Ethiopian Institute of Agricultural Research
Ambo Plant Protection Research Center

Protocols for Race Analysis of Wheat Stem Rust (*Puccinia graminis* f. sp. *tritici*)

Prepared by
Dr. Getaneh Woldeab, Endale Hailu and Dr. Netsanet Bacha

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Photos

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Figure 19: USDA-ARS, Cereal Disease Lab, St. Paul, MN.

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The greenhouse facility at the Ambo Plant Protection Research Center has four compartments for seedling growth and wheat stem rust race analysis.

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Wheat stem rust (*Puccinia graminis* f. sp. *tritici*) and race analysis

Stem rust (black rust), caused by a fungus *Puccinia graminis* f.sp *tritici* of wheat, belongs to the class Basidiomycetes, order Uredinales, family Pucciniaceae and the genus Puccinia. Stem rust has been one of the most significant wheat diseases worldwide, causing famines, economic and even political crises. It is highly specialized biotrophic wheat pathogen with narrow host ranges (Singh et. al., 2012). Stem rust attacks all the above-ground parts of the wheat plant. It is mainly found on the stems, but, at times, on leaves, sheaths, glumes, awns and seeds (Marsalis and Goldberg, 2006). Raised, long and narrow, orange-red pustules occur in early stages of the disease on the stems and leaves of susceptible cultivars (Fig. 1). A crop that appears healthy three weeks before harvest can be devastated by explosive build-up of stem rust if sufficient inoculum arrives from a heavily infected wheat crop in some distant region (Leonard and Szabo, 2005).

Fig. 1: *Puccinia graminis* under field conditions at Ambo, Ethiopia, 2014.

Furthermore, the productivity of wheat in Ethiopia has remained extremely low (2.54 t/ha) due to biotic factors like *Puccinia graminis* f.sp. *tritici* (FAOSTAT, 2015). At times of epidemics, yield losses due to stem rust can reach 100%. Several stem rust epidemics have been recorded in different parts of Ethiopia in recent history that have caused great losses: stem rust epidemics in 1975 on variety Laketch; in 1992/93, on variety Enkoy; in 1994, on variety Kubsa; and, in 2013, on variety Digelu. The epidemics occurred due to the appearance of new races, probably as a result of mutation and/or sexual recombination. So, to minimize the threat of future epidemics, it is important to characterize the race composition of stem rust pathogens and the appearance of new races in the country. The high yield losses caused by this pathogen and frequent appearance of new races demands full-fledged research on wheat stem rust in this country.

This document is a guide for technicians, junior researchers, students and trainees on how to process stem rust races in the greenhouse and how to multiply the races and store the races for use in the future. It also emphasizes the precautions that should be taken during the process.

Race analysis requires organized human resources as well as materials. The activities involved in race analysis are intensive and demand excellent techniques and precautions. Greenhouse and greenhouse related activities for wheat stem rust race analysis are organized in a number of distinct major steps outlined in this document.

Differential lines and controls: seed handling and seedling raising

A separate and rust-free area must be maintained to keep seed materials used for race identification clean and free of contaminants. Seeds of each differential and susceptible check must be stored in clearly labeled, separate glass or plastic containers with lids, and kept in a cool area — cold room or refrigerator, if available (Fig. 2).

For raising seedlings, use sterilized soil composed of three different materials: soil, sand, and farm yard manure mixed at the ratio of 2:1:1 by volume. Seedlings are raised by two methods.

- One method is to pre-germinate seeds by spreading the seeds on filter paper in petri dishes, moisten with water and close the lid. On the third day, the seeds will germinate and the radicles are seen. Then these germinating seeds are planted in pots using forceps (Fig. 3).

Fig. 2: Seeds of stem rust differential and susceptible hosts.

- A second method is direct planting of the seeds in pots filled with sterilized soil.

More uniform seedling germination is normally achieved by using the first method (Fig. 4).

In both methods, at 7-8 days after planting, the primary/first leaf will be fully extended and the second leaf will be 2-4 cm long. This is the right stage for inoculation with rust pathogen spores in the greenhouse.

