

Field manual for rapid and non-destructive shoot biomass evaluations of staked yam

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Summary



A rapid and easy method has been developed to evaluate the shoot biomass of staked yams. This method can reduce the time and cost required to evaluate shoot biomass when compared with destructive sampling methods. Using this new method, more than 200 accessions can be evaluated within 4 h, including the preparation time, while with the conventional methods of shoot sampling, oven drying, and weighing this process would take 3 days. In addition, owing to the non-destructive measurements of the normalized difference vegetation index (NDVI), evaluators can follow the growth of the same plant from planting to plant senescence. This reduces the size of the trial field required for plant replication during time-course of destructive sampling. The new method can be easily introduced into yam breeding programs worldwide. Additionally, no specialist knowledge is required to analyze the spectral reflectance data or record the NDVI value.

The four steps of measurement

- Set the background panel behind the target plant
- Set the guide stick 1.2 m from the target plant
- Perform the NDVI scan (two times from different directions)
- Record the NDVI values and plant height

The biomass is then calculated as follows:

$$\text{Shoot dry weight (g}\cdot\text{plant}^{-1}\text{)} = 29.2 \times \text{NDVI} + 1.3 \times \text{Plant height (m)} + 100.7$$

1. Introduction

Crop phenotyping is a crucial process used to accelerate breeding programs in the era of high-throughput genotyping. The technical challenges associated with yam breeding and selection include a long breeding cycle, an extremely low multiplication ratio for propagules, and the existence of a juvenile phase during the seminal and early clonal stages of selection. One of the difficulties with field-based yam phenotyping is the vine habit of this plant. To avoid interference between neighboring plants, staking has been widely adopted in experimental trials. However, staking makes the application of common remote-sensing techniques for above-ground traits challenging because most techniques have been developed for use with crops that have an erect plant shape and a relatively low plant height. The leaves of staked vine plants, however, are positioned in a vertical direction, which is unsuitable for the horizontal scanning of spectral reflectance from above the plants. In addition, the long growth period, low planting density, and vegetative propagation systems require space, time, and effort for cultivation. Thus, the number of genetic resources has been restricted in previous studies that analyzed growth. To address these limitations, we have developed a non-destructive method to predict shoot biomass by measuring the spectral reflectance of staked yam.

2. Theory

The method developed evaluates the shoot dry weight of the staked yam by using the spectral reflectance of the aboveground plant parts. The normalized difference vegetation index (NDVI) has been used to evaluate shoot biomass in various crops, including rice, barley, and maize. NDVI is calculated using the following equation: $NDVI = (NIR - RED) / (NIR + RED)$, where NIR and RED are the canopy reflectance values for the near infrared and red spectra, respectively (Figure 1). This calculation assumes that the red spectra show low reflectance because of their absorption by chlorophyll, whereas the infrared spectra show high reflectance because of internal leaf scattering and no absorption. The NDVI represents the fractional vegetation cover and leaf area index, with a fully covered canopy value of 0.7–0.8.

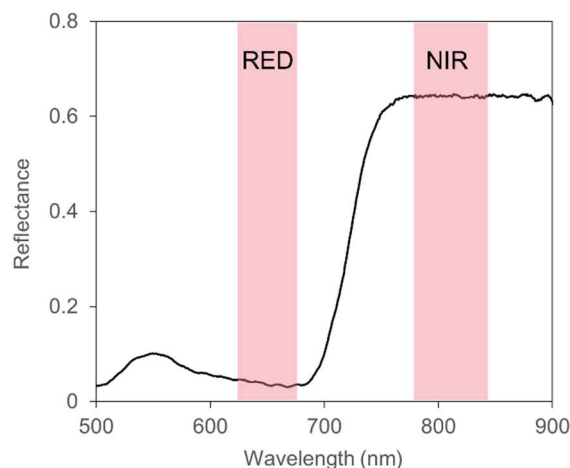


Figure 1. Spectra used for the NDVI calculation

3. Equipment

Several types of devices are available for the measurement of spectral reflectance, but the easiest to use is a handheld NDVI sensor. The GreenSeeker (Nikon-Trimble), as shown in Figure 2, is a device designed for NDVI measurements. The size of the device is 9 cm (width) × 27 cm (length), and it is 310 g, making it suitable for operation with only one hand. The GreenSeeker sensor records the spectral reflectance of an elliptical area with a 50-cm-long axis from a 1.2-m distance, which is the maximum measurable range for the device. It measures spectral reflectance if the trigger remains engaged. NDVI values are automatically calculated and displayed. The battery is rechargeable and lasts for a few days. Full charging is recommended before recording measurements.



