



A protocol for measuring Cassava Bacterial Blight disease progression, using machine-learning image analysis

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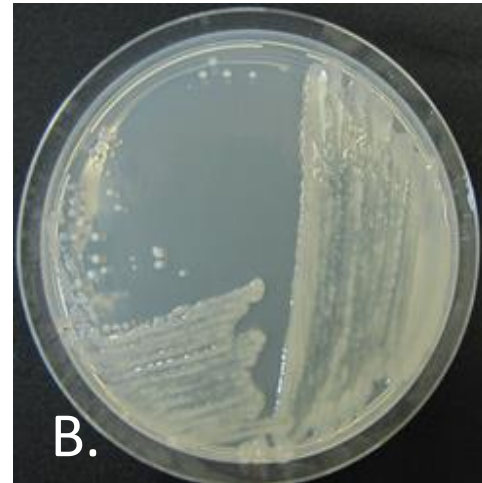
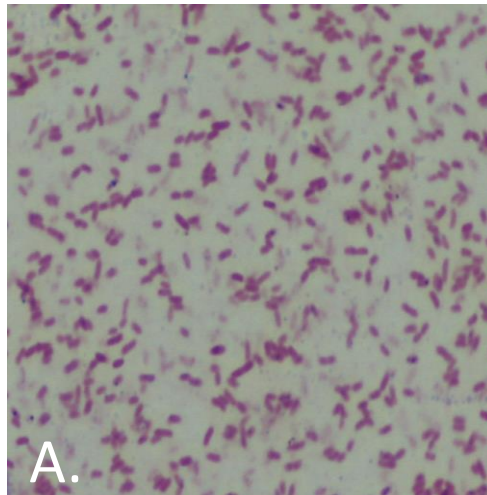
Crop Protection Team, Cassava Program, Crops for Nutrition & Health

Palmira 20/06/11/2023

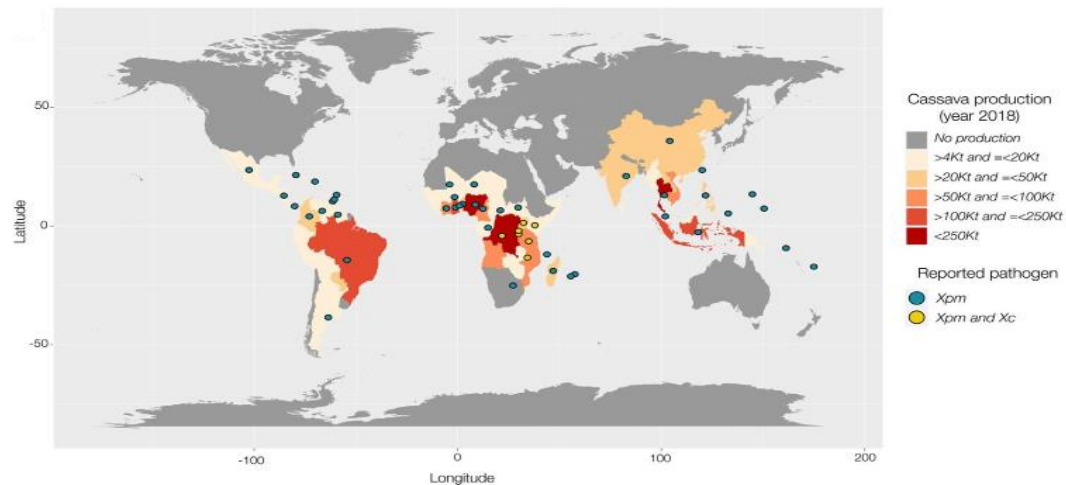
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Bacterial blight (CBB) disease

Caused by *Xanthomonas phaseoli* pv. *manihotis* (Xpm)



A. Gram negative bacillus, **B.** Colonies that look like nonpigmented, circular, raised, convex, creamy-White.



A. Worldwide cassava production (FAO, 2020) by country. Distribution of Xpm and Xc (CABI, 2020). (Zárte-Chaves et al., 2021)