Seeds of a highly susceptible host (for example, McNair) are planted to multiply the rust collected from the field sample to be analyzed. Once the sample is increased sufficiently, the 20 current standard stem-rust differential lines (Table 1) are planted for race designation (Fig. 5). It is critical that the seedling raising-room be free from rust spores. Rust-free protective gowns must be worn when activities are going on in the room.

Different sized clay and plastic pots are used according to the needs of the activity to be conducted. Trays are used to group the pots in order.

Fig. 3: Seedlings planted after germination.

Fig. 4: Primary leaf emergence of direct planted seeds.

Fig 5: Seedlings of stem rust differential lines (listed in Table 1).

Table 1: Wheat Stem Rust Differential Lines (accessions deposited at the National Plant Germplasm-Aberdeen, ID)

Number	*Sr gene*	*Line name*	*Habit*
501	Sr5	ISr5-Ra	Spring
502	Sr21	CnS_T_mono-derivative	Spring
503	Sr9e	Vernstein	Spring
504	Sr7b	ISr7b-Ra	Spring
505	Sr11	ISr11-Ra	Spring
506	Sr6	ISr6-Ra	Spring
507	Sr8a	ISr8a-Ra	Spring
508	Sr9g	Acme	Spring
509	Sr36	W2691SrTt-1	Spring
510	Sr9b	W2691Sr9b	Spring
511	Sr30	BtSr30Wst	Spring
512	Sr17 (+Sr13)	Combination VII	Spring
513	Sr9a	ISr9a-Ra	Spring
514	Sr9d	ISr9d-Ra	Spring
515	Sr10	W2691Sr10	Spring
516	SrTmp	CnsSrTmp	Spring
517	Sr24	LcSr24Ag	Spring
518	Sr31	Sr31/6*LMPG	Spring
519	Sr38	VPM1	Winter
520	SrMcN	McNair 701	Winter

Stem rust collection, processing, and inoculation

Rust samples must be collected and prepared before using them to inoculate the seedlings of the susceptible host or the differential lines. For the survey, required materials are GPS, paper bags, pencil, and alcohol. Samples of stem rust infected stems/leaves are collected at 5-10 km intervals from wheat fields. Separate the infected leaf sheath from the stem and cut the infected stems into small pieces of 5-10 cm length (Fig. 6) and place them in paper bags — this allows the viability of spores to stay reasonably high. Label the paper bags with the name of the zone, district, variety, GPS data, name of collector and date of collection (Fig. 7) and then transport them to the laboratory to be prepared for race analysis.

Fig. 6: Samples of stem rust from wheat fields.

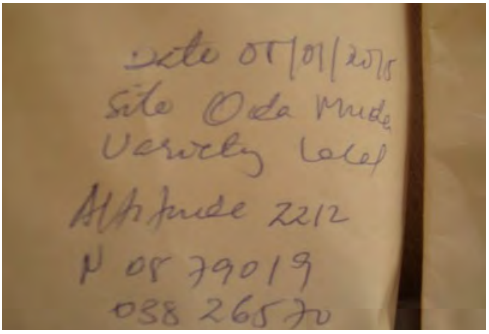


Fig. 7: Labeling of stem rust samples collected from the wheat fields.

After collecting and bringing the infected stem rust samples from the wheat field, the rust spores must be collected into gelatin capsules using a vacuum pump in the cubicle (Fig. 8). If a vacuum pump is not available, spores may be collected on clean glasses/petri dishes by scraping the infected stem with a scalpel. When spore collection of the sample is finished, sterilize hands and other materials with 70% solution of alcohol. Collect the spores from the next sample using a new clean compartment. The same precaution is followed for each sample.

Fig. 8: Collecting stem rust samples using a vacuum pump in the cubicle.

Rust inoculations are performed in an independent room that consists of two inoculation chambers. The pieces of equipment necessary for inoculation are: the inoculation chamber, rotating table, vacuum pump, inoculator, pipette, mineral oil such as Soltrol, gelatin capsules, test tubes, hand-sprayer, distilled water, watch glass, scalpel, cryovial, Tween 20, and labels.

Inoculations in the greenhouse are done either to revitalize the field spores, multiply the isolate, or inoculate the differential lines sets for race identification. Inoculation of the susceptible check McNair, or stem rust differentials, is usually done late in the afternoon when the ambient temperature is low and cool. Cool temperatures help moisture to stay longer on the leaves, thus facilitating the germination of spores resulting in infection.

