J. Jpn. Soc. Soil Phys.
土壌の物理性
No. 95, p. 3～11（2003）

In-situ Measurements of Density and Distribution of Periphery Roots of Tomato Seedling Using Machine Vision System

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Abstract

A new, simple method for evaluating and quantifying the peripheral root and its architecture was developed and applied to tomato (*Lycopersicon esculentum* Mill.) seedlings. This method consists of the use of a transparent tray cell and application of shade in roots, using an image analysis system.

The objectives of this study were to : (1) develop an *in-situ* measurement technique using machine vision technology for the quantification of the peripheral root growth of seedlings using transparent tray cells ; (2) to develop a program to analyze the Peripheral Root Density and Distribution (PRDD) ; and (3) to study the characteristics of the seedling root system in an air-pruning tray cell. The specific PRDD value was calculated for the air-pruning tray cell and a non-air-pruning tray cell and five stages of the seedling. Analysis of the results showed that this method can be useful for evaluating peripheral root architecture in different designs of tray cell and can be used not only to analyze the effect of air-pruning but also to determine the efficient size and design of tray cells.

Key words : Root growth analysis, Machine vision, Peripheral Root Density and Distribution (PRDD), Air-pruning tray cell.

1. Introduction

Healthy seedlings of uniform size are required for transplanting in order to produce a uniform stand in the field. Many studies have indicated that seedling establishment and growth performance depend on the speed of the root system development (Huang *et al.,* 1992). Roots are the underground portions of a plant that interact with the soil. The root system of a plant may have more branches than the shoot system (Wang *et al.,* 1995).

Common problems associated with containerized plant and seedling production are root-tangling and root-spiraling, commonly referred to as “root-binding”. Root-binding deters development of the root system after the plant or seedling is transplanted, resulting in slower growth of the plant. When the plant roots emerge through the holes of a container they shrivel due to exposure with air. This is usually referred to as “root air-pruning”. Once a root has been air-pruned, the plant immediately starts a new root branch. Proper application of air-pruning to a root system therefore promotes secondary root growth and eliminates root-binding, resulting in a large root mass.

Since each variety of plant grows to a more or less recognizable form and size at each stage of growth, modeling and simulation techniques would provide an effective means for exploration and prediction of the pattern of plant growth. A simulation model can be formulated by considering factors which affect the growth and development of root system elements, such as the main roots, root branches and root hairs.

Microcomputerized image analysis (Machine Vision) is a technique which has particular merit for the evaluation of the roots of plant seedlings, since it permits direct, objective, non-intrusive visualization and measurement of both roots. The essential components for machine vision analysis of a seedling root include : 1) a standardized staging environment, 2) an image capture device (e.g., video camera), 3) a digitizer board housed in a microcomputer, and 4) display devices [monitors] for presentation and interactive measurement of the digitized image.

Evaluations of plant root systems are particularly challenging in nature, because roots are buried in soil, sensitive to excavation and intricately arranged within the soil matrix. Image analysis has proven to be an excellent tool for direct analysis of root zone data in plant production and for investigation of root growth rates. The shoot culture imaging was used for examining root zones ; close-up imaging was used in some instances to inspect root surface feature (Smith *et al.,* 1995). Pasian *et al.* (1999) developed a new simple method for evaluating and quantifying the root severity. This method consists of surrounding the root pan with a transparent film and tracing all roots with a marker. Root-length measurement is important for the evaluation of root functions and of influences of the soil environment. Accurate and rapid root-length measurement methods are, however, still under development (Kimura *et al.,* 1999). Minirhizotrons speed up research on root demography, but image quality often hampers standardization of the image-processing method. A simple procedure working within the blue band of the color image was tested on the fibrous roots of sugarbeet (*Beta vulgaris* var. Sacharifera) (Vamerali *et al.,* 1999).

Recently the use of high-resolution digital image analysis based on personal computers has increased in agricultural science, because image analysis has several advantages, including its non-destructive nature, high speed and high accuracy (Box *et al.,* 1996), (Omasa *et al.,* 1998) and (Chikushi *et al.,* 1991).

Smith (1995) indicated that because powerful image-editing software could show the number of pixels in a selected area and the color index of the selected pixels, it could easily obtain the ratio of root spot area to total root area using a simple procedure of image analysis.