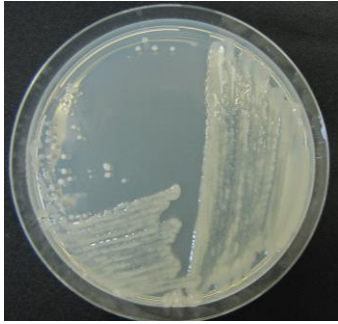
Xpm is a vascular and a foliar pathogen



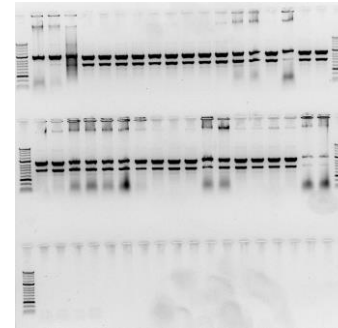
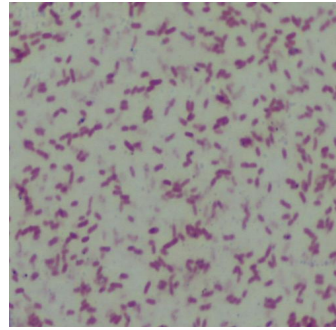
A. Production of exudates; **B.** Wilting and dieback; **C.** Blight or Burning (water soaking symptoms); **D.** Angular leaf spots.

Bacterial Blight disease progression, using machine-learning image analysis

1. Isolate *Xanthomonas phaseoli* pv. *manihotis* (Xpm)



2. Confirm the Xpm identity (Bernal-Galeano et al., 2018)



3. Prepare the inoculum: 3 days at 30°C, dilution 0.05 OD600



4. Choose the three leaf from 4–5-week-old cassava plants



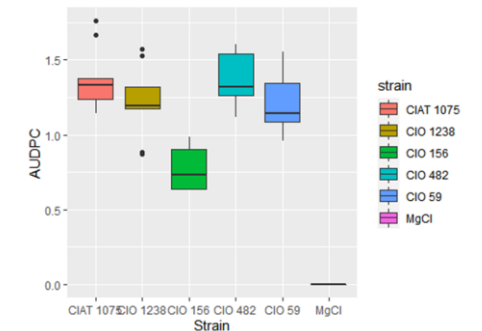
5. Inoculation through infiltration, 3 technical replicates per strain onto the leaf lobe



6. Three infiltration per lobe, central three lobes used, each lobe is a different strain.



7. Measure the spot area advance: phenotyper –Machine learning



Daily AUDPC advance

$$AUDPC = \sum_i [(D_i + D_{i-1}) * (t_i - t_{i-1})] / 2,$$

Relative humidity=80%
Day/night temperature=28/19°C

Per plant one leaf inoculated:
Lobe 1: CIO 81
Lobe 2: CIO 59
Lobe 3: Control (Water)
Per strain 3 plants

Measure in following days: 0, 4, 6, 9, 12, Analyze data on R

Machine learning based quantification of water-soaked symptoms

1. Inoculation.....

2. Take photos (0,4,6,9,12) image device at the same conditions. use X – Rite Passport color card to gray color correction.



3. Choose the best symptoms to create collage.

- Select affected area manually (GIMP or IMAGE J)
- Create a mask: Convert picture to black and white. Only affected area white background black.



