Quantification of Root Density with Digital Camera Photos and GIS

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Abstract

Observation and quantification of plant root growth is an important area of research in plant science because root density reflects overall plant performance. We quantified the root densities of plants grown in containers using digital camera photos and GIS techniques. Photos taken of the surface of roots of mustard *(Brassica juncea)* and wheat *(Triticum aestivum L.)* plants (after containers were removed) were used as input data in a GIS model created by the ModelBuilder tool in ESRI ArcMap. Image enhancement and classification were applied to each image to distinguish background and root pixels using the GIS model. The overall accuracy of the classification procedure was > 80%. This technique was applied to measure the root density of mustard and wheat plants, and the relationship between the root density and the dried biomasses of mustard plant components was investigated. We found that the plant root density estimated by this method could predict the dried stem biomass and the dried branch biomass of mustard plants. This research offered an effective and inexpensive tool for the Plant lab to automatically quantify the root density.

Background and Relevance

Root growth is an important indicator of plant performance. The simplest method to measure root production is to remove selected plants from the growth matrix, wash loose matrix off the roots, and weigh the plant root. This method is time consuming and the loss of fine roots leads to inaccuracy in the quantification of root density. Rhizotron, an underground root observation laboratory for viewing and measuring plant roots through transparent surfaces of root containers, is a nondestructive approach to study characteristics of root growth, including root density. Rhizotrons are difficult and expensive to construct and maintain, and they create an environment for plant growth that is less than natural (Busch et al., 2006, and Box, 1996). A number of computer programs have been developed for plant digital image analysis. WinRHIZO is an image analysis system specifically designed for root measurement in different forms (Arsenault et al. 1995). RootLM is a simple color image analysis program, in combination with a modified marking technique of root growth on the surface of a Petri dish, for root length measurement (Qi et al. 2007). EZ-Rhizo is software for the detection and measurement of plant root system architecture (Armengaud et al. 2009). RootReader2D is a Java Web Start-based program designed to assist with root length measurement from digital images (Clark et al. 2013). Although these computer programs allow the measurement of complex root system traits, they are expensive to maintain and operate.

This paper describes an effective, inexpensive and nondestructive method to quantify root density at laboratory scale using a digital camera and GIS techniques. We estimate the root density of wheat and mustard plants with a GIS technique using control and treatment root density groups. A correlation between estimated root density and dried biomass of mustard plant is included in the results.

Methods and Data

Data

Wheat and mustard plants were each grown in 20 plant pots (10 control and 10 treatment) in the same pod size (11 liter) and potting soil (SM#4 Mix, Sunshine Mix No. 4, Sungro Horticulture Canada Limited). Wheat and mustard crops are grown in a greenhouse and fertilized twice a week during vegetative stage. During reproductive stage additional fertility was applied once per week. Roots were un-potted during ripening stage for photography.

The sample pots were photographed by the same digital camera (Sony DSC-WX300/R 18 MP Digital Camera with Exmor R CMOS sensor 21.1 Megapixels) from five different directions (top, bottom, and horizontal in east, west, north, south directions) (Figure 1). A total of 200 photos of wheat and mustard were taken from different directions and under different light conditions. Each image is composed of three bands (8 bit each), which are red (R), green (G), blue (B). It means that each colour image is stored in an RGB format.

