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TECHNICAL ADVANCE

Tasselyzer, a machine learning method to quantify maize anther exertion, based on PlantCV

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SUMMARY

Maize anthers emerge from male-only florets, a process that involves complex genetic programming and is affected by environmental factors. Quantifying anther exertion provides a key indicator of male fertility; however, traditional manual scoring methods are often subjective and labor-intensive. To address this limitation, we developed *Tasselyzer* — an accessible, cost-effective, and time-saving method for quantifying maize anther exertion. This image-based program uses the PlantCV platform to provide a quantitative assessment of anther exertion by capturing regional differences within the tassel based on the distinct color of anthers. We applied this method to 22 maize lines with six genotypes, showing high precision (F_1 score > 0.8). Furthermore, we demonstrate that customizing the parameters to assay a specific line is straightforward and practical for enhancing precision in additional genotypes. Tasselyzer is a valuable resource for maize research and breeding programs, enabling automated and efficient assessments of anther exertion.

Keywords: maize, anther, tassel, anthesis, male fertility, PlantCV, naïve Bayes classifier.

INTRODUCTION

Anther exertion and pollen viability collectively contribute to male fertility in maize (Weatherwax, 1916). Anther exertion in maize is controlled by complex genetic programs involving many developmental steps (Marchant & Walbot, 2022). Normal development can be negatively impacted by environmental factors such as light, temperature, water, nutrient availability, and mechanical stress such as wind or storm. The ability to regulate anther exertion has substantial agronomic utility; consequently, the ability to evaluate the presence and proportion of exerted anthers is a beneficial technique. Maize anthers emerge from male-only florets, with two florets – an upper and a lower floret in each spikelet – and hundreds of spikelets arrayed on a terminal tassel, well-separated from the female ear. The stamen is a compound organ with a short filament subtending each developing anther. On the day of anthesis, the filament elongates substantially to push the anther through the enclosing spikelet glumes into the air, which initiates pollen shed. The filaments accompanying sterile anthers rarely elongate sufficiently to move the anther out of the spikelet (Egger & Walbot, 2015). Therefore, quantification of anther exertion is likely an indicator that scores the extent of male fertility.

Traditionally, the scoring of anther presence is done manually using a simple scale from 0 = no anther exertion to 5 = full anther exertion (Yadava et al., 2021), however, manual scoring is subjective and varies among personnel evaluating the same tassels. Additionally, if there are regions within the tassel with a distinctive or inconsistent phenotype, this information is not captured; such regions could reflect suppression of anther development for one or a few days. To address these limitations, we developed "Tasselyzer," an automated, image-based color trait analysis tool for maize tassel image segmentation built on the PlantCV naïve Bayes classifier (Abbasi & Fahlgren, 2016; Fahlgren et al., 2015; Gehan et al., 2017; Schuhl et al., 2022). Tasselyzer identifies anthers in two-dimensional tassel images based on distinct anther colors, separates them from other tassel parts, and quantifies the

number of pixels of anthers separately from the other tassel parts, to generate an anther ratio. Then, Tasselyzer mimics human eye perception and calculates anther pixel ratios to evaluate the degree of exertion, thus quantifying the degree of anther exertion in a maize tassel image. In a previous study, Tasselyzer enabled estimates of anther exertion of individual tassels by measuring the ratios of anther pixels to those of the whole tassel, then pinpointed pheno-critical days of temperature treatments in the *dcl5* mutant (Teng et al., 2020). We continued to use Tasselyzer in another study, showing anther exertion is normal in maize *ago18* triple mutant (Zhan et al., 2024). In each case, we imaged and documented tassel images, measured anther exertion with the same set of parameters, and compared these results among genotypes or treatments to rapidly reach relatively unbiased conclusions. These practical applications of Tasselyzer suggested its value and potential in measuring differences in anther exertion.

In this study, we describe Tasselyzer and we examine how well Tasselyzer measures anther ratios compared with manually measuring the width of the main spike, which is a traditional method for tracking the anthesis process. Our goal was to determine if results from Tasselyzer are comparable to those obtained through traditional measurements. Additionally, we tested Tasselyzer's effectiveness across a diverse set of maize lines, including 20 Nested Association Mapping (NAM) founder lines, to assess its applicability beyond specific genetic backgrounds. Finally, we discuss the reasons for using Tasselyzer and its potential for wider applications in research.

RESULTS

Tasselyzer is a color-based tool for maize tassel image analysis

We captured 1438 images of freshly detached tassels from 20 field-grown NAM founder lines and 2 inbred lines grown in greenhouses. These images were taken in an enclosed LED light box, ensuring consistent photo parameters (details provided in Materials and Methods and Table S1).

Tasselyzer segments images into multiple classes based on colors including: (1) dark non-plant background; (2) anthers in yellow or pink; (3) green parts of the tassel, such as glumes, stems of the tassel, and sometimes palea. We selected five tassel images to create parameters, called the probability distribution functions (PDF_1), used by Tasselyzer to analyze the remaining images, performing tasks including segmentation, generating masks for anthers and other tassel parts, and calculating anther ratios (Figure 1a, b). It is worth noting that an anther ratio is a measurement of a two-dimensional full or partial tassel image, but not an actual count of the anther numbers (details in Discussion).

