Puccinia striiformis race analyses 2012, Global Rust Reference Center (GRRC), Aarhus University, Flakkebjerg, DK- 4200 Slagelse, Denmark.

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This is a preliminary report of the non-European *Puccinia striiformis* race analyses activities at GRRC in 2012. The activities are based on an agreement between Aarhus University, CIMMYT and ICARDA to facilitate race analyses of *Puccinia striiformis* infecting wheat and other cereals in Africa and Asia. From 2012-2016, CIMMYT and ICARDA have agreed each to support the activities by a annual contribution of USD 20,000 within the frame of the RUSTFIGHT project. Aarhus University is contributing with quarantine lab and green house facilities, consumables and scientific and technical expertise. RUSTFIGHT, which is focusing on basic research in host-pathogen interactions, is supported by the Danish Strategic Research Council 2012-2016. A summary of the results can be spread within relevant countries and organisations without delay, provided that the author and funding institutions are acknowledged, i.e. "Hovmøller: Preliminary report of yellow rust races 2012, Global Rust Reference Center: Research funded by Aarhus University, CIMMYT and ICARDA". Results from 2009-2011 are available from the GRRC home page, and during 2013 results will also become available via the database facilities provided by the Wheat Rust Toolbox accessible at www.wheatrust.org.

Sampling site focus in 2013 will be selected by staff at ICARDA, CIMMYT and NARCs in Africa and Asia, with a focus on high risk epidemic areas. Since 2011, GRRC also accepted samples of stem rust (*Puccinia graminis tritici*) as agreed upon with the Borlaug Global Rust Initiative and the phase II of the Durable Rust Resistance in Wheat Project (DRRW). GRRC can only process samples according to available space and resources at any time, and we cannot guarantee to process all samples received. This report deals only with yellow rust. Submission procedures are enclosed at the end of this report.

2012 results

A total of 220 rust infected leaf samples from 8 countries entered recovery procedures using susceptible seedlings of Cartago and Morocco. A total of 77 isolates were recovered and multiplied. The recovery rates varied greatly from case to case emphasizing the importance of appropriate sample preparation and submission without delays.

There may be multiple reasons for loss of viability, the main being 1) poor crop status (late, necrotic etc.), 2) too long time between sampling in the field and arrival at GRRC and 3) non-favorable condition after sampling, i.e., during preparation and postage. However, the actual representation of locations and host varieties investigated is considered ok because we try to recover multiple leaf samples from each sampling location/variety. However, successful recovery may involve more than one multiplication step for achieving sufficient amount of spores for storage and race analyses, which were made according to Hovmøller & Justesen (Aust. J. Agr. Research 58, 518-524 (2007).

A subset of 55 Pst isolates were pathotypes using an extended set of wheat differential lines carrying resistance genes to *P. striiformis*. A combination of lines from 'World' and 'European' differential sets and NILs in an Avocet background gave a fairly high resolution in terms of virulence determination despite that

additional previously unreported resistance genes were detected in a number of differential lines including some of the Avocet NILs. For commonly used resistance genes like *Yr1, Yr2, Yr6, Yr7, Yr8, Yr9, Yr17, Yr25, Yr27* and *Yr32*, respectively, at least two differential lines were applied to detect corresponding virulence.

Table 1. Number of *P. striiformis* samples submitted to GRRC between October 2011and December 2012. A total of 77 isolates were recovered, and a subset 53 were analysed for race ID (Table 2).

