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Report for *Puccinia striiformis* race analyses 2013, Global Rust Reference Center (GRRC), Aarhus University, Flakkebjerg, DK- 4200 Slagelse, Denmark.

Mogens Støvring Hovmøller, Manager GRRC, January 31, 2014.
Email: mogens@hovmoller@agrsci.dk

This is a preliminary report of the *Puccinia striiformis* race analyses activities at GRRC in 2013. 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, mainly from Africa and Asia. From 2012-2016, CIMMYT and ICARDA have each agreed to support the research by an 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 substantial scientific and technical expertise. RUSTFIGHT, which is focusing on more 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 organizations without delay, provided that the author of this report is acknowledged, along with funding institutions, i.e. "Hovmøller 2013: Global Rust Reference Center: Research funded by: Aarhus University, Denmark; CIMMYT; ICARDA". Results from previous years are available as Pdf files from the GRRC home page.

From January 2014, the results from 2013 and previous years have also become accessible via the database facilities provided by the Wheat Rust Toolbox accessible at www.wheatrust.org. The site is still under construction and new analytical tools and additional explanations will be made available during 2014. However, already from February 1st, it is possible to get online access to results for specific countries from Europe, South America, Africa and Asia, and for specific years and virulence, and combinations thereof. GRRC would like to interact with user groups and individuals in order to provide the best possible format for displaying results. Later, we expect to provide molecular data to improve the resolution and interpretation of results. For instance, identical or similar races sampled in distant areas (continents) may not necessary imply that these races are closely related in evolutionary terms.

Submission and preparation of samples

Prior to submission of rust infected leaf sample, a request must be sent by e-mail to GRRC to get an import permit issued. Information about details of collector (person), host variety, sampling date, location, disease severity in each plot from where samples were given. Each sample consisted 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 leaves separately (e.g., 5 per plot/site) to avoid curling, with pustules inside the folded leaf. Press leaves while they dry 12-24 hours at room temperature. Put each folded leaf into a pollination bag (or similar), and put together such samples from a single plot/site into a SINGLE paper envelope. After drying, each envelope must be sealed 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.

This permit must be enclosed any sample submission. Samples should be embedded in two additional layers of sealed envelopes (increasing in size) to avoid the risk of spread. Each new envelope layer (absolute clean from rust spores) must be added in physical separate rooms, 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 were avoided for preventing high humidity. Samples are preferably collected from different locations and varieties (e.g., some heavily infected and some light infected), max of 20-25 sites/varieties (i.e., 25

envelopes with individual leaves). The risk of unintentional spread arising from sending the package like this should effectively be zero.

Sampling site focus in 2014 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). Since GRRC can only process samples according to available space and resources at any time, we cannot guarantee to process all wheat rust samples received. This report deals only with yellow rust.

Table 1. Number of *P. striiformis* samples submitted to GRRC January – December 2013. A total of 79 isolates were recovered, and a subset of these were analysed for race ID (Table 2). Additional SSR analyses based on both recovered and non-recovered rust samples are in progress.

Pathogen	P. striiformis			
Crop season (Year)	2013			
Antal af Pathogen			Recovery result	
Country	Location	Sampled by	Failed	Recovered
Afghanistan	Naghrak Village, Surkhrod district, Nangarhar province	Ravij Sharma	2	
	Sheshambagh Research Station, Nangarhar province		7	1
	Zangawee Village, Behsood district, Nangarhar province		5	3
Afghanistan Total			14	4
Azerbaijan	Bilosuvar		1	
	Jalelabad		1	
	Ogiz		1	
	Ogur		1	
	Qobustan exp station		2	
	Shamakhi		1	
	Tar.tar		1	
Azerbaijan Total			8	
Bhutan	Baylangdra	Sonam/Gordon C/ David H/ Thinley	3	
	Chimipang		3	
	Laigai		3	
	Susuna		3	
	Susuna, Paro		6	2
Bhutan Total			18	2
Egypt	Am Alrizk	Dr. Essam Abdelhamid	2	
	Itay Elbaroud	Dr.Walid El.Orabey	1	4
	Nubariya	Dr. Minas Sallam & Dr. Walid El.Orabey	1	2
	Sakha	Dr. Mamdouh Asmawi & Dr. Atef Shahin	5	
	Sherbin	Dr. Essam Abdelhamid	4	
	Tag Elezz		1	
Egypt Total			14	6
Eritrea	ADI BARI	Asmelash Wolday	1	
	ADI ETAY			1
	ADI GOMBELO		1	
	ADI LOGO		1	
	ADI MONGONTI		1	
	ADI QUALA		1	
	AMADR		1	
	BELEZA		1	
	BIHAT		1	
	DANDIER		1	
	DIBDIB		1	
	GEREMI		1	
	GEZA GOBO		1	
	HALHALE			1
	KSAD DAERO		1	
	KUDOFELASI		1	
	LAQIEN		1	
	MAI TSAEDA			1
	MENDEFERA		1	
	METERA		1	
	MEZBA			1
	QUAZIEN		1	
	SERHA		1	
	TAKITA		1	
	TASAT		1	
	TERAEMNI		1	
	TOKONDAE		1	
Eritrea Total			23	4
Ethiopia	Bekoji	Dave Hodson	1	5
	Etaya		1	
	Huruta		1	
	Kulumsa			3
	Meraro		1	3
Ethiopia Total			4	11

