Use of Image Analysis in the Evaluation of Radicular Nodules in Chickpeas

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Method Article

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Abstract

Through the use of computational systems, it is possible to employ a wide range of statistical techniques, available as open-source code, to perform various assessments in plants. This study aims to demonstrate the application of image analysis in the context of evaluating root nodules in chickpea plants, aiming to standardize a methodology. The research was conducted in the field, where roots were collected, cleaned, and photographed in a studio using a camera with ISO320, SPEED 1/1500 F1.5 M0.6, WB490K. Image analyses were carried out using R software. Parameters related to roots and nodules were obtained, including root area (cm2), nodule area (cm^2), the percentage of nodules in relation to roots, and the number of nodules. Comparing the method with conventional approaches showed efficiency, highlighting the effectiveness of this tool for the intended purpose. It is concluded that the use of the developed methodology can be successfully applied to the analysis of nodules and root systems, providing the evaluation of various parameters with precision, reducing labor costs, and saving time.

INTRODUCTION

The use of equipment and software dedicated to analysis has proven to be of paramount importance in the pursuit of precise and expedited results. Employing strategies that enable the acquisition and analysis of data in agricultural environments can play a crucial role in optimizing current agricultural practices. This translates into increased productivity, improved quality control processes, and greater flexibility in agricultural management (Vasconez *et al*., 2020).

Agriculture stands as one of the most prominent activities globally. With the rapid growth of the world population, the demand for food is continually rising. Consequently, new automated methods are being introduced to meet these needs, as traditional methods employed by farmers prove insufficient to address such demands (Zhang *et al*., 2021; Dharmaraj *et al*., 2018). In this scenario, the adoption of alternative high-efficiency phenotyping tools becomes indispensable. In this context, the use of images combined with computer vision deserves attention, as it allows for the assessment of qualitative and productivity attributes with reduced costs, lower labor demand, greater speed, efficiency, and precision (De Mesquita *et al*., 2022).

Agriculture is currently facing significant challenges, including climate change, resource depletion, loss of biodiversity, labor shortages, and the urgent need to increase the production of nutrient-rich foods (Tariq *et al*., 2023). In this regard, chickpea (Cicer arietinum L.) emerges as a legume of special interest, representing an economically accessible source of protein. Moreover, it is considered the second most consumed legume globally, playing a crucial role in regions facing a shortage of animal-origin protein (Zhang *et al*., 2020).

The cultivation of chickpeas exhibits attractive attributes in the context of sustainable production, given its remarkable adaptability to various climatic conditions, along with significant potential in terms of both productivity and economic profitability (Grasso *et al*., 2022). Another important aspect of chickpea cultivation lies in the ability of its roots to establish symbiosis with nitrogen-fixing bacteria, such as those of the Rhizobium ciceri genus, resulting in the formation of nodules capable of fixing atmospheric nitrogen in a form usable by plants. Several research efforts have been directed towards this crop, and the application of high-efficiency phenotyping techniques through image analysis emerges as a tool of significant potential.

The use of images holds considerable potential for enhancing efficiency and precision in phenotyping activities, as indicated by Haque *et al*. (2021). This approach allows for the assessment of qualitative attributes in a more economical, rapid, efficient, and precise manner. As new technologies aim to increase the accuracy and speed of phenotypic measurements, there is a growing interest in research in this field (Massruhá *et al*., 2014).

An advantage of these methodologies is the ability to reproduce results even after the elimination of plant material, as the image database can be preserved. This opens the possibility of obtaining detailed information about root development through image analysis (Haque *et al*., 2021).

Another significant benefit is methodological standardization, which assumes critical relevance by providing consistency, reliability, and a foundation for study comparison. This ensures that processes and procedures are uniformly followed.

METODOLOGY

Objective

The objective of this study is to demonstrate the application of image analysis in the evaluation of root nodules in chickpea plants, aiming to standardize the methodology.