The equipment/materials are organized to do the inoculation by bringing the wheat seedlings from the seedling room into a clean inoculation chamber. The seedlings, on a tray, are placed on a rotating table. To prepare the inoculum, Soltrol oil is added to the spores in the gelatin capsule and mixed by inversion. The solution should be light brown/tea color (Fig. 9). When you suspend the spores with Soltrol in the capsule, pipette the Soltrol not directly on the spore mass, but on the internal wall of the capsule in order to reduce contamination. Tips should be changed between samples or rinsed and dried with alcohol for future use.

Fig. 9: Prepared inoculum in gelatin capsule.

For inoculation, the gelatin capsule containing the inoculum is attached to the inoculator, which is attached to the hose of the electric vacuum pump. During inoculation, the rotating table is rotated clockwise as well as counter-clockwise to ensure the inoculum spray covers all the leaves of the seedlings (Fig. 10). During inoculation with the vacuum pump, do not get too close to the seedlings, but inoculate from a given distance away (6-8 inches/15-20 cm) so that the spread of inoculum covers the whole seedlings. After each inoculation, the pump is turned off and the persons working close to the samples should sterilize their hands with 70% alcohol.

If a vacuum pump is not available, you can inoculate the seedlings using a scalpel, after mixing the spores with Soltrol oil or distilled water with a droplet of Tween 20 surfactant on a watch glass. Or, turn-by-turn, you can deeply rub the seedlings on the watch glass with the spore suspension.

Fig. 10: Inoculation of stem rust on the susceptible check McNair in the inoculation chamber

In order to clean and wash down the suspending spores after inoculation, spray/shower the chamber with water (Fig. 11). After each inoculation, and before doing the next one, technicians should sterilize their hands and other necessary materials with alcohol. The inoculation of another isolate continues in a second chamber and then come back to the first chamber for the third isolate. After all isolates are inoculated, clean the chambers with moistened clothes. The equipment and materials used during inoculation must be sterilized and dried with clean cloths. Participants in this activity should wear gowns.

Upon completion, the inoculated seedlings are placed on a table for 30 minutes until the Soltrol evaporates and leaves have dried out. Following this, the seedlings are moistened by spraying with distilled water and placed in the incubation chamber.

Inoculators and collectors have to be autoclaved periodically in an oven at 120°C for an hour in order to burn the spores that clog the tubes.

Fig. 11: Showering the chamber with water after inoculation.

Stem rust incubation and rust development

Two compartments in the incubation room have light, dew chambers, and humidifiers. The incubation/dew chambers are wooden boxes covered with white polyethylene sheets (Fig. 12). The dew chamber must be cleaned with water before and after the incubation process.

Fig. 12: Incubation/dew chamber in the incubation room.

A black polyethylene sheet should cover the white sheet in order to create darkness in the box; this condition facilitates the initial infection process of stem rust. After drying the inoculated seedlings for 30 minutes on a table, moisten them by spraying with distilled water. Then place them in the dew chamber for a longer period (overnight) to create high relative humidity. Leave the humidifier on for about 1-1:30 hours so the seedlings have enough moisture for the whole dark period, making the infection process successful (Fig. 13). The dew facilitates the germination of the spores on the leaves. If dew is not abundant during the whole dark period, the spores on the seedlings will not germinate and cause infection.

Fig. 13: Stem rust inoculated seedlings placed in the dew chamber for incubation.

For infection, stem rust requires dark period of 16-17 hours, so cover the chamber with the black polyethylene sheet for that period (Fig. 14). After the sheet is removed, the seedlings must be exposed to light for about 4 hours. The light is again turned off and the door of the dew chamber opened so that the seedlings dry gradually. This hardens the seedlings for when they are taken to the outside environment. Once leaves are dried, the seedlings are taken to the growth room/greenhouse until disease develops.

Fig. 14: The dew chamber covered with black polyethylene sheet for about 17 hours.