Equipment to measure root area has been available for some time. A CCD camera to capture an image, and computer software, using mathematical algorithms, are used to measure pixels on the screen and to estimate the area and length of such an image. This computer technology allows easy quantification of some root characteristics and helps remove some of the subjectivity of human analysis. Although it can in theory distinguish between soil and roots based on differences in shading, its use requires the proper lighting balance to maximize contrast.

In this study, image-processing techniques are applied to describe the general growth dynamics of a root system in a tray cell. Since seedlings generally exhibit complex patterns of root development in a tray cell, a peripheral root density and distribution (PRDD) program was developed to measure the number density of roots and their spatial distribution for a seedling at a specific growth stage.

The objectives of this study were :

(1) To develop an *in-situ* measurement technique using machine vision technology for the quantification of peripheral root growth in seedlings using transparent tray cells ;

(2) To develop a program to analyze the

PRDD and ;

(3) To study the characteristics of the seedling root system in an air-pruning tray cell.

2. Materials and Methods

Experimental Apparatus and Image Acquisition

A CCD (SONY, XC-7111) color video camera was used to capture root images. The camera was fitted with a lens (VCL 25 mm, F2.2). Images were captured and digitized with a resolution of 756×486 pixels at 8 bit/pixel for each red (R), green (G) and blue (B) color component. With 8 bit color resolution, the values for each pixel ranged from 0 to 255. Captured images were stored as bit-map (bmp) files in a Gateway (288 MHz Pro) microcomputer and displayed on a Mitsubishi diamontron color monitor. Image-processing and analysis software was developed and executed on the Gateway computer and all algorithms were implemented in the Visual basic (Version 6.0) programming language. Fig. 1 is a schematic diagram of the machine vision system. Light was provided by two 300-W lamps with an input voltage of 100 V. A pneumatic control system rotated the tray cell in a circular (360° degrees) motion and controlled each step (90° degrees). The resulting digitized bmp-format image had a width of 736 pixels, a height of 560 pixels and a size of 1.209 MB. The digitized image was calibrated to record the precise dimensions of the image and relate these dimensions to the actual size of the tray cell. The carefully-calibrated, digitized image of the seedling root were comprised of a pixel array that preserved all of the morphometric (spatial) and photometric (spectral) features. In this experiment the soil (Dega Potgrand Delft, Netherland) was used soil and peat most (1/3 of total volume of the soil).

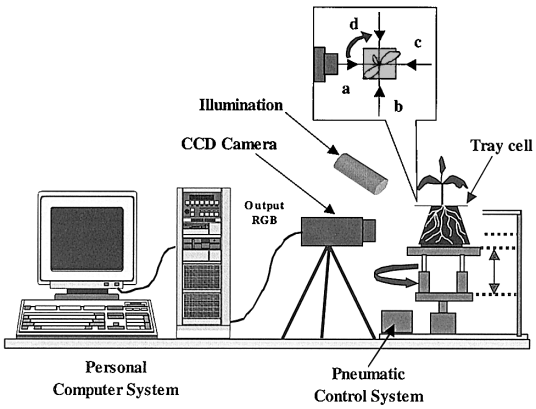


Fig. 1 Schematic of the components of the machine vision system. Artificial light was used for illumination.

Plant Material and Air-pruning Tray Cell

The design of the air-pruning tray cells was developed by Huang in 1973. Fig. 2 shows a transparent air-pruning tray cell and a black-covered air-pruning tray cell. Tomato seedlings (*Lycospersicon esculentum* Mill.) were grown in a growth chamber (Biotron 350) at an air temperature of 23℃, relative humidity 70% and a light intensity of 12,000 Lux (with a 12-h photoperiod). Detached root systems were sampled at 3-day intervals after the one-root stage. Images of the four sides of each tray cell (air-pruning and non air-pruning tray cell) were captured (Fig. 1) on five observation days from germination : 3, 6, 9, 12 and 14 days, thus providing a sample of 512 images.