Study Area and Experimental Conditions

The field study to obtain chickpea root samples was conducted in an experimental area located in the municipality of Montes Claros – MG, at geographical coordinates 16°40’57.5”S 43°50’21.4”W, from June to September 2022, under a microsprinkler irrigation system. The seeding was carried out manually in the planting furrow with 30 seeds per linear meter. The experimental area was divided into 32 plots, each containing four cultivation rows, with one linear meter in length and 40 plants per plot.

In this study, the BRS Cristalino cultivar was used, previously inoculated with symbiotic microorganisms before seeding. Weed control was conducted manually, using hoeing as needed.

Acquisition and Processing of Root System Images

The chickpea plants were harvested at 70 (seventy) days after planting during full bloom. During this period, ten central plants per plot, totaling 320 plants, were collected for image acquisition. After removing the plants from the soil, the roots were carefully separated from the plants, washed with tap water over a sieve (2.0mm mesh) to prevent the loss of nodules.

The dried roots were then placed in a studio for photography using a camera with ISO320, SPEED 1/1500 F1.5 M0.6, WB490K settings, under artificial lighting with a fluorescent lamp (Fig. 1). For each sample (10 plants), three images were taken to capture the ten roots without overlap to avoid errors (a total of 96 images analyzed, with 3 images per plot).

The camera was mounted on a stand in a mini studio with dimensions of 60 x 60 x 60 cm. This strategy was implemented to standardize the image acquisition process, ensuring that all images were captured from the same height and angle. The roots were positioned against a blue background and arranged in a non-overlapping manner, ensuring there was space between them. The analysis of root characteristics was conducted using the R® software, employing the ExpImage package (Azevedo, 2023).

For comparison and evaluation of the method's applicability, a manual count of the nodules present in the roots of the plants was carried out. This was done on the roots that were previously photographed after image capture.

RESULTS

A computational analysis was initiated by resizing the images, originally with dimensions of 2262 x 2896 pixels, to a size of 1000 x 1279 pixels using the "*resize_image*" function. This procedure aimed to reduce the computational analysis time, as illustrated in Fig. 2 (A).

Subsequently, the image was converted to grayscale, as shown in Fig. 2 (B), by extracting the red band with the "*gray_scale*" function for the segmentation of the reference object (2 x 2 cm). The threshold method was then applied through the segmentation function with a value of 0.9, as depicted in Fig. 2 (C).

For the segmentation of roots and nodules (Fig. 2D), the image was converted to grayscale by extracting the blue band. Subsequently, segmentation was performed considering a threshold of 0.6. Two erosions were applied to the images using the "*erode_image*" function, followed by two dilations using the "*dilate_image*" function. This strategy allowed the exclusion of pixels corresponding to small-thickness objects (roots) from the image, followed by restoring the remaining pixels to their original size. The "*mask_pixels*" function was used to highlight the objects created through a mask. The "*measure_image*" function was employed for nodule counting and area estimation (Fig. 2F). The conversion from pixel² to cm² was performed by a simple proportion considering the area and number of pixels of the reference object.

For a better visualization of the efficiency of nodule and root segmentation on the original background (Fig. 2G) and on the black background (Fig. 2H), the "*mask_pixels*" function was also used.

A routine in R allows for the estimation of parameters with different scales, and thus, the functions used were efficient in estimating the following parameters of the chickpea root system: root area (cm²), nodule area (cm²), percentage of nodules in relation to roots, and number of nodules. The analyses of masks followed by segmentation and estimation of the number of nodules through the functions *erode_image,* *dilate_image*, and *measure_image* was also efficient in estimating the area of root nodules. These analyses provide higher quality in the estimation of root parameters by quickly determining living components that undergo rapid decomposition over a short period.

The time optimization in the process of obtaining root parameters stands out as one of the main benefits of using images in this study. This is because roots and root nodules quickly lose viability, dehydrate, and decompose shortly after being removed from the soil. This uniqueness justifies the unprecedented nature of this study, as the computational analysis of root nodules, especially in chickpea cultivation, lacks precedents in the scientific literature. Regarding root analyses, the limitation lies in the complexity of performing manual analyses, as such an approach requires highly specialized labor and is limited to a small number of treatments. However, the use of image analyses offers a solution to these issues, providing precise, fast, and reliable results.