Considering a tassel image from fast-flowering mini-maize (FFMM) as an example, the original input image displayed a tassel with one central main spike and multiple side branches. Yellow anthers exerted from florets of all branches. After processing the image through Tasselyzer, anthers were highlighted in fuchsia and other tassel parts in bright green (Figure 1c,d). In a separate output file, Tasselyzer computed the corresponding anther ratio by summing the pixels of anthers and dividing by the total number of pixels in both anthers and other tassel parts. We propose that this automated measurement closely mirrors a visual assessment made by human observers, irrespective of the size and complexity of maize tassels.

Tasselyzer is a sensitive tool to measure anther exertion of regional or whole maize tassels

Development of a typical tassel spans 2–7 days, with the duration determined by genetic background and growth conditions. Anthers first emerge from upper florets in the center of the main spike on day 1, gradually spreading to lower florets and tips of all branches until the lowest side branch in subsequent days (Egger & Walbot, 2015). To assess if the anther ratio, as determined by Tasselyzer, correlates with the degree of anther exertion, we examined tassel images during anthesis. We captured and analyzed photos of developing inbred line A632 to observe the process of anthesis, and Tasselyzer demonstrated sensitivity in capturing changes in anther exertion during this period (Figure S1).

An intriguing characteristic of inbred maize lines is their highly synchronized flowering time if environmental and artificial impacts are normalized. To further evaluate Tasselyzer across different days during anthesis, we staggered the planting of 6–7 FFMM seeds every day over 2 weeks. We harvested 75 tassels and took 288 images on the same day, providing snapshots of tassels at various stages with differing degrees of anther exertion (Table S2). The width of the main spike of the tassel has traditionally been used as a proxy for tracking anther exertion (Fonseca et al., 2003; Mirnezami et al., 2021), so we manually measured main spike widths in these images using ImageJ (Schindelin et al., 2015; Rueden et al., 2017). Main spike widths of FFMM ranged from 0.38 to 1.45 cm. Notably, plants planted later (days 11–14), in which anthesis had not initiated, exhibited thinner main spikes, while plants planted on days 1–10 showed increasing and fluctuating main spike widths, consistent with anthesis initiation (Figure 2a; Figure S2). This observation aligned with findings from previous studies (Mirnezami et al., 2021).

Subsequently, we applied Tasselyzer to calculate the anther ratios of whole tassels of FFMM and compared these ratios with main spike widths. Plants planted on days 11–14, which had minimal anther presence on the day of photo shooting, exhibited anther ratios close to zero,