Antal af Run no.					Status (failed/recovere	
Sampling year	Country	Location	Sampled by	Collection date	Failed	recovered
2012	Afghanistan	Darulaman Reseach Station, Kabul	Rajiv Sharma	24-06-2012		18
		Gorulana Village, Bayan		13-08-2012	2	
		Mullah Ghulam Research Station, Bamyan		13-08-2012	1	1
		Sarasiab Village, Central Bamyan Province		12-08-2012	2	
	Afghanistan Total				5	19
	Azerbaijan	Gobustan	K.N. & K.A.	01-06-2012	4	1
	Azerbaijan Total				4	1
	Bhutan	Bazangdra	Park	12-04-2012	2	
		Boelangdra		20-04-2012	2	
		Chonju, Tseuto, Paro		20-04-2012	4	4
		Damche	Cisar	18-04-2012	1	1
			Hudson	17-04-2012	1	
		Naja, Susuna	Park	21-04-2012		6
				21-04-2012		6
		Naku Damchi, Kabjisa, Purakha		17-04-2012	3	
		Omthekha, Limbukha, Purakha		18-04-2012		1
		Sali, Dogor, Paro		21-04-2012	3	
		Zamsa, Tshento	Surviellance Team	20-04-2012	1	
	Bhutan Total				17	18
	Eritrea	Adi Abieto		24-10-2012	1	
		Adi Quala		20-10-2012	1	
		Adi Quala Dengold		20-10-2012	1	
		Berik Adi-Laklay		18-10-2012	1	
		Berik Thesnifouga		18-10-2012	1	
		Dekemhare Wekufi		18-10-2012	1	
		Dubarwa Adi-Gada		16-10-2012	1	
		Dubarwa Adi-Keino		16-10-2012	1	
		Dubarwa Kesid Dau		16-10-2012	1	
		Dubarwa Torrniupela		16-10-2012	1	
		Emni haili Meniyaul	Asmelash Welday	20-10-2012	1	
		Geremi Emba-Dermo		24-10-2012	1	
		G-Nefhi Meifano		18-10-2012	1	
		G-Nefhi Mei-Nefti		18-10-2012	1	
		G-Nefhi Selae-Duo		18-10-2012	1	
		G-Nefhi Adi-Gambelo		18-10-2012	1	
		MENDEFERA		22-10-2012	1	
		Mendefera Adi-Zerna		17-10-2012	1	
		Senafe Anfhsi		23-10-2012	1	
		Senafe Emba Ailsa		23-10-2012	1	
		Shimangus-Laelay		24-10-2012		1
	Eritrea Total 2012				20	1
	Eritrea: recovered autumn 2011		Ashmelash Wolday	Oct 2011	46	6
	Ethiopia	Gule	Hodson/Getaneh	09-11-2012	1	
		Gurame Wolka	Hodson/Getaneh	09-11-2012		2
		Sodo	Hodson/Getaneh	08-11-2012	2	2
		off-season SR sampling	Dave Hodson	10-08-2012		3
			Hodson/Getaneh	06-11-2012	1	2
				07-11-2012	5	1
	Ethiopia Total				9	10
	Kenya *	Njoro, Kenya	Kumarse Nasari	02-10-2012		1
	Kenya Total 2012					1
	Kenya: recovered autumn 2011				34	18
	Lebanon	Adaysseh	Rola ElAmil	21-05-2012	2	
		Kounin	Rola ElAmil	21-05-2012	2	
		Terbol	Rola ElAmil	06-01-2012		2
						1
	Lebanon Total				4	3
	Turkey	Urfa	Rola ElAmil	05-09-2012	4	
	Turkey Total				4	
2012 Total					63	53
2011 processed autumn					80	24
	* Additional Kenyan samples 2012 were recieved but not recovered due to uncertainty about origin/ID					

Like previous years we did not detect virulence to *Yr5* and *Yr15* in 2012, whereas virulence for *Yr10* was observed in East Africa (Table 2). Despite that some pathotypes may remain undetected in some areas due to relative low sample sizes, the results were quite consistent across the different sampling areas. Virulence for *Yr10* was common among European samples from Triticale and virulence for *Yr17* was common for European wheat samples (data not shown). For European results, see www.wheatrust.org. The detection of *Yr17*-virulence was restricted in some few isolates, where a potential, additional resistance gene in VPM1 may have been recognized. Inconsistent results were obtained for Avocet x *Yr17* NIL due to lack of homogeneity of seeds, and in some cases inconsistent specificity compared to VPM1.

Table 2. GRRC race analyses of *Puccinia striiformis* in 2012. The figures correspond to virulence matching YR resistance genes, a parenthesis designate 'partial virulence', either due to heterozygosity of the isolate or unknown R-genes in the differentials. *Yr*-genes corresponding to a virulent race are considered ineffective for yellow rust control. Global Rust Reference Center, Aarhus University, Denmark

Country	Pathotype code international	No isolates	Aggressive strain according to Milus et al 2009	Comments
Afghanistan	-,(2),-,(4),-,6,7,8,9,-,-,-,-,-,27,-,AvS	1	x	
	-,2,-,(4),-,6,7,8,-,-,-,-,-,-,27,(32),AvS	5		Additional differentials revealed additional diversity for virulence
	1,2,-,(4),-,6,7,8,9,-,-,-,-,-,27,(32),AvS	3	x	
	1,2,3,(4),-,6,-,-,9,-,-,-,-,25,-,32,AvS	2		
Afghanistan Total		11		
Azerbaijan	1,2,-,-,-,6,7,8,-,-,-,-,-,-,-,-,AvS	1		
Azerbaijan Total		1		
Bhutan	-,(2),-,-,-,6,-,8,-,-,-,-,-,-,-,-,AvS	1		
	-,-,-,-,-,-,-,8,-,-,-,-,-,-,-,-,AvS	3		Additional differentials revealed additional diversity for virulence
	1,2,-,4,-,6,7,8,-,-,-,-,-,-,-,(32),AvS	2		
	1,2,3,4,-,6,7,-,9,-,-,17,-,25,-,(32),AvS	12		
Bhutan Total		18		
Eritrea	-,(2),-,-,-,6,7,8,9,10,-,-,24,25,-,-,AvS	2	x	
	-,2,-,-,-,6,7,8,-,10,-,-,24,-,27,-,AvS	2		
Eritrea Total (sampled autumn 2011)		4		
Ethiopia	-,-,-,-,-,-,(7),-,-,-,-,-,-,-,27,-,-	1		off-season sampling August connected with SR sampling; additional samples from November await testing
	1,(2),-,-,-,6,7,(8),-,-,-,-,-,25,-,-,AvS	1		
	1,2,-,-,-,6,7,-,-,-,-,-,-,25,-,-,AvS	1		
Ethiopia Total		3		
Lebanon	-,(2),-,-,-,6,7,8,9,-,-,-,25,-,27,-,AvS	1	x	
	1,2,-,-,-,6,7,8,9,-,-,-,25,-,27,-,AvS	1	x	
Lebanon Total		2		
Kenya	-,(2),-,-,-,6,7,8,9,-,-,-,-,25,-,-,AvS	2	x	
	-,(2),-,-,-,6,7,8,9,-,-,-,-,25,27,-,AvS	1	x	
	-,2,3,-,-,6,7,8,-,-,-,-,-,25,-,-,AvS	1		
	1,(2),-,-,-,6,7,8,9,-,-,-,-,25,-,-,AvS	2	x	
	1,(2),-,-,-,6,7,8,9,-,-,-,-,25,27,-,AvS	10	x	
Kenya Total (sampled autumn 2011)		16		
Total		55		