The comparative results between nodule counts performed through computational analysis and manual counting highlight the method's effectiveness, as the discrepancy between the two approaches was reduced, as illustrated in Fig. 3 (A), and furthermore, a coefficient of determination close to 1 was obtained (Fig. 3B). It is worth noting that manual counting has some disadvantages, such as the need for more time and the demand for highly specialized labor.

DISCUSSION

Image analysis plays a crucial role in agriculture, providing valuable information and supporting various aspects of crop management. Image analysis enables the monitoring of crops at different growth stages, helping farmers assess crop health, detect diseases, pests, and nutrient deficiencies (Anwar & Polder, 2020). In this study, these analyses allowed for the estimation of valuable root parameters for scientific activities and chickpea production.

In our scientific study, it was possible to estimate root area (cm^2), nodule area (cm^2), percentage of nodules in relation to roots, and number of nodules, indicating the high reliability and efficiency of the method used compared to conventional manual nodule counting methods. Works conducted by Fernandes *et al.* (2023) with sweet potato roots, using R software and the ExpImage package, also yielded satisfactory results. They concluded that image analyses combined with deep learning improve study quality, reducing subjective analyses and phenotyping time for the crop.

These analyses in agriculture offer a non-invasive and efficient means of collecting data for decision-making, allowing agricultural science professionals to optimize resource allocation, minimize inputs, mitigate risks, and improve overall agricultural productivity and sustainability (Ge *et al.*, 2018). Haque *et al.* (2021) and Fernandes *et al.* (2023), working with image analysis in sweet potatoes, verified the high potential of this technology in phenotyping, specifically in predicting root weight. According to Fernandes *et al.* (2022), these images can be stored in a database for future queries and comparisons, enabling simultaneous processing.

Lobet *et al*. (2015) also conducted a scientific study using images to describe root system architecture through the Root System Markup Language. The aim was to enable the portability of root architecture data among different software tools in an easy and interoperable manner and to provide a standard format for root analyses. They concluded that the system accommodates a wide range of root architecture complexities at various levels of detail, storing root topology and any additional information used to describe root segments. This underscores the importance of using artificial intelligence for root analyses.

In works by Bucksch et al. (2014), they also present a high-throughput image-based phenotyping platform for studying roots in field crops. They demonstrate the application of image analysis techniques for characterizing root systems and conclude that the development of image processing algorithms aligned with the needs of current field research practices presents an excellent alternative for estimating root phenotypes in real soil conditions.

Similar to the results found in this study, Bucksch et al. (2014) report that these analyses have a low cost and can automatically classify various root components in a single image. From these studies, many phenotypic assessments can be conducted in a shorter time frame (Haque *et al*., 2021). The use of image analysis for root system-related studies in agriculture provides opportunities to investigate other parameters and a multitude of crops with a short time frame and low cost, enabling high-throughput phenotyping.

CONCLUSION

The parameters obtained through image analyses included root area (cm^2), nodule area (cm^2), the proportion of nodules to the total roots, and the number of nodules. When comparing the nodule counts in estimates from image analyses with traditional counting, a minimal difference was observed, confirming the effectiveness of this approach for the intended purpose. The use of image analysis proved to be a viable strategy for precisely and quickly estimating the quantity of root nodules. This can substantially contribute to research involving chickpea cultivation, underscoring the importance of employing image analyses in agriculture.

Declarations

- Ethical approval and consent to participate: Not applicable.
- Consent for publication: Not applicable
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- Conflicts of interest: The authors declare no conflicting interests.