In addition to preparing seedlings of the differential lines, it is important to grow seedlings of a known susceptible variety—usually this susceptible check variety is McNair. McNair is planted for three purposes: to revitalize the rust spores collected from the field for further investigation; to multiply the rust races; and to be part of the identification set (Fig. 15). Whenever McNair is used as a susceptible check and for multiplying inoculum, it is advisable to use Maleic Hydrazide 99% (1,2 Didydropyridazib-3-6-dion 99%) $C_4H_4N_2O_2$ at the concentration of 0.3g/l, applied when the emerging coleoptile is visible. This Maleic Hydrazide is used as growth regulating agent on the check and to make seedlings more susceptible. To prepare the Maleic Hydrazide solution, allow 0.3g of the chemical to dissolve in 1 litre of water at least for one hour on a stirrer. The solution then is poured on the coleoptile at 10-20 ml per pot. Never apply this chemical on the differential lines.

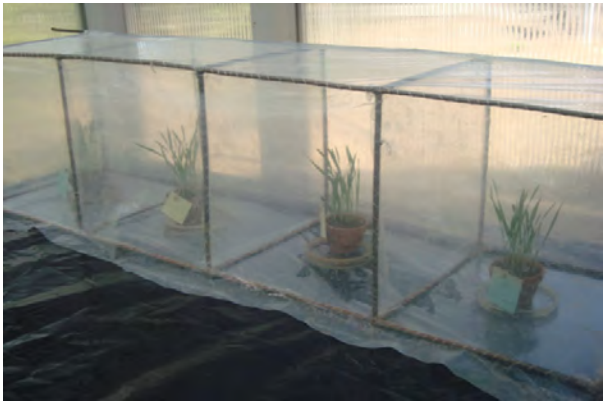


Fig. 15: Isolation of inoculated seedlings of McNair in the cubicle.

After inoculation and incubation, the seedlings are taken to the growth room where they are kept on a table for 14-15 days until disease reaction can be evaluated (Fig. 16). If the season is cold (7-12°C night and morning), the evaluation date can be extended from 14 days to 17 days provided that the pustules have developed well and the leaves of the seedlings are green.

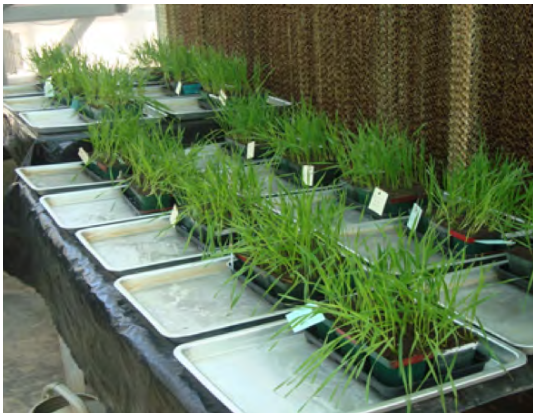


Fig. 16: Stem rust differential sets inoculated with isolates of stem rust from different locations.

When placed in the greenhouse, the seedlings are kept at the temperature of 18-25°C and relative humidity of 60-70%. Each set of inoculated differentials plus one pot of McNair are placed on a table separated from each other (Fig. 17). On the 6th or 7th day after inoculation, flecks/ chlorosis appear on the leaves. The inoculated McNair pot and the differential set are removed and separately placed in a clean cubicle. This is done to reduce contamination of the isolate from the other pots. If the isolate is to be repeated on the differential, the rust isolates from McNair are used for re-inoculation.

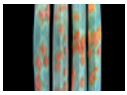
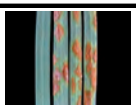
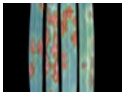
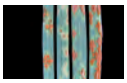
Fig. 17: Inoculated differentials and McNair checks.

When the flecks appear on the leaves, a small amount of urea is applied on the seedlings so that infection continues successfully. It is also important to place isolates inoculated the same day together, but not touching each other, on a bench in the growth room.

Infection types of isolates and race identification

Race identification of stem rust of wheat can be done in three ways: 1) Bring rust samples and inoculated McNair to make pustule isolation, multiply the isolated pustule and then inoculate the differentials; 2) Bring one fresh rust sample and directly inoculate the differential set; and 3) Bring rust samples and inoculate the susceptible host McNair to revitalize the sample and then identify the race.

