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Enhancing Phenotyping Accuracy for Selection of *Urochloa* spp. Tolerant Genotypes to Red Spider Mite (*Oligonychus trichardti*)

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ABSTRACT

The red spider mites are a major biotic limitation to productivity for *Brachiaria* (*Urochloa* spp.) grasses in tropical Eastern Africa, causing severe economic losses mainly in the dry seasons. However, due to the lack of reports of integrated pest management strategies, genetic resistance can play a crucial role in *Brachiaria*-based livestock systems, minimising the impact of this pest. The objective of this study was to develop and validate a methodology for accurately categorising resistance responses in *Urochloa* genotypes to identify potential sources of resistance to the Western Kenya red spider mite *Oligonychus trichardti* (Meyer 1974). Accordingly, 25 genotypes of *Urochloa* spp. were evaluated under the infestation of *O. trichardti*. Response variables included mite count and percentage of plant damage 14 days after infestation. Plant damage quantification involved visual assessment using a severity scale (AVR) and two high-throughput digital image analysis methodologies: one based on the threshold establishment in the RGB colour space (DTR) and another one based on image quantisation using three colour subsets (DQU). A linear regression of these methodologies demonstrated that the DTR methodology is more accurate and has higher concordance with the visual assessments, hence chosen for selecting tolerant genotypes. The genotypes evaluated did not exhibit an antibiotic effect; however, varying levels of tolerance were observed. Ultimately, six tolerant genotypes were identified. CIAT 16125 (cv. Piata) and CIAT 26646 (cv. La Libertad) exhibited the highest levels of tolerance, while CIAT 6426 and CIAT 664 were the most susceptible and are recommended as control candidates for future trials.

1 | Introduction

Improved *Brachiaria* (*Urochloa* spp.) pastures increase the productivity and profitability of livestock systems (Ghimire et al. 2015; Schiek et al. 2018). However, farmers report severe damage in these pastures caused by red spider mites

(Acari: Tetranychidae) during the dry seasons in tropical Eastern Africa (Cheruiyot et al. 2020; Junca Paredes et al. 2023). Integrated pest management strategies for mite control in other crops are based on chemical and/or biological control, which can be costly and have been shown to be inefficient when the pest population develops resistance (De Rouck et al. 2023;

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Mota-Sanchez and Wise 2024), or when exposed to environmental disturbances (Chidawanyika et al. 2019). In addition, red spider mites are multivoltine, migrate easily to other high-value cash crops, and quickly develop strategies to overcome plant defences, which increases their population build-up and pest pressure (Golizadeh et al. 2017; Sperotto et al. 2018). Furthermore, feasible and efficient integrated pest management strategies have not been reported yet for red spider mites in grasses for the region to the best of our knowledge.

Host–plant resistance has proven to be the most suitable alternative to manage pests in pastures as it is a low-cost and easy-adoption tool already incorporated in the seeds (Cardona and Sotelo 2005; Kumari et al. 2022). Breeding schemes usually exploit two categories of resistance for the development of new varieties: antibiosis and tolerance (Peterson et al. 2017). Antibiosis is based on the plant's physical and/or chemical traits that deter herbivory, and tolerance is based on the plant traits that reduce the negative effects of herbivore damage (Mitchell et al. 2016; Smith 2021). Hence, to integrate host–plant resistance traits into early stages of a breeding scheme, it is crucial to deploy a high-throughput methodology that allows the screening of hundreds of experimental units with high accuracy and precision.

Traditionally, plant damage is quantified using severity scales based on visual scoring by trained evaluators. In the interspecific *Urochloa* breeding program, high rates of genetic gain for spittlebug (Hemiptera: Cercopidae) tolerance and antibiosis have been achieved by phenotyping parental and hybrid populations in no-choice tests through visual scoring of the plant damage and the net survival percentage (Cardona et al. 1999; Hernandez et al. 2025; Parsa et al. 2011). However, this method is prone to human bias (experience, inherent ability, instruction), and it is also time-consuming. Therefore, the development of validated standard area diagrams (SADs) and the use of sensor-based image analysis can enhance the accuracy of damage classification and quantification (Bock et al. 2020, 2022). Among the image analysis techniques, RGB images allow the phenotyping of large populations for biotic and abiotic stress in a high-throughput and cost-effective way (Ahmed et al. 2023; Singh et al. 2016).

To further increase the genetic gain rate, a methodology based on digital image analysis developed by Hernández et al. (2022) aims to segment the damaged area by using colour indices in RGB images and Heckbert's median-cut colour quantisation algorithm for three categories and the image analysis software ImageJ. Through colour quantisation, the range of each channel in the RGB colour space, which lies in [0, 255], is narrowed to a user-defined range (Thompson et al. 2020). The median-cut algorithm in the ImageJ 8-bit Colour function uses a size criterion to generate a new image with a predetermined number of colours by splitting iteratively an RGB image in regions based on a greater number of pixels and dividing each of those regions along the longest axis at the median point, until the number of colours is reached (Celebi 2023; Rahkar Farshi 2020). In Hernández et al. (2022), the damage is calculated from the pixel counts of three predetermined plant tissue colour groups defined as background, green, and yellow. Finally, a custom look-up table (LUT) is applied to differentiate each colour group in the image for quality control. This allows an accurate and

precise quantification of spittlebug (Hemiptera: Cercopidae) damage in *Urochloa* plants, replacing the discrete variables of the visual scoring with continuous variables of the image analysis method.

Previous studies identified *Tetranychus urticae* (Koch 1836) and *Oligonychus trichardti* (Meyer 1974) infesting *Urochloa* cultivars in Kenya and determined different levels of resistance in a panel of genotypes through greenhouse tests using a visual severity scale for damage (Cheruiyot et al. 2018; Mutisya et al. 2018). Furthermore, Cheruiyot et al. (2018) reported potential sources of resistance in the agamic complex *U. brizantha*, *U. ruziziensis* and *U. decumbens* for breeding. Thus, the objective of this study was to develop and validate an optimised and scalable methodology for accurately categorising resistance responses in *Urochloa* genotypes. The approach integrated digital imaging for precise quantification of red spider mite-induced plant damage, along with the evaluation of mite survival, enabling high-throughput phenotyping for resistance screening.