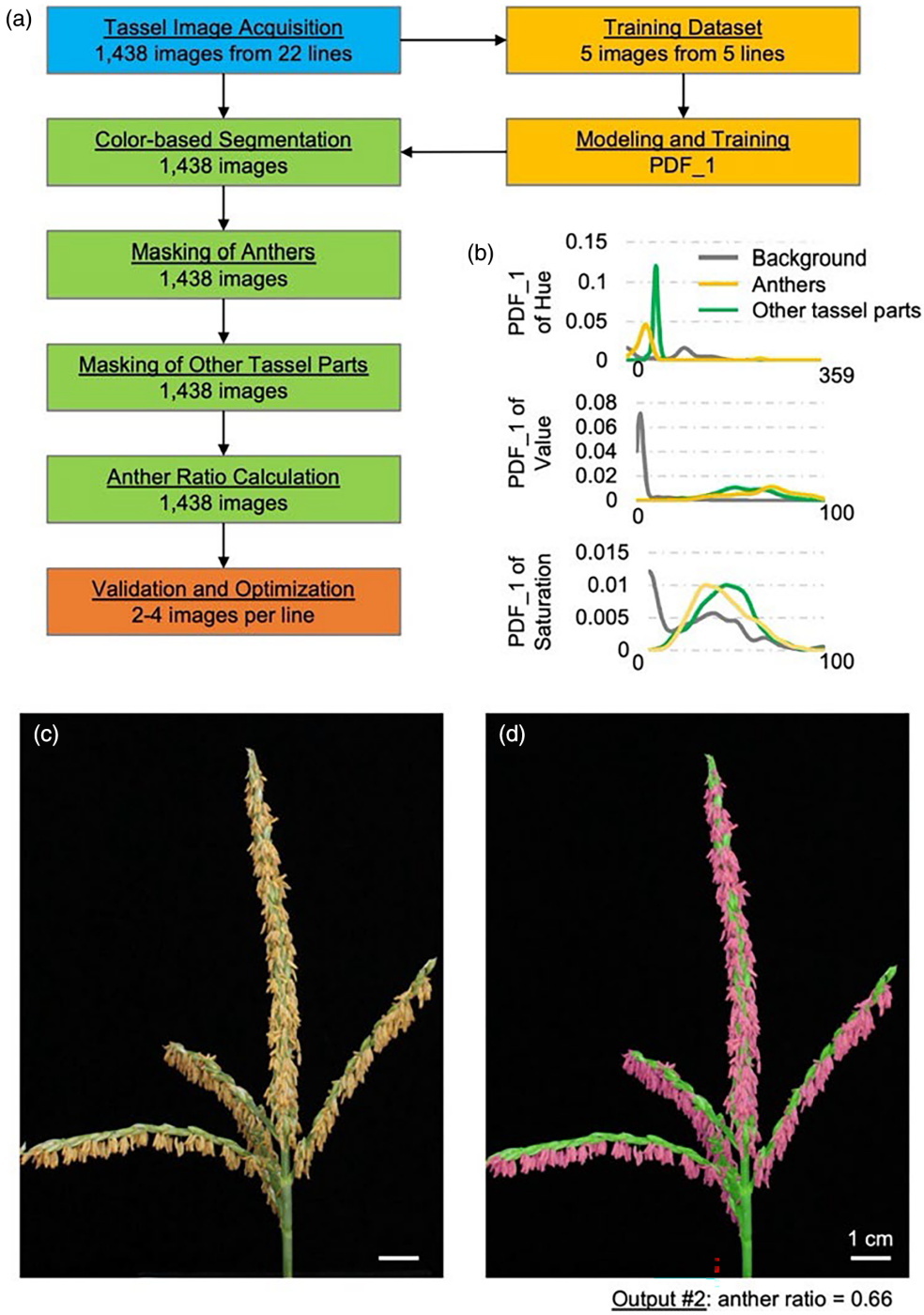

Figure 1. Overview of Tasselyzer.
(a) A workflow of Tasselyzer in this study. A total of 1438 input raw tassel images from 22 maize lines were captured. Five images were selected to generate PDF_1 as parameters for multiclass segmentation into non-plant backgrounds, anthers, and other tassel parts based on color at pixel level. PDF_1 was used to parameterize segmentation, generate masks for anthers (fuchsia) and other tassel parts (green), and calculate anther ratios for all images.
(b) PDF_1 is a three-dimension model with statistical information on hue, saturation, and value (HSV) to parameterize color-based segmentation. An example of a raw tassel image of fast-flowering mini-maize (FFMM) as input in (c), and pseudo-colored output image in (d). Anther ratio of this example image is indicated below panel (d). Bars in (c, d), 1 cm.

consistent with the original images (Figure 2b; Figure S3). As expected, plants planted on days 9 and 10, in which anther exertion commenced, showed anther ratios ranging from 0.18 to 0.50. Plants planted on days 5–7 exhibited medium anther ratios peaking around 0.70. The trend of anther ratio changes in FFMM is similar to that of