Figure 2. A handheld NDVI sensor (GreenSeeker)

Other devices, such as image-based spectral sensors, can also be applied for this method. Multispectral and hyperspectral cameras can extract spectral information only for the plant parts, and image analysis requires specialized skill and knowledge. The high cost of these cameras also makes them impractical for field studies. The recently developed low-cost and small-size spectral sensor (GoSpectro, Goyalab), coupled with smartphones, offers another option but has not been tested with this method.

4. Field application

4-1. Preparation

➤ Spectral sensor

There are several devices available that can detect the reflectance of infrared and red light. The GreenSeeker device is used here, and its battery should be fully charged prior to use.

➤ Background panel

To eliminate the background noise of the reflectance, a wooden board that is 1 m × 2 m is placed behind the plant.

➤ Guide stick

A stick (approximately 2 m) is used to support the scanning device while the vertical scan is performed. A 1.2-m string is attached to the stick to keep a constant distance from the plant.

➤ Recording sheet

GreenSeeker does not have memory to store the data; therefore, a recording sheet is required.

4-2. Measurement

A diagram of the procedure for NDVI measurements using GreenSeeker is shown in Figure 3. A vertical line-scan from top to bottom is performed with a guide stick standing at 1.2 m from the target plant. The scanning is carefully performed for 30 s per measurement, keeping the scan speed constant from top to bottom. The background panel is set behind the plant (Figure 3A). Measurements should be performed twice per plant from different directions (Figure 3B). Plant height is measured at the time of the NDVI scan.

