Low cost, automated high throughput plant phenotyping system to evaluate the response of Menthol mint (*Mentha arvensis*) crop to water stress

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Abstract

Image based high throughput plant phenotyping is a powerful tool to capture and quantify diverse plant traits. The available commercial platforms are often cost-prohibitive. This study describes the development of a low cost, automated plant phenotyping platform, which can acquire images, transfer data, segment the images, extract the traits and perform data analysis using low-cost microcomputers, cameras and IoT irrigation system. Quantifiable plant traits (e.g., shape, area, height, color) were extracted from the plant images using an in-house pipeline developed in R language. An experiment of water stress (waterlogging and drought) on *Mentha arvensis* (Menthol mint) crop (cv. CIM-Kosi) was conducted to demonstrate image traits being used as a proxy for plant response to water stress. It was found that the effect of drought stress on plant height and number of secondary branches could be correlated to color traits of plant canopy images. Also, the effect of waterlogging stress on chlorophyll and flavonoid content could be related to the shape traits of plant canopy images and effect on waterlogging on plant height and canopy width could be associated with color and texture traits. The imaging platforms could successfully demonstrate a viable low-cost solution for incorporating high-throughput plant phenotyping in various plant stress related research applications.

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ABSTRACT

Image based high throughput plant phenotyping is a powerful tool to capture and quantify diverse plant traits. The available commercial platforms are often cost-prohibitive. This study describes the development of a low cost, automated plant phenotyping platform, which can acquire images, transfer data, segment the images, extract the traits and perform data analysis using low-cost microcomputers, cameras and IoT irrigation system. Quantifiable plant traits (e.g., shape, area, height, color) were extracted from the plant images using an in-house pipeline developed in R language. An experiment of water stress (waterlogging and drought) on *Mentha arvensis* (Menthol mint) crop (cv. CIM-Kosi) was conducted to demonstrate image traits being used as a proxy for plant response to water stress. It was found that the effect of drought stress on plant height and number of secondary branches could be correlated to color traits of plant canopy images. Also, the effect of waterlogging stress on chlorophyll and flavonoid content could be related to the shape traits of plant canopy images and effect on waterlogging on plant height and canopy width could be associated with color and texture traits. The imaging platforms could successfully demonstrate a viable low-cost solution for incorporating high-throughput plant phenotyping in various plant stress related research applications.

Keywords: *Mentha arvensis*, Water use efficiency, High throughput phenotyping, Raspberry Pi computers, Image analysis

1. INTRODUCTION

Mentha arvensis (Menthol mint) is a popular short duration crop (90 - 110 days) grown in the Indo-Gangetic plains for its essential oil which is extensively used in pharmaceutical, cosmetic, food and flavor industries. Commercial cultivation of menthol mint requires frequent irrigation (12 - 15 times) for high essential oil yield [1,2,3] and the crop is prone to failure due to waterlogging attributable to early onset of monsoons, making optimum irrigation an important factor in determining the cost of menthol mint cultivation. Studying crop water stress in menthol mint can help improve the irrigation efficiency in its cultivation thereby increasing profit of farmers. Controlled environment greenhouses equipped with state of art information and communication technologies are increasingly being used to study crop water stress by collecting real-time plant growth data continuously, automatically, and non-invasively. The available commercial[4] high throughput plant phenotyping (HTPP) systems are often cost-prohibitive and low-cost HTPP systems[5,6] are developed for definite research projects targeting specific plants with few traits and customized analysis pipelines. The present study thus focuses on development of an automated HTPP system to collect, analyze and visualize longitudinal plant trait data using an open source protocol integrated with an automated irrigation system to maintain precise water stress in plants during experiment.

2. MATERIAL AND METHODS

Experimental setup

An HTPP system was developed in a controlled environment greenhouse of CSIR - Central Institute of Medicinal and Aromatic Plants (CSIR-CIMAP), Lucknow, India to conduct water stress experiments on medicinal and aromatic plants. The system (fig. 1) consists of a Raspberry Pi based imaging platform, and IoT enabled irrigation system, R-based pipeline and R-Shiny based visualization interface. The imaging platform comprises of embedded boards (Raspberry Pi microprocessor) with RPi cameras (RGB and NOIR) to take images at a nadir view 1m above plants. The images get transferred over intranet from the Raspberry Pi to a remote central repository by mirroring the remote hard disk drives. The irrigation system contains IoT enabled soil moisture sensors interfaced with channel relays and motor through a python program running on a Raspberry Pi microcomputer, which regulates the amount and frequency of water flow through pipes mounted on individual pots. The R pipeline processes the images, analyzes image derived data and R Shiny interface (fig. 2) provides a dashboard for displaying the images, soil moisture sensor data, irrigation data and visualization of statistical analysis results.