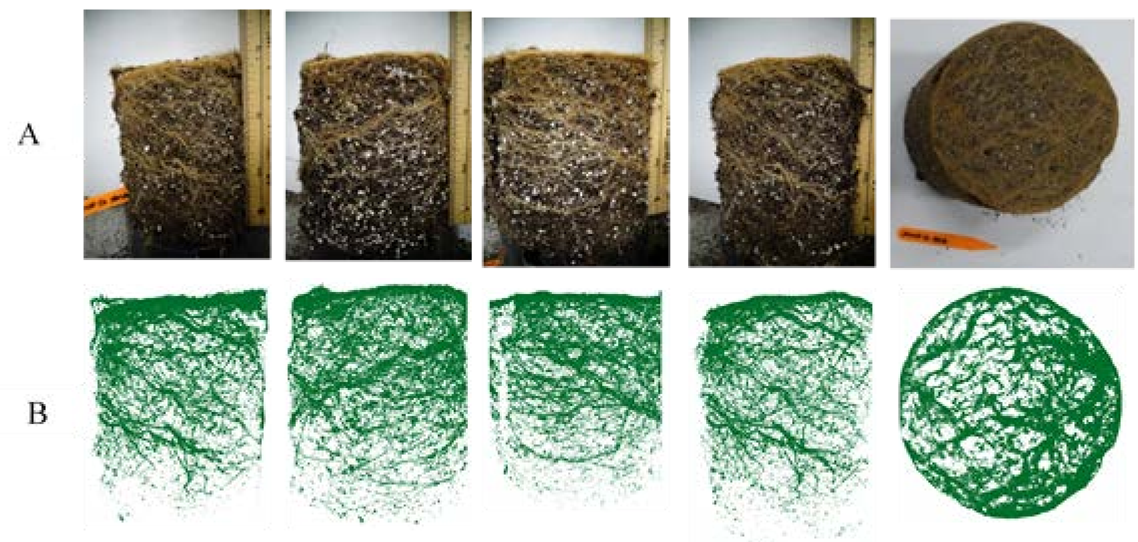

Figure 1: An example of image classification for root density estimation: (A) original photo, (B) output raster file showing root (green) and background (white).

Biomass from the main stem, the branch, and the root of each plant was measured in three steps: a) the plant was separated from the pot and the loose soil was rinsed off, b) the roots were dried, and c) each plant part (leaf, stem, and root) was weighed on a scale. The weight of the plant parts were used to assess the relationship between the biomass and the predicted root density.

Data pre-processing using Photoshop

We use Photoshop to eliminate the background in the 200 photos. Rulers, windows, etc. generate background elements from JPG photos. To clarify the image, cells in the background (except soil and root parts) were set to a unique value, either 255 or 0, which was equated to "no data" in the GIS modelling process.

GIS Modeling

The ModelBuilder tool in ESRI ArcMap was used to create a GIS analysis model. Figure 2 shows the major steps for the model. Input data for the model included the folder with 200 photos preprocessed in Photoshop and a text file with pertinent parameters. The output data included a results folder with 200 raster images that showed the root and background image components (Figure 1B), an excel file that contained the calculated root density for each photo, and a folder containing temporary files.

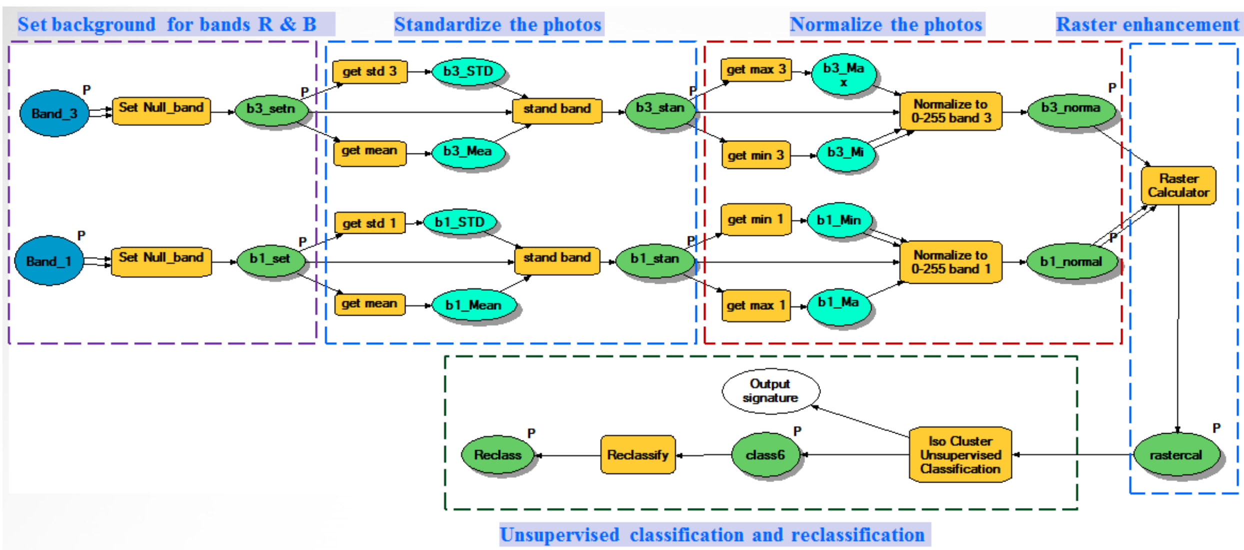

Figure 2: An automated process for data modification using ModelBuilder of ArcMap.

The procedure to create a GIS model is outlined below.