Fourteen days after inoculation, the differentials are evaluated using 0-4 scoring scale (Stackman et al. 1962), where 0-2+ scores are considered avirulent (resistant reactions or effective genes) and 3-4 scores are considered virulent reactions (susceptible or non-effective genes). (See Appendix A.) After reading the infection types and recording on the wheat stem rust differential scoring sheet (Appendix B), the race is identified using the North American nomenclature system of Roelfs and Martens 1988, Jin et al., 2008 (Appendix C). For instance, low infection type (IT) on all four hosts in a set is assigned the letter B, while high IT on the four hosts is assigned T. Hence, if an isolate produces low IT (resistant reaction) on each of the 20 differential lines, the race will be designated with a five letter race code BBBBB. In the same way, an isolate that produces a high IT (susceptible reaction) on the 20 differential lines will have a race code TTTTT. If an isolate produces a low IT on *Sr36*, *SrTmp*, and *Sr24*, but a high infection type on the remaining 17 differential lines, the race will be designated as TTKSK (Ug99). See Fig. 18 for an illustrated example for TTKSP.

Set	Entry	Line	*Sr* gene	Phenotype	IT	Resp	Code
1	1	ISr5-Ra	*5*		4	H	T
	2	Cns_T_mono_deriv	*21*		3+	H	
	3	Vernstein	*9e*		4	H	
	4	ISr7b-Ra	*7b*		4	H	
2	5	ISr11-Ra	*11*		4	H	T
	6	ISr6-Ra	*6*		4	H	
	7	ISr8a-Ra	*8a*		4	H	
	8	Acme	*9g*		4	H	
3	9	W2691SrTt-1	*36*		0;	L	K
	10	W2691Sr9b	*9b*		4	H	
	11	Festiguay	*30*		4	H	
	12	Renown	*17*		4	H	
4	13	ISr9a-Ra	*9a*		4	H	S
	14	ISr9d-Ra	*9d*		4	H	
	15	W2691Sr10	*10*		4	H	
	16	CnsSrTmp	*Tmp*		2	L	
5	17	LcSr24Ag	*24*		3	H	P
	18	Sr31/6*LMPG	*31*		1+	L	
	19	Trident	*38*		3+	H	
	20	McNair 701	*McN*		4	H	

B	C	D	F	G	H	J	K	L	M	N	P	Q	R	S	T
L	L	L	L	L	L	L	L	H	H	H	H	H	H	H	H
L	L	L	L	H	H	H	H	L	L	L	L	H	H	H	H
L	L	H	H	L	L	H	H	L	L	H	H	L	L	H	H
L	H	L	H	L	H	L	H	L	H	L	H	L	H	L	H

Source: Prof. Z.A. Pretorius, University of Free State, South Africa

Fig. 18: Illustrated example for race TTKSP.

Multiplication, collection and storage of stem rust races

The races are multiplied to store in the -80°C refrigerator for future use and to supply inoculum for screening advanced lines at seedling and adult plant growth stages. A set of stem rust differentials, as well as the susceptible check McNair, should be planted and inoculated by the same race during multiplication. The set is planted together to confirm the race we are working with is the one we need (Fig. 19).

Fig. 19: Multiplying stem rust race TTKSK (Ug99) for different purposes.

Equipment needed for collection and storage of the pathogen are petri dish, watch glass, cryovial, capsule, desiccator, marker, freezer and registering book.

Fig. 21: Deep freezer for isolate storage at Ambo.

Collect rust spores from infected leaves on aluminum foil and then to petri dish, watch glass or cryovials (Figs. 20a, b, c, d); or collect with the vacuum pump directly into capsules and dry them in a desiccator with silica gel for 5-7 days. Then store in clean, dry, clearly labeled cryovials in a deep freezer (-80°C). For large amount of spores, disperse spores evenly in the bottom of a petri dish and leave it to dry in desiccator. After drying, move the spores to a cryovial, close cap tight and place in the -80°C freezer (Fig. 21).

Fig. 20a: Collecting spores onto aluminum foil.

Fig. 20b: Spores are grouped for petri dishes.

Fig. 20c: Spores are spread evenly in petri dish.

Fig. 20d: Petri dishes are placed in desiccator for drying.