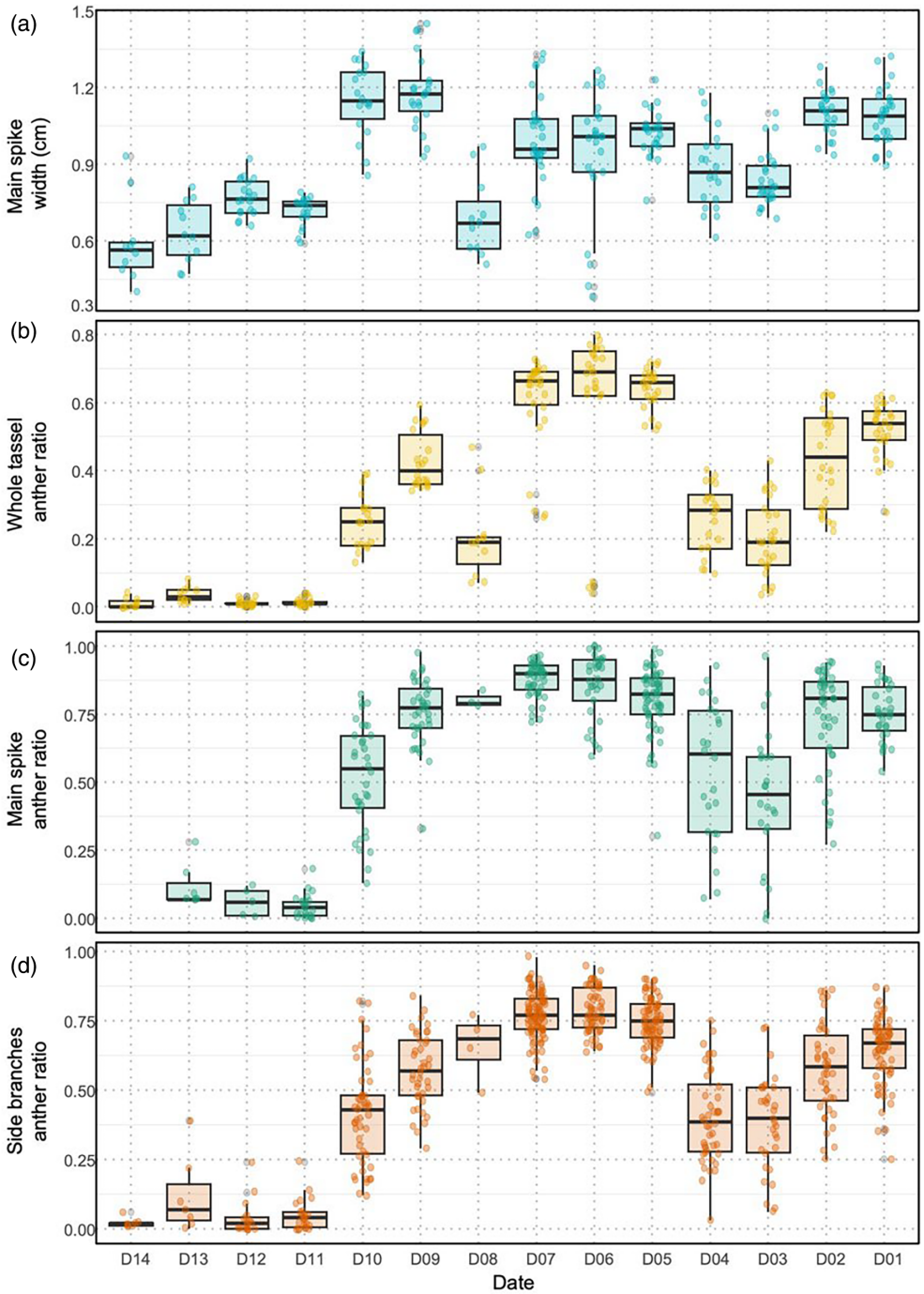

Figure 2. Comparison of measurements of anther exertion in FFMM on different days of anthesis.
(a) Box and scatter plots of main spike widths were measured from tassel images with different planting dates, with D01 the earliest and D14 the latest. Box and scatter plots of anther ratios of whole tassels (b), main spikes (c), and side branches (d) were calculated using Tasselyzer. Each dot represents a value extracted from one tassel image and each box depicts a distribution of values on a planting date. All values were extracted from the same set of 288 tassel images of FFMM prepared as indicated in Materials and Methods and Table S2.

developing tassels of A632 and A619 (Figures S1 and S4). Comparing anther ratios with main spike widths, while both measurements were consistently low on days 11–14, their peaks occurred on different days. Main spike widths were less sensitive to anthesis stages, whereas anther ratios exhibited greater sensitivity to changes in anther

exertion (Figure S5a). Notably, unexpectedly low values of main spike widths and anther ratios on day 8 were consistent with each other but were outliers, likely due to an error or delay in germination or growth on that day.

Furthermore, analyzing either the main spike width or the entire tassel ignores regional phenotypic differences resulting from short-term developmental and environmental factors affecting anther development temporarily. To assess the performance of Tasselyzer in capturing this information, we cropped the photos of the main spike and side branches from FFMM whole tassel images during anthesis and analyzed them separately with Tasselyzer (Figure 2c–d; Figure S6). For plants planted on days 11–14, anther ratios of main spikes and side branches ranged from 0 to 0.28 and 0 to 0.39, respectively. Anther ratios increased to 0.13–0.79 and 0.12–0.82 for the main spike and side branches of plants planted on day 10, right after initiation of anthesis. Both trends in regional anther ratios were comparable but more sensitive in capturing the degrees of anther exertion over anthesis, compared with the measurements of main spike widths or anther ratios of whole tassels (Figure S5b–e).

Tasselyzer distinguishes anthers with distinct colors from other tassel parts

We then examined whether Tasselyzer could be extended to a broader selection of maize lines, using 1438 tassel photos from 22 maize lines including 20 NAM founder lines (Table S1). To evaluate the performance of Tasselyzer, we employed standard metrics of recall, precision, and F_1 score, a balanced score of precision and recall, through manual curation of subsets of pseudo-colored output images from each maize line (Materials and Methods and Figure S7). Our finding revealed variation in Tasselyzer's performance across these maize lines, with scores ranging from 0.28 to 0.97. Notably, six lines — CML69, A619, FFMM, P39, Ky21, and Oh43 —exhibited high F_1 scores, indicating robust performance (Figure 3a, Figures S8–S10). Notably, no P39, Ky21, and Oh43 images were used in training. These lines share a common color pattern in their anthers, facilitating accurate segmentation by Tasselyzer with PDF_1. In general, recall scores were high, around 0.9, except for Mo17, in which anthers were poorly identified due to their green color, making them difficult to distinguish from other tassel parts (Figure S9). However, precision varied across the other 16 lines, ranging from 0.16 to 0.67, owing to different and variable color patterns (Figure S10).

Assuming consistent color patterns within each maize line, a line-specific PDF (probability distribution function) could potentially enhance segmentation accuracy. To test this hypothesis, we selected one out of 30 images from NC358 and generated an NC358-specific PDF (PDF_2) for parameterizing segmentation. PDF_2 notably improved precision from 0.05–0.45 to 0.4–1.0 and F_1 score from 0.15–0.79 to 0.46–0.93, while maintaining comparable levels of recall (Figure 4; Figure S11). The improvement of line-customized PDF in precision indicates that Tasselyzer can be effectively used to analyze additional genotypes if users customize the PDF.

DISCUSSION

The degree of anther exertion serves as a crucial indicator of male fertility in maize; however, the lack of an automated tool for quantification has presented a challenge. To address this gap, we developed an image-based system capable of recording both whole and regional tassel phenotypes. Thus, we introduced Tasselyzer, a user-friendly and cost-effective machine learning method for extracting and quantifying anther exertion information based on pixel color. This system has the capacity to process thousands of images using parameters generated from simple training steps. Like any method, Tasselyzer has advantages and limitations which users should consider when deploying it. Below, we discuss the shortcomings and potential applications of Tasselyzer in various scenarios.

