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ARTICLE

A Shovel-omics Facelift: Exploring Inexpensive and Simple Root Phenotyping Techniques in Peanut

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ABSTRACT

Accurately characterizing root systems in mature, field-grown crops presents a significant challenge due to the complexity of root architecture and the limitations of existing phenotyping techniques. While recent technological advancements, such as in-situ photographing, have improved root assessment, widely accessible and cost-effective methods remain scarce. Root phenotyping in peanut is typically limited to terminal excavation or expensive, spatially constrained imaging systems. In this exploratory methods paper, we describe a simplified, low-cost approach—referred to as the 'root box method'—to visualize and characterize root architecture in peanut under controlled conditions. We document the construction and use of a box system made from widely available materials, enabling manual root washing, high-contrast imaging, and basic image-based phenotyping. We compare root metrics obtained with this system to those generated by a commercial root scanner to explore its utility as an accessible alternative for small-scale or early-stage research. While the method has clear limitations, it offers a practical starting point for observing root architectural variation in peanut genotypes. Our goal is to provide a transparent evaluation of this approach to support broader participation in root research and tool development in resource-limited contexts. Additionally, it offers significant potential for breeding, agronomy, and extension research, particularly in studies related to drought resilience, nutrient acquisition, and soil health. The root box method serves as an accessible tool for researchers, agronomists, and growers, providing critical insights into root architecture that can inform crop management strategies and improve agricultural sustainability.

INTRODUCTION

This study began by critically examining a fundamental question that transcends multiple disciplines within crop and soil science: How do roots influence and interact with their environment to drive plant and ecosystem function? In the context of plant water uptake, soil nutrient dynamics, biotic interactions with insect and fungal pathogens, and biomass allocation strategies, plant root systems serve as a critical component needed to better understand the mechanisms governing plant performance and ecosystem processes.

However, as questions on this topic continue to evolve in complexity and scope, quantitatively assessing root architectural characteristics in mature, field-grown plants remains a significant challenge. Recent technological advances have addressed this challenge, with in-situ phenotyping methods enabling the capture of root responses to environmental variability in field conditions. These advancements provide critical insights for optimizing crop performance in real-world agricultural systems (Burridge *et al.,* 2016). Even with these

advancements, accurately quantifying root architectural phenotypes in mature field-grown plants remains challenging and is still not as widely adopted as other methods that have been utilized over the past fifty years. Researchers originally coined the term "shovelomics" to describe various crop/plant specific methods used to visually score root architectural traits and morphophysiological characteristics in the field (Trachsel *et al.,* 2011; York *et al.,* 2018; Fenta *et al.,* 2014). The oldest and most common method, shovelomics, has been routinely used among crops such as corn, wheat, and soybean in which a hollow cylinder is used to excavate a standard diameter of soil at a standard depth (both dependent on the crop and previously described methods for the specified crop), using the plant base and the horizonal center point. Soil is loosely shaken to reveal excavated root crowns, gently soaked to further remove larger clumps of adhered particles and physically agitated to remove fine particles before drying (Vijay, 2016; Boudiar *et al.,* 2021). Crowns are then photographed on scaling boards where metrics such as rooting angle, length, and nodulation can be recorded (Böhm, 2012; Bucksch *et al.,* 2014) and visual rating systems for belowground architecture (shape), and branching density can be implemented (Walters *et al.,* 1994; Lynch 1995). This method provides researchers with basic data that can be extrapolated to better understand water/nutrient uptake, and adaptability to varying soil/environmental conditions (Burridge *et al.,* 2016). Although widely cited and standardized, this method is labor-intensive, prone to sampling error, and subject to rating bias.