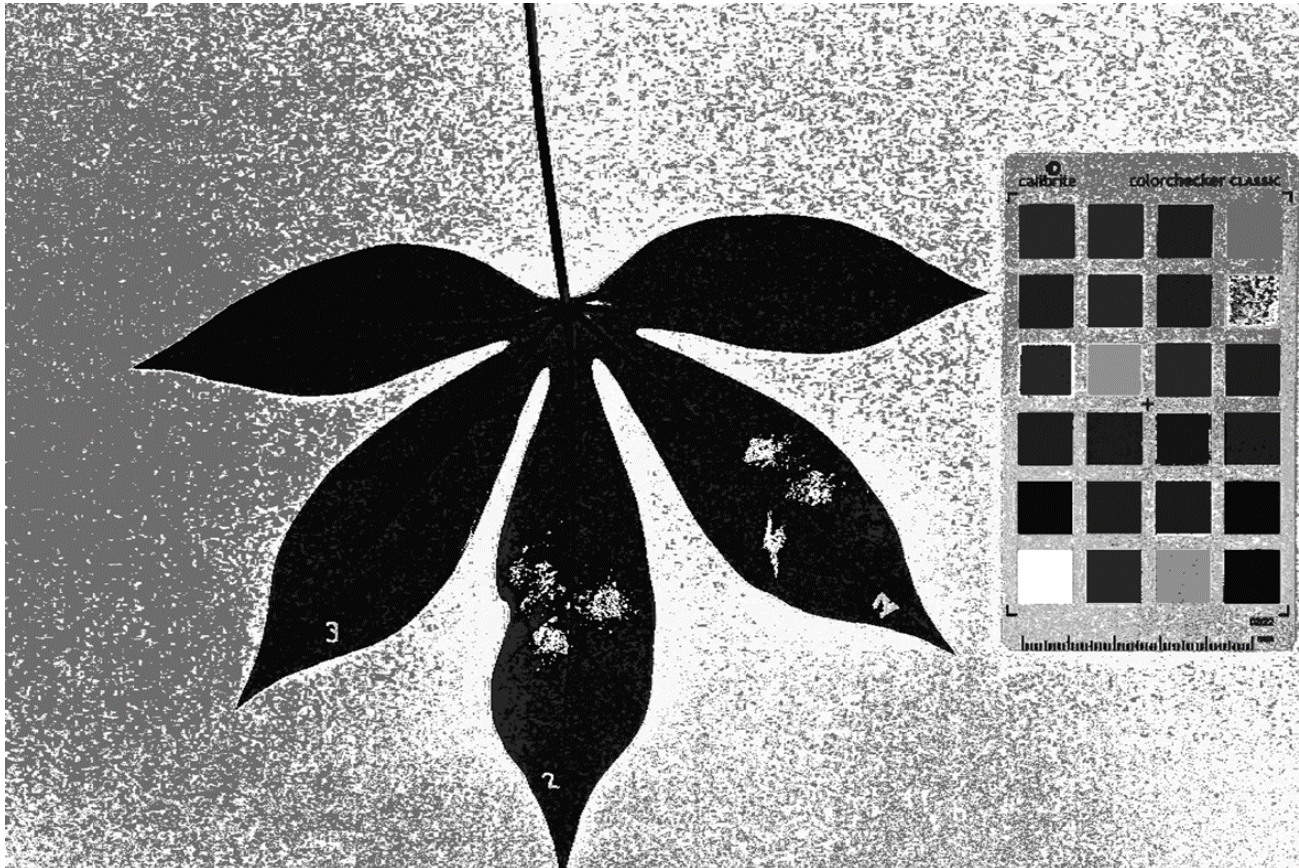
Machine learning based quantification of water-soaked symptoms

5. Machine training:

- Use phenotyper software (Berry et al., 2018).
- Train naive Bayes or SVM using ML_CREATE specifying classifier type:

```
PhenotyperCV -m=ML_CREATE -method=svm -i=collage.png -b=mask.png -class=output_bayes_classifier.yaml
```

- Check the classifier prediction using either ML_PRED specifying classifier type