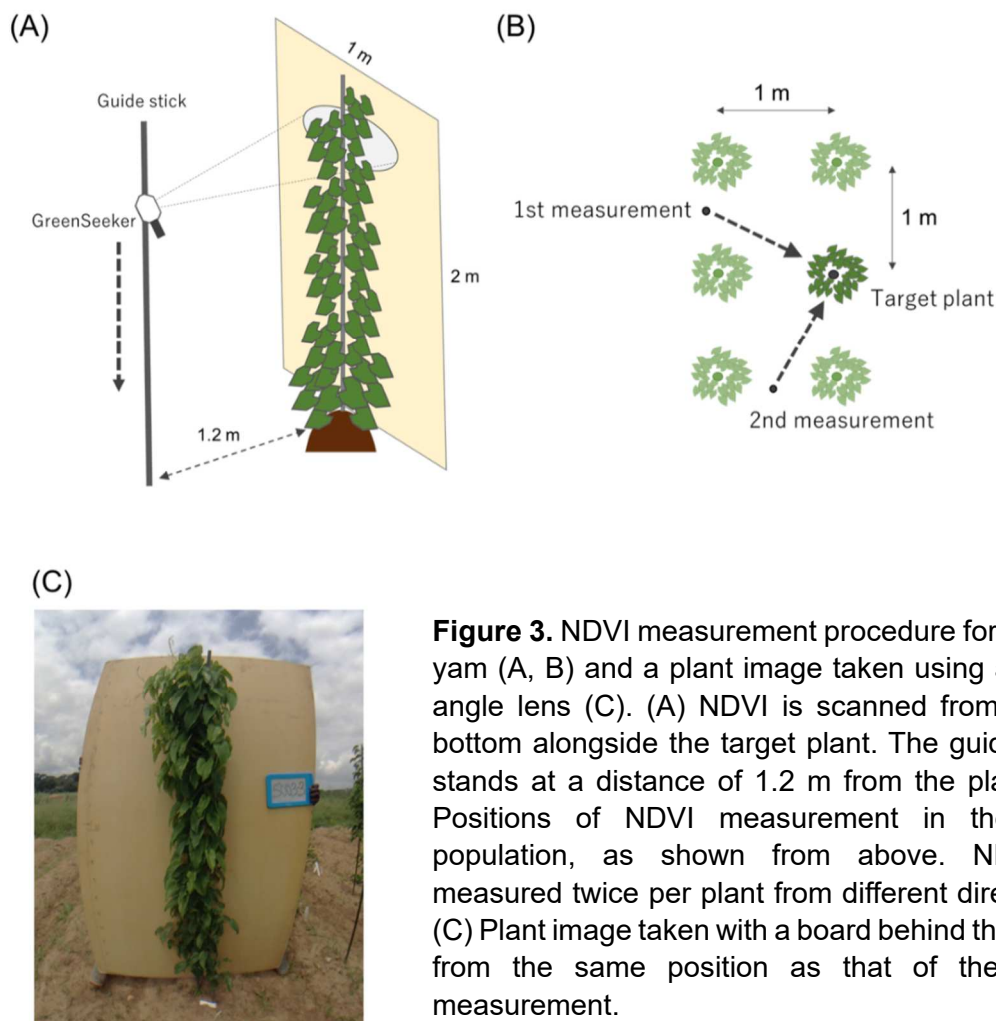


Figure 3. NDVI measurement procedure for staked yam (A, B) and a plant image taken using a wide-angle lens (C). (A) NDVI is scanned from top to bottom alongside the target plant. The guide stick stands at a distance of 1.2 m from the plant. (B) Positions of NDVI measurement in the yam population, as shown from above. NDVI is measured twice per plant from different directions. (C) Plant image taken with a board behind the plant, from the same position as that of the NDVI measurement.

4-3. Calculation

The shoot dry weight can be calculated using the following equation:

$$\text{Shoot dry weight (g·plant}^{-1}\text{)} = 29.2 \times \text{NDVI} + 1.3 \times \text{Plant height (m)} + 100.7$$

Example data are given in Figure 4. The estimation model was applied to 125 data samples obtained from yam plants with different shoot biomasses. The correlation coefficient was $r = 0.83$, and the standard deviation of the prediction was $\pm 32 \text{ g·plant}^{-1}$. Note that shoot biomass estimation is also possible using a simpler method without plant height (Iseki & Matsumoto, 2018).

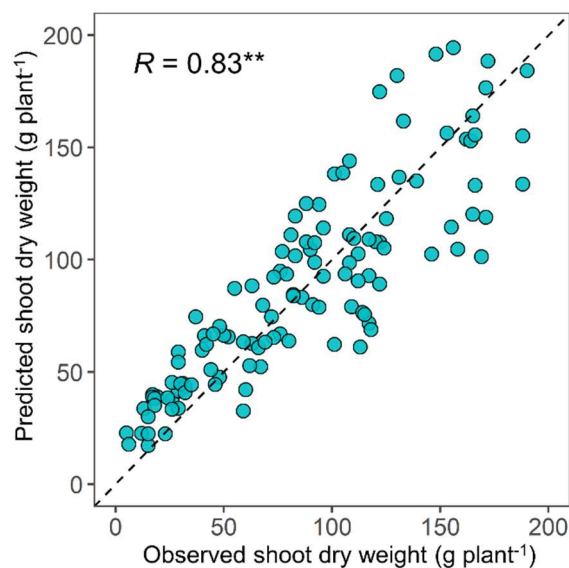


Figure 4. Correlations between the predicted and observed values of shoot biomass. The dashed line is an equal line between the predicted and observed values. The correlation coefficient was statistically significant at $p < 0.05$.

4-4. Plants not suitable for this application

We found that predictions were likely to be underestimated when the shoot biomass exceeded 150 g·plant^{-1} . This is because NDVI is calculated from the spectral reflectance of the plant surface, which cannot be assessed inside the leaf layer. Therefore, the NDVI value plateaus after the scanning area is covered by leaves.

Underestimation also occurs in the case of senescence of the plants. This is because NDVI represents plant greenness, i.e., chlorophyll content. Therefore, senescent plants (with yellowish leaves) are not suitable for shoot dry weight prediction using this method.

In addition, uneven leaf distribution can prevent a reliable prediction of shoot biomass, as shown in Figure 5. Such plants were observed during the late period of yam growth and caused a failure in the NDVI measurements. However, plant images might improve their predictions, and other scanning methods, such as point scans instead of line scans, might also be suitable.

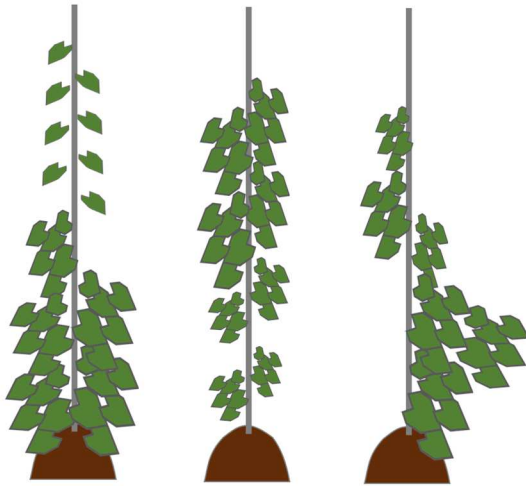


Figure 5. Plant shapes of staked yam not suitable for shoot biomass prediction using the normalized difference vegetation index (NDVI). Left and middle: leaves are dense in the top or bottom part. Right: leaves are inclined towards one side.

