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RGB low-cost imaging for plant phenotyping: index calculation and analysis

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Field Phenotyping with Image Analyses and Open Source Software: University of Barcelona Plugins FIJI + MaizeScanner, CerealScanner and MosaicTool

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- **Folder containing FIJI software, University of Barcelona plugins (MaizeScanner, CerealScanner and MosaicTool), guidelines and relevant literature.**

https://drive.google.com/file/d/1VtTqUQrlMZYGEuhC7Gs-lievqCuaqnXt/view?usp=drivesdk

- **Using FIJI to select and measure images based on pixel colour counts. Manual calculation of the Green Area index based on the parameter Hue.**

1. Open the image. Must be in format of 24 bits (RGB Colour, 8-bits x 8-bits x 8-bits = 24).

2. Converts the type of image: Image - > Type - > HSB Stack

3. Separate images by selecting Images - > Stacks - > Stack to Images.
4. Close the images of Saturation and Brightness.

5. Select the image's Hue (hue). Apply the Threshold: Go to Image - > Adjust - > Threshold.

6. To calculate the GA we have to select the Hue values according to the values of green and green/yellow indicated by the HSB model, that are values of 0 to 360, but we have pictures of 8-bit values from 0 to 255, because we have to do some calculations...

GA = Green Area (360: pixels with 60 < Hue < 120; for 255: 42.5 < Hue < 85)
GGA = Greener Area (360: pixels with 80 < Hue < 120 360 are 57 < Hue < 85)

7. Select the area of interest. Go to Edit -> Selection -> Create Selection.

8. Measure the area of interest. Go to Analyse - > Measure.

- **Using FIJI to measure vegetation indices based on RGB indices. Manual calculation of the Normalized Green Red Difference Vegetation index (NGRDVI) based on the parameter Hue.**

1. Separate the three bands of the RGB image: Image -> Type -> RGB Stack and Image -> Stack -> Stacks to Images

2. We can now do the math between the spectral "bands" of an RGB image. The calculation is:

$$NGRDVI = \frac{(Green - Red)}{(Green + Red)}$$

3. Process - > Image Calculator.

4. Anlyze -> Set Measurements -> “mean gray value"; Redirect to "None"; Decimal places "3”.

5. Analyze -> Measure

- ***Using the University of Barcelona MosaicTool Plugin in FIJI for assessing maize disease impacts and general crop physiological status: Breedpix Tab***

1. Open the Breedpix Tab on the MosaicTool.

2. In Options insert photos in Batch Inputs to analyze.

3. In CSV Results Files select where want to download the Excel template. As this function will produce a number of different indices, you should also select which delimiter will be used.

4. You also have the option to save image visualizations of the indices for Green Area (GA) and Greener Area (GGA).

5. Then click on Process and after a few minutes the output data and optionally images will be completed.