Phenotyping for Scab Resistance

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In this Standard Operating Procedure (SOP), we describe how to visually phenotype scab resistance on plots. Visually phenotyping scab resistance is done in two ways, in-field and post-harvest. In-field phenotyping for scab resistance is done by evaluating “Incidence” and “Severity” on plots in the field. Post-harvest phenotyping for scab resistance is done by evaluating the trait “Fusarium Damaged Kernels” on grain samples after plots are harvested.

1. In-field phenotyping: Incidence and Severity

1.1. Definition and purpose

The purpose is to evaluate the wheat genotype's resistance to Fusarium head blight (FHB)/scab, by measuring disease incidence, the visual percentage of infected spikes in a plot, and disease severity, the visual percentage of infected spikelets in one spike.

1.2. Prerequisite steps and knowledge

- The evaluation should be done on research plots that were maintained in a humid environment during the infection period. Ideally, the plots being evaluated should be most-irrigated two weeks before and after flowering to ensure all plots experience conditions conducive to disease development.
- To obtain uniform and high levels of disease, plots should have been inoculated with *Fusarium graminearum* inoculum (see protocols at: https://scabusa.org/protocols).
- Days-to-heading should have been evaluated on all plots.
- Care needs to be taken to ensure each plot is evaluated at a time when symptoms are at their maximum, but prior to senescence when symptoms are not clearly visible. Based on heading dates and considering how quickly the plots are reaching maturity, a research supervisor will have grouped the plots into two to four groups and prepared separate field book files for each group. The plots within the same group will have similar phenology and can be evaluated on the same date.

1.3. Requirements

- Plants within 21 days after flowering (24 days post heading) or 4 to 5 days before physiological maturity. Due to the impact of genetics and the environment on the onset

of senescence, a research supervisor should monitor the plots to be evaluated twice per week after heading to determine the ideal time for phenotyping.

- One tablet/smartphone for data collection.
- 2 staff members.
- About 1 hour of time for every 100 plots evaluated.
- Plots belonging to the same experiment should be evaluated by the same two people.

1.4. Work procedure

- First, select the proper group of plots for phenotyping, according to the maturity time as described above. Use the field book corresponding to the group that should be phenotyped that day. Two staff members will work together on the plot's evaluation. One person will focus on the severity rating, while the other one will focus on the incidence rating and data registration.

- For the incidence (INC) rating:
  - Make a visual evaluation of the entire plot and estimate the percentage of infected spikes. A spike is considered infected if *any* spikelet within the spike has symptoms.
  - Scab symptoms include tan or light brown lesions in one or more spikelet (Figure 1). It is also possible to find spikelet with an orange fungal mass in the lower part of the glumes.
  - There is another spike infection disease called *Stagonospora nodorum* blotch or just glume blotch, which is frequently present in wheat fields. Different from scab, the glume blotch symptoms are dark brown or purple lesions on heads (Figure 2). It is important not to confuse glume blotch and scab and consider glume blotch in the scab incidence and severity rating.

Figure 1: Scab symptoms.

Figure 2: Glume blotch symptoms.

- For the severity (SEV) rating:
  - Make a visual evaluation of the average percent of infected spikelet per spike across the entire plot. For low levels of infection, one spikelet infected per spike means 7%, two spikelets mean 14% and three spikelets means 21%. When more than three spikelets are affected, the percentage of the spike that is infected is estimated directly. See a picture scale below (Figure 3):

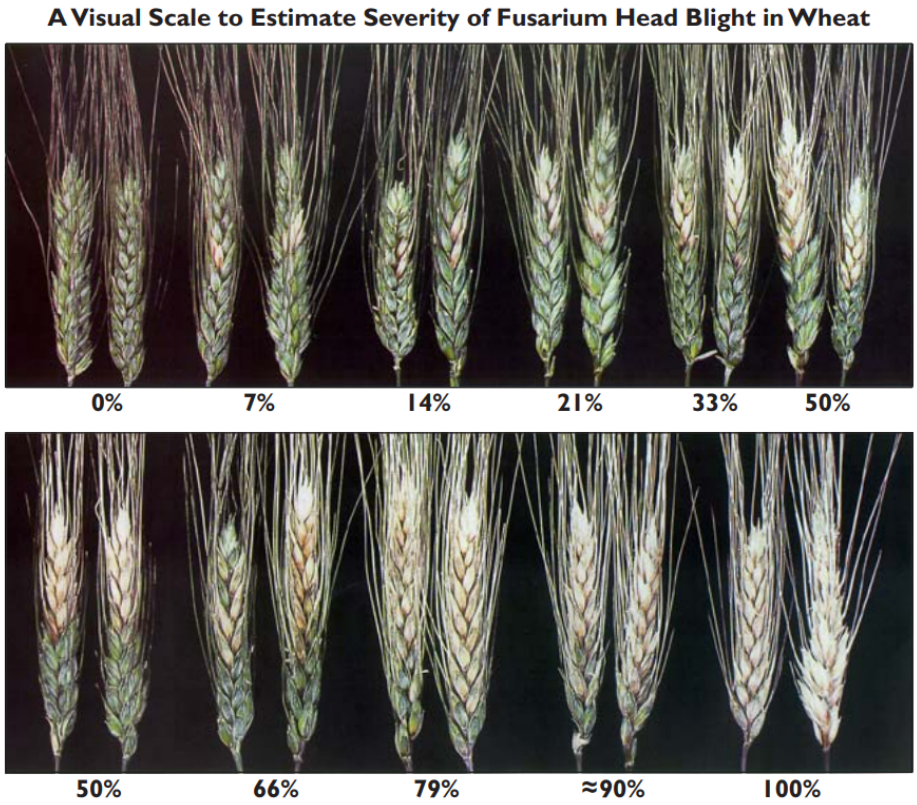

Figure 3: Visual Scale to estimate the FHB severity in wheat.
Credits: Robert W. Stack and Marcia P. McMullen, NDSU Extension Service.
https://scabusa.org/pdfs/StackMcMullen_2011_pp1095.pdf

