REVIEW ARTICLE Agricultural Reviews, Volume 46 Issue 4: 676-680 (August 2025)

Rhizotron: A Unique Technique for Root Study: A Review

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10.18805/ag.R-2671

ABSTRACT

Roots, which holds the plant in the soil and responsible for water and nutrient uptake is the most unique plant part essential for survival. Root characters like length, diameter and surface area are the important parameters that have to be measured for evaluating root growth. Most of the methods used to study root development are extremely time consuming and tedious. Many techniques have been used to increase the accessibility of plant roots. Rhizotrons have been introduced to get over these restrictions. Rhizotron is a tool for observing the roots of the plants without destructing it. Research teams interested in root-shoot physiological relationships, root system reactions to local soil conditions, cultivar comparisons and mass balance studies of harvested soils find rhizotrons to be useful tools for studying plant roots. They often contain substantial, field-like amounts of soil, sensitive instrumentation and may offer some degree of control over root environmental parameters like temperature. Additionally, they may allow access to specific roots for testing and measurement. As more advanced non-destructive measurement techniques are employed to assess the root and soil parameters, the value of rhizotrons will rise. Modern minirhizotrons are well equipped with colour video camera and processor with improved quality. This review critically examines the numerous rhizotrons designed to provide real-time, non-destructive monitoring and sampling of rhizosphere ecosystems during a plant's developmental stages.

Key words: Minirhizotron, Rhizosphere, Rhizotron, Roots.

Roots, which hold the plant in the soil and responsible for water and nutrient uptake are the most important plant part essential for its survival (Waisel *et al*., 2002) mentioned the roots as the hidden half of the plant body since the research on roots is limited or due to the various difficulties and constraints existing in root research. Field phenotyping, especially incorporating below ground information is crucial for scientists to evolve the information about soil heterogeneity for field observations on quantitative traits, particularly those related to root features that influence drought stress tolerance and understanding the distribution in soil water and nutrient content. But the expenses involved in studying the roots and the difficulties in understanding the root activities limit the root research (Carlson, 1965). Rewald *et al*. (2014) also opined that analyzing the root systems requires the use of specialized approaches and techniques.

A rhizotron is a tool used to observe plant roots over time without causing any damage. A facility or structure for observing and measuring plant subterranean components through transparent surfaces is known as a rhizotron. The name was created from the Greek words rhizos for root and tron for instrument (Klepper and Kasper, 1994). These facilities give a researcher access to both the roots and the shoots of plants that are growing in a setting a field like surroundings. A permanent installation known as a rhizotron is made up of compartments that are either segregated from the native soil profile and kept apart from one another or transparent walls that are placed against a continuous native soil profile. The use of large, field-like volumes of soil, access to electricity, water and other utilities with a protected below-ground environment for sensitive instrumentation and horizontal access to the root-soil system are all significant characteristics of rhizotrons. They also include visual access through transparent surfaces (Klepper and Kasper, 1994).

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How to cite this article: Reni, F., Bindhu, J.S. and Pillai, P.S. (2025). Rhizotron: A Unique Technique for Root Study: A Review. *Agricultural Reviews*. **46(4):** 676-680. doi: 10.18805/ag.R-2671.

Submitted: 25-09-2023 **Accepted:** 13-04-2024 **Online:** 04-07-2024

Rhizotron-past developments

Klepper and Kasper (1994) classified rhizotrons into four parts based on the nature of the work, accessibility of resources and other inputs for construction. The first rhizotron was constructed at East Malling Research Station, Kent, England from 1960-1961. It was constructed to research how woody perennials, especially fruit trees and shrubs, respond to seasonal fluctuations in root development and root turnover. The rhizotron which is constructed primarily to study of roots of annual plants was

Auburn rhizotron (Taylor, 1969). Ames rhizotron conducted study of the rate of soybean root growth at various phases of shoot development (Kaspar *et al*., 1978).

Similar structures have been constructed for research on sugarcane in South Africa (Glover, 1967), coffee in Kenya (Huxley and Turk, 1967), tea in Malawi (Fordham, 1972) and grape vines in Australia (Freeman and Smart, 1976). The Guelph rhizotron, erected in Ontario, Canada, in 1967 shared the same concept and objectives as the East Malling facility, with the distinction that it was separated into discrete compartments with a rebuilt native soil profile (Hilton *et al*., 1969). One of the four main root observation laboratories in the world is the Auburn rhizotron. It is the only one that is particularly focused on researching the root systems of annual plants (Taylor, 1969). Studies on the growth rate of soybean roots at various phases of shoot development have been conducted at the Ames rhizotron (Kaspar *et al*., 1978). In a controlled environment facility housed inside a building, the National Soil Tilth Laboratoryconducted research on soil tilth (Kaspar *et al*., 1992).