2 | Materials and Methods

2.1 | Plant Material and Red Spider Mite Colony

This study was conducted at the International Centre of Insect Physiology and Ecology (Icipe), Thomas Odhiambo Campus, Mbita Point, Kenya (0°25'S, 34°12'E; 1200 m.a.s.l.) under greenhouse conditions (27°C, 64% R.H, 12 h photoperiod).

The panel of 25 *Urochloa* tetraploid genotypes evaluated in this study included 13 hybrids from the Alliance Bioversity-CIAT breeding programme, eight GenBank accessions, and four cultivars from germplasm selection (Table 1, Supporting Information). The seeds of the accessions and hybrids were provided by the CIAT GenBank and the interspecific *Urochloa* breeding programme in Cali, Colombia, while the cultivars were obtained from Simlaw Seeds in Kisumu, Kenya.

The red spider mites were collected from an interspecific *Urochloa* hybrid cv. Cayman pastures at a livestock farm in Mbita Point, Kenya, for mass-rearing. A group of 80 adult females with similar morphological characteristics was selected

TABLE 1 | Damage visual scale of red spider mite infestation for each leaf of the experimental units of *Urochloa* spp.

Score	Upper limit (%)	Lower limit (%)	Average (%)
6	100		100
5	75	99	87.5
4	50	75	62.5
3	25	50	37.5
2	5	25	15
1	0	5	2.5
0	0		0

Note: 0 is a completely green leaf portion and 6 is a completely necrotic leaf portion.

under a stereoscope and used to infest the interspecific *Urochloa* hybrid cv. Mulato II clean plants in cages. Female and male individuals were mounted in Hoyer's medium following Razuvaeva et al. (2023) and identified as *Oligonychus trichardti* (Meyer 1974) by taxonomists in the Grupo Red Diagnóstico Fitosanitario in Colombia. The colony was reared for 2 months before the no-choice tests began.

2.2 | No-Choice Tests

No-choice tests were conducted for red spider mites on the *Urochloa* panel in three independent trials. The experimental treatments were defined by two factors: genotypes with 25 levels, and infestation status with two levels (i.e., infested and uninfested). All treatment combinations were included in each of the three trials. The basic experimental design was a completely randomised design including the 25 genotypes, with four replicates for the infested level and two replicates for the uninfested level. The unbalanced replicate number follows the assumption that no damage would occur in the uninfested treatments, thus requiring fewer replications to capture variability in that

group. The experimental units were modified from Aguirre-Rojas et al. (2019) and Cardona et al. (1999), consisting of single-stemmed plants with three fully expanded leaves, planted in a substrate of vertisol soil and sand (1:1) in PVC tubes (5.3 cm diameter, 6.5 cm long), and caged with an acetate tube with three openings (3.5 cm diameter) to prevent mite migration and to control humidity inside the experimental unit (Figure 1).

Two weeks after planting, the experimental units were infested with 30 adult females from the red spider mite colony. Digital images of the units and mite counting were conducted 14 days after infestation (DAI).

2.3 | Digital Image Acquisition

Each experimental unit was placed in a white, enclosed wooden chamber (dimensions: 1 × 1 × 1 m), with a strip of LED day white lights (6000k) for continuous illumination. Images were captured using a NIKON D7500 reflex camera with the following set-up: manual mode, focus mode AF-A single point, white balance set to 0.0 in the fluorescent mode, ISO speed set to Lo 0.7,