Tasselyzer is a sensitive color-based tool to identify anthers

Tasselyzer, as a sensitive color-based tool, effectively distinguishes anthers from other parts of the tassel and a dark, non-plant background. However, its performance may vary depending on the distinctiveness of the anther color compared with other tassel parts. In our testing with 22 maize lines, Tasselyzer demonstrated acceptable performance in terms of recall, precision, and F_1 score, with potential for improvement through optimized segmentation parameters, except for the Mo17 line. Anthers of Mo17 were green like other tassel parts, which presented a challenge to Tasselyzer; this may be the case for other lines with indistinguishable anther colors. Additionally, Tasselyzer is limited by its inability to exclude leaves or remove stems beneath the lowest branch. Currently, manually, stem trimming and removal or masking of leaves before imaging is required. Theoretically, this limitation could be addressed through pre-staining anthers with specific chemicals or using specialized cameras.

Moreover, deep learning models like the Segment Anything Model (SAM) potentially could address issues in distinguishing anthers (Kirillov et al., 2023). Initial trials with SAM showed potential, although SAM tends to treat the entire tassel as a single object rather than segmenting out anthers (Figure S12). While this capability is useful for separating tassels from the background, it does not meet the requirement for quantifying anther exertion directly. Cropping whole tassel images into parts partially alleviates this limitation, and SAM performed similarly to the current

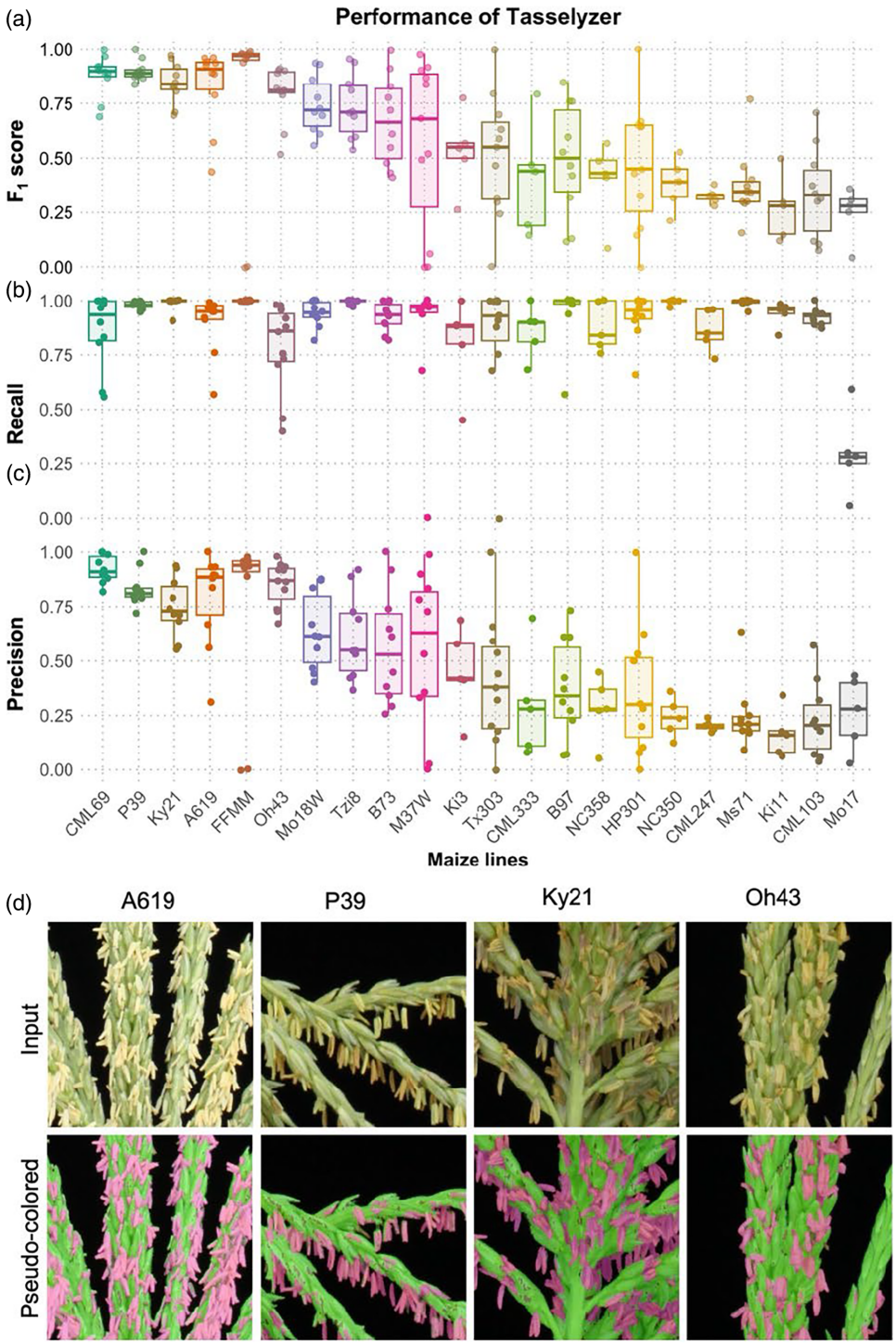

Figure 3. Segmentation performance of Tasselyzer on maize lines.
Box and scatter plots of F_1 score (a), recall (b), and precision (c) of Tasselyzer on individual maize lines with scores ranging from high to low (left to right). Each dot represents a value extracted from one image and each box depicts a distribution of values of an individual line.
(d) Examples of input (upper) and pseudo-colored (lower) tassel images of A619, P39, Ky21, and Oh43. More examples are in Figures S7 and S8.

version of Tasselyzer in terms of segmenting anthers and excluding leaf or extra stem areas (Figure S13). While SAM exhibits promise for future enhancements of Tasselyzer, its primary function is not tailored to quantifying anther exertion. Therefore, it remains outside the main scope of this study.