Image Processing (Win-Roof)

Figure 3 a shows the procedures using the Win-Roof (Mitani Corporation Inc.) image-processing system. The original image of the root underwent the following processes. (1) Color measurement : measuring the average color of the pixels within an on-screen region. (2) Color extraction : counting the pixels of a specified color within an on-screen region and thereby obtaining the area. (3) Calibration : the root image was adjusted to give a clear contrast between the tray cell area and the root area. (4) The root image was obtained by extracting the root area from the tray cell and all exposed roots were traced in green. (5) Shading applied to seedling root. The brightness of the root in the original image varied with the position of the root. In the binary image, roots were seen clearly, even for areas of low bright-

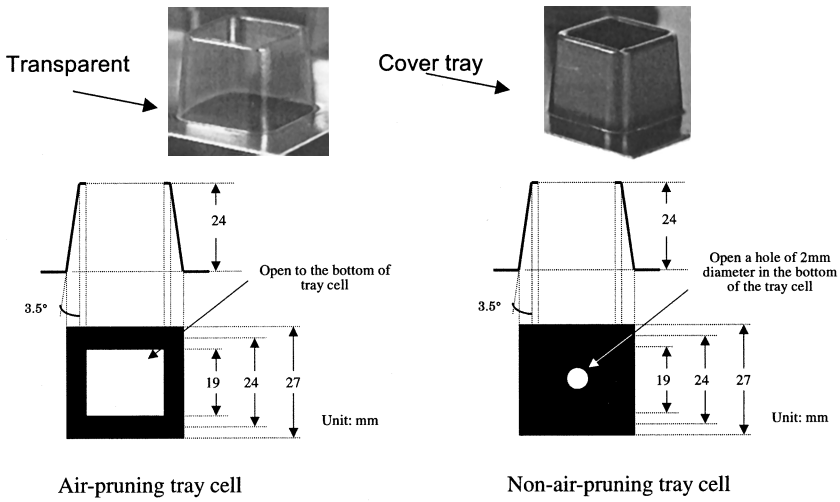


Fig. 2 The configuration of the air pruning and non-air-pruning tray cells used in the study.

ness. In the original image, the tangled roots at the base of the root system were transformed into clearer lines than those in the original or binary image. Although partial root image was lost by this operation, the loss was relatively small when compared to the total area of the root system.

Algorithm Development (PRDD program)

(Figure 3 b) shows a flow-chart of the PRDD program that was developed. The next procedure was as follows : (1) The starting point was the image of the root already processed using the Win-Roof program ; (2) Input of image data ; (3) Determination of range of image processing ; (4) Setting of conditions for root (selecting only root images from the background and counting the number of pixels in the total root area) ; (5) Setting range for all pixels ; (6) Extraction conditions ; (7) Calculation of the number of pixels (selecting only the root area and counting the number of pixels) ; (8) Repetition ; (9) Determination of root distribution for each layer and the ratio between total number of pixels for the root and total number of the pixels for one layer ; (10) Calculation of the root area as a percentage of the total layer area (pixels) ; and (11) Measurement of the total root area after evaluating the distribution of root area in the periphery of the tray cell.

Peripheral root density (PRDD) was calculated as follows :

$$PRDD\ (\%) = (TRA/TTA) \times 100$$

Where TRA is the total root area in one layer and TTA is the total area of one layer of the tray cell (both measured in mm^2). Fig. 4 showed a processed image using the PRDD program showing the distribution of root ratio in the tray cell of the eight layers.

3. Results and Discussion

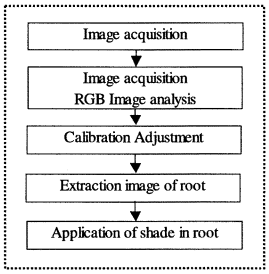
Soil luminance varied only weakly with time, depending on soil moisture and on the illumination intensity, neither of which were constant, whereas root luminance decreased progressively with time, due to senescence.

The root image obtained using the transparent tray cell method has the advantages of being less subjective and less dependent on evaluator experience. It also allows a longer period of evaluation because of its non-destructive nature. On the other hand, it re-

a)

Win-Roof

(Mitani Corporation Inc.)



b)

Peripheral root density and distribution (PRDD) program

(Program in Visual Basic 6.0)

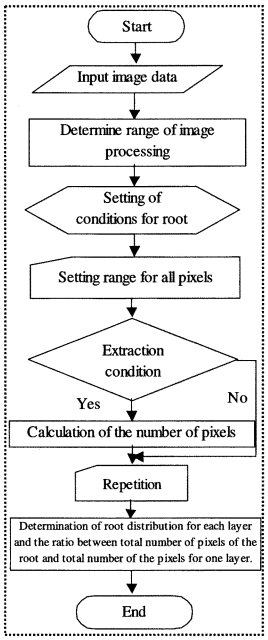


Fig. 3 The procedure involved image capture and processing, followed by measurement using an image analysis system. Flow-chart for (a) Win-Roof (Mitani Corporation Inc.) and (b) the PRDD program used to determine the root distribution in each layer (one tray cell separated into eight layers) ; see the text for detailed explanation.

