Instituto Nacional de Investigación Agropecuaria
URUGUAY

CGIAR RESEARCH PROGRAM ON Wheat

Adapting automated image analysis to breeding programs constraints for the characterization of the resistance to leaf rust and other diseases

Jade Pezard[1], Peter Fernandez[2], Silvia Pereyra[2], Martín Quincke[2], Carolina Saint-Pierre[3], Pawan K. Singh[3], Gustavo Azzimonti[2]

[1] *AgroParisTech, Paris, France*

[2] *National Institute for Agricultural Research (INIA), La Estanzuela, Uruguay*

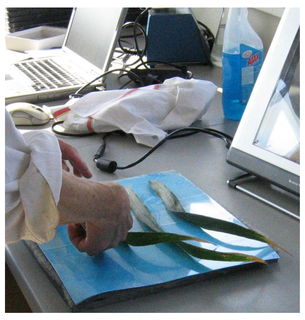
[3] *International Maize and Wheat Improvement Center (CIMMYT), El Batán, México*

gazzimonti@inia.org.uy

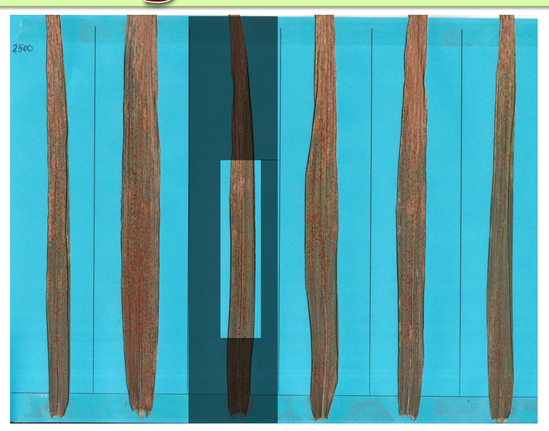
Is it possible to replace visual scores with automated image analysis for the characterization of resistance to leaf diseases in field trials of wheat breeding programs?

Data collection: scan of flag leaves

1. Cutting flag leaves of wheat genotypes in a field trial inoculated with *Puccinia triticina* isolates



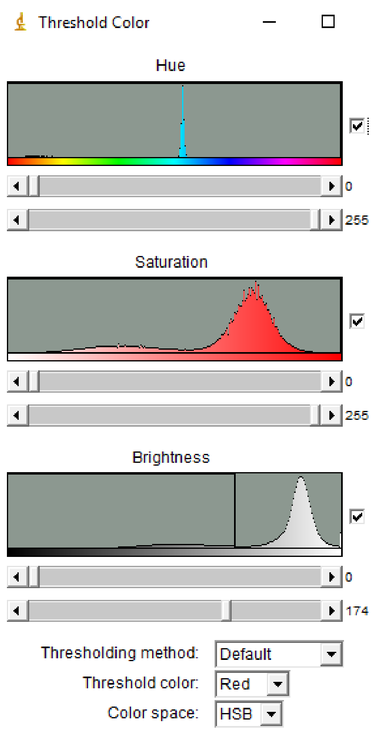
2. Scanning flag leaves with a flatbed scanner



