The EuroBiotech Journal

RESEARCH ARTICLE
Plant Biotechnology

Growth of pineapple plantlets during acclimatisation can be monitored through automated image analysis of the canopy

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Abstract

Pineapple is an economically important tropical fruit crop, but the lack of adequate planting material limits its productivity. A range of micropropagation protocols has been developed over the years to address this shortfall. Still, the final stage of micropropagation, i.e. acclimatisation, remains a challenge as pineapple plantlets grow very slowly. Several studies have been conducted focusing on this phase and attempting to improve plantlet growth and establishment, which requires tools for the non-destructive evaluation of growth during acclimatisation. This report describes the use of semi-automated and automated image analysis to quantify canopy growth of pineapple plantlets, during five months of acclimatisation. The canopy area progressively increased during acclimatisation, particularly after 90 days. Regression analyses were performed to determine the relationships between the automated image analysis and morphological indicators of growth. The mathematical relationships between estimations of the canopy area and the fresh and dry weights of intact plantlets, middle-aged leaves (D leaves) and roots showed determination coefficients (R2) between 0.84 and 0.92. We propose an appropriate tool for the simple, objective and non-destructive evaluation of pineapple plantlets growth, which can be generally applied for plant phenotyping, to reduce costs and develop streamlined pipelines for the assessment of plant growth.

Keywords Ananas comosus (L.) Merr; image analysis; acclimatisation; large-scale propagation; micropropagation

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DOI: 10.2478/ebtj-2020-0026

Introduction

Pineapple [Ananas comosus (L.) Merr] is a monocotyledonous, herbaceous perennial crop valued for its fruit. It is the only species in the Bromeliaceae family that is cultivated commercially. In this regard, pineapple represents the third most important tropical fruit worldwide, in terms of production, after banana and mango (1-3). At present, pineapple is grown on more than one million hectares, with most of the harvested fruit destined for the fresh produce market in Europe and North America. The economic importance of pineapple is emphasised by the fact that the gross production value of the 24.8 million metric tons of fruit that are produced annually, is in the region of 9 x 109 US$ (4). However, a severe restriction that hampers production is inadequate access to pineapple planting material of good quality (5, 6). To address this problem, alternative propagation methods have been investigated. In this regard, micropropagation techniques in tissue culture have been considered to rapidly multiply superior genotypes under controlled conditions in the laboratory (5, 7-12). The final stage of micropropagation, i.e. acclimatisation, presents a challenge in the case of pineapple since plants grow at a very slow rate during this process, which increases the time needed to release the plants and has associated cost implications (13-15). Several strategies have been employed to promote faster and more efficient acclimatisation of pineapple plants, including the use of nitrogen-fixing microorganisms (16) and modifications in light supply, irrigation and fertilisation (17-19). Assessing the efficacy of these and other growth stimulatory treatments would be greatly facilitated if simple, non-destructive methods were available for the evaluation of pineapple growth during the acclimatisation process.

Image analysis has been used for a range of diverse applications in plant sciences. For example, in a plant phenotyping study, semi-automated image analysis was combined with machine learning algorithms to quantify root system architectural traits (20). Similarly, fully automated image analysis has been used to identify quantitative trait loci in roots for plant phenotyping (21). Other authors showed that digital image analysis could be used for the real-time estimation of the leaf chlorophyll content of micropropagated potato plants, and suggested that this parameter could represent a quality control indicator to assess the photosynthetic and hyperhydric status of tissue-cultured plants during acclimatisation (22). Niazian et al. (23) used image analysis to determine the physical properties of ajowan embryogenic callus; subsequently, artificial neural network models were applied to predict the properties of the produced calli, depending on culture media and other input variables. In the context of plant diseases, the severity of Fusarium Head Blight on the surface of grains has been quantified by an image analysis method that was recommended as being inexpensive, objective and fast, in comparison with current methods involving visual detection (24). Also, Wang et al. (25) described a combination of image analysis and deep learning to estimate the severity of apple black rot. The effects of abiotic stress on plants can also be evaluated by image analysis, as shown in a detailed study undertaken to assess the efficiency of hyperspectral imaging in a high-throughput phenotyping platform for early detection of water stress in plants (26). However, to our knowledge, canopy growth of pineapple in vitro-plantlets during acclimatisation has not yet been monitored through automated image analysis.

