PLANT DAMAGE ASSESSMENT IN *Urochloa* spp. TO CATEGORIZE RESISTANCE TO SPIDER MITES (Acari: Tetranychidae) ATTACK

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Report

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Summary

Despite improved brachiaria (*Urochloa* spp.) grasses increase the productivity in African livestock systems, its adoption and establishment in the region is challenged for the attack of spider mites (Acari: Tetranychidae) during dry seasons. Breeding for resistance to key pests is one of the principal objectives in the *Urochloa* scheme for East Africa. Thus, developing high-throughput phenotyping methodologies for screening the high diversity of the tropical forages' gene banks is crucial to identify potential sources of resistance, categorize the current available germplasm and optimize the selection process. 26 accessions of *Urochloa* spp. were assessed for resistance to red spider mites under greenhouse conditions in Mbita, Kenya with no-choice tests. For this report, we compared two techniques of analyzing digital images for plant damage to decide which is more accurate for categorizing tolerance.

Objective

Develop a methodology to categorize the plant damage in *Urochloa* spp. to red spider mites' attack

Background

Despite East Africa is the center of origin and diversity of *Urochloa* grasses, the improved cultivars developed by CIAT for the Americas are severely damaged in dry seasons for heavy spider mites' attack (Acari: Tetranychidae). Thus, it is needed further research focusing on this key pest as its biology allows them to migrate easily from and to other high-value cash crops (e.g. maize, tomato), to have many generations per year and a large offspring. Furthermore, feasible and efficient integrated pest management strategies have not been reported yet for spider mites in grasses for the region.

Host-plant resistance has proven to be the most suitable alternative to manage pests in pastures for its low-cost and easy-adoption, being already incorporated in the seeds. Exploiting spittlebug (Hemiptera: Cercopidae) resistance has been one of the most important aims in brachiaria interspecific breeding scheme in America, selecting hybrids expressing antibiosis and tolerance. Similarly, CIAT and ICIPE have advanced on the development of a quick, accurate and precise methodology for screening for resistance to spider mites under greenhouse and laboratory conditions in Western Kenya. These methods are mostly based on plant damage assessment by experts, using severity scales in no-choice tests. However, visual scoring is prone to the subjectivity and expertise of the evaluator and may not be accurate enough in

quantitative traits (Walter et al., 2012; Hernandez et al., 2022). To overcome this limitation when categorizing brachiaria hybrids for resistance to spittlebugs, our group developed a straight-forward pipeline to estimate a damage index (yellowness) using digital images analysis in ImageJ (Hernandez et al., 2022). This technique based on quantifying yellow pixels, allows to measure more experimental units in less time without requiring a high level of expertise, delivering accurate data for the selection process within the current breeding scheme of brachiaria interspecific.

Considering that different levels of resistance were found among a narrow panel of brachiaria accessions (Cheruiyot et al., 2018), the next step would be exploring the diversity conserved in the gene banks to identify spider mites' resistant genotypes within the tetraploid agamic complex *U. brizantha, U. ruziziensis and U. decumbens*.

Data

A panel of 26 accessions of *Urochloa* spp. that includes CIAT's gene bank accessions and hybrids of the breeding program, was used for testing the methodologies (Table 1). No-choice tests were performed to assess the plant damage caused by spider mites (Fig. 1).

Table 1. Panel of *Urochloa* spp. to develop a methodology for plant damage assessment

Accession number	Species
CIAT 606	*U. decumbens* (cv. Basilisk)
CIAT BR02/1752	*U. ruziziensis* x *U. decumbens* x *U. brizantha* (cv. Cayman)
CIAT 16125	*U. brizantha* (cv. Piata)
CIAT 26110	*U. brizantha* (cv. Xaraes/Toledo)
CIAT 36087	*U. ruziziensis* x *U. decumbens* x *U. brizantha* (cv. Mulato II)
CIAT 16107	*U. brizantha*
CIAT 664	*U. decumbens*
CIAT 6370	*U. decumbens*
CIAT 6426	*U. brizantha*
CIAT 6702	*U. decumbens*
CIAT 6735	*U. brizantha*
CIAT 16122	*U. brizantha*
CIAT 26133	*U. brizantha*
CIAT 26646	*U. brizantha*
CIAT BR02/1794	*U. ruziziensis* x *U. decumbens* x *U. brizantha*
CIAT BR02/0465	*U. ruziziensis* x *U. decumbens* x *U. brizantha*
CIAT BR04/3025	*U. ruziziensis* x *U. decumbens* x *U. brizantha*
CIAT BR04/3207	*U. ruziziensis* x *U. decumbens* x *U. brizantha*
CIAT BR06/0423	*U. ruziziensis* x *U. decumbens* x *U. brizantha*
CIAT BR05/1435	*U. ruziziensis* x *U. decumbens* x *U. brizantha*
CIAT BR05/1467	*U. ruziziensis* x *U. decumbens* x *U. brizantha*
CIAT BR09/3660	*U. ruziziensis* x *U. decumbens* x *U. brizantha*