3. Scan of six flag leaves per genotype. The leaf portion to be analyzed is showed

Data processing: script parameterization and image analysis

4. Components of color (Hue, Saturation and Brightness) used to select and recognize the different diseases



5. Threshold max. and min. values determined for each component to recognize each disease

	Min	Max
Leaf Rust	0	25
Stripe Rust	20	75
Septoria	0	55

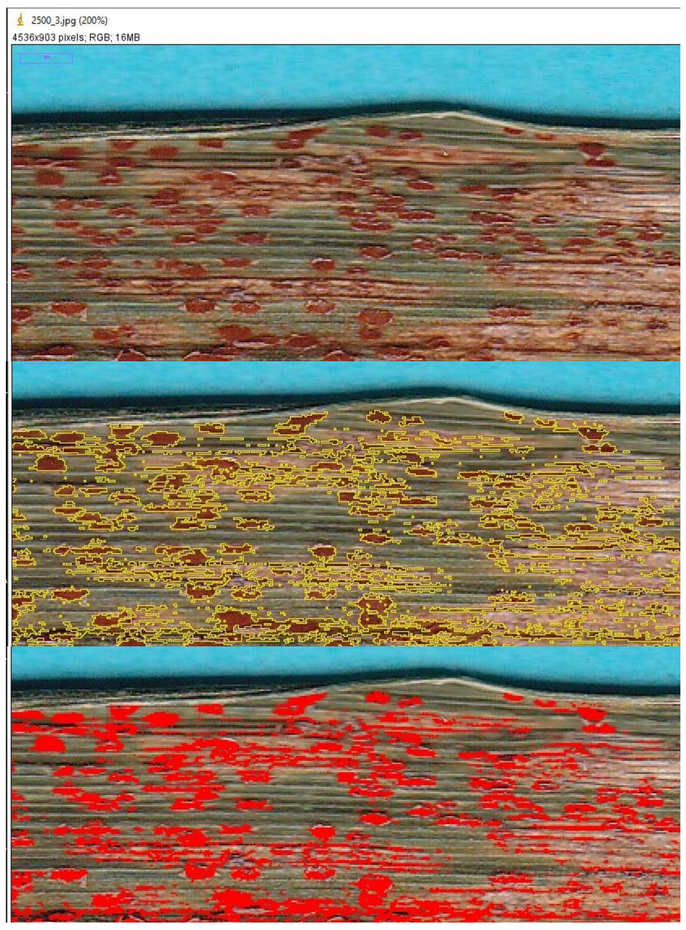
	Min	Max
Leaf Rust	140	255
Stripe Rust	150	255
Septoria	55	130

	Min	Max
Leaf Rust	70	160
Stripe Rust	150	205
Septoria	140	255

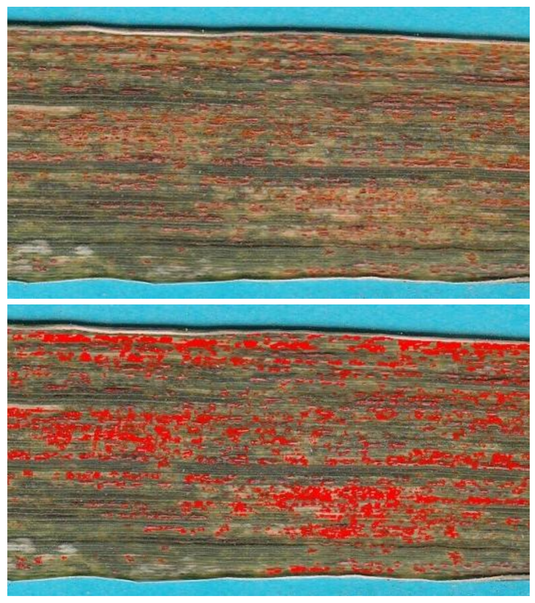
6. Script developed in the ImageJ software to autonomously recognize and measure the leaf diseased surface (only for leaf rust showed here)

```
/*************************************
Fonction 3 : Leaf Rust AIA
*************************************/
function action3(input, output, filename) {
    open(input + filename);

makeLine(1056, 1872, 894, 1872);
run("Set Scale...", "known=1 unit=cm");

run("Color Threshold...");
min=newArray(3); max=newArray(3);
filter=newArray(3);
a=getTitle();
run("HSB Stack");
run("Convert Stack to Images");
selectWindow("Hue"); rename("0");
selectWindow("Saturation"); rename("1");
selectWindow("Brightness"); rename("2");

//Leaf Rust parameters

min[0]=0; max[0]=25;
filter[0]="pass";
min[1]=140; max[1]=255;
filter[1]="pass";
min[2]=70; max[2]=160;
filter[2]="pass";
for (i=0;i<3;i++){selectWindow(""+i);
 setThreshold(min[i], max[i]);
 run("Convert to Mask");
 if (filter[i]=="stop")  run("Invert");
}
imageCalculator("AND create", "0","1");
imageCalculator("AND create", "Result of 0","2");
for (i=0;i<3;i++){selectWindow(""+i);close();
}
selectWindow("Result of 0");
close();
selectWindow("Result of Result of 0");
rename(a);
run("Analyze Particles...", "display clear summarize");}
```

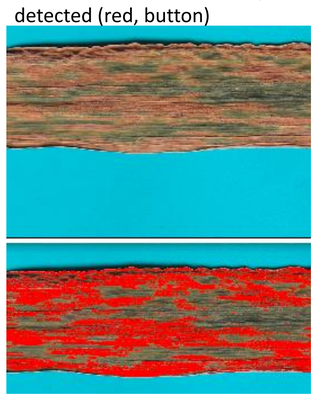
7. Once the threshold parameters were chosen for each disease, all the leaves were analyzed autonomously with the ImageJ scrpipt. Original image (up), leaf rust lesions autonomously detected (yellow, middle) and lesion surface determined (red, button)