Prediction image: Affected area showed in white

Machine learning based quantification of water-soaked symptoms

6. Routinary measuring process on Phenotyper software

1. Click only the gray scale color chips from black to white to isolate features

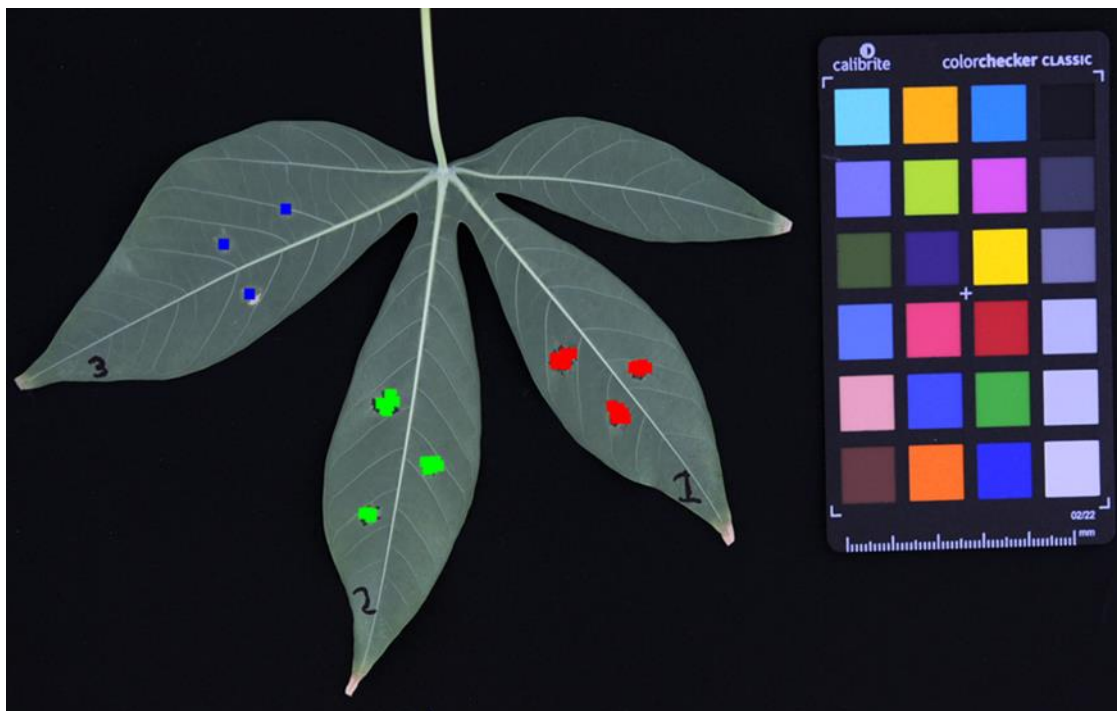


2. Feature prediction of input image, Threshold the prediction

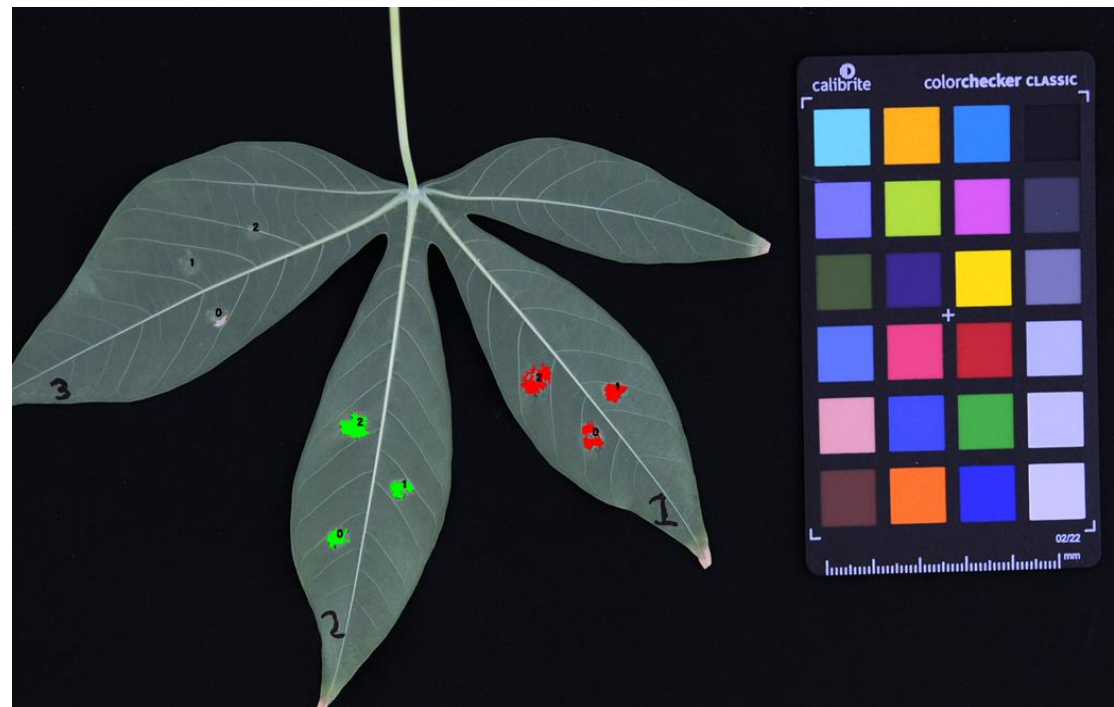


Per plant one leaf inoculated:
Lobe 1: CIAT1075
Lobe 2: CIO 59
Lobe 3: Control
(MgCl 10 mM)