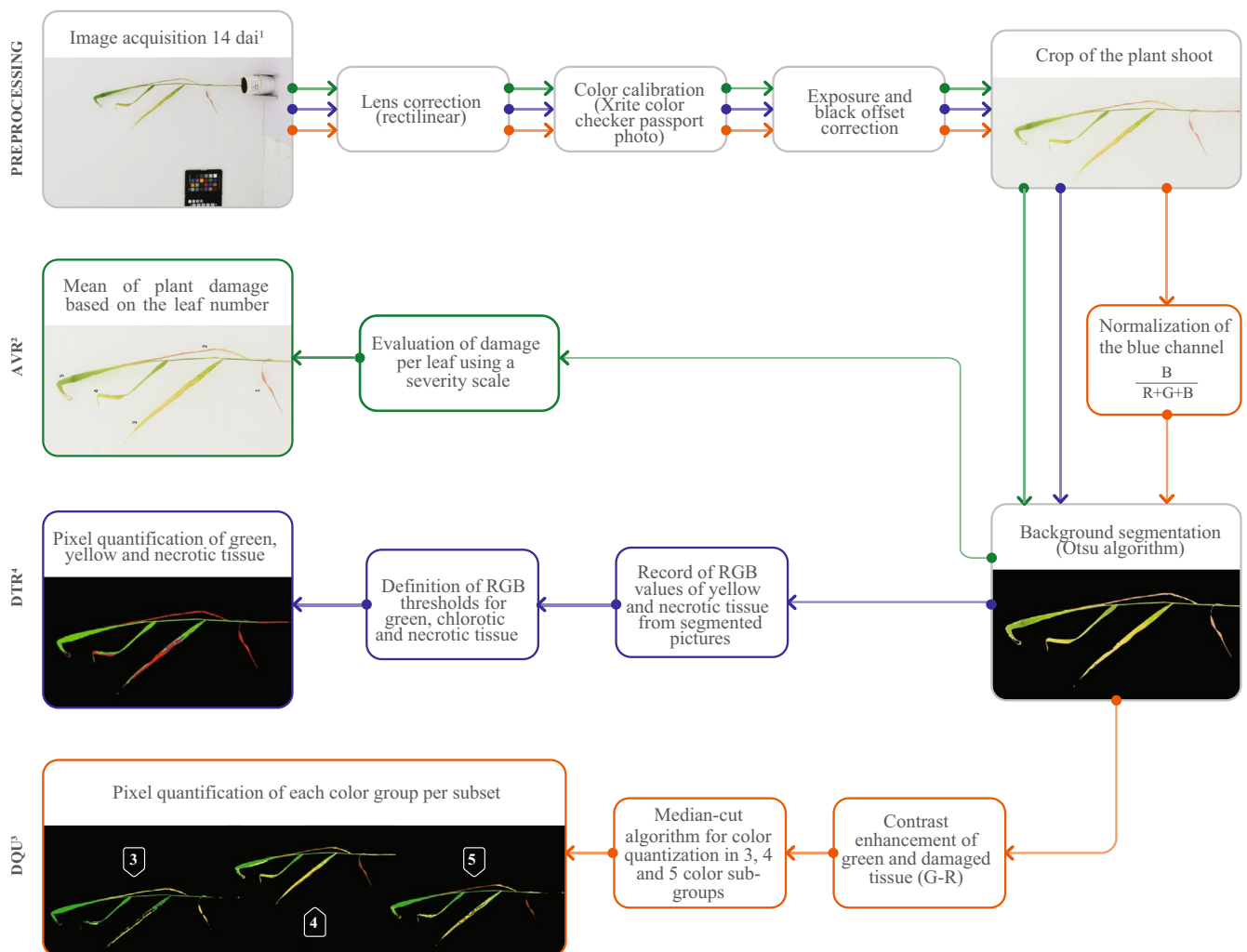


FIGURE 1 | Workflow for image analysis of plant damage: Preprocessing and plant damage quantification methodologies. The arrows indicate the sequential flow of data and operations within the workflow. ¹Days after infestation. ²Plant damage estimation by Average Visual Damage Rating (AVR). ³Plant Damage per colour Quantisation (DQU). ⁴Plant Damage per colour Thresholding (DTR).

shutter speed set to 1/1.6s, and aperture set to F14. All images were saved in both 14-bit RAW image files and high-quality JPEG formats. To calibrate the colour of the images, an X-Rite Colour Checker Passport scale was positioned beside the experimental units in all pictures.

2.4 | Preprocessing: Colour Calibration and Segmentation

RAW images were used for lens correction (rectilinear) and colour calibration in the open-source software darktable (The Darktable Team 2023). A new colour profile was generated using the Xrite Colour Checker photo 2 chart option, ensuring that the average delta E was less than 2.3, approximately one JND (just noticeable difference) (Mokrzycki and Tatol 2014). The corrected exposure and black levels were applied to adjust these parameters. Subsequently, the images were cropped to retain the plant shoot portion and were saved in a 16-bit TIFF format.

For background segmentation, we followed the methodology reported by Hernández et al. (2022) in ImageJ (IJ 1.46r). Otsu's clustering algorithm was employed, and outliers (2.5 pixels) were removed to create a mask. This mask was applied to the unsegmented image using the AND logic operation. The resulting segmented images were saved in a 16-bit TIFF format.

2.5 | Quantification of the Plant Damage by Red Spider Mites

The preprocessed images were used to quantify plant damage using two methods based on segmentation: one implemented in ImageJ (Rasband 2012), adapting the workflow from Hernández et al. (2022), denominated as Damage per colour Quantisation (DQU); and the other based on colour thresholding using the RGB values of the green, yellow, and necrotic tissue, denominated Damage per colour Thresholding (DTR) developed using the tiff package (Urbanek and Johnson 2022) in the R environment (R Core Team 2023).

2.5.1 | Plant Damage Quantification Through Colour Quantisation (DQU Technique)

In the DQU methodology, before preprocessing, each image was split into its red (R), green (G), and blue (B) channels, with a normalisation of the blue channel ($B/[R + G + B]$). This normalisation involves adjusting the pixel values in the blue channel relative to the sum of the red, green, and blue channels. Following background segmentation as previously described, a subtraction operation was performed between the green and red channels ($G - R$). This operation entails subtracting the pixel values in the red channel from the values in the green channel to enhance the contrast between the green and senescent colours (Hernández et al. 2022).

Next, a colour quantisation using Heckbert's median-cut colour quantisation algorithm in the ImageJ function "8-bit Colour" (Heckbert 1982) was performed to reduce the number of colours in the images to 3, 4 and 5 colour subsets (DQU_3, DQU_4 and

DQU_5, respectively). This was done to compare the accuracy across different colour subsets. Pixel counts from the histograms of the processed images are then used to categorise pixels into background, green and yellow based on intensity levels. Finally, the damage was quantified with the severity equation (Olivoto et al. 2022):

$$\text{Damage (\%)} = 1 - \frac{\text{Green pixels}}{\text{Total pixels} - \text{Background pixels}} \times 100$$

2.5.2 | Plant Damage Quantification Through R, G and B Threshold Values (DTR)

In the DTR methodology, 20 preprocessed images of the experimental units were randomly selected to record 20 RGB values for the chlorotic (yellow) and necrotic tissue. From these 400 records, the lower and upper bounds for the two categories were obtained to set the threshold for those categories. For the green tissue, we used the reported value for the app Canopeo (Patrignani and Ochsner 2015).