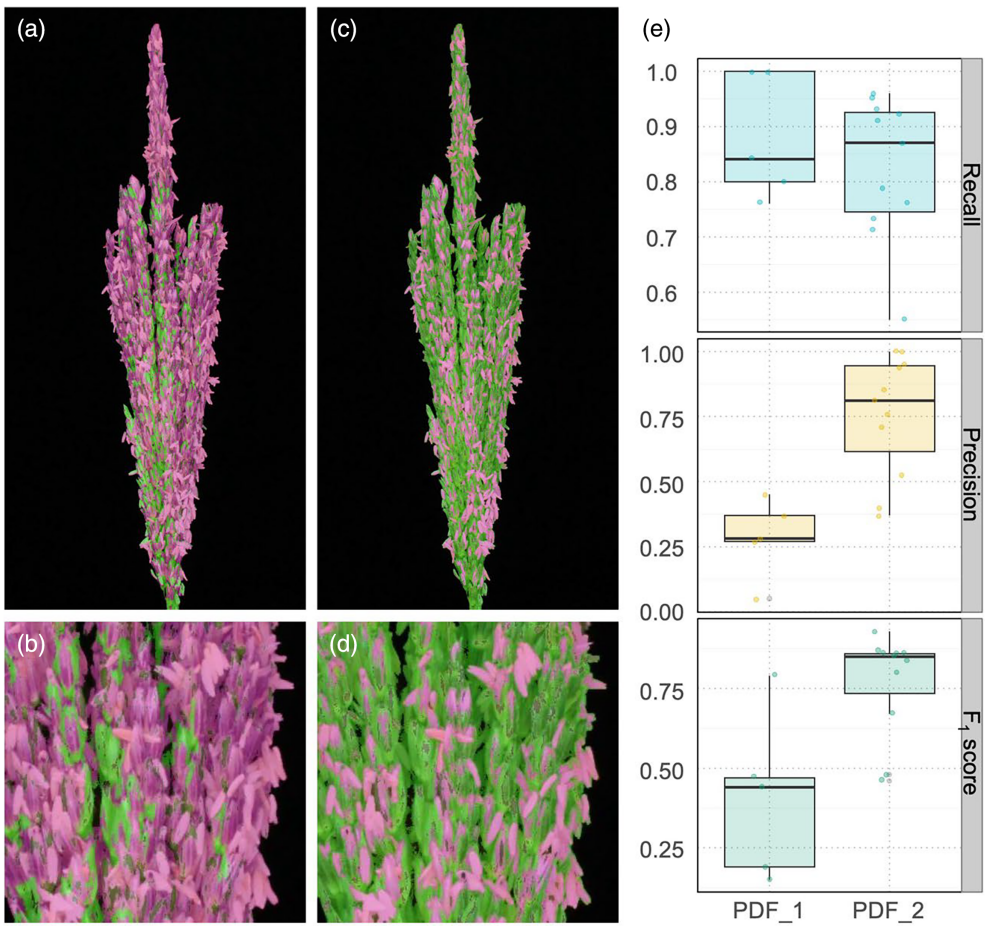

Figure 4. Segmentation optimization with line-specific parameter in maize line NC358.
Overall and magnified images of segmentation in NC358 before (a, b) and after (c, d) optimization.
(e) Comparison of recall, precision, and F_1 score before (PDF_1) and after optimization with NC358 line-specific parameters (PDF_2). Scores before and after optimization with NC358-specific parameters PDF_2 were subjected to statistical analysis for significance using a one-way ANOVA test. *P*-value of difference in recall is 0.3991. *P*-value of difference in precision is 0.00101. *P*-value of difference in F_1 score is 0.00377. More example images are in Figure S11.

Tasselyzer has potential as a non-destructive method for field studies

In this study, most of the tassel images were captured from detached tassels from either field or greenhouse-grown plants and were taken in a closed photo booth to ensure uniformity of the imaging background and simplify downstream performance evaluation. To further enhance its applicability in field settings, a color card could be included during image acquisition, and additional color correction methods could be employed for color and size normalization in field-captured photos. This approach would be particularly valuable when combined with SAM or similar tools, which can segment tassel parts from the background before employing Tasselyzer to segment anthers.

Meaning and usage of anther ratio

The size and fitness of anthers depend on genetic and environmental factors and vary among individual plants. Furthermore, old anthers drop off the tassel or senescence, and young anthers exert during a 2- to 7-day process of anthesis. All these factors contribute to difficulties in accurate quantification of anther exertion, unlike many other biological measurements including width, length, weight, etc. Thus, we propose recording and measuring anther exertion by taking snapshots during anthesis and calculating anther ratio. The anther ratio represents the proportion of anthers in an image compared with the entire tassel at a pixel level. Conceptually, it ranges from 0 to 1, where 0 signifies no anther exertion and 1 indicates that anthers completely cover other tassel parts. It is important to note that the anther ratio does not provide an exact count of individual anthers; rather, it offers a proportion of anthers to the whole or partial tassel for comparative purposes. For instance, it can be used to compare mutant phenotypes under different environmental conditions or to track changes during different stages of anthesis.