Considering the ideas and examples above, the present study evaluated the use of semi-automated and automated image analysis as tools to quantify canopy growth (superior projected area) in pineapple in vitro cultured plantlets, during five months of acclimatisation. The applicability of these methods was assessed using regression analyses to determine the relationship between the image analysis methods and morphological growth indicators

Materials and Methods

Plant material and growth conditions

Pineapple buds (cv. MD-2) were initiated from field-grown plants, according to Daquinta and Benegas (7). Following a year of micropropagation, plantlets were acclimatised (13) in 250 plastic bags with Ferralitic red soil and sugarcane filter cake (1:1, v:v). Relative humidity was maintained at 80 ± 3%, the temperature at 25.5 ± 2˚C, and the photosynthetic photon flow at 400 ± 25 μmol m−2s−1. Daily microjet irrigation for 30 min (8:00 - 8:30 a.m.) was applied. The canopies of three blocks of 56 plantlets each were photographed (Cannon EOS 600D) from a height of 1.3 m at 0, 1, 2, 3, 4 and 5 months. A separate group of plantlets, grown in parallel under the same conditions, were destructively harvested to determine fresh and dry weights (including all leaves, stem and roots) and D leaf (middle-aged leaf) (27). Ten plantlets were harvested each month for destructive measurements (n = 60 plantlets in total).

Image analysis

For canopy image analysis, two procedures were compared: semi-automated and automated analysis. For the semi-automated method, Paint.net (v4.013, tools: magic wand - contiguous and global saturation mode; bucket of paint and eraser) were used. On the other hand, image analysis for the automated system included image capturing in RGB format (Red, Green, Blue; 5,184-pixel x 3,456-pixel; vertical: 72 pixels per inch; horizontal: 72 pixels per inch), followed by a series of processing steps. The image of the RGB colour system was first converted to the HSI (Hue, Saturation, Intensity) colour system, which disregards the effect of lighting conditions when photos were taken. Then, images were segmented, allowing for differentiation of pineapple leaves from the picture background by the H component. After that, small 'holes' detected in the leaves were filled, and finally, the number of pixels belonging to the pineapple plantlet leaves were counted and converted to cm2, which represented the canopy area.

Statistical analysis

Data were analysed using SPSS (Version 8.0 for Windows, SPSS Inc., New York, NY) to perform One-Way ANOVA and Tukey tests. Regression analyses and R2 were calculated in Microsoft Excel.

Results

The use of image analysis to monitor canopy development represents one of the many applications of this technique, as mentioned above. In the present study, observation of the canopy of pineapple in vitro plantlets during acclimatisation showed that the canopy area progressively increased during the first five months of ex vitro growth (Fig. 1).

MMA common trend was observed for the fresh and dry mass of whole plantlets, D leaves and roots, i.e. there was little mass gain at first, followed by an exponential increase in mass for the subsequent months (Fig. 2A-F). A sharp increase in mass was found after one month for fresh and dry weight of whole plantlets and following two to three months of growth for the other parameters measured.

A linear regression was performed to evaluate the relationship between the canopy area data obtained using the semi-automated (x) and automated (y) methods (Fig. 3A). The results from this regression analysis showed that 99.15% of variations in automated data (y) resulted from variations in semi-automated data (x) [determination coefficient (R2) = 0.9915]. Fig. 3B shows the progression of canopy development over the five months monitored with automated image analysis. These results indicate that canopy area increased significantly at each sampling stage except for the second month (where there was a non-significant increase in canopy area).

