International Symposium "Root Research and Applications"
RootRAP, 2–4 September 2009, Boku – Vienna, Austria

Digital image analysis: Simplifying quantification of traits for root morphology and architecture for different sorghum varieties

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ABSTRACT

Rhizotrons are effective for screening traits of root morphology and architecture prior to field experimentation. The objectives of this study were to improve the Rhizotron method by comparing digital imaging of root systems with conventional root scans and to apply it to five different sorghum varieties. Plants were grown in duplicate in inclined, slim rhizotrons filled with a sandy top and subsoil at field capacity (FC) and 50% available water content (AWC) in a split-plot design in the glasshouse (16/24 °C). Root growth was monitored weekly and roots were washed out after 42 and 90 days using a pin-board (25 mm grid). After digital imaging of the root architecture the roots were sub-sampled for scanning with WinRHIZO to quantify the baseline root parameters. Undisturbed rhizotron images showed differences in root advancement rates but revealed little of root architecture. Digital images of increasing resolution (9.1 to 12 Megapixel) and optimized contrast between roots and background, improved the recovery rate of scanned roots from <50% (r^2=0.12) to >70% (r^2=0.70). Root size distributions from pin-board images moved towards larger root diameters when compared to the root scans. Sorghum varieties bred for rainy and post-rainy season showed very different rooting patterns (angle of adventitious roots; root distribution in the profile) but similar root development (1st and 2nd order roots). Further analysis, e.g. links and development, using the permanent records of pin-board images, is discussed.

KEYWORDS (6): Digital image, Root morphology, Root architecture, Sorghum, Rhizotrons

1. INTRODUCTION

Spatial and temporal soil heterogeneity generates plasticity of the root system for efficient resource capture, especially in water-limited environments. Generally, it is cumbersome to study root architecture under field conditions as the labor-intensive profile method may destroy the root pattern. Root scans from destructive samples allow quantification of a large number of root morphological parameters like root length, diameter classes, surface area, number of root tips, branching angle etc (Bauhus and Messier, 1999). Slim rhizotrons allow semi-destructive sampling, e.g. for screening different lines of upland rice for their root distribution (Price *et al*., 2002) and quantifying the effect of compaction on maize and triticale seedlings (Grzesiak, 2009).

We adopted a method of growing plants in rhizotrons and washing roots on black pin-boards to maintain their spatial arrangement (semi-destructive). Photos of high resolution were taken for digital image analysis before (destructive) sampling for root scanning. Our objective was to compare root traits of different sorghum varieties bred to be grown during the rainy and post-rainy seasons in India using these digital images with the conventional root scans.

2. METHODS

Experimental design

Two treatments of water availability were imposed on two sorghum (M35-1, CSV-15) and three sweet sorghum cultivars (ICSV-25267, ICSV-25274 and SSV-84), replicated twice in a split plot design, sampled 42 and 90 days after sowing. The greenhouse temperature regime was 16/24°C night and day; daylength was 16 hours providing additional illumination at 360 µmol m^{-2} s^{-1}.

The slim rhizotrons (500 x 700 x 15 mm) were built from “sandwiched” 3 mm glass panes, a polystyrene base with a drainage pipe and 70 mm duct-tape holding the panes together. The rhizotrons were filled with air-dried, sieved sandy top and sub soil (Butt Close soil series at Woburn Farm). Soil was loosely packed to an effective dry bulk density of 1.4 to 1.53 g cm^{-3}. Water retention curves were measured at high and low bulk density to enable us to set and maintain water contents at FC and 50% AWC, respectively.

The rhizotrons were (1) monitored daily for root extension rate (MRER) and pattern, (2) sampled semi-destructively at 42, 90 days after sowing mounting the root systems on pin-boards to take digital images (PBI), and (3) sub-sampled destructively at different soil depths to be scanned with an A4 flat-bed scanner (RSCN). The parameters of root architecture were obtained from the scans and pin-board images to be analyzed using Win Rhizo.

Technical issues

Sample preparation: During semi-destructive sampling special care needs to be exercised to prepare the root samples for digital photography. The roots on the pin boards were floated in a water pan and a light water spray was applied to disperse the fine roots and avoid root clusters along the pin raster. Dispersal in the water and higher resolution improved the root recovery from PBI from 1st sampling to 2nd sampling.

Photography and image analysis: Resolution of the camera was enhanced from 9.1 to 12 Megapixels, illumination (studio flash) and object distance (~1.5m) were standardized to increase detection of fine roots. All samples (PBI, RSCN) were analyzed using WinRhizo (Pro version, 2008a). For the evaluation it is critical to optimize the grey threshold value distinguishing the root from the background, aided by its graphical representation in this software. Reflectance and debris, which affect the photograph quality, were filtered using a length to width ratio (default 4).

