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A Minirhizotron Method for Measuring Root System of Soybean Plants Growing in the Field*

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Abstract: In order to develop a simple and accurate system for measuring the root system of soybean plants growing in the field, a fiber-optic scope with a portable color video tape recorder was employed in a minirhizotron system. A transparent acrylic tube of 60 mm outer diameter and 54 mm inner diameter as the observation tube was installed into the soil at an angle of 30° from the vertical. The accuracy and the utility of the system were examined.

(1) The length of all tap and branch roots at the observation tube-soil interface was measured with an accuracy of 5% using this system. (2) The observation tube did not significantly disturb root penetration into the soil. This was also suggested by the results that the changes in root length with the increase of soil depth showed a reasonable tendency without an unusual increase or decrease. (3) There was a significant linear correlation between the root length measured with the minirhizotron system and the root length density measured by a soil-core sampling method in the plants growing in the field. However, variation in the measurements taken with the minirhizotron system was as large as those by the soil-core sampling method, indicating that the root distribution in soil was extremely non-uniform. From these results, it was possible to estimate root growth in the field well using this system. Moreover, taking as many measurements as in the soil-core sampling method, root distribution in the field can be estimated quantitatively with less labor and time with the minirhizotron system than the conventional method.
Key words: Fiber-optic scope, Minirhizotron, Root length, Root length density, Root system, Soil-core sampling method, Soil moisture, Soybean.

圃場に生育するダイズの根系をミニリゾトロン法を用いて測定する方法の検討：平沢　正・武居理英・石原　邦（東京農工大学農学部）

要　旨：圃場に生育する作物の根系の発達程度を簡易に測定するために，ミニリゾトロン法にファイバースコープとカラービデオを採用し，ダイズを用いて測定精度の検討を行った．圃場には外径 60 mm，内径 54 mm の透明アクリル管を 30 度の角度で埋設した．

(1) 本装置によって管壁に現れたすべての主根，分枝根の長さを約 5% の精度で測定できた．(2) 圃場に埋設したアクリル管は根の伸長方向に著しく影響を及ぼすことはなかった．このことは，根長は土壌が深くなるに伴ってある一定の傾向をもって変化し，不規則に増減することがないことからも推察された．(3) 圃場に生育するダイズについて，コアサンプリング法で測定した根長密度と本装置で測定した根長を比較したところ，両者には密接な直線関係が認められた．両方法による測定値はほぼ同じ程度に大きく変動した．このことは土壌中の根の分布がかなり不均一であることを示している．以上の結果から，本装置によって圃場に生育するダイズの根の生長パターンを推定することができ，さらに，コアサンプリング法と同程度に測定数を増せば，従来の方法に比較して簡易に根系の発達程度を量的に測定することができると考えられた．
キーワード：コアサンプリング法，根系，根長，根長密度，ダイズ，土壌水分，ファイバースコープ，ミニリゾトロン．

Roots have important functions for the performance of crop plants[18,23]. Not only root physiological activities but also root system development affect the root function of a whole plant. For example, root system development was responsible for the difference in drought resistance among species[1], among cultivars[3,8,28] and between plants grown under different conditions[10]. Root system development might affect leaf senescence[9,14]. Therefore, many researches have been done on root system development of crop plants. Estimating root system development of crop plants grow-

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ing in the field by a conventional method requires a great deal of labor and time[2]. Observing the root system of crop plants like rhizotron under field conditions also requires extremely expensive and large facilities.

The minirhizotron system is a simple method for observing roots which intersect the surface of a transparent tube inserted in the soil[2,4,5,13,21,24,27]. The minirhizotron method was designed more than 50 years ago and had been developed by many researches[21]. However, it has not been employed often in measuring root systems in the field probably because of a lack of precise investigation into how to employ it and on accuracy. Root distribution patterns in the field and morphology of roots constituting root systems differ among crop plant species[15,16,19,20,23,25]. In order to employ a method for estimating root growth and distribution in the field, the accuracy of root length measurement and the effect of the method on root system development should be investigated in each crop under investigation[21]. Unfortunately, such a research has not been done yet, apart from a few reports[4,21,27], although many reports on the equipment have been carried out.