To address the limitations of traditional root observation techniques and improve the reliability, standardization, and throughput of root phenotyping in field settings, a variety of new field-based approaches have emerged (Table 1). These include minirhizotron imaging systems, in situ trenching, coring combined with imaging software, and more recently, ground-penetrating radar and electrical resistance tomography. While each of these methods provides important advances in spatial and temporal resolution of root data, they also introduce new challenges. Chief among these are high costs for equipment and data processing, limited spatial coverage or field of view, and significant labor requirements for installation, maintenance, or image analysis. In addition, many of these methods still struggle to capture dynamic root traits in real time and across the full soil profile in diverse field environments. As a result, researchers often face trade-offs between resolution, scalability, and affordability. This ongoing need for practical, high-throughput, and cost-effective root phenotyping tools, especially in under-resourced or applied agricultural settings, has led to increased interest in hybrid approaches that balance control, replication, and realism. In this context, semi-controlled, low-cost root observation systems such as root boxes or rhizoboxes deployed in field conditions offer a promising compromise between full-scale field experimentation and controlled-environment studies, enabling direct observation of root development with higher throughput and better comparability across treatments and genotypes.

Table 1. Methods for measuring plant root systems under field conditions.

Method	Pros	Cons	Citation
Soil Cores & Auguring	Simple, cost effective, widely cited across literature	Destructive, labor intensive, misses fine root structures	Böhm *et al.*, 2012
Minirhizotron Cameras	Allows for repeated measurements at same location, nondestructive	Limited field of view, cannot move after instillation, expensive and requires specialized camera	Mohamed *et al.*, 2017
Pit Excavation & Washing	Direct measurement of root structure	Destructive, labor intensive, misses fine root structures	Böhm *et al.*, 2012
Monolith/Profile Wall Sampling	Good visualization of root distribution	Destructive, labor intensive	Böhm *et al.*, 2012
Mesh Bags/Ingrowth Cores	Used for quantifying root turnover and growth dynamics	Focuses on new root growth and not representative on whole root system	Steingrobe *et al.*, 2000
Ground Penetrating Radar	Non-destructive, provides spatial root mapping	Expensive and requires technical expertise	Guo *et al.*, 2013
Root Image Analysis from soil cores (WinRHIZO)	Provides detailed root trait measurements	Root extraction may not be representative, requires extensive lab and image processing	Himmelbauer *et al.*, 2004

In this study we created a protocol similar to the "pinboard method" (Kano-Nakata *et al.,* 2012; Pandey *et al.,* 2017) in order to capture an accurate and intact profile of the root system of an individual peanut plant. The goal was to allow enough room for the plant to occupy and proliferate naturally across soil horizons during the growing season, as well as hold the roots within those spatial horizons upon harvest/data collection. We also compared analysis techniques to find a simple and cost-effective way to analyze and extract data from these root architectures. We found that this root box method has large scale applicability and streamlined methodology that allows for a data rich and accurate approach to studying belowground characteristics of peanut. Root phenotyping remains a critical yet challenging component of peanut research, with field-based methods often requiring substantial resources, labor, and specialized equipment. To address these constraints, we present a simplified wood box method designed to provide full root profile visualization in a controlled setting. While this method offers a relatively low-cost and accessible alternative for researchers and students lacking extensive resources, it is important to acknowledge its limitations.

Specifically, the soil medium used—a mixture of field-collected topsoil and potting soil compacted to approximate field bulk density—does not fully replicate the natural soil profile heterogeneity encountered in peanut fields, where variations in topsoil and subsoil characteristics significantly

influence root growth dynamics. Consequently, results obtained using this system should be interpreted as indicative rather than directly representative of in-field root development. This method is intended primarily as a preliminary screening tool or for educational purposes, complementing but not replacing detailed field phenotyping approaches.