This process allowed for the definition of colour thresholds for the green, chlorotic, and necrotic categories in the RGB colour space. The decision rules were as follows:

Green tissue:

$$R < (0.9 \times G)$$

$$B < (0.9 \times G)$$

$$(2 \times G) > (20 / 255) \times (B + R)$$

Yellow:

$$(141 / 255) < R < (218 / 255)$$

$$(164 / 255) < G < (215 / 255)$$

$$(47 / 255) < B < (122 / 255)$$

Necrotic:

$$(160 / 255) < R < (250 / 255)$$

$$(137 / 255) < G < (240 / 255)$$

$$(58 / 255) < B < (220 / 255)$$

The damage was quantified with the equation:

$$\text{Damage (\%)} = \frac{\text{Yellow pixels} + \text{Necrotic pixels}}{\text{Yellow pixels} + \text{Necrotic pixels} + \text{Green pixels}} \times 100$$

2.5.3 | Plant Damage Quantification Through Visual Severity Scale (AVR)

A scale based on yellowing and necrosis was developed for validation through photo interpretation of damage caused by red spider mites in the experimental units at 14 DAI in the segmented images. Initially, the fully expanded leaves were counted, and each leaf was horizontally divided into four sections, with each

section representing 25% of the leaf. Subsequently, each leaf was scored from 0 to 6 based on the percentage of chlorosis and/or necrosis (Table 1). Finally, these scores were replaced by the average visual damage rating (AVR), with the mean of AVRs per leaf representing the total percentage of damage for the plant (Table 1). The assessment was conducted twice to minimise potential biases.

2.6 | Validation of the Methodologies

To validate the damage quantification methodologies using image analysis with visual scoring, the following model was employed:

$$\text{AVR} = \hat{\beta}_0 + \hat{\beta}_1 x + \varepsilon$$

where AVR is the vector of visual scale observations of size n (416), both $\hat{\beta}_0$ and $\hat{\beta}_1$ are scalars representing the intercept and the linear regression coefficient, respectively; x is the vector of size n with measurements from the DQU or DTR methodologies; and ε is the vector of size n corresponding to the residual random effect following a normal distribution with mean zero and variance σ_ε^2 . Additionally, the coefficient of determination (R^2), Lin's concordance coefficient (ρ_c), and 95% confidence intervals for $\hat{\beta}_0$ and $\hat{\beta}_1$ were obtained. The level of discrepancy between visual assessment and the measurements by the two image analysis methodologies is given by $\hat{\beta}_1$. Perfect fit is considered when $\hat{\beta}_0$ and $\hat{\beta}_1$ (Alves et al. 2022). For these analyses, the R statistical environment (R Core Team 2023) and the concordance package (Cross 2023) were used.

2.7 | Statistical Analysis

For the analysis, we considered a experiment ($i = 1, 2, a$), t levels of infestation ($j = 1, t$), g genotypes ($k = 1, 2, \dots, g$), n_{ijk} observations in each combination of experiment, infestation, and genotype, and $n = \sum \sum \sum n_{ijk}$ as the total of observations. Thus, for the $n \times 1$ y vector of observations, the following mixed model is defined:

$$y = X\beta + Zu + 1_{n_i} \otimes e_i \quad (1)$$

where $\beta = [\mu_1, \dots, \mu_{tg}]$ and u are vectors of fixed effects of genotypes-infestation and the random effects between experiments; X ($n \times tg$) and Z ($n \times a$) are the corresponding incidence matrices for the effects in these vectors. The residual e vector is $e = [e_1, \dots, e_{atg}]'$, where e_{ijk} is the $n_{ijk} \times 1$ vector of the residual effects (experimental error) of the k th genotype and j th infestation within i th experiment, and e is the $n \times 1$ vector of the residual effects. In this analysis, normal priors for both the fixed and random effects of the mixed model (Equation 1) were used in the Bayesian inference.

2.7.1 | Bayesian Prior and Posterior Density of the Parameters of the Mixed Model

Prior distribution for the vectors of random effects u and e are normal distributions with zero mean and variances $\sigma_{u_m}^2$ for

$m = 1.1, 1.2, \dots, j.k, \dots, tg$, and $\sigma_{e_{ijk}}^2$, respectively. Thus, the vector of parameters is $\theta = [\beta, u, \sigma_{e_{111}}^2, \dots, \sigma_{e_{atg}}^2]'$, and the conditional distribution of $y|\theta$, is the multivariate normal distribution.

$y | \beta, u, \sigma_{e_{111}}^2, \dots, \sigma_{e_{atg}}^2 \sim N(X\beta + Zu, R)$, where $R = \left\{ \sigma_{e_{ijk}}^2 I_{n_{ijk}} \right\}_d$ and $I_{n_{ijk}}$ is the identity matrix of order n_{ijk} , and d is used to indicate a diagonal matrix.

We further need to define prior distributions for β , as well as prior distributions or prior values for the variance components.

1. The vector of genotypic-treatment effects was assumed to have a multivariate normal truncated distribution within the range (0, 1) for DQU and DTR, and (0, 600) for mite counts (all genotype-treatment effects non-negative); the non-negative constraint was imposed to ensure that genotypic-treatment means are always positive. In the case of mite counts, an upper limit was established, as counts were only conducted up to 600 mites due to practical constraints. Constraining percentage variables ensures that statistical inference remains within the natural parameter space, as recommended by Box and Tiao (1992). For mite counts, the upper bound was set at 600, which represents 20 times the initial infestation level and corresponds to a high population growth for measuring antibiosis.

Thus, the vector of genotype-treatment effects is given by

$$\beta | B_0, \sigma_0^2 \sim N - \text{truncated} (B_0, I_m \sigma_0^2) \quad \begin{array}{l} 0 < \beta < 1 \text{ for DQU and DTR} \\ 0 < \beta < 600 \text{ for mite counts} \end{array}$$

where B_0 is the prior vector of the genotype-treatment effects, namely genotypic-treatment means, and $I_m \sigma_0^2$ is the prior variance matrix of the genotypic-treatment means.

2. The vector of between-experiment effects was assumed to have a multivariate normal distribution $u_{jk} | \sigma_{u_{jk}}^2 \sim N(0, I_{q_m} \sigma_{u_m}^2)$ where q_m and $\sigma_{u_m}^2$ are the number of levels and the variance of the effects (u_m), respectively.