4-5. Improving the precision of estimations

We found that in addition to the NDVI and plant height, the use of plant images improved the predictions for plants with high values for their shoot dry weight (Figure 6). Plant images were obtained at the same time as the NDVI measurements in August 2017. A wide-angle lens (PL-WD02, Aukey, Shenzhen, China) was attached to a camera installed on an iPad mini (Apple, Cupertino, CA, USA), and the camera direction was set as vertical to the board behind the plant. Whole plant images were obtained from the middle point of the guide stick for the NDVI measurement, approximately 1 m above the ground, standing 1.2 m from the target plant (Figure 3C). The projected green area in the image was calculated using the image analysis software ImageJ1 version 1.50i (National Institute of Health; <http://rsb.info.nih.gov/ij/>). The width of the board was used as the reference length for each image. The time to take the images and the direction of sunlight did not affect the image analysis. Barrel distortion due to the use of a wide-angle lens was not corrected, and thus the calculated green area was different from the actual value.

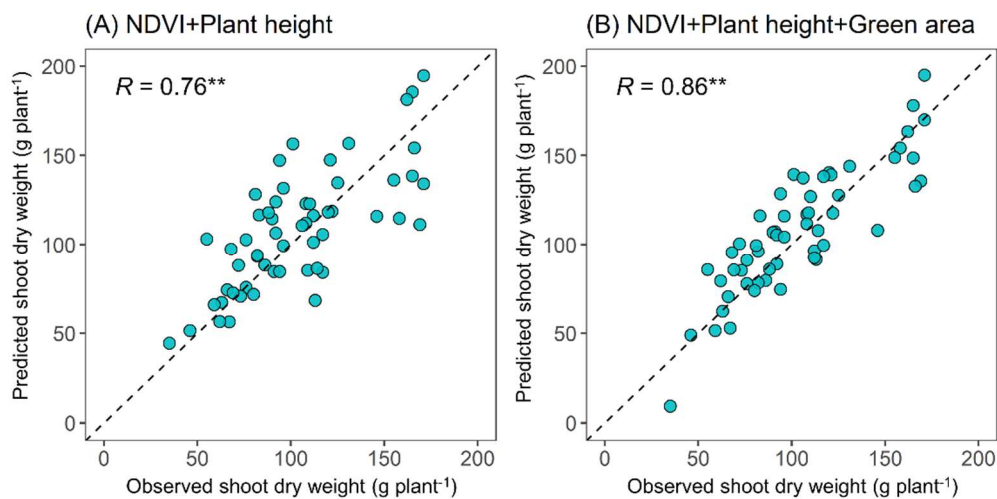


Figure 6. Relationships between predicted and observed shoot dry weight. The prediction can be improved by the equation including green area in the explanatory variable. Shoot dry weight = $12.2 \times \text{NDVI} - 5.9 \times \text{Plant height (m)} + 25.4 \times \text{Green area} + 100.7$ (Iseki & Matsumoto, 2019).

5. Examples of field applications

The method was applied for shoot biomass predictions of the F1 cross population of 210 plants, and a strong correlation was found between shoot biomass and tuber yield (Figure 7). This indicates that the information on shoot biomass is also applicable for tuber yield prediction. The strong relationship between shoot biomass and tuber yield in both years also indicates the reliability of this method.