MATERIALS AND METHODS

Root Box Construction and Setup

Small root growth chambers were made from wood, filled with a soil mixture, and irrigated using a small drip irrigation system. The size of the wood structure was sized to minimize wood loss. A 122 by 244-cm sheet of 1.9-cm plywood was cut in half both horizontally and vertically resulting with four 61 by 122-cm sized pieces. In addition, a 4.4-cm by 15-cm by 244-cm board was cut in half and one piece of plywood was attached to these two boards to make 3/4 of a box. The plywood was attached using nails six inches apart to reduce warping. This is the back of the box. The internal part of the box was painted white to aid in taking pictures of the roots when the soil is removed at the end of the project. After painting, the final piece of plywood was attached to the boards using screws making an opened end narrow tall box. The back part of the plywood was attached to the boards using nails while the front portion of the plywood was attached with screws so it could be removed. Inside dimensions of the box was 15 by 53 by 122 cm (96,990 cm3 or about 1 m^3 volume) (Figure 1.). For this research a total of nine boxes were made to have two peanut cultivars replicated three times. The approximate cost for materials to build each wood box was $60, which includes wood, mesh, and hardware.

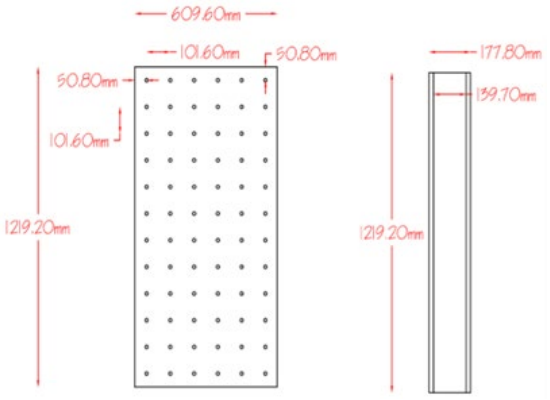

Figure 1. Dimensions of constructed root box.

Soil was collected from a local farm classified as a Red Bay sandy loam (Fine-loamy, kaolinitic, thermic Rhodic Kandiudults). Only the top 15 cm of soil was collected and used in this project. Approximately 0.95m^3 of soil was placed into a soil mixer (Bouldin & Lawson, Model 12103, McMinnville, TN, USA) along with 0.05 m^3 potting soil (ProMix BX, Premier Horticulture Inc., Quakertown, PA, USA) and allowed to mix for a minimum of 60 minutes. The soil blend was transferred to a soil box. The soil box was stood long side vertical on end with a piece of metal window screen beneath the box to retain soil but allow excess water drainage. The box was placed on a plastic pallet and held in place with ratchet straps to keep it from falling over during filling. As the box was being filled, the soil was lightly tamped down with each 15 to 20 cm of soil depth to an approximate bulk density of 1.2 to 1.4 g/cm^3 to simulate a loam soil. Once the box was filled with soil, nails were inserted into front part of the plywood using a pneumatic nail gun on a 10 by 10 cm grid that was marked on the plywood. The nails were full round head, hot dipped galvanized, ring shank 101 mm long and 2.87 mm diameter. It was agreed that a 10-cm grid arranged nails close enough to support the roots, but not too many to interfere with the software during root determinations (Figure 2).

Figure 2. Photographs of root box assembly. Left side displays half assembled boxes with the main wall covered in white paint. Right side displays completed boxes with closed face and nails inserted through the painted white side.

Construction, filling and setup of each box required two people. Boxes were arranged vertically on pallets for storage and movement, with three boxes fitting per pallet. Movement was facilitated using a forklift. The boxes were arranged front to back so the front of the box could be removed at the end of the project. The boxes were held in place using rachet straps both around and over the top to prevent tipping over when the pallet was moved to its final location beneath a shelter. The shelter was open sided with a clear plastic top to allow sunlight in but prevent rainfall so we could control irrigation water applied to each box (Figure 3).

Figure 3. Root boxes placed in outdoor shelters. Left side displays how emitters were arranged around the germinated plant for the duration of the experiment. Right side displays how boxes were organized for structural stability simple management throughout the season.