3. Click on the features to measure. Select region of interest specify them by which type of mouse click you use. Left = red, right = green, middle = blue



4. Machine learning output (Data in Pixels). Color map on classified pixels (0,1,2).



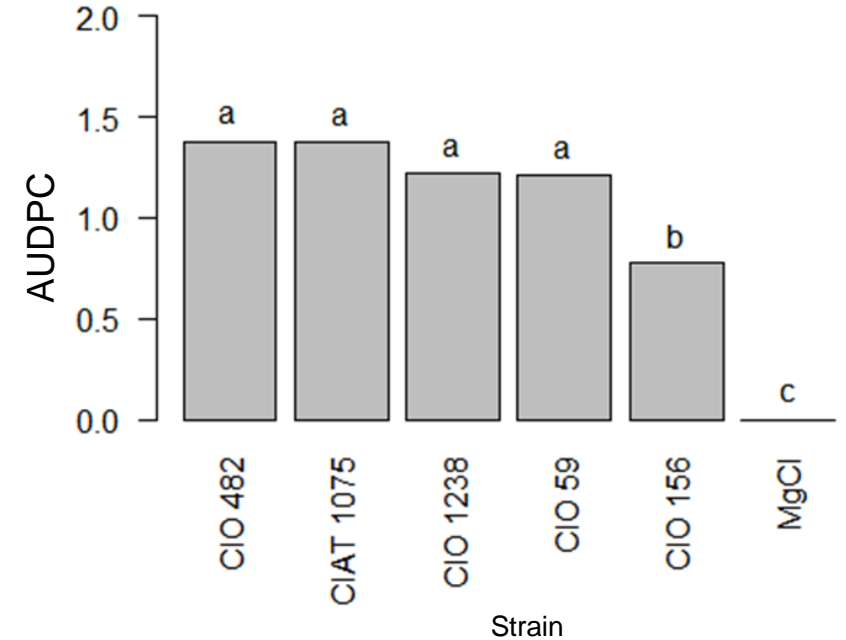
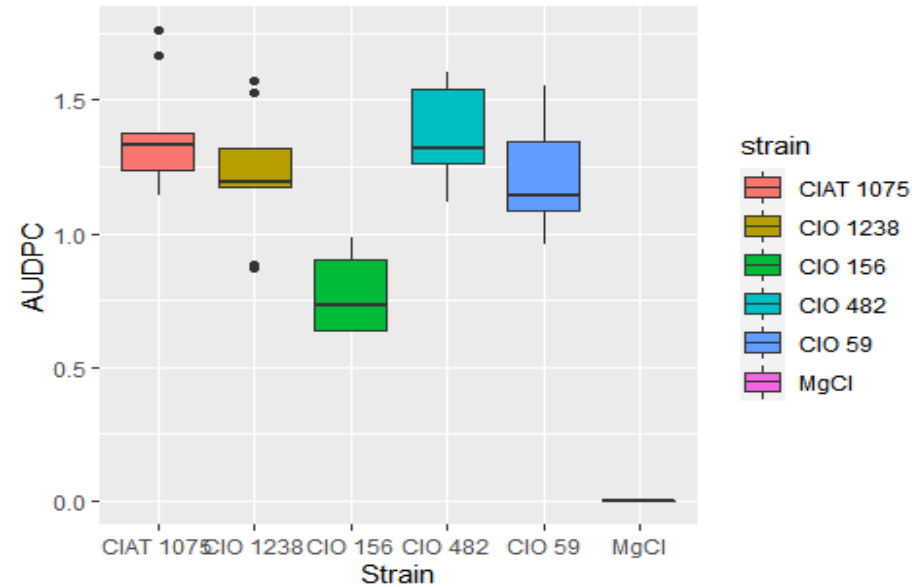
Results and data analysis

Pathogenicity test

- Experimental design: Randomized Blocks. (Three Blocks, 9 observations per strain). Negative control MgCl. Genotype CM6740-7 (Reina).