In this study, a fiber-optic scope, which has been well developed for many purposes, with a portable color video tape recorder, was employed in the minirhizotron system. The accuracy of measuring the length of root intersecting the surface of the observation tube and the effect of the tube on root elongation and on root system formation were investigated in the field. Moreover, rooting density estimated with this system was compared with root length density measured by the conventional method in the field. Based on these results, we discussed the accuracy and the utility of the minirhizotron system for researching root system development in soybean plants growing in the field.

Materials and Methods

1. Plant Materials

Soybean plants (*Glycine max* (L.) Merr. cv. Enrei) were grown in 1/2,000 a Wagner pots and in the field. The plants were grown at the rate of 2 plants in a pot filled with Tama River alluvial and Kanto diluvial soils (1 : 1, v/v). Fertilizer was applied at the rate of 1.0, 1.0 and 1.0 g/pot of N, P_2O_5 and K_2O, respectively. At the 7th leaf expanding stage, the soil was washed away gently and roots were fixed with formalin-acetic-alcohol (FAA). In the field, the plants were grown at the rate of 10 or 20 plants per m in N-S rows at 0.60 m spacing. Fertilizer was applied at the rate of 30, 100, 100 Kg/ha for N, P_2O_5 and K_2O, respectively.

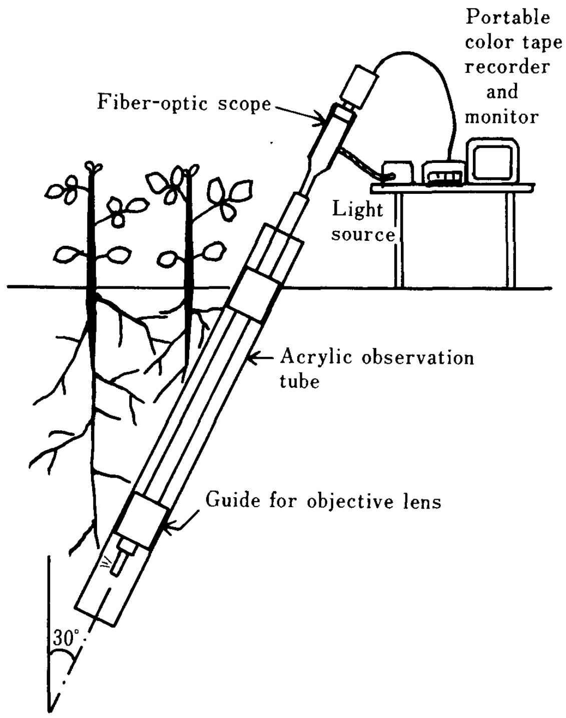

Fig. 1. Minirhizotron system examined.

2. Minirhizotron system

(1) Equipment : A root observation apparatus is shown in Fig. 1. The larger the visual field of objective lens, the smaller the magnification in a fiber-optic scope (model IF11D4 ; Olympus). Because this system was for measuring length of tap and branch roots, rather than for observing the fine structure of a root, such as a root hair, we perferred an objective lens with a larger visual field. A lens with 120° field was loaded into the fiber scope. A transparent acrylic tube of 60 mm outer diameter and 54 mm inner diameter was used as the minirhizotron observation tube. The distance between the inner surface of the observation tube and the fiber-scope lens was kept constant at 22.5 mm using a lens guide apparatus. According to an industry construction manual, magnification in this system was about × 1. Each picture was recorded on a videotape with a portable color videotape recorder (model BR-1600 ; Victor).