A drip irrigation was installed with emitters installed in each box (3.785 L/hr). Prior to planting peanut, irrigation water was applied to fill the soil profile. Peanuts were planted between 1 to 15 May as recommended by normal planting recommendations. Three seeds were planted in each box of the two most planted commercial cultivars in GA (Georgia 06G, and Georgia 18RU). After germination and emergence, excess peanut plants were removed to maintain only one plant per box. During the growing season, pesticides were applied at recommend timing and rates to maintain best management practices. Irrigation amounts were applied weekly for about 45 minutes/event. If leaf wilting occurred between these weekly events another 30 minutes of irrigated was applied.

Root Washing

Typical peanut maturity is between 135 and 145 days after planting which is when we dismantled the root boxes. One pallet (3 root boxes) was moved to a washing location and the ratchet straps were removed from one box at a time leaving the other two strapped together and secured so they wouldn't tip over while manipulating the single box. The single box was gently moved such that it was laying on its back with a slight horizontal slope and the top (side) screws were removed and the top removed to expose the soil and roots in the box. The soil in the box was washed using a garden hose and sprinkler attachment to lightly shower the soil leaving the roots to "hang" on the exposed nails (Figure 4). This light sprinkling occurred until all the soil was removed leaving the roots. The box was stood back up on its end, and pictures were taken with a high-resolution digital camera. This process was used for all nine boxes to get digital pictures of the roots for each of the three peanut cultivars and replications.

Figure 4. Removing box face at full maturity. Left and center photo shows intact root system as well as identifiable soil horizons. Right photo demonstrates root washing technique.

Processing Root Photos

High resolution .jpg images were first cleaned for noise in Adobe photoshop (Adobe, 2024). Nails, rocks and other small debris were removed from the photo to reduce error in root segmentation further into the analysis. These modified images were uploaded to RhizoVison Explorer (Seethepalli, Version 2.0, 2024), a freely available and highly validated software for root image analysis and measurement extraction (Figure 5) (Seethepalli *et al.,* 2021). Additional validation of data extracted from these photos was completed by scanning sections of the physical root systems on a root scanner (STD4800 scanner, calibrated for WinRHIZO image analyses, Regent Instruments Inc., Quebec, Canada) and processed through the software WhinRHIZO (Regent Instruments, 2024).