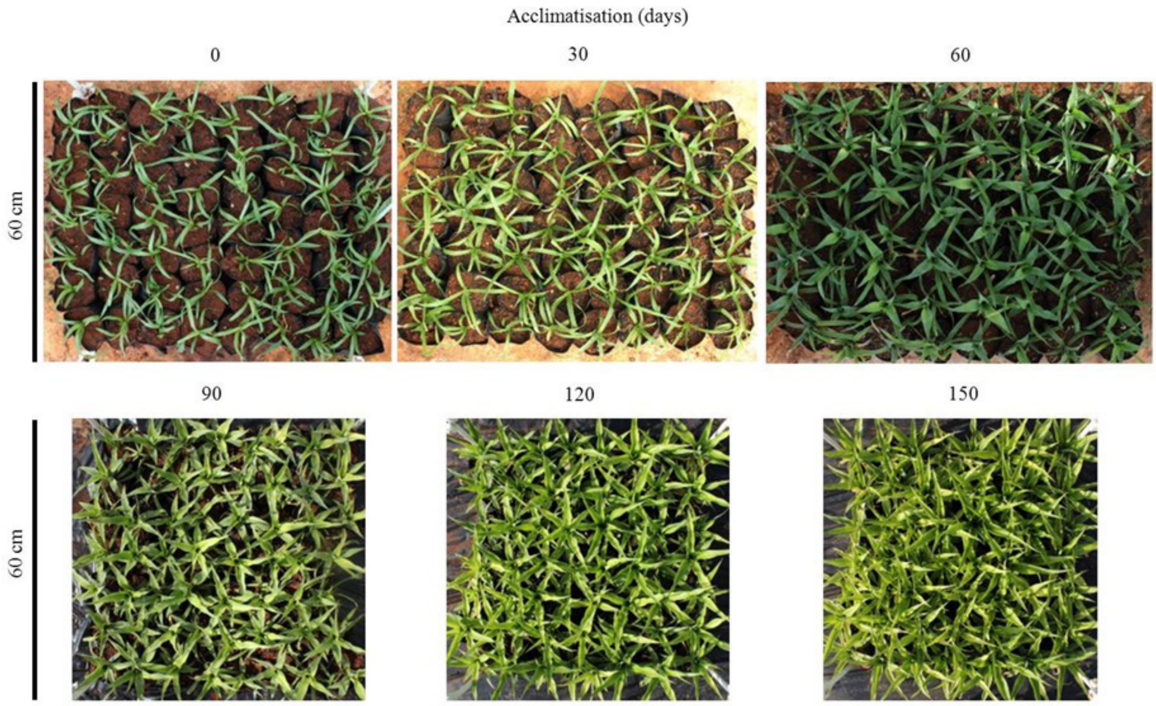

Fig. 1: Canopy growth of 56 pineapple plantlets during acclimatisation .

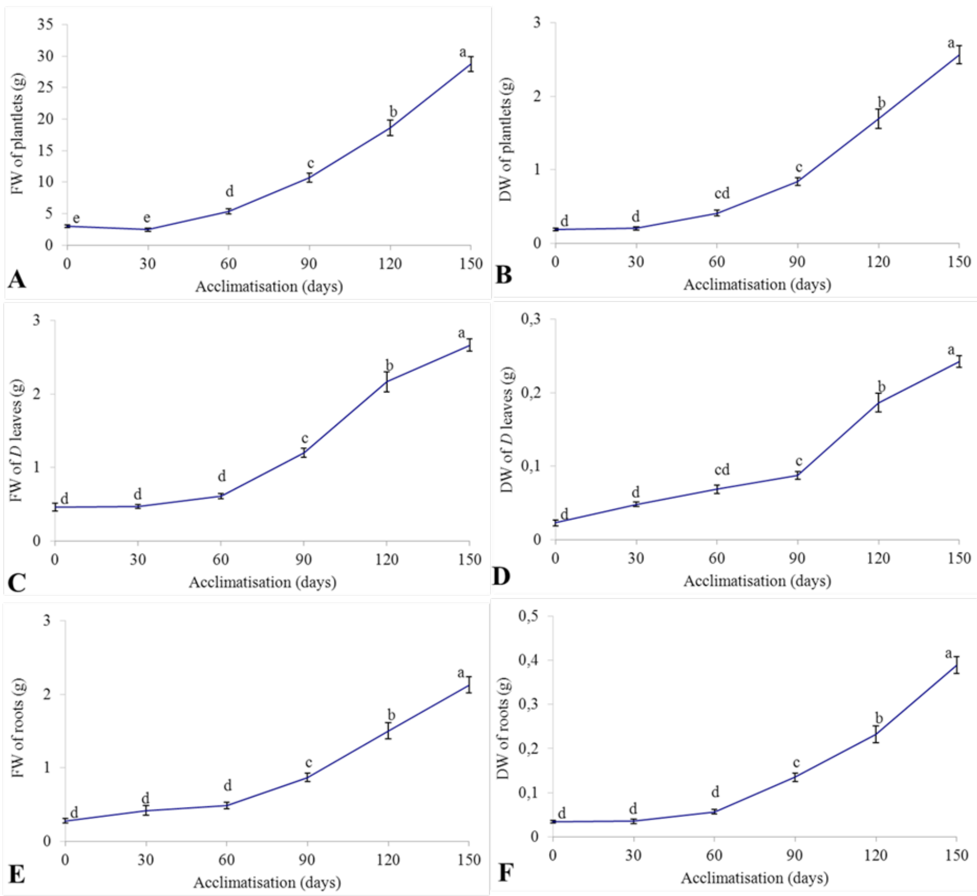

Fig. 2: Growth of individual pineapple plantlets during acclimatisation. A, C, E: Fresh weights (FW) of plantlets, leaves and roots, respectively. B, D, F: Dry weights (DW) of plantlets, leaves and roots, respectively. Results with the same *letters* are not statistically different (One-Way ANOVA, Tukey, p > 0,05). Vertical bars represent SE.