CIAT BR09/4467	*U. ruziziensis* x *U. decumbens* x *U. brizantha*
GP/0549	*U. ruziziensis* x *U. decumbens* x *U. brizantha*
GP/2090	*U. ruziziensis* x *U. decumbens* x *U. brizantha*
MG4	*U. brizantha*

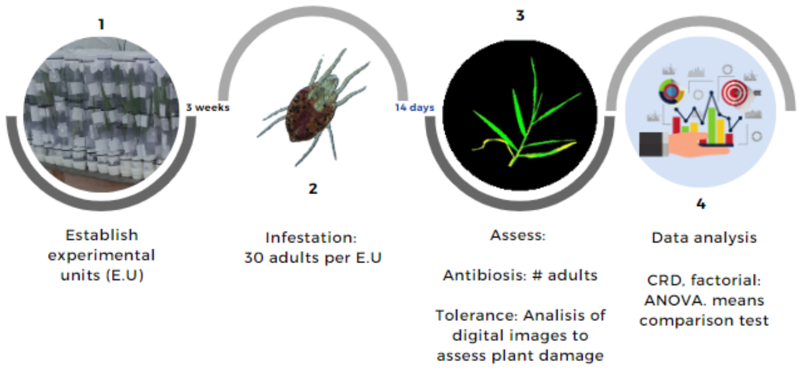


Figure 1. Workflow for no-choice tests

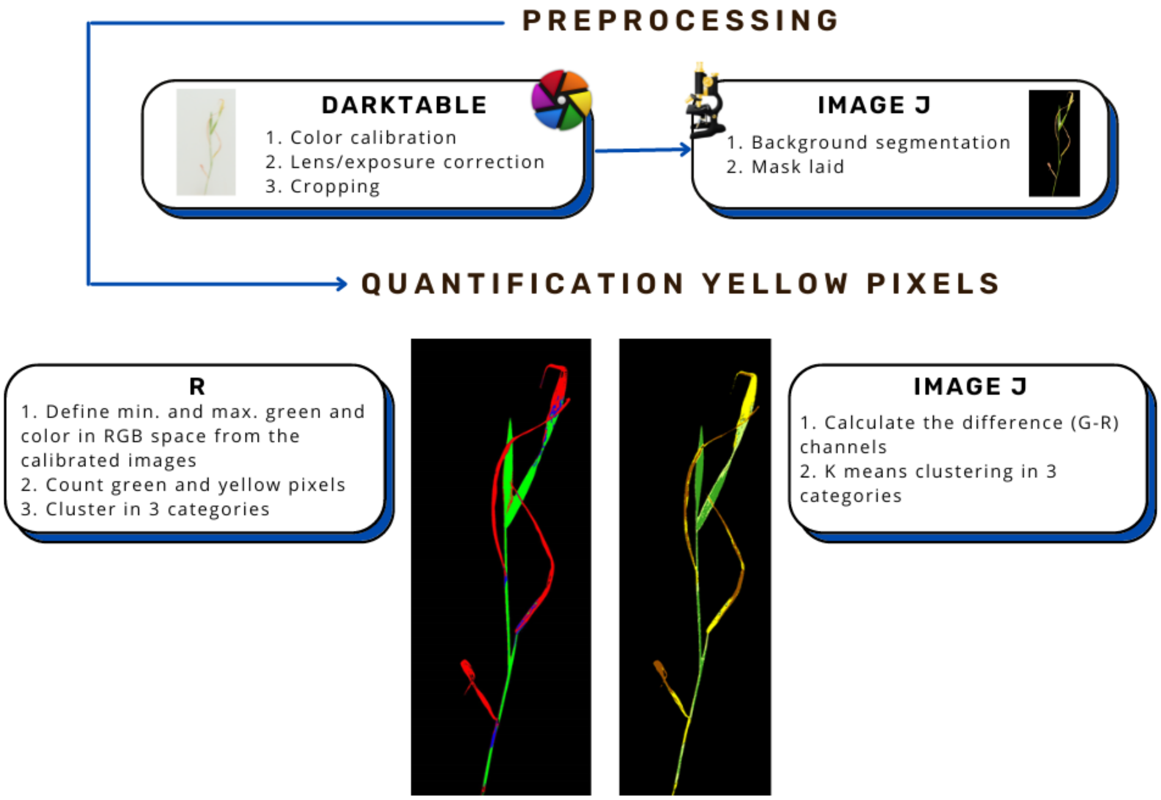
Images acquisition

Each experimental unit was photographed with a professional camera (NIKON D7500) under controlled light conditions with the following set-up: ISO Lo 0.7, shutter speed 1/1.6, aperture F14, white balance 0.0 in mode 4 fluorescent. The images were saved with high quality JPEG and RAW formats.

Figure 2. Photo of an experimental unit 14 days after infestation under light controlled conditions

Images analysis workflow

Based on our previous experience (Hernández et al., 2022), we designed a pipeline for analyzing the data and obtain the damage percentage (% yellow pixels) from the yellow pixels proportion in each image (number yellow pixels / number of total pixels):



Visual rating

All the images were classified by a visual mean of the damage percentage in the experimental units after the photo calibration considering the number of fully expanded leaves. To estimate the damage percentage, each leaf was divided into four parts and check the chlorosis or wilt, categorizing it into a class (Table 2).

Table 2. Visual scale to assess spider mites' damage in each leaf of a single stemmed experimental units of *Urochloa* spp.

Class	Upper limit (%)	Lower limit (%)	Average (%)
6	100		100
5	76	99	87,5
4	51	75	62,5
3	26	50	37,5
2	6	25	15
1	0	5	2,5
0	0		0