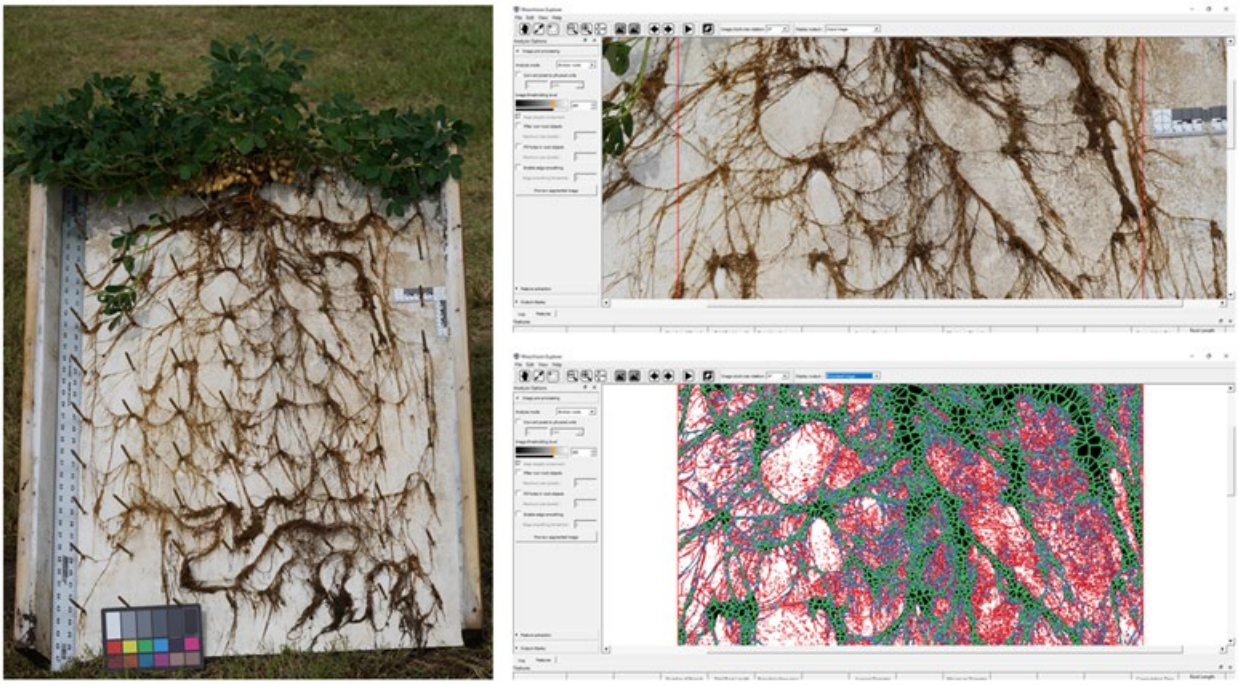

Figure 5. Left side is a photograph of a final washed root system with a standardization color pallet as well as reference rulers. The right side displays screenshots of a close up section of the photograph that is being processed via RhizoVison Explorer image segmentation.

Statistics

Statistical analyses were limited to two-sample *t*-tests, which were used to compare selected root trait outputs between WinRHIZO and RhizoVision Explorer in order to assess consistency between the two software platforms. Given that this is a methods-focused study rather than a hypothesis-driven experiment, no additional inferential statistics were performed. The goal of these comparisons was to validate general agreement in trait quantification across platforms, rather than to conduct extensive quantitative benchmarking.

RESULTS/DISCUSSION

This study was conducted twice, once in the summer of 2023, and once in the summer of 2024 in order to allow for modifications to be made in box construction and to validate the accuracy of image analysis. In both years with both genotypes, we found that the individual peanut plants exhibited rooting architecture that took advantage of the entirety of space in the box. In 2023 our boxes were approximately 122cm tall, and after 130 days we found roots proliferating out of the open bottoms and continuing to grow. The following year, we added an additional 40cm in height, which seemed to be just about the maximum root depth at R7. This single plant profile was consistent in both depth, architecture, and branching frequency as measured using other methods in the field (Yuan *et al.,* 2019, Huck and Taylor, 1982). Variety Georgia-06G had an average rooting depth of 82cm whereas Gorgia-18RU had an average depth of 71cm (Figure 6).

Figure 6. Intact root systems of peanut cultivar Ga06G (left) and Ga18RU (right).

Cost, setup, maintenance, and data collection were also extremely low compared to other techniques. Material costs for each box cost approximately $60 USD and consisted only of wood, nails, and white paint. Box assembly averaged 30 minutes per box (after all wood was cut to size), and root washing averaged about 45 minutes per box. After roots were imaged, they were easily removed from the pegs, and the boxes were briefly rinsed to be used the following year. We were able to remove the root systems fully intact and processed these systems destructively to obtain "ground truth" data to compare to the image analysis that we conducted (Figure 7). Horizons were cut into 22cm sections, stored in an equal parts ethanol/water solution to maintain root turgor and avoid shrinkage. These root subsections were sorted and scanned using a root scanner hooked up to the commercially popular software WhinRHIZO. This technique and software system has been considered the most reliable high throughput standard used in many root studies (Himmelbauer *et al.,* 2004). However, processing one root system in this scanner took about 8 hours and requires specialized equipment, software, and training to use. Data collected from this method included surface area, volume, and total root length, which were then compared to our free to use root analysis software RhizoVision Explorer.