The following varietal parameters were quantified: Total dry matter in root and shoot (Root/Shoot-ratio), root extension rates (MRER, cm d^{-1}), branching angle (º) of adventitious root (measured on PBI; defined as the angle between soil surface and root at 15 cm depth), total root length (TRL) and root length density (cm cm^{-3}), distribution of root length in the soil profile, number of tips and distribution of root diameters.

3. RESULTS AND DISCUSSION

3.1 Simplifying the root analysis using Pin Board Images, PBI (semi-destructive)

By improving camera resolution, sample preparation and detection levels (contrast thresholds) increasing amounts of root (TRL) were quantified from PBI. The average “recovery rate” rose from 47 to 72% of the baseline data (RSCN) from the first to second sampling. Differences of TRL between varieties were highly correlated between the two methods (Figure 1a; r^2 = 0.70) proving the reliability and usefulness of the PBI. Comparing the root size distribution, however, showed a shift to larger root diameters in the PBI compared to the RSCN (Figure 1b). Very fine roots could not be identified because of lack of contrast and resolution. The change of contrast threshold as suggested (WinRhizo Pro, 2008a), however, did not improve the resolution or recovery despite pale roots being visible. The PBI analysis significantly improved when the pixel size was reduced by changing the scanner resolution from 200 to 800 DPI. More work is needed to increase the precision of the image analysis.

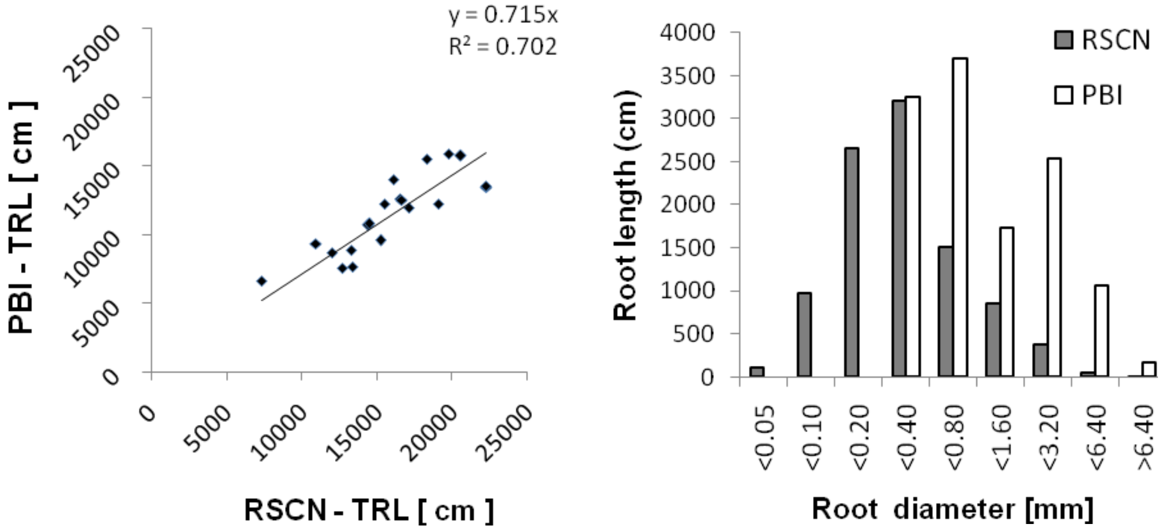

Figure 1: (a) Root recovery (TRL) and (b) root size distribution (mm Ø) comparing PBI to RSCN

3.2 Detecting differences of root architecture using Pin Board Images

The angle/spread of the root system, root distribution in the profile and the number of root tips are the most critical root architectural parameters for resource acquisition by the plant. The preservation of the root image in the PBI allows quantification of the architectural parameters of the varieties in a near undisturbed state with respect to branching angle, spread in two dimensions and distribution of roots in the profile. Similar to the RSCN data, one can distinguish different orders of roots. The branching angle of adventitious roots is a critical parameter for understanding the acclimatization of cultivars to drought stress. The post-rainy season variety M35-1 recorded the largest (steepest) branching angle emphasizing its potential to survive under late moisture stress conditions by developing deep fast growing roots (Figure 2). The trend towards larger branching angle at 50%AWC was found to be common across all cultivars except ICSV-25274.