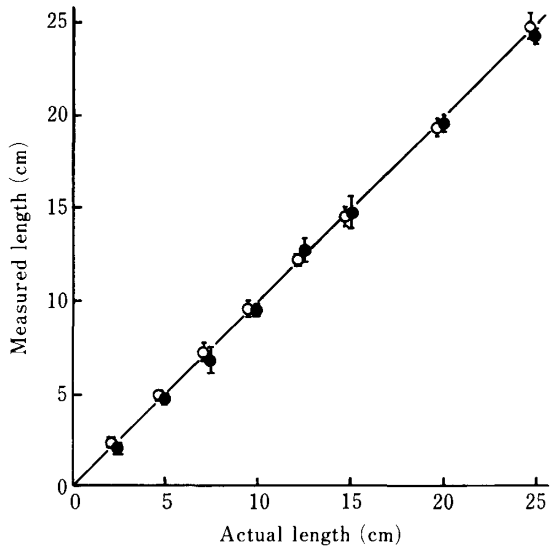

Fig. 2. Length of copper wire* on the observation tube measured with the minirhizotron apparatus. Solid and open circles represent 0.32 mm and 0.11 mm of wire diameter, respectively. Vertical bars represent the standard deviations calculated from five repeated measurements. The line represents values of equal length.

*Wire segments used were from 2.5 to 5.0 cm in length.

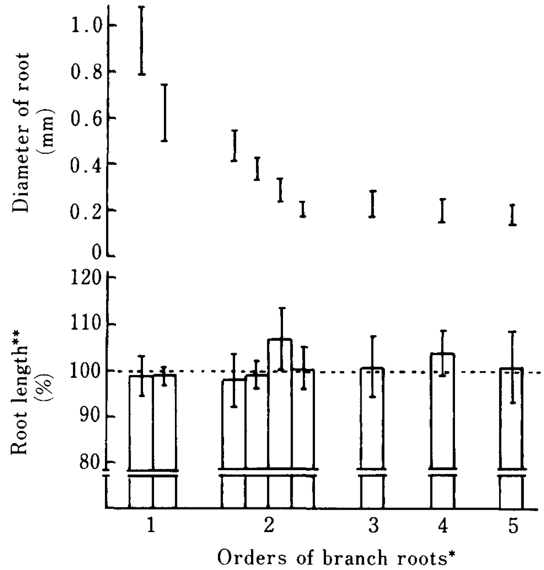

Fig. 3. Length of washed roots on the observation tube measured with the minirhizotron apparatus.

*1, 2, 3, 4 and 5 represent the branch roots of the first, secondary, tertiary, fourth and fifth order, respectively.

**The ratio of the length measured with the minirhizotron apparatus to the actual length measured by the line intersect method. Vertical bars represent the standard deviations calculated from three repeated measurements.

(2) **Root length measurement** : Length of roots in a given central portion (12 cm^2 (40 by 30 mm)) of the visual field was measured by the modified line intersect method[26] as follows : A piece of white paper with 5 × 5 mm grid squares was put on the outer surface of the observation tube, which was recorded with the videotape recorder. A picture of the squares was reproduced on a TV monitor and traced on a thin transparent plastic film. Putting the film on the monitor, the picture recorded in the field through the fiber scope was reproduced on the monitor. Root length within a given central portion of the picture was estimated by counting the number of intersections of roots with grid lines. Root length measured with this system was expressed as root length (mm) per unit surface area of observation tube (cm^2).

(3) **Observation tube installation into a field** : The observation tubes were equipped in the row before planting. The tubes were installed into the trenches in the field at a 30° angle from the vertical[4]. Field soil was returned carefully into the trench in order to minimize voids at soil-tube interface. The above-ground part of each tube was covered with aluminum foil to prevent light reaching the roots.

3. Root length density and soil water potential

Soil cores taken from the field with a sampling tube of 32 mm diameter[6] were washed gently with tap water for root collection. Root length was measured by the line-intersect method[26] and expressed as root length (mm) per unit soil volume (cm^3). Soil water potential was measured with a tensiometer.