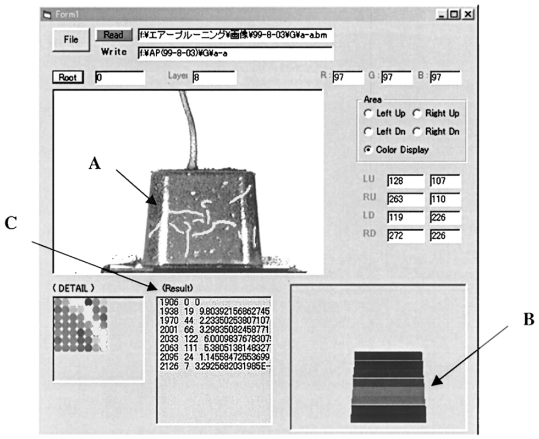


Fig. 4 A processed image using the PRDD program showing (A) a preprocessed root image, (B) the distribution of roots in the eight layers, and (C) the results of peripheral root density and distribution calculated for roots in the eight layers.

quires an expensive, although easy to use, video camera that may not be available to everyone.

The method takes into account only roots at the periphery of the tray-cell, ignoring roots located at the center. This should not be a concern because most actively growing roots of plants grown in containers tend to be at the periphery of the root area. Furthermore, in order to image roots at the center, the growing medium must be removed which can also dislodge damaged roots causing an increase in the evaluation error. This problem may be aggravated with vegetable plants, such as the tomato used in this study. In the case of root areas where there are numerous fine roots too close to each other to trace individually, the root image analysis method allows for area measurement of only total root areas of high and low root growth.

The results of image analysis demonstrate that shortly after the first branching, a second branching occurs midway through the tray depth in a pattern similar to the first branching. Stage 3 [air-pruning - preprocessing image (AP-B)] shows dominant downward and diagonal root growth. At stage 5 (AP-B) the cell shows “filling” of the upper soil layer with roots are ready to produce new roots as soon as the seedlings are transplanted. This process can be observed using the PRDD program by relating the root growth, the root growth rate, cell size, branching and sub-branching rates, as shown in several stages of air-pruned root development in Fig. 5. Seedling production can