Before using stem rust spores from the -80°C freezer, the rust must be "heat shocked." Make sure the cryovial is tightly closed and then place it in a water bath at 48°C. Leave the vial in the water for 6 minutes. Heat shocked spores can be used as inoculum as usual.

To know whether the varieties/advanced lines are resistant in particular, they have to be tested with the dominant and virulent races at seedling and adult plant stages. During the testing, there should be positive and negative controls for the races (Figs. 22a, b).

Fig. 22a: Seedling is labeled for screening

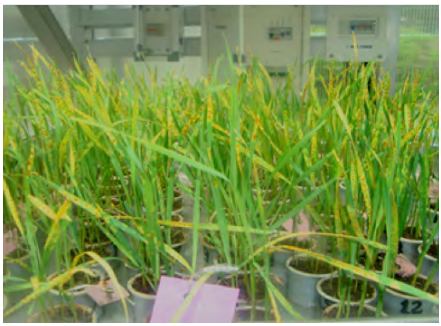


Fig. 22b. Screening germplasm with different races at seedling stage.

Race analysis requires organized human resources as well as materials. The activities involved in race analysis and the collection and storage of stem rust are intensive and demand excellent techniques and precautions. Following the steps outlined in this document will ensure that you can conduct safe and successful research to identify the pathogens that affect the wheat crop.

References

Dagnachew Yirgu. 1967. Plant diseases of economic importance in Ethiopia. Experiment Station Bulletin No. 50. College of Agriculture. Debre Zeit, Ethiopia. H. S. I. U.

FAOSTAT. 2015. Statistical Database of the Food and Agricultural Organization of the United Nations. (http://www.faoststat.org). Accessed on 10/04/2016

Hailu, G, Tanner, DG and Mengistu, H (eds). 1991. Wheat research in Ethiopia: A historical perspective, IAR and CIMMYT, Addis Ababa. pp.392.

Jin Y, Szabo LJ. Pretorius ZA, Singh RP, Ward R, Fetch T Jr. 2008. Detection of virulence to resistance gene *Sr24* within TTKS of *Puccinia graminis* f. sp. *tritici*. Plant Disease 92: 923-26.

Leonard, KJ and Szabo, JA 2005. Rust of small grains and grasses caused by *Puccinia graminis*. Molecular Plant Pathology, 6:99-111.

Marsalis, MA and Goldberg, NP. 2006. leaf, stem, stipe rust diseases of wheat. New Mexico State University Guide A-415. http://www.cahe.nmsu.edu.

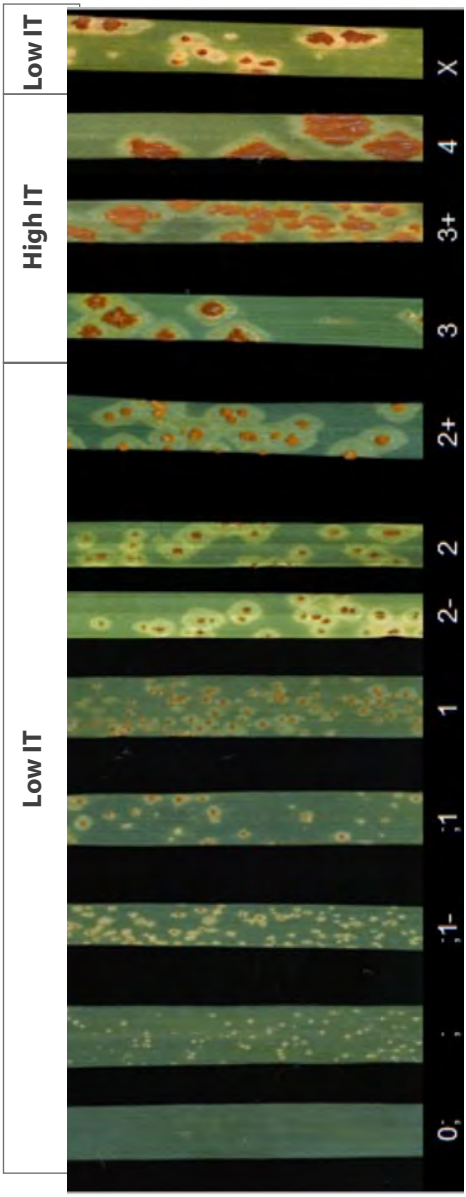
Roelfs, AP and Martens, JW. 1988. An international system of nomenclature for *P. graminis* f. sp. *tritici*. Phytopathology, 78:526-533.