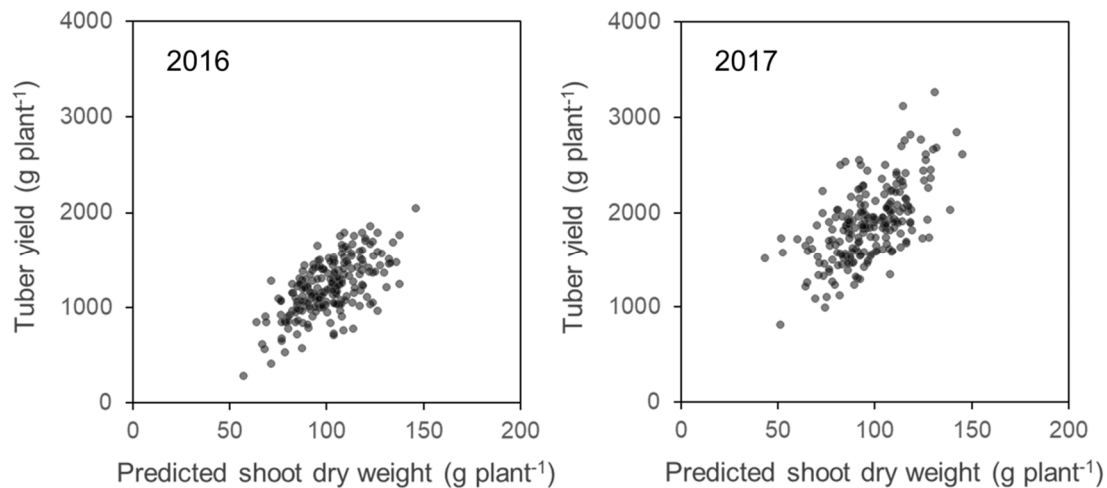


Figure 7. Relationships between the predicted shoot biomass and observed tuber yield in the cross population. Correlations were statistically significant at $p < 0.05$ in both years. Data represent the means of three replicates.

Another example of the usefulness of the method is the analysis of the effects of seed sett size on shoot growth. The shoot dry weight in a total of 300 plants consisting of five accessions, three sett sizes, four plants, and five replicates was evaluated every 2 weeks. The vigorous shoot growth in the plants from the larger setts, which showed a higher tuber yield, was noticeable during the early growth period and was maintained until plant senescence (Figure 8). This indicates that early shoot growth is crucial for yield formation.

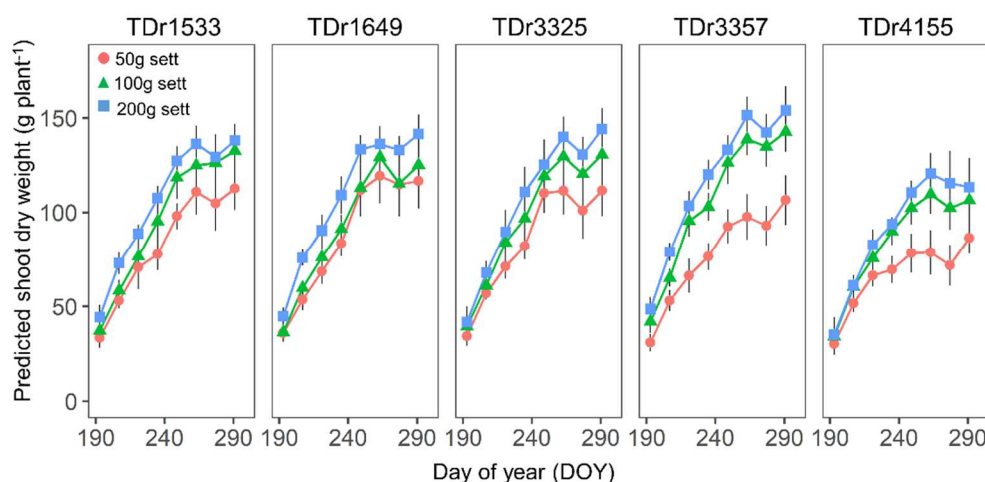


Figure 8. The time-course changes in shoot dry weight for the plants established from different sett sizes. Data represent the means \pm standard errors of 20 samples (4 plants \times 5 replications).

The value of this non-destructive shoot biomass evaluation method has been shown in the aforementioned case studies. The method can be easily applied to large populations of plants comprising multiple varieties, treatments, and replications. Such application is not feasible for conventional destructive sampling owing to the time, cost, effort, and field space required for conventional shoot biomass evaluation.

Publications

- Iseki, K., & Matsumoto, R. (2019). Non-destructive shoot biomass evaluation using a handheld NDVI sensor for field-grown staking yam (*Dioscorea rotundata* Poir.). *Plant Production Science*, 22(2), 301–310. <https://doi.org/10.1080/1343943X.2018.1540278>
- Iseki, K., & Matsumoto, R. (2020). Effect of seed sett size on sprouting, shoot growth, and tuber yield of white guinea yam (*Dioscorea rotundata* Poir.). *Plant Production Science*, 23(1), 75–80. <https://doi.org/10.1080/1343943X.2019.1667835>
- Iseki, K., & Matsumoto, R. (2018). Non-destructive shoot biomass evaluation for field-grown staking yam (*Dioscorea rotundata* Poir.). *JIRCAS Research Highlights*, https://www.iircas.go.jp/en/publication/research_results/2018_b01.

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