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- Author Contributions: KSMAP: Conducting the experiment, data collection, statistics, analysis and interpretation of results, writing. PVS: Writing the manuscript. AMA: Methodology, statistics, text review. RFP: Analysis and interpretation of results, text review. AMFP: Conducting the experiment, data collection. VASFS: Conducting the experiment, data collection. SFB: data collection. All authors read and approved the final manuscript.
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Figures

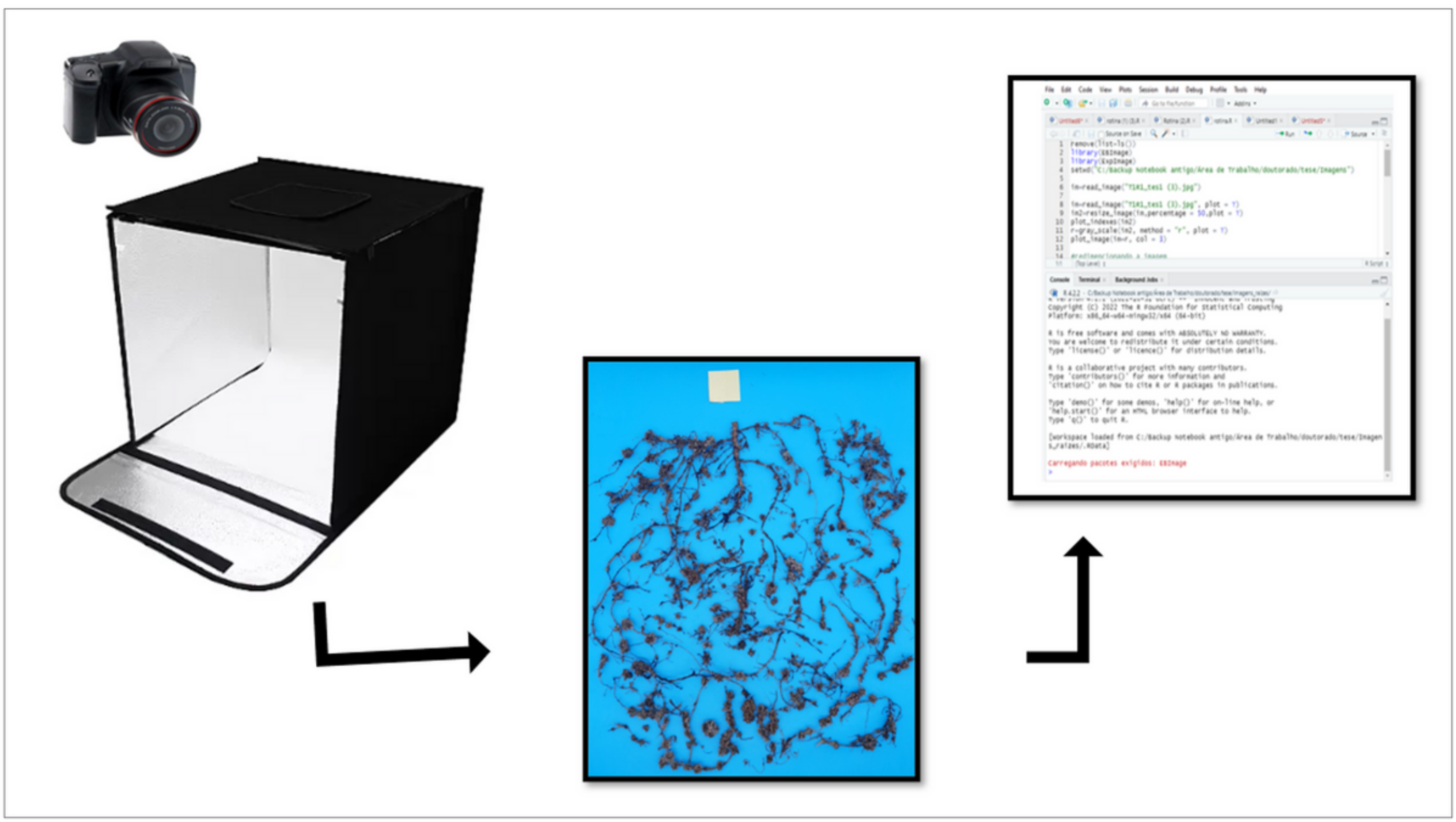

Figure 1

Image Acquisition: Photographic studio for image acquisition with artificial lighting using a fluorescent lamp; Root image acquired in the studio; Analysis using R® software.