Singh, S, Singh, RP, and Huerta-Espino, J. 2012. Stem rust. Pp. 18-32. In: Sharma, I (ed.). Disease resistance in wheat. Cabi Plant Protection Series. CAB International, Cambridge, MA, USA.

Stackman EC, Stewart DM, Loegring WQ. 1962. Identification of physiological races of *Puccinia graminis* var. *tritici*. Washington, USA: US Department of Agriculture, Agricultural Research Services, E617 (revised).

Appendix A

Infection types of *P. graminis* f. sp. *tritici* stem rust and host response



Source: Prof. Z.A. Pretorius, University of Free State, South Africa

Appendix B

Wheat Stem Rust Race Analysis Differentials Scoring Sheet

Wheat Stem Rust Race Analysis Differentials Scoring Sheet

Region _____ Zone _____ District _____ Kebele _____

Center _____ Altitude _____ Inoculation date _____ Recording date _____

Set	Diff #	Line	Seed Source	Gene	Expected Low IT	Field # Infection type	Iso- H vs L	Iso- Name	Field # Infection type	Iso- H vs L	Iso- Name
I	1	ISr5-Ra		*Sr5*	0,0;						
	2	CnS_T_mono		*Sr21*	;,23-						
	3	Vernstine		*Sr9e*	1-,2+						
	4	ISr7b-Ra		*Sr7b*	2,3-						
II	5	ISr11-Ra		*Sr11*	;,2-						
	6	ISr6-Ra		*Sr6*	0;,X						
	7	ISr8a-Ra		*Sr8a*	2-,2						
	8	CnSr9g		*Sr9g*	2=,2-						
III	9	W2691SrTt-1		*Sr36*	0;=X(LIF)						
	10	W2691Sr9b		*Sr9b*	1+,2						
	11	BtSr30Wst		*Sr30*	1+,2						
	12	Combination VII		*Sr17 (+Sr13)*	0,;,X,X+						
IV	13	ISr9a-Ra		*Sr9a*	1-,2-						
	14	ISr9d-Ra		*Sr9d*	1-2+						
	15	W2691Sr10		*Sr10*	0;,1N,3C						
	16	CnsSrTmp		*SrTmp*	2=,23						
V	17	LcSr24Ag		*Sr24*	1-,22+						
	18	Sr31/6*LMPG		*Sr31*	1-,2						
	19	VPM1		*Sr38*	X						
	20	McNair 701		*SrMcN*	2-						
	21	Siouxland									
	22	DK42									

Note: For Expected Low Infection rates, some variation may occur depending on the entire host or pathogen genotype, as well as with changes in the environment; *Source:* R McIntosh et al, *Wheat Rusts: an Atlas of Resistance Genes*, CSIRO and ZA Pretorius, University of Free State, South Aftrica.

Appendix C

North American nomenclature of *P. graminis* f. sp. *tritici* based on 20 differential lines

Wheat *Pgt* gene differential sets and infection phenotype coding				
Set	**Differential lines identified by Pgt resistance gene**			
Set 1	5	21	9e	7b
Set 2	11	6	8a	9g
Set 3	36	9b	30	17
Set 4	9a	9d	10	Tmp
Set 5	24	31	38	McN
***gt*-code**	***Infection phenotype: High = virulent reaction (susceptible); low = avirulent reaction (resistant)***			
B	Low	Low	Low	Low
C	Low	Low	Low	High
D	Low	Low	High	Low
F	Low	Low	High	High
G	Low	High	Low	Low
H	Low	High	Low	High
J	Low	High	High	Low
K	Low	High	High	High
L	High	Low	Low	Low
M	High	Low	Low	High
N	High	Low	High	Low
P	High	Low	High	High
Q	High	High	Low	Low
R	High	High	Low	High
S	High	High	High	Low
T	High	High	High	High

Source: Roelfs and Martens, 1988; Jin et al., 2008

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