

Appendix F: Schema for Stem Rust DNA sample, single pustule protocol

Collect samples from as widely dispersed and representative locations as possible (cover as many environmental zones as possible. 20-25 samples are usually a good representative sample). Include as many farmer field samples as possible. If possible, collect single pustule sample from the same stem (plant) as a live sample sent for race analysis. [NB: Label the live sample envelope with the same code as used for the dead DNA sample!] Include the same sample code on the field survey form and indicate on the survey form that live and/or dead DNA samples were collected.

1. Collect single pustule isolate

- a. Identify a distinct single pustule (largest unique pustule possible)
- b. Using sharp scissors, cut stem immediately above and below pustule. Cut sheath tissue at sides of pustule, remove core stem tissue. [NB: Single pustules excised from leaves are also fine]
- c. Put sheath tissue with pustule into tube containing 80% ethanol {NB: tube filled to 75-80% volume}
- d. Label tube, with permanent marker, using following naming convention (NB: use pre-labelled tubes if possible): 2 number year, 3 letter country code, 3 number code e.g., 11YEM001 – year =2011, country = Yemen, sample = 001
- e. Clean scissors thoroughly after each sample –use alcohol wipes, wipe scissors thoroughly and discard wipes
- f. Repeat above process for each sample

NB: ONLY ADD 1 UNIQUE SINGLE PUSTULE TO EACH INDIVIDUAL SAMPLE TUBE

2. Store samples in ethanol for at least 7 days

3. Pour off ethanol, air-dry tubes for 1-2 days, seal tubes

4. Sending samples

Before sending samples email Dr Szabo (Les.Szabo@ARS.USDA.GOV) and Dr Hodson (d.hodson@cgiar.org) to inform them of intent to send.

- Place sample tubes inside 2 sealed plastic ziplock bags
- Send samples via DHL (or equivalent) to:

Dr. Les Szabo

Cereal Disease Laboratory

U.S. Department of Agriculture

Agricultural Research Service

1551 Lindig St, University of Minnesota

St. Paul, MN 55108-6052

(612) 625-3780 FAX (651) 649-5054

Email: Les.Szabo@ARS.USDA.GOV

- Describe contents on package as: "Herbarium samples (dead, sterilized) for DNA analysis".

NOTE: ONLY DEAD, STERILIZED SAMPLES CAN BE SENT TO THE USDA-ARS CEREALS DISEASE LABORATORY, MINNESOTA

DNA Sampling Protocol

[NB: Clean scissors + hands with Ethanol after each sample]



1. Select a single, large, isolated pustule. Cut the stem either side of the pustule. [NB: **Only 1 pustule per tube**]

2. Remove the core stem tissue



3. Place pustule (and sheath tissue) into a cryovial. Add 70% Ethanol (or absolute alcohol) to $\frac{3}{4}$ fill the cryovial (ensure pustule is completely covered). Label tube + record unique code on survey form



4. Store in ethanol for 7 days. Decant the ethanol, air dry (with tube cap off) for 12-24 hours. When dry, seal the tube, place in zip lock bag and send via courier to Cereals Disease Lab, Minnesota