Isolates recovered from Afghanistan and Bhutan appeared relatively diverse and additional fine-scale diversity based on modified infection types were observed in between (data not shown). In Bhutan we

detected pathotypes ranging from very 'simple' virulence spectrum to quite diverse virulence spectra. Based on virulence phenotype and previous studies of aggressiveness (Milus et al. 2009: Phytopathology 99, 89-94) high frequencies of isolates were considered to belong to the aggressive strain previously reported in many parts of the world. The aggressive strain has been detected frequently in the Middle East and East Africa in recent years with additional virulence for *Yr1, Yr10,* or *Yr27*. Thus, the combination of virulence for *Yr27* and aggressiveness has proven to increase the epidemic risks in many areas. Molecular PCR based-markers to detect the aggressive strains are currently being developed by CRRC which will greatly facilitate rapid detection of such isolates (Walter et al. 2013, in preparation). Another *Yr27*-virulent race was predominant in several areas, e.g., Ethiopia in 2010 (data not shown) and in Afghanistan in 2012.

Our general observation is that typical pathotype data, where 'infection types' on the appropriate differentials may be classified into 'virulence' and 'avirulence' phenotypes do not cover the pathogenic variability observed in *Puccinia striiformis* of different geographical origins. We expect to look further into the processes of evolution of virulence by investigating appropriate isolates showing various degree of 'virulence'. New insights based on investigating samples from historic Stubbs collection via an ongoing PhD project at GRRC and others may show additional light on this issue. Collaboration with other European and international research groups, we are developing robust DNA-based markers which will facilitate a more rigorous analysis of genetic variability among *Pst* isolates and how and where the pathogen may spread.

GRRC expect to provide more services on-line in the future via the GRCC home page www.wheatrust.org, facilitated by further development of the Wheat Rust Toolbox, which is hosted by GRRC. Among others, this activity is part of a new Danish driven initiative, RUSTFIGHT, focusing yellow rust aggressiveness, transcripts and genetics.

Submission procedures for wheat rust samples to GRRC

Prior to submission of rust infected leaf sample, a request must be sent by e-mail to GRRC to get an import permit issued. Sampling details, e.g., about collector (person), sampling date, location, geographical coordinates, host variety, disease severity in plot/fields from where samples, are requested. Each sample should consist of 4- 5 leaves/stems from each field plot investigated, younger (upper) leaves are usually better than older leaves (more viable and green). Whenever possible take leaves/stems with clearly separated lesions/ postules. Fold individual leaves to avoid curling, with pustules inside the folded leaf, and allow to dry individually in a pollination bag (or similar) 12-24 hours at room temperature. Put samples from a single plot/site into a SINGLE paper envelope and seal with tape. To increase diversity, GRRC recommend taking samples from different locations and varieties (e.g., some heavily infected and some light infected), up to 20-25 sites/varieties, i.e., up to 25 envelopes with 4-5 individual leaves.

The permit from GRRC must be enclosed the submission of any rust infected plant sample. Samples should be embedded in two additional layers of sealed envelopes (increasing in size) to avoid the risk of spread fungal spores. Each new envelope layer (absolute clean from rust spores) must be added and handled in a lab bench/clean environment using separate lab coats and clean gloves. The final envelope/package should be wiped using 70% ethanol to avoid unintentional spread of spores during transportation. Use of plastic bags and storage in fridge at any point must be avoided for preventing loss of viability. The risk of unintentional spread arising from sending the package like this should effectively be zero.