Priors used for the variance components $\sigma_{u_m}^2, \sigma_{e_1}^2, \dots, \sigma_{e_a}^2$ were used a scaled inverse chi-square distribution Scaled - Inv - χ^2 (Gelman et al. 2013). Each Scaled - Inv - χ^2 has two hyperparameters that can be obtained from experimental data or from the researcher's own experience, that is, $\sigma_{u_m}^2 | v_{u_m}, s_{u_m}^2 \sim \text{Scaled - Inv - } \chi^2(v_{u_m}, s_{u_m}^2)$, where $v_{u_m}, s_{u_m}^2$ are the degrees of belief and the variance (scale factor) for u_m , respectively.

3. For the vector of residual variances, the priors were $\sigma_{e_{ijk}}^2 | v_{e_{ijk}}, s_{e_{ijk}}^2 \sim \text{Scaled - Inv - } \chi^2(v_{e_{ijk}}, s_{e_{ijk}}^2)$, where $v_{e_{ijk}}$ is named, following Sorensen and Gianola (2002), the degrees of belief, and $s_{e_{ijk}}^2$ is the prior value for the error variance, $\sigma_{e_{ijk}}^2$, within the i th experiment of the k th genotype and j th treatment, respectively.

Parameters $B_0 = 0$, $\sigma_0^2 = 110^8$, $v_{u_m} = 1$, $s_{u_m} = 0$, $v_{e_{ijk}} = 1$, and $s_{e_{ijk}}^2 = 0$, are called hyperparameters as these were not estimated.

Furthermore, a multiple trait linear mixed-effects model, based on the univariate model described in Equation (1), was also

considered to assess the relationship between the best computational damage estimation technique and the mite counts, to estimate the covariance and correlation between these two variables. For this purpose, we followed the approach described in chap. 13 of Sorensen and Gianola (2002), replacing the Scaled – Inv – χ^2 distribution with the appropriate inverse-Wishart distribution, which includes not only the variance parameters but also their respective covariances.

All analyses were conducted in the R environment (R Core Team 2023) using the MCMCglmm package (Hadfield 2017). Highest posterior density (HPD) intervals at a 0.85 probability level were estimated using the coda package (Plummer et al. 2006). This non-conventional probability threshold was selected given the preliminary nature of the trial, with the aim of providing an initial screening of accession responses to red spider mite infestation.

For each model, 2.5×10^6 samples were drawn from the marginal posterior distribution of each parameter. The mean of the posterior distribution was used as the Bayes estimator, as it minimises the quadratic loss function according to estimation theory.

3 | Results

3.1 | Methodologies for Plant Damage Quantification

A total of 416 digital images from the three experiments were used for the analyses, excluding dead plants. The DTR method outperformed the three DQU methods, demonstrating superior fit and precision, as indicated by a higher coefficient of determination, narrower confidence interval for the slope, an intercept closer to 0, and greater concordance with visual measurements (Table 2, Figure 2). This method also has greater flexibility to generate and adjust each part directly from the code itself as it is programmed in R, which is particularly useful if subsequent trials involve changes in image capture conditions (e.g., lighting), or require adding more categories (e.g., anthocyanin expression), or just requires recomputing the RGB colour thresholds for the existent categories. However, the execution time for DTR was longer (4 min and 46 s) compared to DQU_4 (3 min and 21 s) when processing 100 segmented digital images in tiff format, using a single processor core.

In contrast, colour quantisation in the DQU methodologies resulted in an underestimation of plant damage with DQU_3 and

DQU_4, and an overestimation with DQU_5 (Figure 2). While the algorithm of DTR measures yellow pixels using predefined RGB values obtained from a subset of the images in this study, the algorithm of DQU measures the number of pixels in pre-determined colour groups (3, 4 and 5) without explicit rules. According to the regression parameters and Lin's concordance coefficient, colour quantisation to four colours had a better precision, suggesting that with five colours, more green pixels are grouped with yellow pixels, resulting in higher severity values, and that with three colours more yellow pixels are grouped with the green pixels, leading to lower severity values. Furthermore, colour groups may vary among the images as the algorithm uses the parameters of each of them, resulting in less consistent severity calculation. The AVR showed a higher mean per genotype than those calculated by the DTR (Figure 3), suggesting that visual scoring overestimated the damage.

3.2 | Selection of the *Urochloa* spp. Genotypes by Their Response to Red Spider Mite Infestation

The *Urochloa* genotypes showed variability in their response to red spider mites after 14 DAI. Mite counts ranged from 264.59 (200.33–325.32) for accession CIAT 664 to 552.48 (529.40–581.61) for the hybrid GP/0549 (Figure 4). Plant damage among infested genotypes varied from 30.73% (24.55–36.98) for cultivar Piatá, CIAT 16125, to 80.24% (72.18–88.85) for accession CIAT 6426 (Figure 3). Six genotypes exhibited plant damage levels below 40% (Figure 4). The population of *Oligonychus trichardti* in all genotypes showed substantial growth, with CIAT 664 increasing to 9.5 times its initial size, suggesting a lack of significant antibiotic effect.

4 | Discussion

Image segmentation allows to define subareas based on the R, G and B values, useful in plant damage quantification. In a similar approach, Olivoto et al. (2022) developed a workflow based on the manual delimitation for 'affected', 'healthy' and 'background' classes, and the posterior use of those values as predictive variables in a logistic regression in the pliman R package (Olivoto 2022). The processed images were used to calculate plant severity using the APS Assess software (Lamari 2008). The authors reported a high agreement ρ_c ranging from 0.49 to 1, and Pearson correlations between 0.6 and 1. These values are similar to those obtained in this study. Furthermore, the same authors found that when symptoms

TABLE 2 | Damage visual scale of red spider mite infestation for each leaf of the experimental units of *Urochloa* spp. with 95% confidence interval limits in parentheses.