Pathogen	P. striiformis			
Crop season (Year)	2013			
Antal af Pathogen			Recovery result	
Country	Location	Sampled by	Failed	Recovered
Iraq	Abbasia Queri, Erbil	Dr. Emad M. al.Maaroof	2	
	Bakrajo, Sulaimania		3	
	Duhok		1	
	Fayda Hakar, Ninawa	Abid Al.Hameed Fayadh	1	
	Fayda Rahmaniya, Ninawa		1	
	Minara, Erbil	Dr. Emad M. al.Maaroof	1	
	Musol, Ninawa	Laith Husain	4	2
	Perdi, Kerkuk	Dr. Emad M. al.Maaroof	1	
	Qara hanjir, Kerkuk		1	
	Qudis/Kabelka, Kerkuk		3	
	Rashidya, Ninawa	Abid Al.Hameed Fayadh	1	1
	Takia, Sulaimania	Dr. Emad M. al.Maaroof	1	
	Tilkef, Ninawa	Abid Al.Hameed Fayadh	1	
Iraq Total			21	3
Kenya	Katakala	Wanyera	1	
	Kipsombe		1	
	Lorian burnt forest		1	
	Mau Summit		1	
	Ngata		1	
	Njoro			5
	Ntulele		2	
	UasoNarok		1	
Kenya Total			8	5
Morocco	Ain Toto (between Meknes and Fes)	Ramdani		2
	Exit Khemisset to Meknés		2	
	JEMAAT RIAH (Near BERRACHDI)		2	
	Marchouch to Rabat (just near CT)			2
	Mhaya (between Meknes and Fes)		1	3
Morocco Total			5	7
Tajikistan	Gnonchi		1	
	Isfara		4	1
	Istaravshan		4	
	J. Rasulov		1	
	Konibodom		1	
	Kurush, Spit		1	
	Spitamen		1	
	Sughd Fl		1	
Tajikistan Total			14	1
Tanzania	Bunotu			1
	Bura Kitanyesh			1
	Enguik			1
	Lemangalo			1
	Mbeya Rural, Tembele, Galijembe		3	
	Njombe Hagatilo		1	
	Njombe, Igagals Wembwe		1	
	Njombe, Igosi		1	
	Njombe, Kipengere		1	1
	Njombe, Mafinga		1	
	Njombe, Ngaudi			1
	Njombe, Ramadhan		1	
	Njombe, Wembwe,usalule village		1	
	Reasearch plot Mbeya urban, Iyunga		8	
Tanzania Total			18	6
Uzbekistan	Jizzakh region, Gallaral district		1	
	Kashkadarya region, Yakkabog district		1	
	Surhandarya region			1
	Surhandarya region, Angor district			1
	Surhandarya region, Denov district			1
	Surhandarya region, Jarkurgan district			1
	Surhandarya region, Sherobod district		1	
	Tashkent region			26
Uzbekistan Total			3	30
Grand total			150	79

2013 results

A total of 229 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 sampling methodology and rapid handling and submission without delays. We have had relatively poor recovery results from Eritrean samples. 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).

Table 2. GRRC race analyses of *Puccinia striiformis* in 2012 and 2013. 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