1. Set background for bands red and blue: There were three bands (Band 1= Red; Band 2= Green; Band 3= Blue) for each image and only bands R and B were useful for this model. In band R, soil was a dark tone, compost was in a light tone, and root was also in a light tone as well. In band B, the tones for soil and compost were similar, but the tone for root was much darker than the tone for root in band G, and much darker than the tong for root in band R.
2. Standardize the photos: Because the images were photographed under different conditions, the images needed to be standardized to set the pixel values of root at the same level for the photos of a single crop. Standardization was performed with the following equation:

$$\hat{x} = \frac{(x - \bar{x})}{std(x)} \qquad E.q.1$$

Where $\hat{x}$ is the standardized pixel value, x is the original pixel value, $\bar{x}$ is the mean value of all the pixel values in one photo, and $std(x)$ is the standard deviation for all the pixel values in a single image.

3. Normalize the photos: In the standardized images, all the pixel values fit in a range of -1 to 1. We enhanced the image to 8 bits so that the image was normalized to a range of 0-255, using

$$\hat{x} = \frac{(x - \min(x))}{(\max(x) - \min(x))} * 255 \qquad E.q.2$$

Where $\hat{x}$ is the normalized pixel value, x is the standardized pixel value, $min(x)$ is the minimum value of all the pixel values in the standardized photo, and $max(x)$ is the maximum of all the pixel values in the standardized photo.

4. Raster enhancement: To enhance the contrast between roots and soil or compost, raster calculation were conducted using the following equation:

$$(R - B) \times R \qquad E.q.3$$

Where R is the Red band, B is blue band

5. Unsupervised classification and reclassification: Unsupervised classification was applied to classify each image into six classes, two classes each for roots, soil, and compost. To obtain the final outputs, reclassification was implemented to narrow the six classes into two classes (Figure 3). In the reclassification scheme, class 1 is background and class 2 is root.
6. Root density calculation: The root density for each image was calculated and exported into a Microsoft Excel file.

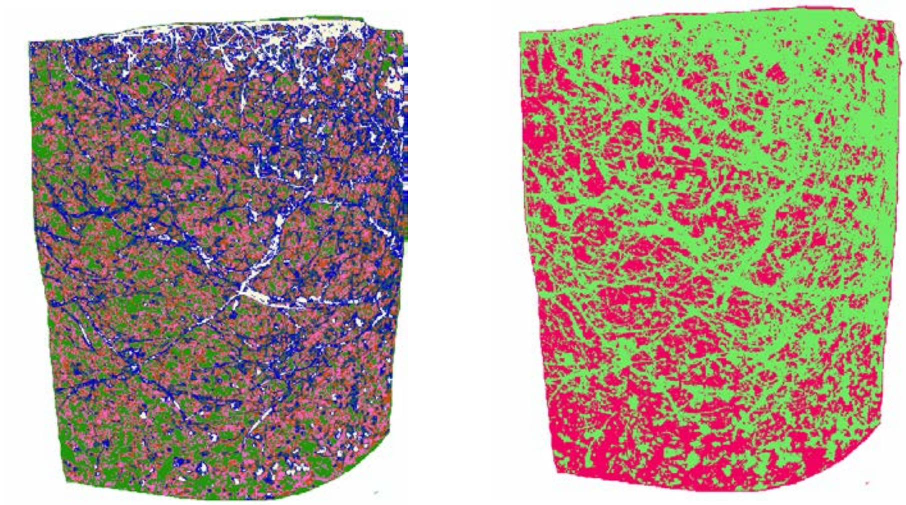

Figure 3: An example of the classification of plant parts: Left: original image, Middle: 6 classes, Right: final classification results.

Classification Accuracy Assessment

In the GIS model in Figure 2, each pixel in the 200 images is classified into one of two categories, root or background. Classification error occurs when a pixel belonging to one category (e.g., root) is assigned to another category (e.g., background). An accuracy assessment was performed to evaluate the agreement between a standard assumed to be correct and an estimated result generated from image classification. Classification accuracy is the percentage (%) that the classifier (the GIS model) has labeled an image pixel into the truth class. A common method for accuracy assessment of a classification image is through the use of an error matrix (Congalton, 1991; Foody, 2002). In this study, we used the *Create Random Points* in ESRI ArcMap to generate 10 random points for each of the 200 images, and then manually assigned these 2000 points into root or background by visual means. An accuracy assessment was then performed for each image using *Compute Confusion Matrix* tool, which is available on ArcGIS 10.4. Accordingly, some important measures such as overall accuracy and Kappa coefficient were calculated. The overall accuracy, in percentage (%), is the proportion of the total number of predictions that were correct, which is the major accuracy indicator. Kappa coefficient (k) reflects the difference between actual agreement and the agreement expected by chance (Foody, 2002; Landis and Koch, 1977). This additional measure is more robust measure than overall accuracy as it takes into account the agreement occurring by chance, and it is calculated as (McHugh, 2012):

$$k = (OA - OC)/(1 - AC) \qquad E.q.4$$

Where OA is observed agreement; AC is agreement by chance; k > 0.80 represents strong agreement and good accuracy, 0.40 < k < 0.80 represents approximate accuracy, and k < 0.40 represents poor accuracy.