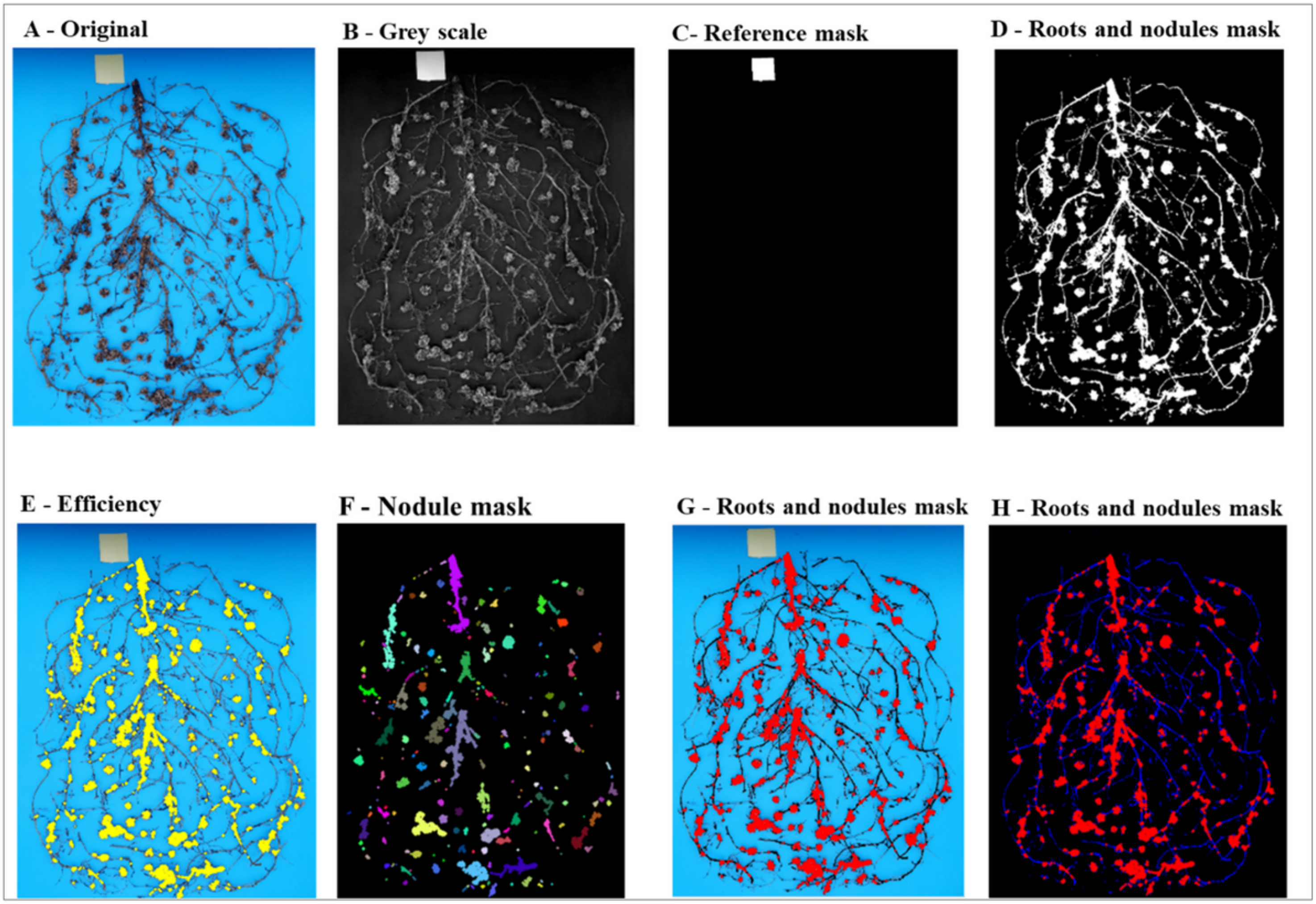

Figure 2

Image Acquisition: original image and reference masks for roots and nodules, and efficiency of the analyses in R® software.