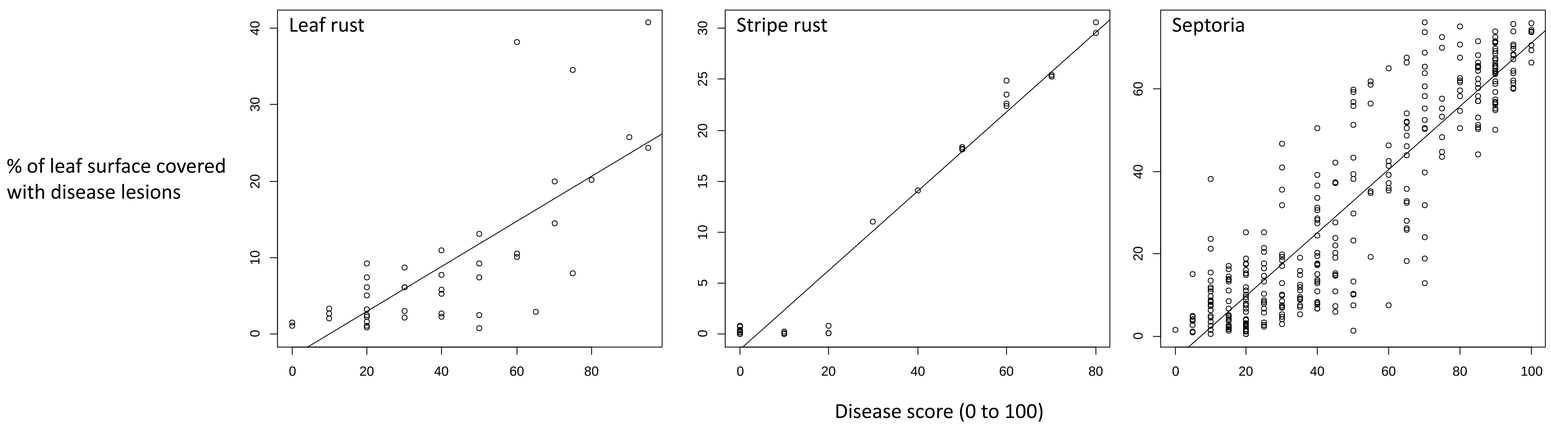
7. Original image (up), stripe rust lesion surface autonomously detected (red, button)



7. Original image (up), Septoria lesion surface autonomously detected (red, button)



Data analysis: AIA reliability estimation (in progress...)



8. Diseased leaf surfaces obtained with automated image analysis vs. disease scores obtained in the field by visual scoring. Linear regression was positive and significant for the three diseases (P<5%)

INTRODUCTION

Disease phenotyping methods used in breeding programs to characterize the level of resistance of breeding materials usually consist on visual scores (VS) of disease symptoms determined in field trials. VS are considered as high time-consuming and rely on experienced operators. Nevertheless, up to date, it is the only method that has an efficient time/effort relationship considering breeding constrains.

The objective was to develop a phenotyping methodology based on automated image analysis (AIA) for leaf diseases, adapted to the constraints of a breeding program.

MATERIALS & METHODS

410 wheat lines from 5 different breeding programs were sowed in three field trials, as part of the materials tested in 2017 at the multi-disease phenotyping platform INIA-CIMMYT, Uruguay. One trial was inoculated with *Puccinia triticina* isolates the second with *Zymoseptoria tritici* isolates and the third had natural infection of *P. striiformis* f. sp. *tritici*. Six flag leaves per genotype were cut and scanned with a flatbed scanner. A script was developed in the ImageJ software to autonomously recognize and measure the leaf diseased surface. Disease recognition and surface measurements were based on the different threshold color patterns of each disease. Host response was also determined for leaf and stripe rust, measuring the ratio of necrosis-chlorosis/sporulation area of lesions.

RESULTS

AIA recognized the different diseases (error<5%). The diseased surfaces obtained by AIA correlated significantly and positively with the VS measured for the three diseases. Host responses estimated by AIA were the same as determined visually, (error<5%). AIA was fast, a mean of 214 leaves/hour analyzed, taking into account the adjustments of color thresholds and the validation of AIA. However, the time to prepare and scan the leaves was higher than the VS: a mean of 205 lines could be scanned per person/day while a mean of 402 lines per person/day could be visually scored.

DISCUSSION

Adjustments to the scan methodology are being carried out to enhance the speed at this step. Nevertheless, AIA can be a performing alternative to VS in limited panels or mapping populations that undergo QTL analysis, where precise measurements of quantitative resistance variables are required to detect QTL with moderate effects and QTL interactions.