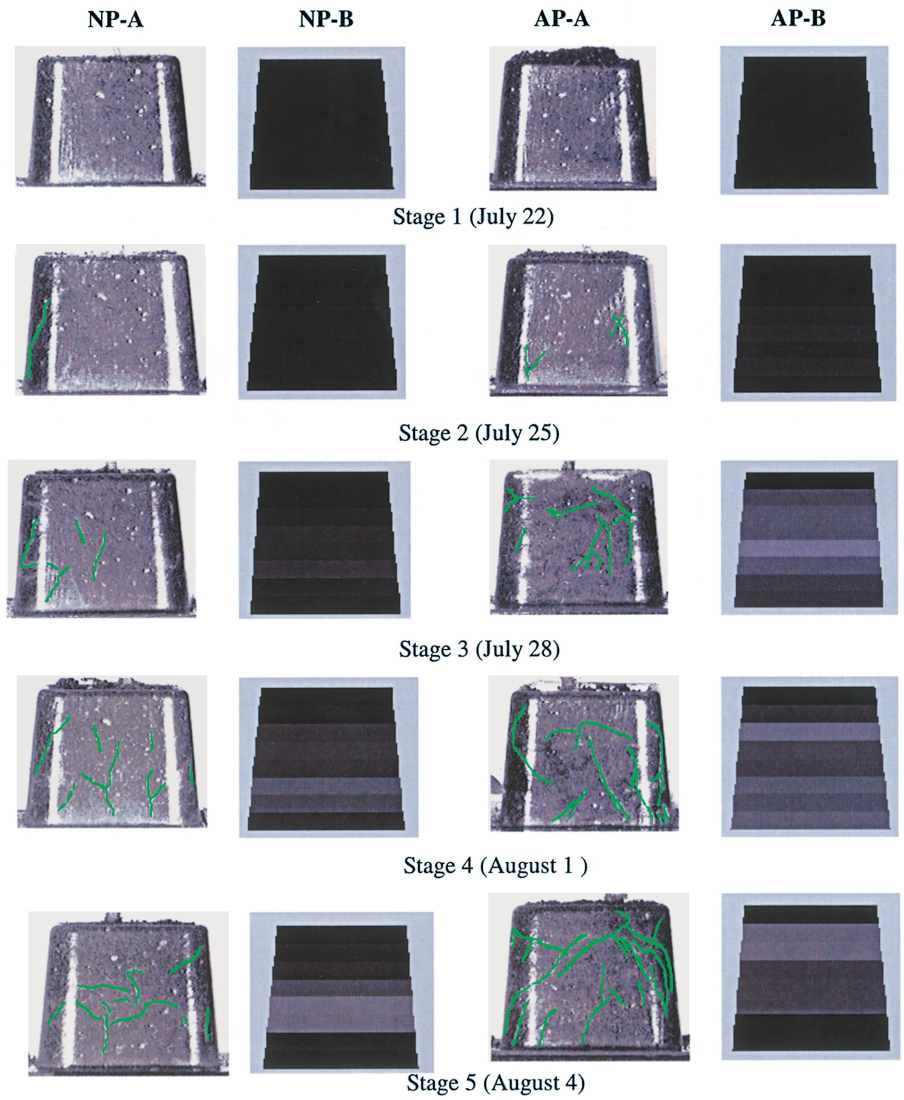


Fig. 5 Five stages of shoot and root development of tomato (*Lycospesicon esculentum* Mill.) seedlings using an air-pruning (AP) and non-air-pruning (NP) tray cells. The image processed using the PRDD program is shown for air-pruning (AP-B) and non-air-pruning (NP-B) tray cells. Stage 5 (NP-B) shows dominant downward and diagonal root growth while stage 5 (AP-B) shows "filling" of the upper soil layer with roots, stage 3 (AP-B) shows rapid elongation of the stem while stage 3 (NP-B) shows low growth.

be optimized according to plant species, minimum time requirement and cell size. For the non-air-pruning tray cell at stage 3 (NP-A), the first root continues to elongate, spiraling around inside the base of the container cell. At stage 5 (NP-B) the peripheral root can be seen to be concentrated around the base of the tray cell. This would result in severe root-binding.

The results using the PRDD program to calculate peripheral root area distribution for the air-pruning tray cell. The program determined the distribution of the roots among eight layers for each cell. The value of the area of the root is the average of four repetitions. Images of

each cell were captured for all four sides.

Figure 6 shows the root distribution profile of the air-pruning tray cell with depth. The results demonstrated a significant development of the root 9 days after germination (August 1). The air-pruning effect could be observed by the development of the root through the center of the tray cell (depth : 1.5–4.0 cm).

Figure 7 shows the root distribution profile of the non-air-pruning tray cell and depth. The results demonstrated non-significant development of the root on all data collection dates. The difference between the maximum area of the root using the air-pruning and non-air-pruning tray cells was 67.85%. The root growth process illustrates that root development within a air-pruning tray cell is far superior to non air-pruning tray cell.

The advantage of the present system is its

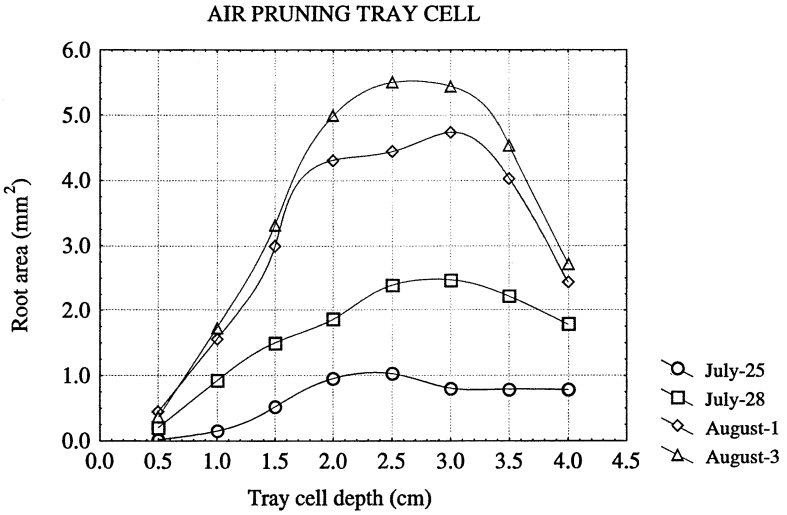


Fig. 6 Root distribution profiles and depth of tomato seedling in air-pruning tray cell.