In our prior work and its initial application, Tasselyzer was successfully deployed to assess the impact of growth temperature on specific stages of maize development, such as over 9 days of meiosis and post-meiosis in a maize *dcl5* mutant (Teng et al., 2020). Similarly, Tasselyzer was applied to compare the degree of exerted anthers of a maize *ago18* triple mutant, relative to wildtype siblings (Zhan et al., 2024). In this study, the anther ratio was used to monitor the process of anthesis across multiple inbred lines (Figure 2). Theoretically, comparisons of anther ratios among different maize inbred lines can be made when F_1 scores are 0.8 or higher. However, users should exercise caution when significant variations in tassel color or architecture exist, as these factors may affect the reliability of the comparison. As addressed in Figure 4, an additional training process with a customized PDF was straightforward. Generating a line-specific PDF required only about an hour (Materials and Methods), and it resulted in improved precision for the tassel images of NC358 line. A customized PDF could be applied to maize materials sharing common colors in anthers and other tassel parts distinct from the typical pattern shown in Figure 3. The precision is largely determined by the choice of PDF, which can be easily optimized by users, indicating a customizing PDF is a practical solution to widen the applications of Tasselyzer.

CONCLUSION

In summary, Tasselyzer provides a sensitive tool to analyze images of tassels at various stages of anthesis, whether from whole tassels or tassel parts, across a diverse collection of maize lines. It enables the study of environmental or stress impacts on anthesis by quantifying degrees of anthesis, despite limitations when applied to tassels with complex tassel architectures or color patterns. These limitations can be mitigated through parameter optimization and analyzing partial tassel images instead of whole tassels. Additionally, there is potential for improvement by limited effort in customizing PDF for additional assays by users or integrating more sophisticated algorithms, such as deep learning methods, with extensive training efforts. Overall, Tasselyzer is an accessible, cost-effective, time-saving, and automated tool for quantifying maize anther exertion across a diverse range of maize lines, offering valuable insights into plant development and responses to environmental factors.

MATERIALS AND METHODS

Plant material and growing conditions

Maize (*Zea mays*) NAM founder lines (McMullen et al., 2009), including B73, B97, Ky21, M37W, Mo17, Ms71, Oh43, CML103, CML247, CML69, CML333, Ki3, Ki11, NC350, Tx303, Tzi8, HP301, P39, Mo18W, and NC358 were cultivated at the field research site of the Donald Danforth Plant Science Center (4195 MO-94, St Charles, MO, 63301, USA) in the summer of 2023. Inbred lines including A619, A632, and FFMM (McCaw et al., 2016) were grown in optimized greenhouses for maize (28°C/22°C, 14 h/10 h or 16 h/8 h day/night temperatures, ~ 500 μmol/m^2/s) in the Plant Growth Facility at the Donald Danforth Plant Science Center. To stagger planting for FFMM and A619 lines in the greenhouse, 6–7 seeds were planted daily over a 2-week period. Anther exertion was monitored daily, and tassels of FFMM and A619 were detached and images were captured on the same day when anthers from plants planted on "day 10" started exerting from the center of main spikes. A summary table of lines and images is in Table S1 and S2.

Tassel imaging

We implemented an imaging system designed to capture RGB images of detached tassels with multiple options of camera options and conditions, aiming to optimize image quality while minimizing cost, time, and computing power investment. Our final setup featured an enclosed box illuminated by LED lights (Neewer 32-inch shooting tent, Amazon), with black fabric serving as the background. A DSLR camera (Canon EOS Rebel T1i) equipped with an EF-S 18–55 mm f/3.5–5.6 IS lens was employed, controlled by ggphoto2 software installed on a Raspberry Pi computer. This setup ensured consistent imaging settings, including manual mode with ISO at 800, aperture at 16, shutter speed at 1/80th of a second, and resolution at 15.1 megapixel, facilitating downstream analysis and providing descriptive image names. For potential color calibration and size measurement purposes, a color card with a ruler (X-Rite ColorChecker classic mini) was included in all tassel images. Each tassel was photographed at least four times, capturing variation from approximately 90-degree rotations to provide comprehensive coverage from different angles.

Measurements of tassel main spike width

The measurement of the tassel main spike width was conducted using a ruler reference provided by the X-Rite ColorChecker classic mini card in each image. The measurement was achieved by employing "straight line" and "measure" tools available in ImageJ (Schindelin et al., 2015; Rueden et al., 2017). The main spike widths of each group of FFMM plants from different planting days were subjected to statistical analysis for significance using a one-way ANOVA test, followed by *post hoc t*-tests with Holm corrections.