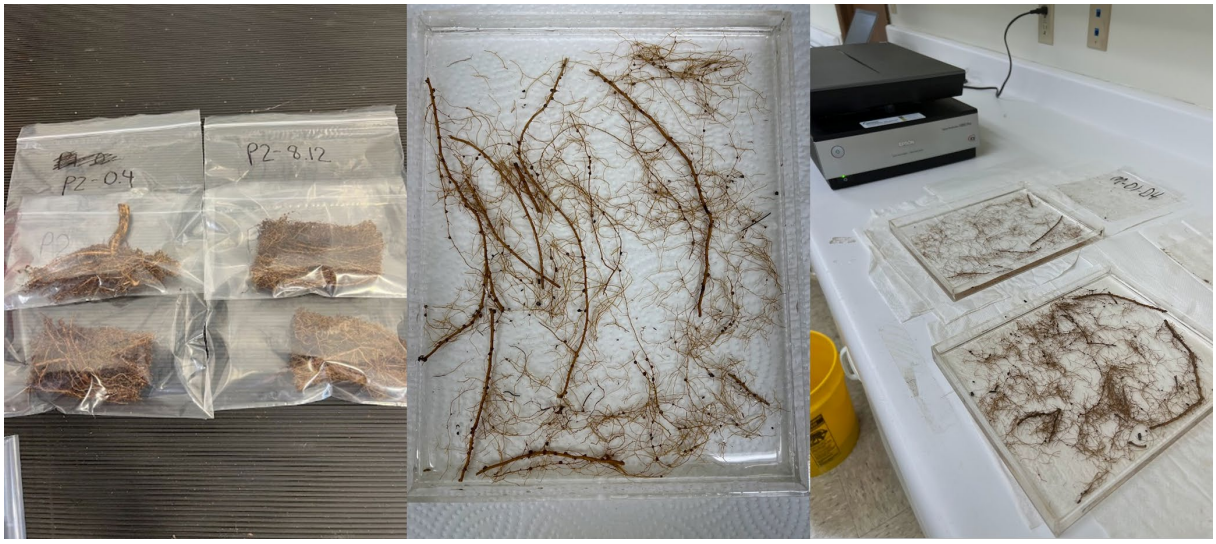

Figure 7. 22-cm samples separated from imaged root systems, cleaned and suspended in water to be scanned on WhinRHIZO scanner.

Data analysis using RhizoVision was straightforward and accurately matched the ground truth data that we collected from the physical root systems with only about a 4% error rate for GA06G and 5.2% error in Ga18RU. It quickly provided us with morphology metrics such as root length, surface area, and volume; architecture metrics such as number of root tips, and number of root crossings, as well as color-based metrics which can be used to infer root health. This software is free, open source, regularly updated by the research community and requires very minimal system requirements to run, compared to WhinRHIZO which requires a paid license and is only compatible with specific root scanning systems. In terms of quick and dirty high throughput data collection of root systems, we found RhizoVision easy to use while providing reliable metrics.

This data collection and analysis combination has the potential to provide a wide range utility for breeders, agronomists, soil and other crop scientists. Breeders can use this as an effective phenotyping tool to select root traits that improve crop performance such as rooting depth, nutrient efficiency, and lodging resistance (Clarke *et al.,* 1993; Wu *et al.,* 2022). Agronomists can gain deeper insight into crop management practices including fertilization efficiency, irrigation strategy, and tillage practice (McGrail *et al.,* 2020; White *et al.,* 2013). Soil scientists may better understand soil structure and compaction and well as carbon sequestration and microbiome dynamics. In addition, this method serves as a powerful tool for extension work and agricultural outreach by visually demonstrating large differences in crop root traits. It can be utilized for field demonstrations where growers can see how different genotypes or soil treatments affect root development (Figure 8). Images from studies can also be used in extension bulletins and fact sheets to visually communicate key findings of ongoing research studies. It allows farmers to gain a clear understanding of how root dynamics influence drought tolerance, nutrient uptake and soil stabilization to make more informed management decisions.

Figure 8. Right side depicts a washed versus unwashed root box for use in research presentations. Left side depicts the same boxes being used as an education and outreach tool during the 2024 Georgia Peanut Tour.