Methodology	Slope ($\hat{\beta}_1$)	Intercept ($\hat{\beta}_0$)	Coefficient of determination (R^2)	Lin's concordance coefficient (ρ_c)
DTR	0.739 (0.703–0.775)	2.743 (0.660–4.826)	0.819	0.837
DQU_3	0.599 (0.549–0.650)	–5.015 (–7.955 to –2.075)	0.598	0.545
DQU_4	0.663 (0.617–0.709)	–4.375 (–7.051 to –1.699)	0.687	0.640
DQU_5	0.376 (0.342–0.411)	37.0321 (35.030–39.034)	0.559	0.579

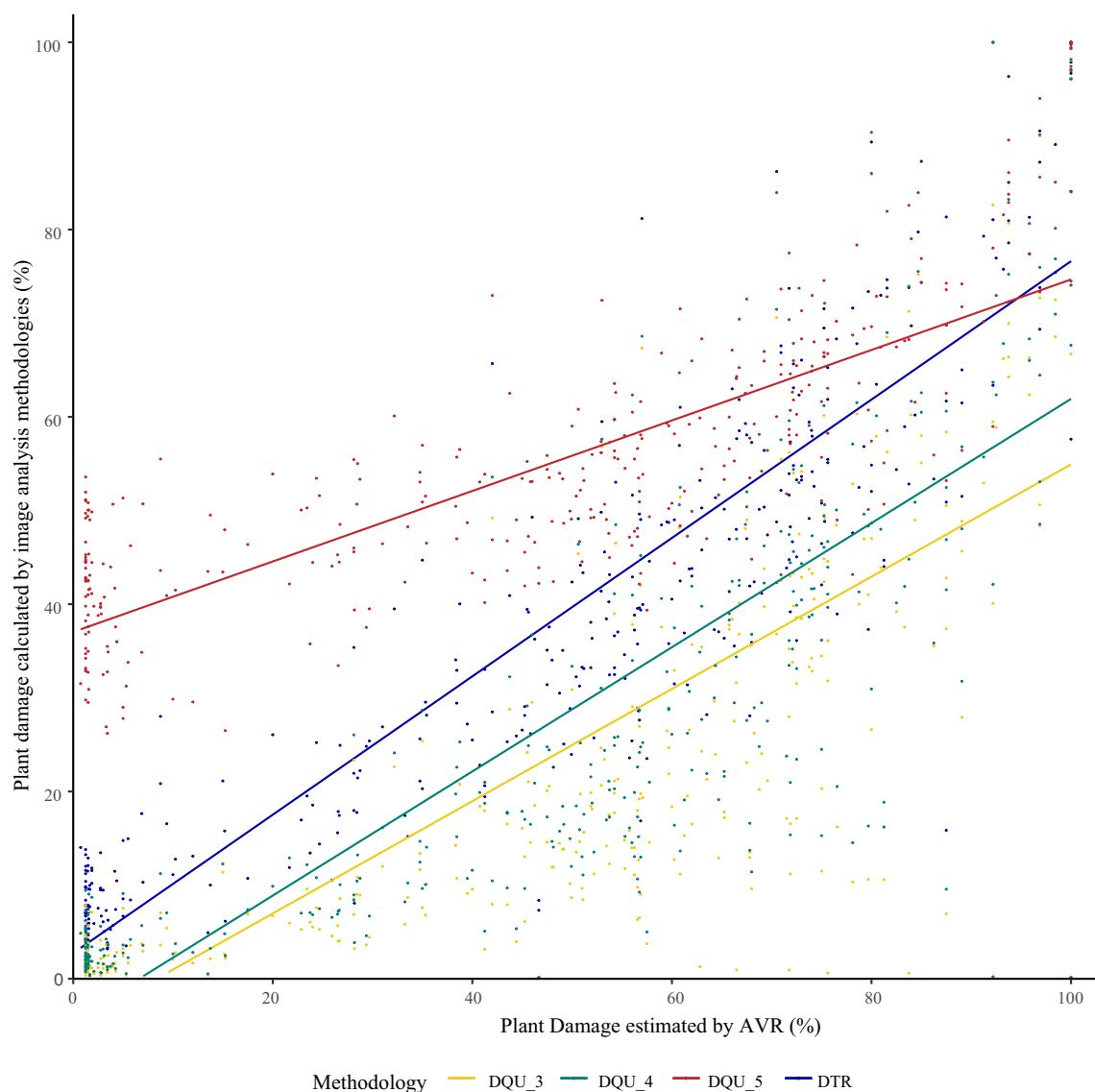


FIGURE 2 | Linear regression of plant damage calculated by image analysis techniques, DQU, and DTR, against the average visual damage rating (AVR).

were incipient, the delimitation of the damaged areas was more complex in the software, suggesting that the areas with subtle chlorosis may be underestimated.

Similarly, the visual scale used in the plant damage rating by the AVR method was discrete and ordinal, resulting in the assignment of damage scores that overestimate the affected area. A scale based on SADs and having more than one expert evaluator scoring the genotypes may increase the accuracy and precision of the visual scoring; however, it would require more time for developing the SADs and for training the evaluators, along with a greater investment (Araus et al. 2018; Barbedo 2016; Bock et al. 2022; Shi et al. 2023).

Differences were observed in the genotype tolerance ranking compared to those reported by Cheruiyot et al. (2018) for *Urochloa* accessions assessed to *O. trichardti* (Meyer) in Mbita, Kenya using a visual scale of six categories. The authors reported damage of less than 20% for the genotypes CIAT 16107, CIAT 16125, and CIAT 26110; damage ranging from 40% to 59%

for genotype CIAT 16122; and damage between 60% and 79% for genotypes CIAT BR02/1752, CIAT 606, and CIAT 36087. Although five of the genotypes in our study had a similar ranking, cultivars CIAT 26110 cv. Toledo and CIAT 36087 cv. Mulato II exhibited divergent responses, as Mulato II showed higher damage than Toledo. Thus, Mulato II is considered susceptible and Toledo moderately tolerant in livestock and push-pull systems (Cheruiyot et al. 2020, 2022; Mutisya et al. 2018). Additionally, 19 accessions were identified as more susceptible than Mulato II, and hybrids CIAT BR02/1752 cv. Cayman and CIAT 606 cv. Basilisk showed approximately 10% higher damage quantification.

