated via a wide range of public outlets to ensure maximum accessibility and utility to the global rust community.

Whilst the Wheat Rust Toolbox and the GCRMS in general has a focus on the pathogen it is essential that linkages exist to the host, so that surveillance and monitoring information can be used to guide control and mitigation efforts. Some progress has also been made on this more holistic and integrated approach. Recent developments include collaborative work between the developers of the Genetic Resources Information System (GRIS www.wheatpedigree.net) and the CIMMYT Wheat Atlas (wheatatlas.cimmyt.org) development team. The GRIS system already includes information on all published references to known resistance genes, and these are tagged to cultivars and pedigrees. Work is in progress to consolidate, integrate and enhance the cultivar databases currently held in the respective systems. A direct linkage between the CIMMYT Wheat Atlas cultivar data and RustTracker.org has already been established. Additional activities are on-going to update the information on areas planted to major wheat cultivars at the sub-national level. Once consolidated, this cultivar area data will be used to update responses to important races such as those within the "Ug99 race group". Direct linkages to an International Rust Screening Nursery database being developed by the DRRW project team at Cornell University is also under development. Consolidation into a single global database of all current information on rust resistance genes present in major commercial cultivars is also planned as a future activity.

Future activities

Although the GCRMS has developed into what can now be regarded as a fully operational global disease monitoring system, several areas are targeted for improvement and enhancement. The current system has a strong focus on stem rust, but it is clear that more information on the other two rusts, particularly stripe rust is needed. Timing of surveys is

often geared towards the optimum for stem rust, but ways have to be found to encourage multiple surveys to obtain reliable data on stripe rust outbreaks. Improved host information and associated rust resistance genes have already been mentioned as priority areas. Expansion of the existing core databases and enhanced functionality of the Wheat Rust Toolbox are other areas on which significant progress is anticipated on a relatively short timescale. Other aspects in which it is hoped to make further progress are disease early warning and mitigation planning advice. Both these areas are under consideration to be tackled through an epidemiological modelling approach as part of a planned collaboration with Cambridge University and Rothamsted Research Institute in the UK.

Conclusion

Considerable progress has been made towards the goal of establishing a GCRMS. Within a relatively short time period, the system has now reached the point where it can be regarded as a fully operational global disease monitoring system, at least for stem rust. Creation of a functional surveillance and monitoring network, covering 35 countries and a substantial proportion of the developing world wheat area is a significant achievement. Through the efforts of all contributing partners in this network it has been possible to track the spread and status of important *Pgt* races such as those within the "Ug99 race group". The mere fact that no major changes in the current geographical distribution of the "Ug99 race group" can be reported with a large degree of confidence indicates that the system is working. The current system has a strong focus on stem rust, but it is clear that more information on the other two rusts, particularly stripe rust, is needed. New technologies are playing an increasingly important role in the GCRMS. These are already having an impact in several different areas, from data collection to diagnostics, and their role is likely to increase in the future. Considerable attention has been given to developing a robust and functional data management system. Having the Wheat Rust Toolbox fully functional, databases populated with considerable amounts of data, and a range of operational visualization tools is a major advance. The rich, consolidated global data resource is now permitting data mining and value addition in the form of rust risk maps. New web resources are providing access to a wealth of information regarding rust surveillance and monitoring in ways that were not previously possible. Even more encouraging is that numerous groups working on rust related information systems are working collaboratively to ensure that single source databases are shared and integrated into different information platforms. This level of global collaboration around a major disease is highly commendable and likely to result in major benefits to the global wheat community. Despite good progress, several areas are seen as priorities for further work to advance the current GCRMS. These include; expansion of the data management system, increased information relating to the host and to rust resistance genes, early warning systems, and disease mitigation planning. Activities in all these areas are planned and hopefully will be initiated in the near future. A bigger challenge is to find effective mechanisms to ensure long-term, in-country sustainability of rust monitoring work versus reliance on international support. Building capacity, implementing tools and information systems to enhance efficiency will help, but sustained commitments will be needed by governments and research institutes to address the challenges posed by wheat rusts.

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