Pathogen	P. striiformis					
		Crop season (Year)			Aggressive strain according	
Country	**Race by January 2014 [AvS: Avocet S; Amb: Ambition]**	**2012**	**2013**	**Total**	**to Milus et al.**	**Comments**
Afghanistan	-,2,-,4,-,6,7,8,-,-,-,-,-,-,27,32,-,AvS,-	4		4		
	-,2,-,4,-,6,7,8,-,-,-,17,-,-,27,32,-,AvS,-	3	3	6		
	-,2,-,4,-,6,7,8,9,-,-,17,-,-,27,32,-,AvS,-	2		2		May represent recombining population (cf. Ali et al. 2014). V17 revealed by Avocet/Yr17 only.
	1,2,-,-,-,6,7,-,9,-,-,17,-,-,27,-,-,AvS,-	1		1		
	1,2,-,4,-,6,7,8,9,-,-,17,-,-,27,32,-,AvS,-	4	1	5		
	1,2,3,4,-,6,-,-,9,-,-,17,-,25,-,32,-,AvS,Amb	1		1		
	1,2,3,4,-,6,-,8,9,-,-,-,-,25,-,32,-,AvS,Amb	1		1		
Afghanistan Total		**16**	**4**	**20**		
Azerbaijan	1,2,-,-,-,6,7,8,-,-,-,-,-,-,-,-,-,AvS,-	1		1		
Azerbaijan Total		**1**		**1**		
Bhutan	-,-,-,-,-,-,-,8,-,-,-,-,-,-,-,-,-,AvS,-	3		3		
	-,2,-,-,-,6,-,8,-,-,-,-,-,-,-,-,Sp,AvS,-	1		1		The isolates with many virulences originate from a single epidemic site. Clearly different from 'Warrior/Ambition' race in Europe based on SSR
	-,2,-,-,-,6,7,-,-,-,-,-,-,25,-,-,-,AvS,-		1	1		
	1,2,-,4,-,6,7,8,-,-,-,-,-,-,-,-,-,AvS,-	2		2		
	1,2,3,4,-,6,7,-,9,-,-,17,-,25,-,-,Sp,AvS,Amb	12		12		
Bhutan Total		**18**	**1**	**19**		
Egypt	-,-,-,-,-,6,7,8,-,-,-,-,-,-,-,-,-,AvS,-		1	1		
	-,2,-,-,-,6,7,8,-,-,-,-,-,-,-,-,-,AvS,-		2	2		
	-,(2),-,-,-,6,7,8,9,-,-,-,-,25,27,-,-,AvS,-		2	2	x	
Egypt Total			**5**	**5**		
Eritrea	-,2,-,-,-,6,7,8,9,10,-,-,-,-,-,-,-,-,-	1		1		
Eritrea Total		**1**		**1**		
Ethiopia	-,-,-,-,-,-,(7),-,-,-,-,-,-,-,27,-,-,-,-	1		1		
	1,2,-,-,-,6,7,-,-,-,-,-,-,25,-,-,-,AvS,-	1		1		
	1,2,-,-,-,6,7,-,-,-,-,17,-,25,-,-,-,AvS,-	1		1		
	1,2,-,-,-,6,7,-,9,-,-,17,-,25,27,-,-,AvS,-	2		2		
Ethiopia Total		**5**		**5**		
Iraq	-,(2),-,-,-,6,7,8,9,-,-,-,-,25,-,-,-,AvS,-		1	1	x	
	-,(2),-,-,-,6,7,8,9,-,-,-,-,25,27,-,-,AvS,-		9	9	x	
Iraq Total			**10**	**10**		
Lebanon	-,(2),-,-,-,6,7,8,9,-,-,-,-,25,27,-,-,AvS,-	1		1	x	
	1,(2),-,-,-,6,7,8,9,-,-,-,-,25,27,-,-,AvS,-	1		1	x	
Lebanon Total		**2**		**2**		
Morocco	-,(2),-,-,-,6,7,8,9,-,-,-,-,25,27,-,-,AvS,-		2	2	x	
	1,2,3,4,-,6,7,-,9,-,-,17,-,25,-,32,Sp,AvS,Amb		5	5		Identical to 'Warrior/Ambition' race in Europe based on SSR
Morocco Total			**7**	**7**		
Tajikistan	1,2,3,4,-,6,-,-,9,-,-,17,-,25,27,32,-,AvS,Amb		1	1		
Tajikistan Total			**1**	**1**		
Tanzania	-,(2),3,-,-,6,7,8,9,-,-,-,-,25,27,-,-,AvS,-		1	1	x	
	1,2,-,-,-,6,7,-,9,-,-,-,-,25,-,-,-,AvS,-		1	1	lack of V8 to be confirmed	
	1,2,-,-,-,6,7,8,9,-,-,-,-,25,-,-,-,AvS,-		1	1	x	
	1,(2),-,-,-,6,7,8,9,-,-,-,-,25,27,-,-,AvS,-		1	1	x	
Tanzania Total			**4**	**4**		
Uzbekistan	1,2,3,4,-,6,-,-,9,-,-,-,-,25,-,32,-,AvS,Amb		3	3		
	1,2,3,4,-,6,-,-,9,-,-,-,-,25,27,32,(Sp),AvS,Amb		1	1		Preliminary SSR results suggest larger diversity
	1,2,3,4,-,6,-,-,9,-,-,-,-,25,27,32,-,AvS,Amb		22	22		
Uzbekistan Total			**26**	**26**		
Hovedtotal		**43**	**58**	**101**		

A subset of 58 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.

Like in previous years, we did not detect virulence to Yr5 and *Yr15*, whereas virulence for Yr10 was observed in East Africa. Despite that some races 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.

Isolates recovered from Afghanistan appeared relatively diverse and additional fine-scale diversity based on modified infection types were quite often observed (data not shown). Based on race and previous studies of aggressiveness (Milus et al. 2009: Phytopathology 99, 89-94), isolates considered to belong to the aggressive strain previously reported in many parts of the world were still common. The aggressive strain was also detected frequently in Kenya and in epidemic areas in Ethiopia with additional virulence for *Yr1, Yr10,* and *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 GRRC which will greatly facilitate rapid detection of such isolates (Walter et al. 2014, in preparation). *Yr27*-virulent races were detected in many areas, e.g., Central and South Asia, East Africa and the Middle East.

Our general observation is that typical *P. striiformis* race data, where 'infection types' on the appropriate differentials may be classified into 'virulence' and 'avirulence' phenotypes do not cover the pathogenic variability observed in isolates of different geographical origin. 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.