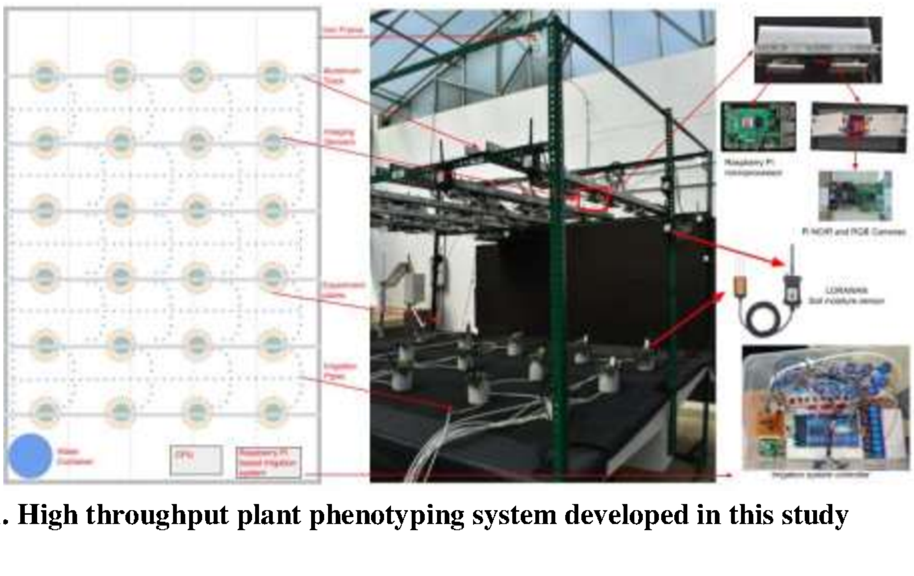

Figure 1. High throughput plant phenotyping system developed in this study

Figure 2. User interface of developed HTPP for visualization

Experimental Data acquisition

An experiment of water stress (waterlogging and drought) on Menthol mint plants (40 days after sowing), *cv.* CIM-Kosi was undertaken to study plant canopy imaging traits as a proxy for plant response to water stress. Individual plants were imaged every 2 hours through the day (7 AM to 5 PM) every day of the experiment, acquiring 28,800 images (170 GB) over the Menthol mint growth period of 100 days with water stress applied 56th day after transplant. R pipeline (fig. 2) processed the acquired images starting with dimensionality reduction where daily images of each plant were analyzed for highest PAV (Photo-synthetically Active Vegetation) to account for dynamic sunlight exposure to each plant in a glasshouse setting.

Figure 2. Flowchart depicting image processing by R-pipeline

Normalized Vegetation Index (NDVI) [7,8,9] given by equation (1) was calculated using images of red and blue channels from Raspberry Pi RGB and NOIR camera and NDVI image with maximum PAV each day was used to extract image traits.

$$NDVI = \frac{RGB.R - NOIR.B}{NOIR.R - NOIR.B} \quad \text{-- (1)}$$

Where RGB.R is red channel image from RGB camera; NOIR.R and NOIR.B are red and blue channel images from NOIR camera.

The pipeline then performed plant canopy extraction followed by application of image transformations pertaining to images traits (table 1), extraction of numeric image traits datasets and integration with the daily physiological plant data. The resultant big data was subjected to cause and effect statistical analysis in the R-pipeline using two-way ANOVA test with post hoc simple. effects, Tuckey's and Pairwise comparison test to correlate the image derived data with changes in manually measured physiological and pigment data for determination of image traits which can be used as proxy for plant response to water stress.

Table 1. Plant canopy traits considered for the study

Physiological	Plant height, Canopy width, No. of secondary branches
Pigment	Chlorophyll content, Flavonoid content, Anthocyanin content, Nitrogen Flavonoid Index (NFI)
Canopy Color	Visible Band Difference (VD), Green Red Ratio (GRR), Triangulated Green Index (TGI), Red Green Blue Vegetation Index (RGBVI), Normalized Green Red Difference Index (NGRD), Normalized Green Blue Difference Index (NGBD)
Canopy Shape	Plant area, Convexity, Circularity, Rectangularity, Eccentricity, Solidity, Centroid distance
Canopy Texture	Mean, Variance, Homogeneity, Contrast, Dissimilarity, Entropy, Second moment

3. RESULTS

It was found that out of 27 imaging traits only 10 could proxy the water stress response in Menthol mint viz. rectangularity, circularity, centroid distance, plant area, VD, NGBD, RGBVI, NGRD, mean and variance. Waterlogging stress induced change in chlorophyll and flavonoid content was characterized by rectangularity, circularity and centroid; change in plant height and canopy width by VD, RGBVI, NGBD, mean and variance. On the other hand, drought stress induced change in plant height was characterized by VD, RGBVI, NGRD; change in number of secondary branches by NGRD.

4. DISCUSSION AND CONCLUSIONS

Based on the above results, it is evident that HTPP system developed in present study is capable of automatically collect, analyze and visualize plant traits along with maintaining precise water stress for a longitudinal plant stress study in glasshouse environment. Compared to employing traditional stress phenotyping in Menthol mint which enables monitoring variables recorded using invasive measurements on few days[10,11], the data sampling rate and temporal resolution of data collection performed in this study were superior enough to find the relation between the physiological traits and image traits. The developed HTPP system can be used further for quantification of the water use efficiency in Menthol mint.

DATA AVAILABILITY STATEMENT

The datasets generated during and analyzed during the current study are available from the corresponding author on request.

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