Figure 3. Experimental unit with 72.6% of damage according to the visual scale. Red lines represent the division of a leaf

For instance, the plant of the Fig. 3 has five leaves with the next classification from the bottom to the flag leaf: 6, 6, 6, 4 and 0. The mean would be:

$$\frac{(100+100+100+63+0)}{5} = 72.6\%$$

Preliminary results

In a preliminary analysis, the correlation between the visual scoring and the damage calculated with the R pipeline is higher that the one calculated by ImageJ (Fig. 4).

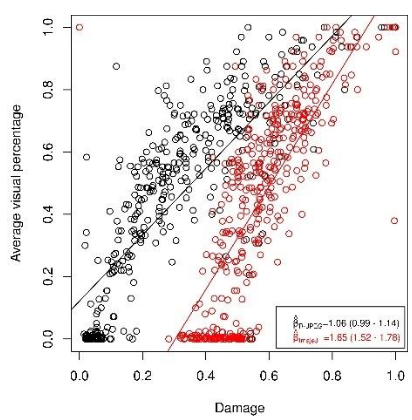


Figure 4. Correlation between visual scoring and R or Image J methods to calculate plant damage.

Considering this, each experimental unit was compared with the number of mites in the 14th day after infestation and the damage percentage estimated with R (Fig. 5). Most of the genotypes evaluated are susceptible to the infestation rate of 30 female adult mites.

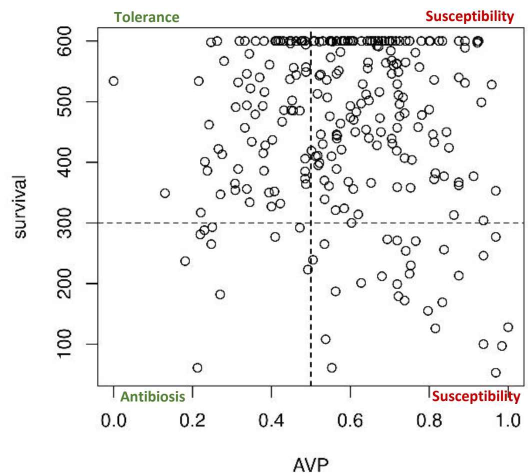


Figure 5. Classification of the experimental units of 26 Urochloa genotypes according to the number of mites 14 days after infestation and the average damage percentage (AVP)

Future outcomes

1. Set thresholds to categorization of genotypes
2. Categorize the genotypes with the experimental design
3. Test the most tolerant and antibiotic genotypes in field conditions

References

Hernández, L., Espitia, P., & Cardoso, J. A. (2022). Digital imaging outperforms traditional scoring methods for spittlebug tolerance in Urochloa humidicola hybrids. *Tropical Grasslands-Forrajes Tropicales*, *10*(3), 271–279. https://doi.org/10.17138/TGFT(10)271-279