The methodologies to evaluate plant damage differ in both studies, as well as in the experimental units and the level of infestation of *O. trichardti*. In the methodology employed by Cheruiyot et al. (2018), the experimental units were plants with several tillers planted in pots, and the number of individuals in the infestation was not controlled. In our trials, we sought to homogenise all experimental units with a single stem and three fully expanded

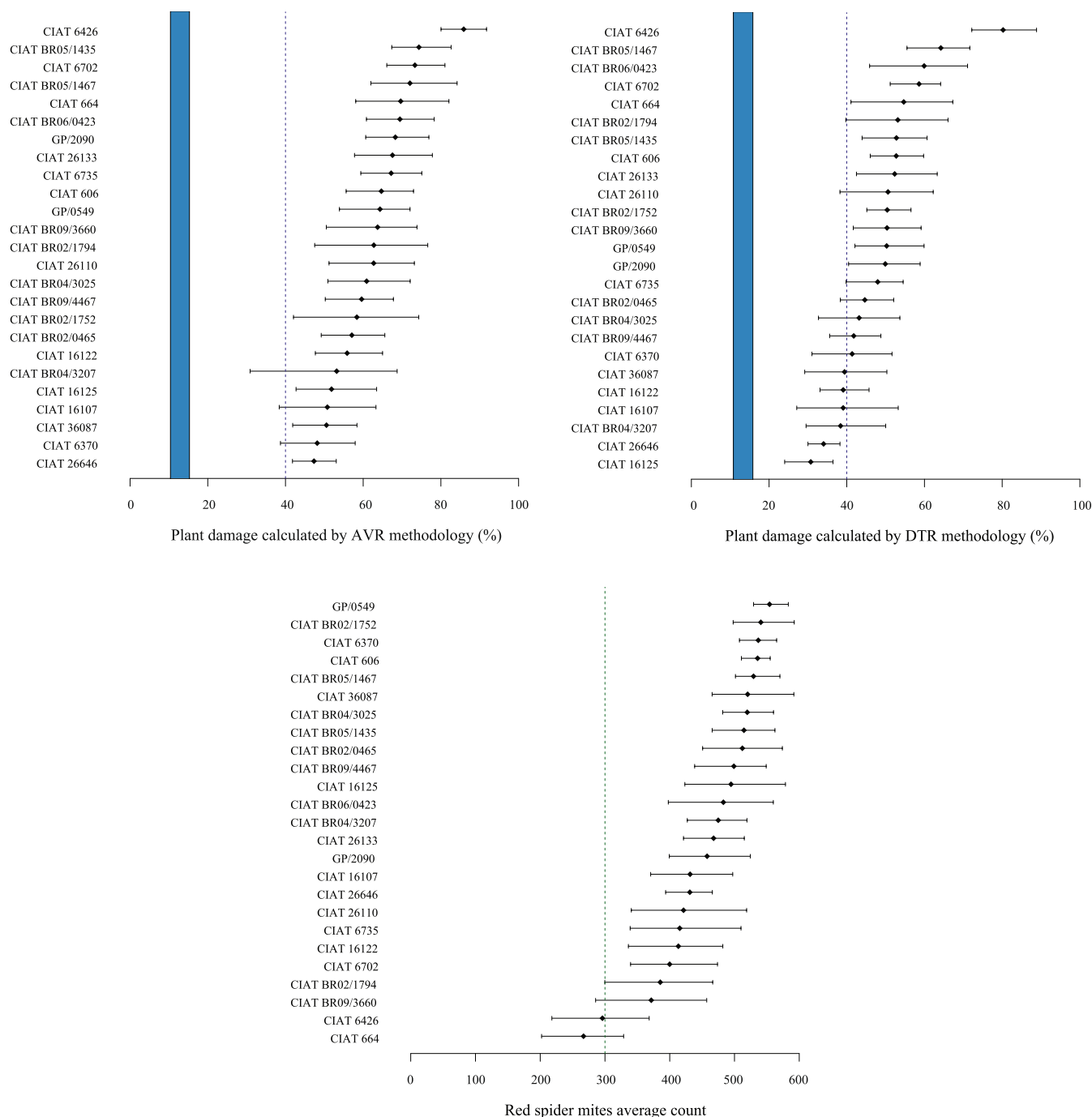


FIGURE 3 | Damage percentage caused by the red spider mite on 25 *Urochloa* spp. genotypes estimated using two methodologies: (A) The average visual damage rating (AVR); (B) Segmentation with RGB threshold methodology (DTR); (C) The average count of red spider mites 14 days after infestation. The solid dark points are the Bayes estimate (mean of the posterior distribution), and the whiskers show the limits of the highest posterior density interval at 85% for the infested replicates. The blue bar represents the lower and upper limits of the highest posterior density interval at 85% for the non-infested replicates. The dashed line represents the tolerance threshold for selection.

leaves, subjecting them to the same biotic pressure through infestation with 30 adult females. This approach aimed to eliminate biases associated with uneven plant growth or differences in the number of mites colonising the plant, thus reducing interaction with the environment, as arthropod activity is highly influenced by prevailing conditions at any given time (Obermeier et al. 2022).