Statistics

A Pearson product-moment correlation coefficient was used to test whether there was a linear relationship between the root density variable predicted from the image classification approach and the dried biomass variable.

Results

Classification Accuracy Assessment

The objective of the GIS model is to distinguish between pixels that represent root and pixels that represent background (not root) so that root density can be calculated from root pixels. The classification accuracy assessment evaluates how well the GIS model works. Overall accuracy of results for wheat ranged from 82.0% to 97.5% in the control group and 73.3% to 92.5% in the treatment group (Table 1). The average accuracy, 85.9%, in the control group was higher than the average accuracy, 83.6%, in treatment group. Overall accuracy results for mustard ranged from

Table 1: Classification accuracy assessment results for wheat and mustard plants

Pot Group	Wheat Control		Wheat Treatment		Mustard Control		Mustard Treatment	
	Overall accuracy	Kappa Value	Overall accuracy	Kappa Value	Overall accuracy	Kappa Value	Overall accuracy	Kappa Value
1	82.5%	0.63	80.0%	0.59	76.0%	0.37	82.0%	0.62
2	97.5%	0.95	82.0%	0.58	70.0%	0.37	88.0%	0.75
3	84.0%	0.63	90.0%	0.80	80.0%	0.43	86.0%	0.69
4	84.0%	0.69	92.5%	0.85	82.0%	0.62	88.4%	0.69
5	80.0%	0.56	77.5%	0.48	86.0%	0.71	80.0%	0.56
6	87.5%	0.74	73.3%	0.47	76.0%	0.51	82.0%	0.45
7	82.0%	0.64	86.0%	0.66	86.0%	0.69	80.0%	0.55
8	88.0%	0.73	90.0%	0.79	76.0%	0.53	80.0%	0.60
9	90.0%	0.78	80.0%	0.53	84.0%	0.69	81.7%	0.58
10	83.3%	0.67	85.0%	0.69	88.0%	0.70	78.0%	0.46
Average	**85.9%**	**0.70**	**83.6%**	**0.64**	**80.4%**	**0.56**	**82.6%**	**0.60**

76.0% to 86.0% in the control group and 78.0% to 88.4% in the treatment group. The average accuracy, 80.4%, in the control group was higher than the average accuracy, 82.6%, in treatment group. The average overall accuracy in this study was over 80%. Kappa coefficient values of the two groups were high, with average values of 0.56 to 0.70. It shows that agreement between sample points and classified results is reliable.

Estimated root density results

Root densities of wheat and mustard plants in control and treatment groups are presented in Figure 4. The root densities were 39.9% to 46.6% in the wheat control group (average 43.0%) and 40.2% to 44.3% in the wheat treatment group (average 42.0%). The absolute value of root density in the wheat treatment group was lower than the absolute value of root density in the wheat control group. The root densities were 46.6% to 62.3% in the mustard control group and 47.7% to 63.6% in the mustard treatment group. The average root density (60.0%) in the mustard treatment group was higher than the average root density (56.0%) in the mustard control group. In general, root density on the pod surfaces is inhomogeneous and ranges from 39.9 to 63.6%.