Tassel segmentation by machine learning

The Tasselyzer pipeline was implemented using naïve Bayes classifier module from the open-source software package PlantCV (Abbasi & Fahlgren, 2016; Fahlgren et al., 2015; Gehan et al., 2017; Schuhl et al., 2022). It was assumed that the colors of the multiclass in tassel images (background, anthers, and other tassel parts) are distinct. RGB values for each class were sampled using the pixel inspection tool of ImageJ (Schindelin et al., 2015; Rueden et al., 2017) to extract color features and statistical information for generating the probability distribution functions (PDF) for training. The maximum likelihood ratio test was then employed as the naïve Bayes approach to identify pixels, using the corresponding PDF. In the initial phase of this study, 4294 pixels were sampled for each class from five training images to generate PDF_1 (see training image information in Table S1), which was subsequently applied to segment all tassel images in this study, unless otherwise specified. To enhance segmentation performance in the NC358 line, 76 pixels were sampled for each class from one

training image to generate PDF_2, which was then applied to segment the remaining NC358 images.

Anther ratio calculation

Tasselyzer generates masks for anther and other tassel parts based on the segmentation results. These masks are then overlaid onto the original input images, with anthers depicted in pseudo-colored fuchsia and other tassel parts in green. This visualization serves for visual inspection and demonstration purposes. To calculate the anther ratio, Tasselyzer sums up the total number of pixels corresponding to anther (n1) and other tassel parts (n2). The anther ratio is then computed by dividing the pixel count of anthers by the total number pixels in the tassel [Anther ratio = n1/(n1 + n2)]. The anther ratios of each group of plants were subjected to statistical analysis for significance using a one-way ANOVA test, followed by *post hoc t*-tests with Holm corrections.

Performance evaluation of Tasselyzer

We employed standard measures including recall, precision, and F_1 score. This assessment involved randomly selecting 2–4 pseudo-colored tassel images from the results of the testing dataset. These images were cropped into smaller segments (320 × 320 pixels), focusing on main spikes, side branches, and branch zones. Anther pixels were manually labeled into regions of interests (ROIs) as true-positive (TP) within anther regions with fuchsia mask, false-positive (FP) of other tassel regions with fuchsia mask, false-negative (FN) of anther regions with green mask using the "freehand selection" and "measure" tools of ImageJ (Schindelin et al., 2015; Rueden et al., 2017). Yellow outlines were used to highlight all ROIs (Figure S7). Subsequently, we annotated, measured, and summed the pixel numbers to calculate recall, precision, and F_1 scores. Recall represents the fraction of correctly identified anther areas of the actual anther areas [Recall = TP/(TP + FN)], indicating the ability of Tasselyzer to locate anthers in random tassel images. Precision denotes the fraction of correctly identified anther areas among all Tasselyzer-identified anther areas [Precision = TP/(TP + FP)], indicating the ability of Tasselyzer to identify anthers accurately without including other tassel parts. The F_1 score, a balanced metric, combines recall and precision to evaluate the overall performance of Tasselyzer [F_1 score = 2 × TP/(2 × TP + FP + FN)]. These scores range between 0 and 1, where scores above 0.9 are considered excellent, scores between 0.8 and 0.9 are good, scores between 0.5 and 0.8 are considered average, and scores below 0.5 indicate poor performance. The scores of each group of plants were subjected to statistical analysis for significance using a one-way ANOVA test, followed by *post hoc t*-tests with Holm corrections.

AUTHOR CONTRIBUTIONS

CT, NF, and BCM conceived of the project, NF programmed the scripts, and CT performed and interpreted experiments, CT authored the manuscript, with editing by NF and BCM.

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CONFLICT OF INTEREST

The authors declare no competing interests.

DATA AVAILABILITY STATEMENT

Tasselyzer is based on PlantCV and developed by integrating Matplotlib v 3.2.1, PlantCV v4, OpenCV v 3.4.9, NumPy v 1.18.1, Python v 3.7.7, and skimage v 0.14.3 modules. The code, the original and pseudo-colored images in this study are available in an open-access folder within the GitHub of https://github.com/danforthcenter/plantcv-tasselyzer-tutorial; also within Zenodo of https://doi.org/10.5281/zenodo.5524971 (Teng et al., 2021). The full image sets used in this study are available within a Zenodo repository at https://doi.org/10.5281/zenodo.14553158 (Teng et al., 2024).

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article.

Figure S1. Segmentation and anther ratios during anthesis in A632 line.

Figure S2. Pairwise comparisons of main spike widths among groups of plants.

Figure S3. Segmentation of anthers during anthesis in FFMM line and pairwise comparison of whole tassel anther ratios among groups of plants.

Figure S4. Segmentation of anthers during anthesis in A619 line.

Figure S5. Comparison and linear regression of anther exertion measurements.

Figure S6. Segmentation of anthers in the tassel parts during anthesis in FFMM line.

Figure S7. Regions of interest (ROI) and sums in processed tassel images for performance evaluation with PDF_1 for individual maize lines.

Figure S8. F_1 score varies among maize lines.

Figure S9. Comparison of CML69 with good segmentation and Mo17 with poor segmentation using PDF_1.

Figure S10. Performance of Tasselyzer varied due to different color patterns of maize lines.

Figure S11. Regions of interest (ROI) and sums in processed tassel images for performance evaluation with PDF_2 for NC358.

Figure S12. Performances of SAM on automated segmentation of tassels.

Figure S13. Performances of SAM on automated segmentation of partial tassel images of maize line FFMM, Mo17 and NC358.

Table S1. Summary of maize lines and images.

Table S2. Summary of planting date and image numbers of A619 and FFMM.

Dataset S1. Analysis of variance tables.

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