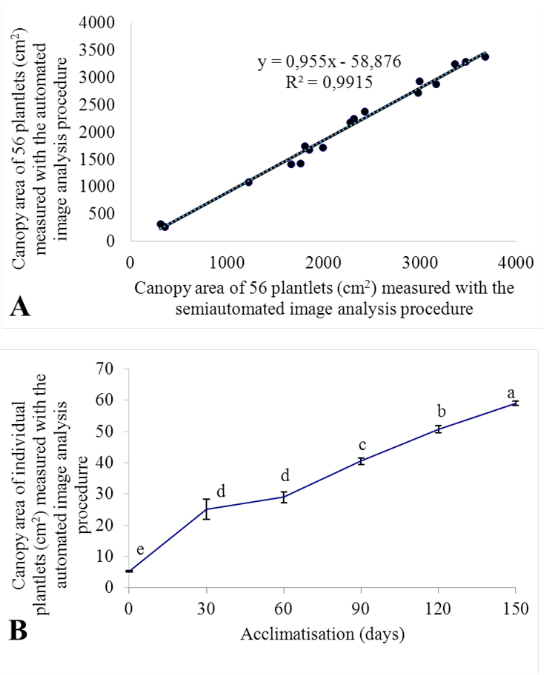

Fig. 3: Relationship between canopy area of 56 pineapple plantlets measured with the automated and semiautomated image analysis procedures (A); and canopy area of individual plantlet during acclimatisation (B). In A, R^2 is statistically different from cero ($p \leq 0,05$; n = 18). In B, results with the same *letters* are not statistically different (One-Way ANOVA, Tukey, $p > 0,05$) and vertical bars represent SE.