Future Use in Peanut

While this method can be used across disciplines and modified for other crop species, data collected from this study has been used as a starting point for answering some of the current pressing questions in peanut science. Much of the current research done in peanut across varieties and environments focuses on identifying mechanisms and traits that facilitate drought resilience and yield maintenance in peanut. Key traits we need information on include root depth, length, surface area, diameter distribution, and lateral root density. Integration of this data with other physiological traits such as sap flow or leaf water potential allow us to link root structure/dynamics with water use efficiency and whole plant plasticity under drought conditions and heat stress. Another avenue in which can accelerate understanding is in terms of peanut risk to aspergillus infiltration and aflatoxin mitigation. Fine root production as well as lateral spread dynamics have a significant effect on microbial interactions and fugal colonization risk (Hartmann *et al.,* 2009). Whole root system architectures can also be compared to soil moisture levels to correlate if certain root systems and maintained soil moisture can naturally buffer aspergills proliferation in the field (Hill *et al.,* 1983, Bowen *et al.,* 2015). Peanuts are also an essential part of many crop rotation systems as they provide ample nitrogen fixation to soils and require a relatively low amount of applied fertilizer in most systems (Li *et al.,* 2022, Rachaputi *et al.,* 2021). Looking at root traits can help researchers identify which varieties are better at improving nutrient acquisition in low fertility soils, as well as comparing root responses to biological nitrogen fixation in relation to nodulation efficiency. While the uses of root biology are extensive, this study provides a starting point for researchers, agronomists, and extension agents to take advantage of some of the practical applications of these techniques.

Additional Considerations

While the wood box root phenotyping method provides a cost-effective and accessible approach for visualizing and measuring peanut root profiles, several important considerations should be noted. The soil mixture and compaction used approximate field bulk density but cannot replicate the complex vertical and horizontal heterogeneity of field soil profiles, including subsoil layers and localized compaction zones. This limitation may affect root growth patterns and restrict the natural exploration of soil volume, potentially altering root architecture compared to field conditions. Environmental factors are also simplified under the open-sided shelter, reducing variability but limiting the ability to fully simulate field microclimates and stochastic stressors such as rainfall fluctuations, soil biota, or pathogens. Consequently, plant responses to drought or nutrient stress observed in this system may differ from those in situ. Although the method is relatively low-cost and straightforward, manual construction, soil preparation, and handling requirements may constrain throughput for large breeding programs; thus, potential automation or mechanization should be explored. Importantly, this method should be viewed as complementary to field phenotyping, useful primarily for initial mechanistic insights or screening, with subsequent validation in field trials needed to establish trait relevance under realistic growing conditions. Future improvements could include layering soils to better mimic field profiles, incorporation of subsoil material, or integration of sensors to monitor root growth and soil moisture dynamically, thereby enhancing ecological relevance and data richness. Nevertheless, the wood box system provides a valuable, low-barrier approach for researchers to explore root traits, especially in resource-limited settings.

CONCLUSION

This technique is not only affordable and accessible but also adaptable for a wide range of agronomic, ecological, and breeding research applications. Unlike traditional excavation methods that risk damaging fine root structures, this approach preserves root integrity, allowing for precise measurements of root depth, branching patterns, and lateral spread - key factors in water conservation, disease resistance, and yield stability. Additionally, simple and visual tools such as this play a key role in bridging the gap between research and on-farm decision-making. By providing growers, agronomists, and extension specialists with clear, tangible insights into root system dynamics, this method enhances the adoption of improved cultivars, optimized irrigation strategies, and conservation practices. It can also be used as a powerful outreach tool during field days, workshops, and participatory research programs, fostering direct engagement with stakeholders. Furthermore, this approach proves to be a valuable tool for phenotyping, allowing researchers to assess and compare large numbers of samples in real-world conditions. Given its practicality and efficiency, it holds immense potential for advancing peanut breeding programs, optimizing soil health management, and enhancing agricultural resilience, particularly in the face of climate variability and extreme weather events.

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