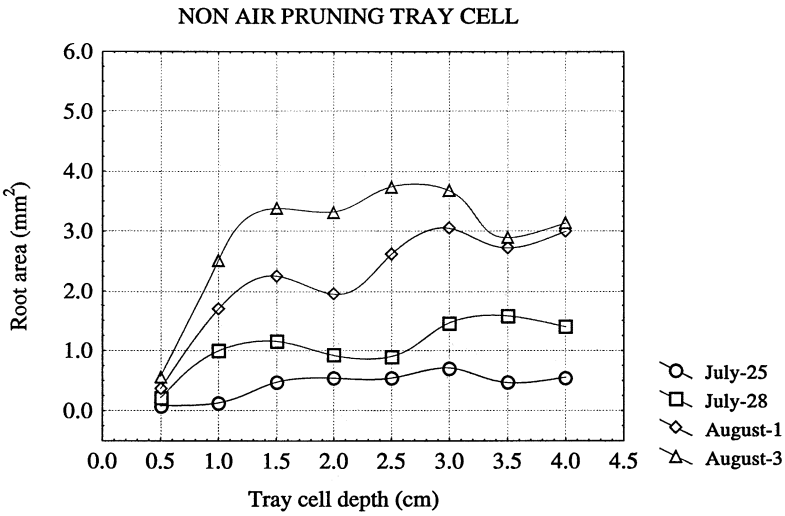


Fig. 7 Root distribution profiles and depth of tomato seedling in non-air-pruning tray cell.

rapid processing time. Measuring a series of original color images and reconstructing the corresponding surface image with color texture can be accomplished automatically in a few minutes, which is probably less time than is required by other methods. Moreover, by incorporating the software that we developed, the resulting surface image can be observed the distribution of the root in layers (8 layers).

4. Conclusion

The newly developed method using machine vision provides good results when used to measure peripheral root area. The most important information discovered from the study was :

(1) The results analysis showed that the present method can be useful for the measurement of peripheral root density (PRDD) and architecture in different designs of tray cell, in order to understand the characteristics of the seedling root system ;

(2) The use of transmitted light allowed us to acquire a very clear image, by which we could quantify, the root spot severity precisely and easily. This result suggested that image analysis using transmitted light could be a very useful tool to quantify root area in plants with very complex root growth patterns, such as tomato seedlings.

(3) The method takes into account only roots at the periphery of the root area, ignoring roots located at the center.

Future work might investigate a discrimination analysis taking into account features such as root texture and soil color. The fundamental study to discover new and efficient designs of tray cells will be necessary.

Acknowledgments

The authors would like to thank Mr. Ryuichi Ikuta (Dia-Topy Inc., Japan), for his cooperation in the design of the transparent tray cell used in this study. Special thanks go to Mr. Hiroyuki Okawa and Hiroko Nakazato (Graduate student, Department of Bioenvironmental & Agricultural Engineering, Nihon University), for his assistance in acquiring seedling root images.

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マシンビジョンによる苗の根量と根系構造の評価法に関する研究

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要　　旨

苗の根系構造を評価，定量する簡単な方法を検討した。これは，透明なトレイセルを試作し，画像解析システムで測定する方法である。本実験で対象にした苗はトマト（*Lycopersicon esculentum* Mill.）苗である。本研究の目的は ⑴透明なトレイセルを用いてマシンビジョンにより育苗中の根の生長を定量的に計測する技術の開発 ; ⑵トレイセルの縦壁面で観測される根の面積（PRDD）を解析するプログラムの開発 ; ⑶エアプルーニングトレイセル内における苗の根の生長を 5 段階に分けて各段階での PRD 値の特徴の把握などをすることにある。計測結果の解析から，根の発育状況を表すための PRD 測定や，形状の違うセル内での根端構造の解析のために，この方法が使用できることが分かった。また本法はエアプルーニングの効果やトレイセルの適切なサイズおよび形状の設計にも利用できると考えられる。

キーワード : マシンビジョン，根の測定，根の密度，エアプルーニング・トレイ・セル

受稿年月日 : 2000 年 6 月 20 日
受理年月日 : 2001 年 9 月 3 日