- Notes:
  - It is important that the severity rate represents the plot.
  - The INC rating is simpler and faster than the SEV rating, because of this, whoever is taking the INC will make their evaluation, record the INC data and record the SEV rating dictated by their partner.
  - During phenotyping, distractions should be minimized. Conversation should be avoided, and headphones should not be worn.
  - After registering the data, it is important to export the data to Google Drive in the folder pertaining to the person who recorded the data.

2. Post-harvest phenotyping: Fusarium-damaged kernel (FDK)

2.1. Definition and purpose

The Fusarium-damaged kernels (FDK) is a trait that represents the percentage of kernels damaged by FHB infection. This trait is correlated to the deoxynivalenol (DON) level and helps to select genotypes more resistant to scab.

2.2. Prerequisite steps and knowledge

- Thresh, clean, and sort the wheat kernels samples.
- If you don't have a set of standard samples, it is essential to make one. For this, you will need one sample of wheat kernels representative of the entire field where your samples are from. With this sample, take *each* kernel and classify it as infected and non-infected. Do not avoid kernels that are difficult to classify. Then, using kernels from the two classes, combine them in small petri plates to generate plates with 'known' percentages of damaged kernels. For example, if you need a 30% infected kernel rate standard, join 30 infected kernels with 70 non-infected kernels on the petri plate. Recommended rates for the standard are 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100%.
    - Grain from plants infected by FHB is generally shriveled and/or with a white chalky appearance. Some kernels can have a pink discoloration representing the *F. graminearum* mycelium (Figure 4).

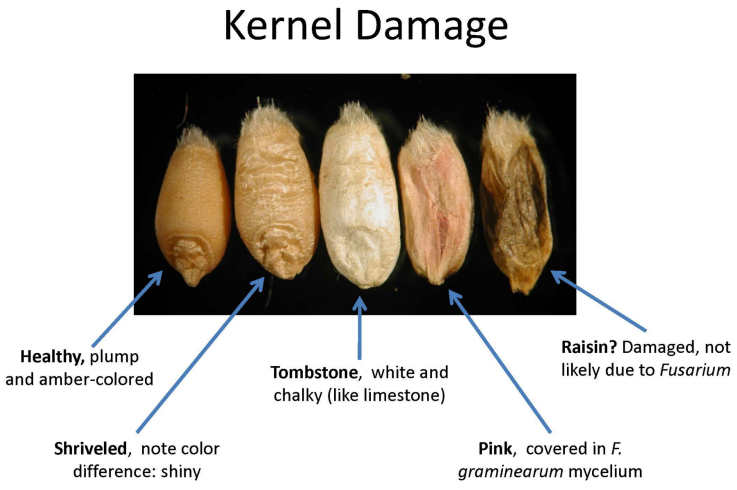

Figure 4: Different types of kernel damage in wheat.
Credits: Pierce Paul, Ohio State University.

- These grain symptoms are variable and can be combined or not, depending on the sample or season. Because of this, it is recommended to have more than one standard, contemplating as much as possible the kinds of symptoms.

2.3. Requirements

- A set of FDK standards.
- Cleaned and sorted wheat kernels samples.
- One tablet/smartphone for data collection.
- Approximately one hour for every 40 samples.
- Each experiment must be evaluated by only one person.

2.4. Work procedure

- Shake the envelope, homogenizing the sample.
- Collect a subsample in a petri plate ensuring a single layer of kernels (Figure 5).

Figure 5: Wheat kernels in a petri plate.

- Compare the subsample with the standard rate samples (Figure 6).

Figure 6: Interns comparing subsample with the standard rate samples.

- Estimate an FDK percentage.
    - If comparing the subsample with the standard proves to be challenging, an alternative approach is to segregate the infected from non-infected kernels, count both groups, divide the number of infected grains by the total number of grains, and multiply this number by 100.
- Record the data in the electronic field book (Figure 7).

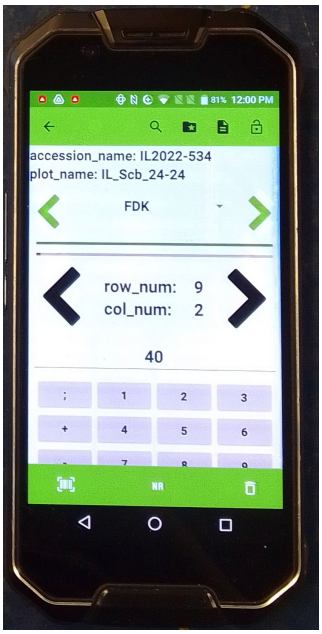

Figure 7: Data recording in the electronic field book.

- Return the subsample to the envelope before moving on the next grain sample.
- Each day that phenotyping is done, export the data to the Google Drive folder pertaining to the person recording the data.
- Notes:
    - During phenotyping, distractions should be minimized. Conversation should be avoided, and headphones or earbuds should not be worn.
    - Infected kernels are toxic to humans and animals if ingested in sufficient quantities.