Strain	Edaphoclimatic zones
CIO156	2-acid soil savanas*
CIO 59	1-Subhumid tropics
CIO 1238	2-acid soil savanas
CIO 482	1-Subhumid tropics
CIAT 1075	5-High altitude

*Verdier et al., 2000



A. duncan test for difference of means p-values < 0.05, strains with different letter have statistical differences B. Boxplot graph showing differences between strains.

Results and data analysis

Susceptibility test

- Experimental design: Split plots . (4 genotypes, 2 strains, three replicates, 9 observations per strain X Genotype). Negative control MgCl.

Strain	Edaphoclimatic zones	Genotype	Field Response
CIO 59	1-Subhumid tropics	CM681-2	S
CIAT 1075	5-High altitude	KU50	R
		CM4919-1	R
		CM6740-7	R

Analysis of variance (ANOVA) for Split plot model showing statistically significant differences between varieties and strains

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
genotype	3	4.885	1.6282	10.377	6.24e-06 ***
stra:genotype	6	2.545	0.4242	2.704	0.0185 *
Residuals	90	14.122	0.1569		

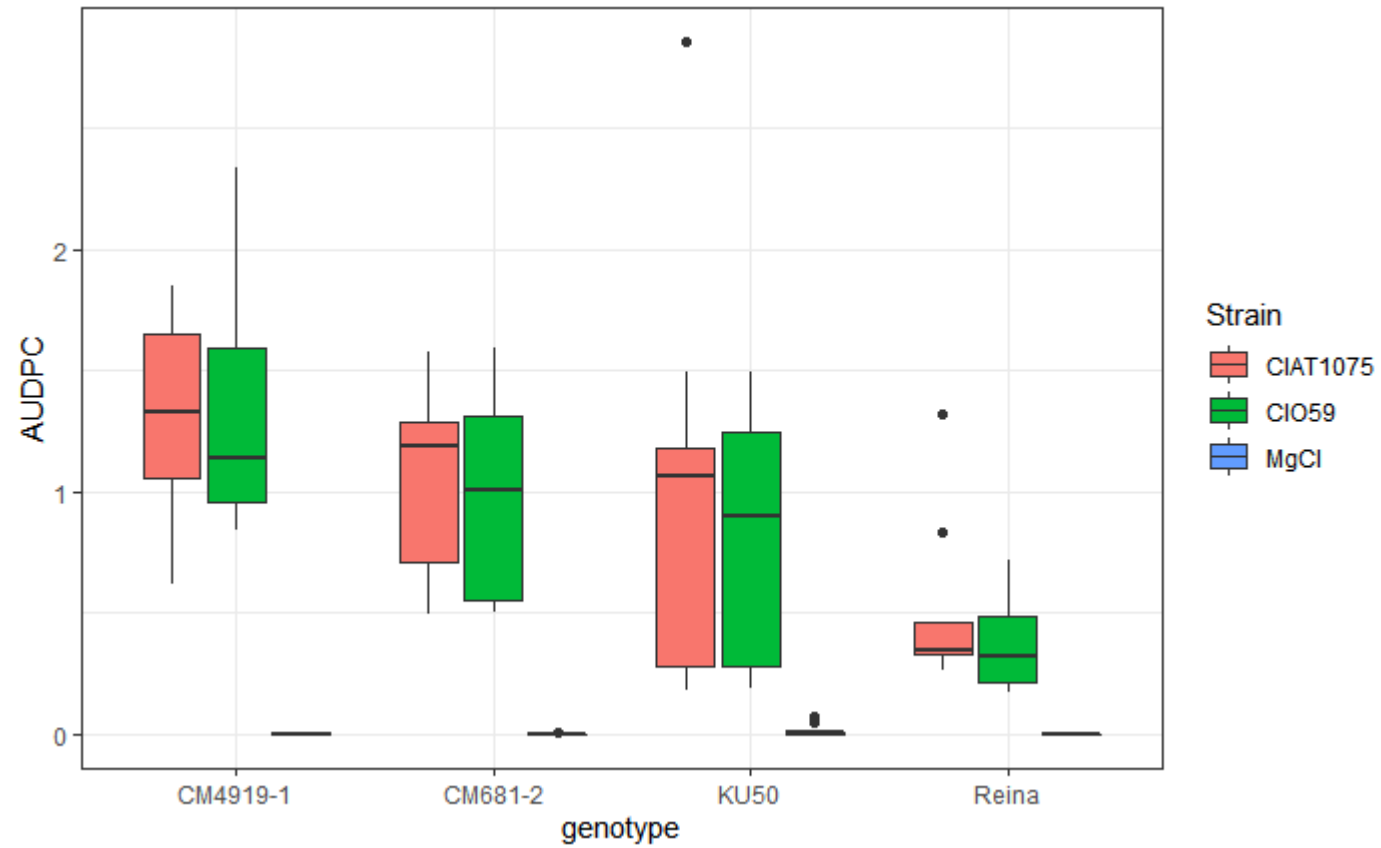
Difference of means p-values < 0.05, Genotypes with different letter have statistical differences