Five methods for acquiring images of root systems growing in rhizotrons (Mohamed *et al*., 2017)

Flatbed scanner

Two common types includes the compact, less power required CIS (Contact Image Sensor) and the higher-resolution CCD (Charge Coupled Device) scanners capable of scanning with a good depth of field. Four horizontal scans and a resolution of 300 dpi are needed for one 50 × 50 cm rhizotron.

Handheld scanner

Scans were taken by moving the scanner manually downwards on the surface of the rhizotron window which is lightweight and portable.

Manual tracing

Roots can be manually drawn using permanent color pens onto a transparent sheet that is placed over the rhizotron window if there are no electronic instruments accessible in the field. Different colors correspond to different observation times, while the clear sheet notes the date of the observation. Transparent sheets are then scanned in the laboratory.

Smartphone scanner application

Using image-processing technology, the Cam Scanner program automatically recognizes object edges and eliminates background noise. This program may return processed data in a JPG or PDF format and modifies brightness, contrast and image details. The smartphone must be held at a specific distance in order to run the program on a rhizotron in the field. In order to calibrate the scan, a fixed scale must also be scanned simultaneously.

Time- lapse camera

It allow users to pre-set regular intervals at which to shoot pictures. The cameras were positioned ninety centimeters apart from the rhizotron on a wooden cleat. Every 30 seconds, pictures can be captured. With an alkaline battery, time-lapse cameras can operate without human assistance for several months.

Different types

Minirhizotron

Bohm (1974) was the first to use the term “minirhizotron.” Bates (1937) put forth the concept and design of minirhizotron. Bates utilized lamp glass pieces that were put into the ground to observe the roots that crossed the glass. Bohm further modified Bates technique in the year 1974 utilizing a battery-powered light source. With the help of a fibre optic system, Waddington (1971) later improved the method.

Modern minirhizotrons are well equipped with colour video camera and images can be recorded on photographs or video with improved quality. Villordon *et al*. (2011) studied a scanner based minirhizotron system in sweet potato to study the adventitious root development. The system documented the transition of adventious root into pencil roots and storage roots.

Now a days, small size CD mini-rhizotrons, was the best applied to young plants and small herbaceous plants allowing for the long-term tracking of root morphologies (Cassidy *et al*., 2020). It is a creative, affordable and reusable replacement for the conventional mini-rhizotron. The thinness of the CD cover reduces the possibility that rhizotrons will conceal root phenotypic measurements in the middle of the growth media, particularly for plants with large root systems.

Above-ground rhizotrons

Apart from the under ground rhizotrons which are expensive and labour intensive, new rhizotrons which are above ground and are user friendly developed over time. The first above ground rhizotron was developed by Wright and Wright (2004). This has eight glass panels and is star-shaped; it can be used both for greenhouse or a field condition. A large volume above-ground rhizotron was built to investigate the growth of roots naturally in woody plants (Silva and Beeson, 2011). It had drainage capabilities and could be used to determine how low soil moisture levels affected root development.

Rhizobox

Wenzel *et al*. (2001) conducted studies in controlled environments using rhizoboxes. Rhizoboxes are chambers for the soil and roots that mimic naturalistic settings for studying the root system structures. Through the acrylic pane attached to it, a digital camera can be used to see the growth and spread of roots.

Rhizoponics

Hydroponic rhizotron system that provides the benefits of both access to the roots for measurements and control over the root environment. It may be utilized in controlled cabinets due to its portability and inexpensive cost. Mathieu *et al*. (2015) conducted a study on rhizoponics to understand the root system architecture of Arabidopsis adult plants.

Wetland rhizotron

In wetlands also it is possible to study the growth of roots using rhizotrons. *Eleocharis cellulosa* and *Rhynchospora tracyi*, two Cyperaceae species, were studied in wetlands to determine how they grew at various water levels and phosphate availability. The study found that while *R. tracyi* experienced smaller increases in root density, root biomass, total shoot length and shoot biomass with rising water level, *E. cellulosa* displayed bigger increases in these traits (Busch *et al*., 2006).