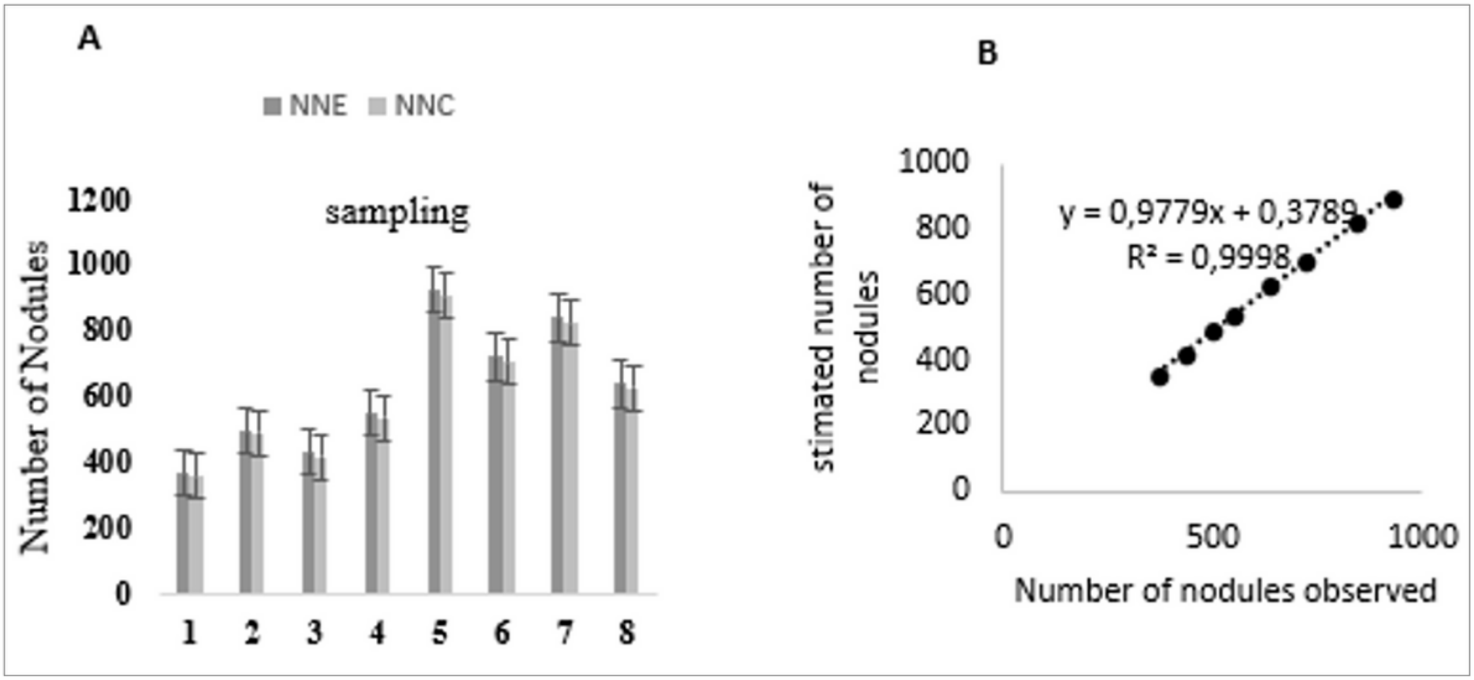

Figure 3

Comparison between the estimated number of nodules (NNE) through image analysis and the traditional method (NNC) using a bar chart (A) and regression analysis (B).