AUDPC groups		
CM4919-1	0.8877260	a
CM681-2	0.6804157	ab
KU50	0.5966230	b
Reina	0.2957532	c

Difference of means p-values < 0.05, interaction genotype x strain with different letter have statistical differences .

Interaction	AUDPC	groups
CIO59:CM4919-1	1.3440826	a
CIAT1075:CM4919-1	1.3173175	a
CIAT1075:CM681-2	1.0565354	ab
CIO59:CM681-2	0.9840317	ab
CIAT1075:KU50	0.9731702	ab
CIO59:KU50	0.8025246	bc
CIAT1075:Reina	0.5140739	cd
CIO59:Reina	0.3726708	de
MgCl:KU50	0.0141743	e
MgCl:CM4919-1	0.0017779	e
MgCl:CM681-2	0.0006801	e
MgCl:Reina	0.0005149	e

Results and data analysis



Boxplot graph showing differences between genotypes.

References

- Illiott, K., Berry, J.C., Kim, H. *et al.* A comparison of ImageJ and machine learning based image analysis methods to measure cassava bacterial blight disease severity. *Plant Methods* **18**, 86 (2022).
- Berry JC, Fahlgren N, Pokorny AA, Bart RS, Velez KM. An automated, highthroughput method for standardizing image color profiles to improve image-based plant phenotyping. *PeerJ*. 2018. <https://doi.org/10.7717/peerj.5727> .
- Restrepo, S., Duque, M. C., & Verdier, V. (2000). Characterization of pathotypes among isolates of *Xanthomonas axonopodis* pv. *manihotis* in Colombia. *Plant Pathology*, 49(6), 680-687.
- Zárate-Chaves, C. A., Gómez de la Cruz, D., Verdier, V., López, C. E., Bernal, A., & Szurek, B. (2021). Cassava diseases caused by *Xanthomonas phaseoli* pv. *manihotis* and *Xanthomonas cassavae*. *Molecular plant pathology*, 22(12), 1520-1537.
- CABI. (2020) *Xanthomonas axonopodis* pv. *manihotis* (cassava bacterial blight). In: *Invasive species compendium*. Available at: <https://www.cabi.org/isc/datasheet/56952>.
- Bernal-Galeano, V., Ochoa, J. C., Trujillo, C., Rache, L., Bernal, A., & López, C. A. (2018). Development of a multiplex nested PCR method for detection of *Xanthomonas axonopodis* pv. *manihotis* in cassava. *Tropical Plant Pathology*, 43(4), 341-350.

Acknowledgement





Thank you!