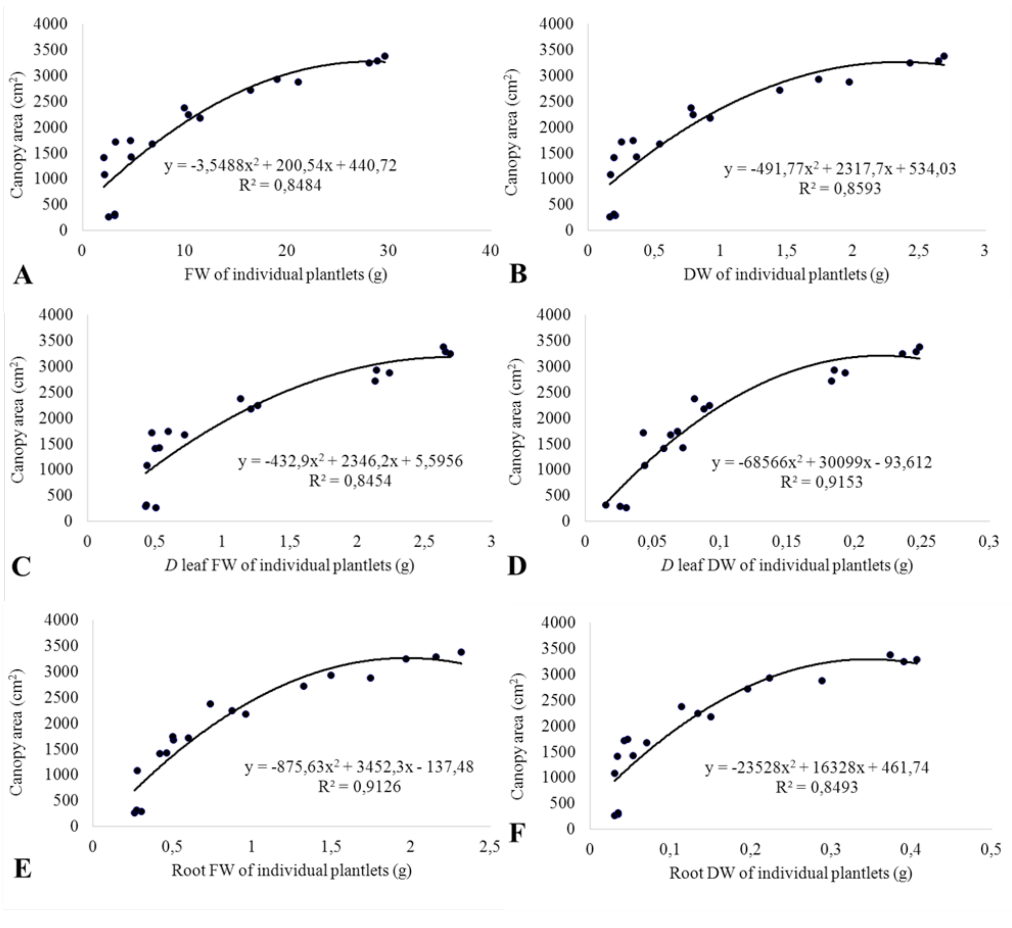

Fig. 4: Relationship between canopy area of 56 pineapple plantlets measured with the automated image analysis procedure (y) and individual plantlet growth during acclimatization. R^2 are statistically different from cero ($p \leq 0,05$; n = 18). FW: fresh weight. DW: dry weight.

Fig. 4 show the mathematical relationships (parabolas) between automated canopy area assessments and the fresh and dry weights of individual plantlets, including roots, stem and leaves (Fig. 4A, B); fresh and dry weights of D leaves (Fig. 4C, D), and fresh and dry weights of roots (Fig. 4E, F). The determination coefficients (R2) ranged between 0.84 and 0.92, which are regarded as high in biological research (28).

Discussion

There have been several published reports on the use of automated image analysis techniques to determine the area of plant canopies. Aguilar et al. (29) studied the leaf area index in tomato plants in a greenhouse and reported an R2 value of 0.75 with the manual method. Minervini et al. (30) established an automated image analysis method for Arabidopsis phenotyping with 96.7% accuracy. Subsequently, these authors developed Phenotiki, an open-source hardware and software platform for image-based phenotyping of rosette-shaped plants (31). Similarly, Ubbens et al. (32) also reported on the application of image analysis phenotyping in rosette plants and achieved R2 values of 0.82. Rincon Guerrero et al. (33) used different types of cameras for image analysis, to study leaf area in several species, namely Syngonium podophyllum, Codiaeum variegatum, Citrus spp., Tradescantia zebrina and Malviscus arboreus. These authors obtained R2 coefficients in the region of 0.98. Finally, Guo et al. (34) developed a novel algorithm for image analysis to investigate canopy coverage in a rice paddy, reporting R2 values of 0.99. These are just some examples of research demonstrating the effectiveness of image analysis in studies investigating canopy dynamics in a diverse range of crops.

Our work describes the application of image analysis to monitor the progression of canopy development in micropropagated pineapple during the first five months of acclimatisation. This method is easy to carry out, non-destructive and objective. In the current study, blocks of 56 plantlets were photographed every month, and their canopy area was automatically determined. Strong correlations were found between canopy area measurements from image analysis and destructive measurements of plant fresh and dry mass for intact plantlets, D leaves and roots. These data indicated the efficacy of image analysis for canopy estimations in pineapple.

There was also an excellent correlation between the data obtained by the semi-automated and automated image analysis, but with a substantial difference in the time required for each of the two methods The the semi-automated procedure took approximately 4 hours per image, whereas the automated method could be completed in 0.27 seconds (Intel(R) Core(TM) I3-4160 3.6 GHz and 8 GB RAM).

An additional advantage of the automated system is that the HSI colour system of this method is closer to how humans perceive colours and is less affected by lighting changes that generally occur in acclimatisation greenhouses (35). These observations make the automated method a preferred option when compared with the semi-automated technique. In the context of H values, values between 30 and 150 were recorded for the pineapple leaves, ranging from pure yellow to the green-cyan border. As these values were not present in the background, it was possible to discriminate between the background and the leaves.

The automated procedure has the potential to save time and reduce research costs in plant phenotyping studies, which could have diverse applications such as investigating the effect of different growth conditions, fertilisation and irrigation regimes, or soil treatments. Furthermore, in plant breeding programmes, automated image analysis has the potential to allow for fast and efficient phenotyping of superior genotypes (or identification of inferior genotypes), particularly when large numbers of samples are evaluated. In the context of pineapple production, image analysis can be used to improve the efficiency of pineapple micropropagation and to design strategies to promote plantlet growth and establishment during the acclimatisation stage.

Acknowledgements

This research was not covered by any specific grant but supported by internal funds from the Bioplant Centre (Cuba), the Agricultural Research Council-Tropical and Subtropical Crops (South Africa), and the Universitat Politècnica de València (Spain). Authors are also grateful to Mrs Lelurlis Nápoles for her experienced technical assistance.

Conflict of interest statement

The authors declare no conflict of interest.

Ethical compliance

This article does not contain any studies involving human participants or animals

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