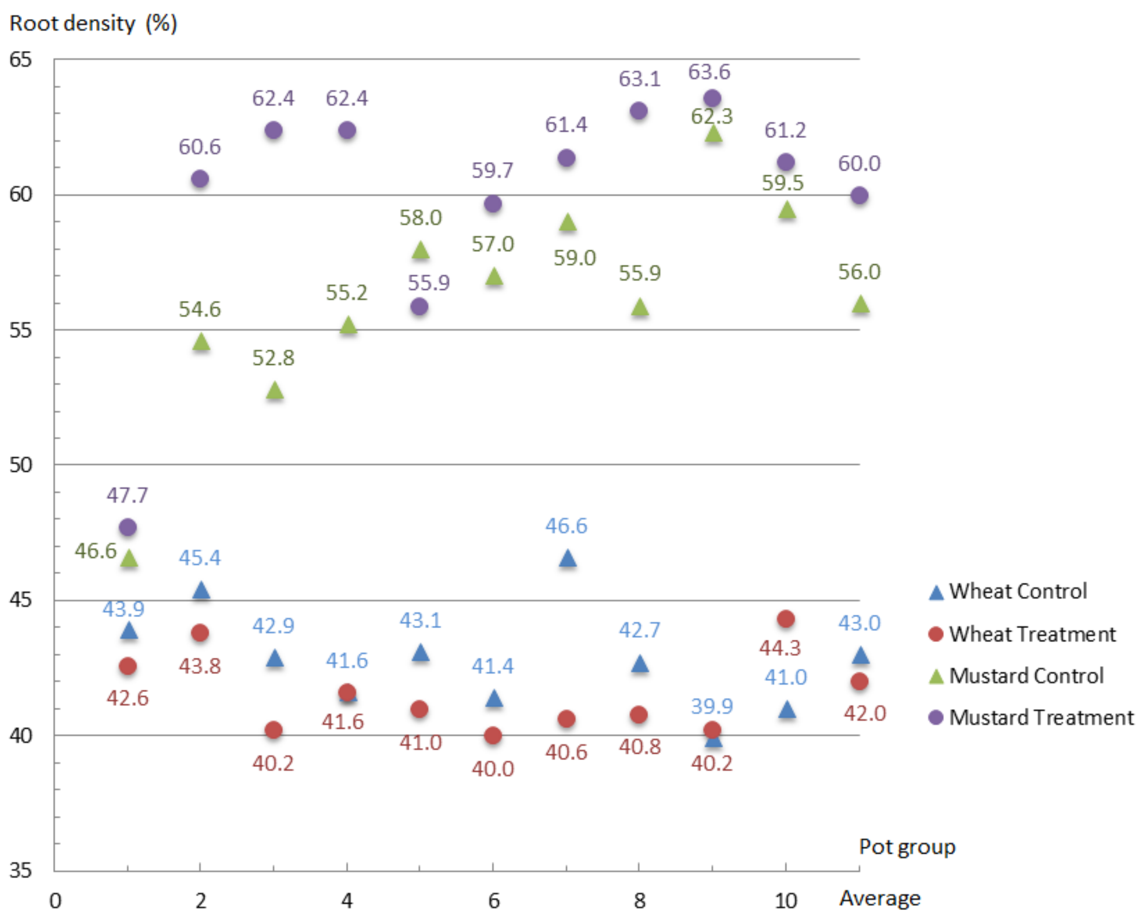

Figure 4: Estimations of root density in wheat and mustard plants

Pearson correlation between root density and plant biomass

A Pearson product-moment correlation coefficient was computed to assess the relationship between the predicted root density of mustard and the dried weight biomass of main stem, branch, and root adapted from the laboratory report. The results in Table 5 indicate that there was a strong, positive correlation between the Root Density and the dried weight of Main stem Biomass, which was statistically significant, r (20)=.69, p <.05. Similarly, Root density and the dried weight of Branch Biomass variables were significantly correlated, r (20) = 0.573, p < .05. Meanwhile, There was a nonsignificant correlation of r (20)=.38 between Root Density and the dried weight of Root Biomass variables. While high accuracy was achieved using this protocol, a large portion of the primary root distributed mainly in the center of the pots might not be captured and used as input data. The linear correlation between root density and the dried root biomass were, therefore, not significant. Mustard has a taproot system, and the dried root biomass would distribute mainly in the center of the containers (e.g., where the primary root located). By including photos of the cross-section (so that primary roots in the center of the pots can be captured), this relationship would definitely be enhanced.

Table 5: Pearson correlation coefficient among variables (Mustard)

		Root Density	Main Stem Biomass	Branch Biomass	Root Biomass
Root Density	Pearson Correlation	1	.690**	.573**	.383
	Sig. (2-tailed)		.001	.008	.096
	N	20	20	20	20

**Correlation is significant at the 0.01 level (2-tailed).

Conclusion and Future Work

Root densities of wheat and mustard plants were estimated using GIS techniques. The average values of root density in our study ranged from 43% to 60%. The average overall accuracy in this study was over 80%. Kappa coefficient values of the wheat and mustard plants range from from 0.56 to 0.70 indicating a reliable classification results. A Pearson correlation revealed that the estimated root density correlated with the dried biomass in the main stem and with the dried biomass in the branch.

The technique used in this study is fast, accurate, and able to deal with a large data set. However, consistency in the data collection process, such as brightness, distance, and angle between the camera and objects, should be refined in future work. Addition to images retrieved from pot's surface, cross-section images will be included. Root staining is suggested to provide a contrast between the background (soil) and the root. The study was carried out with limited pot size, which may alter biomass allocation, including the biomass in the root zone (Poorter, et al., 2012); investigations with larger pot size are needed in the future.

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