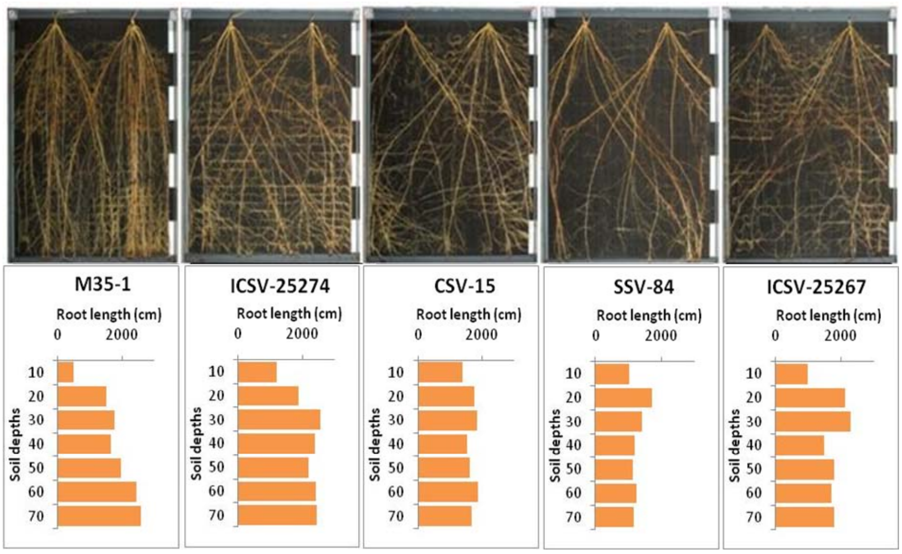

Figure 2: PBI for root systems of sorghum and sweet sorghum cultivars and RL in the profile

3.3 Monitoring rhizotrons (Non-destructive)

Although root growth rate was monitored continuously the images were not utilizable for evaluating the rooting pattern (angle, branching) due to a lack of consistency in root contact with the glass pane and poor contrast between soil and roots. The mean root extension rate (MRER) showed marked differences between cultivars and water treatments. The cultivar SSV-84 has the fastest root growth while ICSV-25274 recorded the slowest root extension rate. However, CSV-15 and ICSV-25267 roots grew faster when exposed to moisture stress at 50% AWC over FC.

3.4 Analysis of RSCN (destructive)

The RSCN data are the most reliable and were taken as the baseline. However, they do not illustrate the root architecture and exhibit more root tips due to destructive sub-sampling. M35-1 recorded more root length, and more fine roots compared to other varieties (Figure 3). Both sweet sorghum varieties (ICRISAT; ICS) show a similar root size distribution while SSV-84 has fewer roots. Number of root tips show that the single evaluation of PBI may differ by a factor of 2.5 to 4 compared to conventional root scans, partly due to sub sampling of the root system. Therefore, PBI data must be improved to be reliable and be comparable with the conventional root scans.

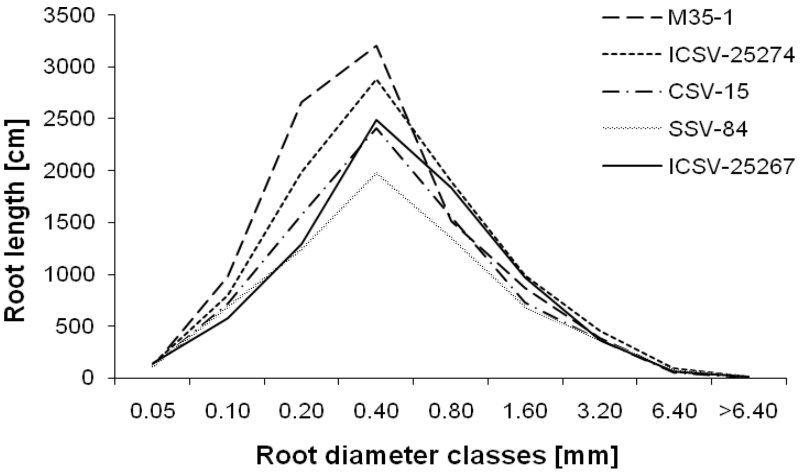

Figure 3: Root length [90 DAS] at different diameter classes for sorghum cultivars

4. REFERENCES

Grzesiak, M. T., 2009. Impact of soil compaction on root architecture, leaf water status, gas exchange and growth of maize and triticale seedlings. Plant Root 3: 10-16.

Bauhus, J. and C. Messier (1999). "Evaluation of fine root length and diameter measurements obtained using RHIZO image analysis." Agronomy Journal **91**(1): 142-147.

Price, A.H., K.A. Steele, et al. 2002. Upland rice grown in soil-filled chambers and exposed to contrasting water-deficit regimes I. Root distribution, water use and plant water status. Field Crops Res 76: 11-24.

Acknowledgements

This work was funded by Rothamsted International. The senior author gratefully acknowledges the support of her institute, Central Research Institute for Dryland Agriculture (ICAR) in Hyderabad and ICRISAT, India, for supplying of seed material. Rothamsted Research is an Institute of the Biotechnology and Biological Sciences Research Council (BBSRC) of the UK.