The differential responses in greenhouse tests of this study and the field trials suggest that environmental conditions mediate

the genotype–mite interactions, thereby causing the differences in the response of the genotypes in these observed study variables. Steel et al. (2023) report that in insects, no-choice tests tend to overestimate their ecological host range as the pre-contact and post-contact cues that are used to find a host plant are limited compared to the complex environment in the field. Similarly, this phenomenon is observed in Cheruiyot et al. (2018) study, where the average damage of Mulato II and Toledo cultivars from evaluations in two seasons and two locations under

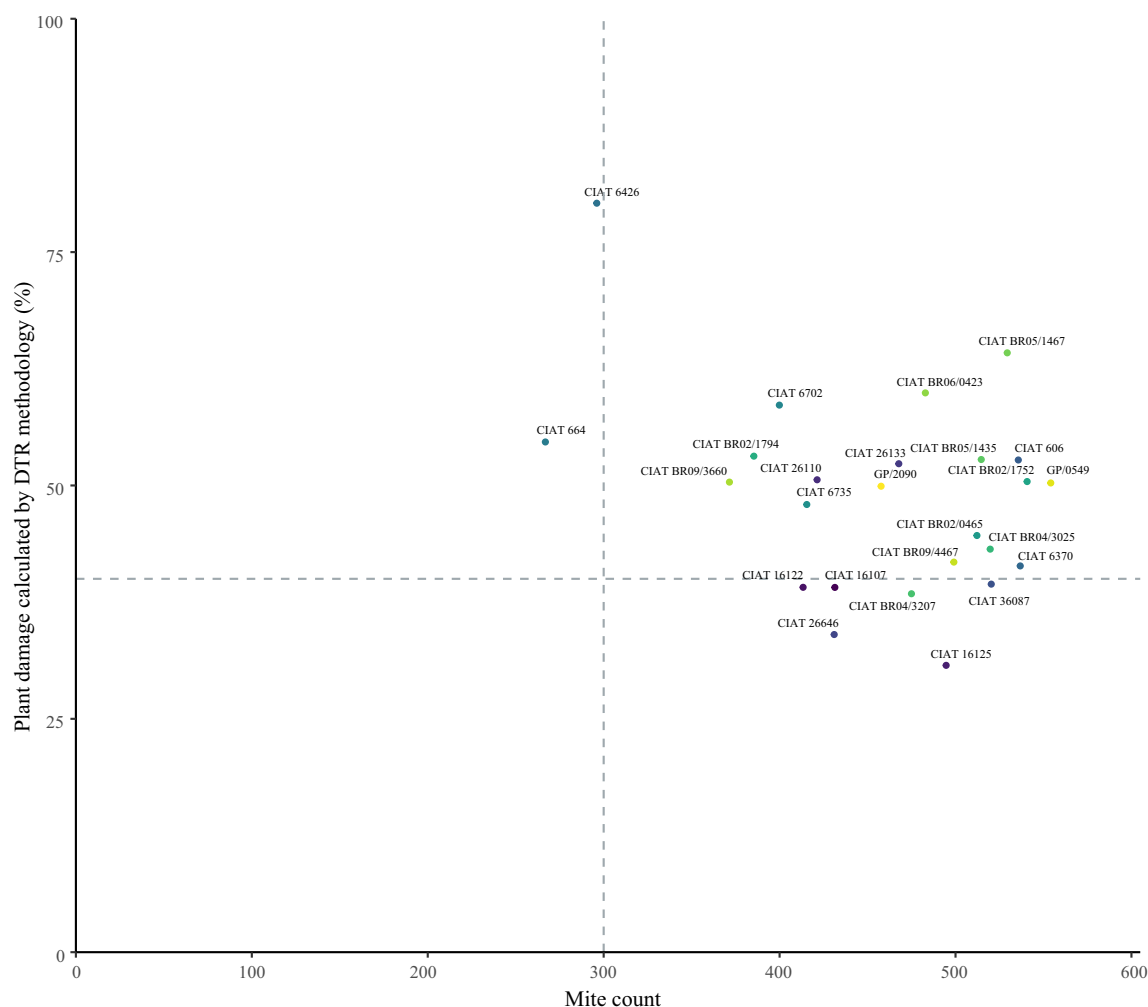


FIGURE 4 | Biplot of mite counts and plant damage calculated by the DTR methodology (%) of 25 *Urochloa* spp. genotypes after the red spider mite infestation, analysed using a multiple characteristic linear mixed-effects model.

natural field infestations was lower than that observed in greenhouse trials conducted in this study.

It should also be considered that resistance or tolerance levels in the plant vary as different species of phytophagous mites have variable responses to the same tolerance factor (Blaazer et al. 2018). Studies investigating the antibiotic effect of benzoxazinoids produced in maize on *T. urticae* and *O. pratensis* (Bui et al. 2018) and of cystatins produced in barley on *T. urticae* and *Brevipalpus chilensis* (Baker) (Carrillo et al. 2011) report such variability in the behaviour and life cycle of the mentioned species. Therefore, it is of great importance to correctly identify the red spider mite species associated with *Urochloa* grasses in Kenya.

5 | Conclusion

The methodology developed in this research demonstrates the effectiveness of digital image analysis and the scalability for no-choice trials involving hundreds of experimental units, accurately quantifying red spider mite-induced damage in *Urochloa* spp. The methodology based on RGB threshold values (DTR)

outperformed the unsupervised clustering approach of the colour quantisation technique (DQU), showing strong agreement with visual assessments, offering a reliable, high-throughput tool for phenotyping tolerance and the further classification of *Brachiaria* genotypes based on their resistance responses. Given the absence of antibiosis, this finding highlights the importance of accurately detecting tolerance traits through image-based methods. Leveraging the DTR approach, six tolerant genotypes were identified, with CIAT 16125 and CIAT 26646 emerging as the most promising candidates for use as resistant checks in breeding programmes. Conversely, CIAT 6426 and CIAT 664 were confirmed as susceptible controls. Overall, the integration of this imaging pipeline supports more accurate and scalable selection of red spider mite-tolerant *Urochloa* genotypes.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.