For measuring the root length, marking the locations on the outerside of viewing panel was initially practised. Later the grid system which include rectangular grids and imbedded wire grids are used (Huck and Taylor, 1982). Diameter and surface area of roots can be measured with greater accuracy using a microscope.

Rhizotron in soil-plant studies

A field experiment on two *Eucalyptus* stands using rhizotrons revealed that there is a strong correlation between the lengthening of fine roots and the water content of the soil at all depths (M'Bou *et al*., 2008). In order to identify which of the three species is most adapted to water stress, Dhief *et al.* (2011) examined the adaptation capacity of *Callogonium* spp. with a variety of developmental parameters. *Callogonium arich* had the highest root length, elongation rate and biomass output among the three *Callogonium* species. Rhizotrons were used by Schmidt *et al.* (2010) to track the root zone of wetland rice as it grew. On the basis of digital image analysis in a submerged soil, redox conditions were also identified using redox electrodes in paddy soil rhizotrons. Root dry weight demonstrated the most consistent genotypic performance across investigations of the root variables evaluated in over 20 root trials (Wade, 2015).

Siebers *et al.* (2019) labelled the algae *Chlorella vulgaris* with the radioisotope ^{33}P to study the algal fertiliser transformation in soil and its efficacy for plant nutrient uptake. The rhizotron tests revealed that the release and uptake of algal ^{33}P by plants, proving the appropriateness of algal fertiliser for plant growth. Schreider *et al.* (2022) carried out a rhizotron study using *Populus* × *canescens* and its compatible ectomycorrhizal fungus *Paxillus involutus*. The results indicated that an ectomycorrhiza has the potential to fill essential niches of different P sources that range in their bioavailability, showing that being a generalist in P nutrition can ease adaptation to different nutritional situations in soil.

Root system architecture

Root length, width, spread and number are structural elements of the root system architecture (RSA) that give spatial arrangement (Khan *et al*., 2016) and is a crucial rhizosphere factor in controlling soil porosity as well as the effectiveness of plant nutrient and water uptake (Helliwell *et al*., 2017; Fang *et al*., 2019).

With the help of rhizotrons Yuan *et al.* (2016) investigated the root development and root system architecture of oil seed rape under low and high soil phosphorous condition. Total root length, root tip number and root dry weight of oil seed rape were significantly reduced in phosphorous deficient condition. Gandullo *et al.* (2021) developed an economic rhizotron platform for two dimensional, non invasive method of root system architecture for tomato seedlings. The study reported that salt had a considerable impact on the overall root architecture, especially in terms of where the lateral roots were located in the soil. The soil's surface, where salt concentrates, was the area where lateral root emergence was most severely constrained.

Bagnall *et al*. (2022) reported that plant breeders can create cultivars that are more resilient to drought, have larger root biomass and utilize nutrients more effectively by using the vital information through root phenotyping. It is crucial to phenotype roots in their native habitat in order to comprehend how the soil environment influences the genotypic manifestations of roots. They developed Low-field magnetic resonance imaging rhizotron for visualizing roots in moderate to high clay soils for sorghum, demonstrating the potential for this technology for root phenotyping.

Advantages of rhizotron

- Non-destructive approach.
- Measurements taken repeatedly on the same root.
- Measure soil parameters and take time-lapse pictures.
- Information on root development rate and root density is provided.

Limitations of rhizotrons

- Lack of structural mobility.
- Finite number of replications.
- Change in soil conditions.
- The roots in bulk soil are not reflected in this.
- After each experiment, new soil is added.
- May alter population of soil micro organisms.
- Costly and labor-intensive.
- Long-term commitments and a collaborative team required.

CONCLUSION

The rhizosphere is difficult to study because of the complex interactions and also by the obscure soil. Specialised plant chambers have been and continue to be a crucial tool in studying rhizosphere in soil. The soil dynamics can be

in situ and continuously tracked by a variety of imaging techniques in rhizotron chambers with a visibly apparent rhizosphere. Rhizotrons helps to measure the rate of root growth or death as well as water and nutrient uptake in plants. Using rhizotron systems changes in soil properties have been studied with respect to root growth and its dynamics.

Conflict of interest

All authors declared that there is no conflict of interest.

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