Results and Discussion

1. Accuracy of the system

First of all, we measured the length of bare copper wire segments using the minirhizotron system. They were put randomly within a square of 4 by 3 cm on the outer surface of the observation tube. The mean of the measurements for each length of the wires of 0.32 and 0.11 mm diameter closed very well to the actual length and the standard deviation was very low for each measurement (Fig. 2). It was also clear that the location of the wire in the visual field did not affect the accuracy. The length of the wire was able to be measured accurately both in the marginal and central portions in the square (data are not shown).

FAA-fixed roots of soybean plants grown in a pot were classified into 9 groups according to branch order and thickness. After the length of the root segments was measured by the line-intersect method for each group, the segments were put within the square on the outer surface of the observation tube and their length was measured with the minirhizotron system. The diameter of soybean roots ranged from more than 0.5 mm for branch roots of the first order to about 0.2 mm for branch roots of the tertiary, fourth and the fifth orders (Fig. 3). For every root group, the ratio of the length measured with the minirhizotron system to the actual length was from 96% to 105% (Fig. 3).

The accuracy of the *in situ* measurements of root length of soybean plants growing in the soil was examined. The plants were grown in a styrofoam box filled with soil and the observation tube was equipped horizontally in the soil before planting. When several roots intersected the surface of the tube, the length of roots wihin the square area of 4 by 3 cm, previously indicated with water-insoluble paint, was measured using the minirhizotron system. The length of the roots was measured as follows: (1) the tube was split longitudinally into upper and lower halves without disturbing soil and roots; (2) roots were traced on the inner wall of the observation tube with a fine pen; (3) soil was removed from the observation tube; (4) a piece of white paper with 5 × 5 mm grid squares was put on the outer surface of the tube; (5) the legth of the traced line was measured by the line-intersect method. For every measurement, the ratio of length measured with the minirhizotron system to the length by the line-intersect method was from 95% to 106% (Table 1). The roots observed on the surface of the tube consisted of a tap root and the roots of the first to the fifth branch orders. These results indicated that the minirhizotron system could measure the length of all tap and branch roots intersecting the observation tube.

Table 1. Root length of soybean plants measured with the minirhizotron system installed in the soil of a styrofoam box.

Box number	A* (cm)	B** (cm)	(A/B) × 100 (%)
1	9.8	10.2	96.1
2	15.7	16.5	95.2
3	15.3	15.7	97.5
4	7.1	6.7	106.0
5	7.1	7.1	100.0

*Length of roots in 12cm^2 of a given area of the observation tube measured with the minirhizotron system.

**Length of roots in the same area measured by the line intersect method.

2. Field measurements

The soil around the observation tube was removed carefully to investigate the influence of the observation tube on the root system development of the plants growing in the field. Nearly all roots, coming across the tube, elongated around the tube temporarilly and then went away. Few roots continued to elongate along the tube. No higher concentration of roots at the tube-soil interface than that in bulk soil[20] was observed. The observation tube might not significantly affect the root penetration into the soil, therefore, root system development.

Repeated measurements can be taken on the same plants with the minirhizotron system. This will be of great advantage in the investigation of the root growth for soybean plants growing in the field. In order to examine this, the development of the soybean root system was measured with the minirhizotron system by changing field conditions. Fig. 4 shows vertical profiles of root distribution at various growth stages. Root length was measured at every 5 cm of soil depth on upper half of the observation tube and two measurements were averaged. Root length changed with the increase of soil depth. Root length was high at 5 cm in soil depth from the surface at each growth stage. With the increase of soil depth, root length decreased. At the 6th leaf expanding stage, there was another peak of root length at 45 cm in soil depth. At the flowering and the ripening stages, there were another two peaks of root length at 45 cm and 85 cm in depth. Before reaching the peak and after leaving it, root length increased and decreased gradually, respectively. No unusual increase or decrease was observed in the vertical profiles

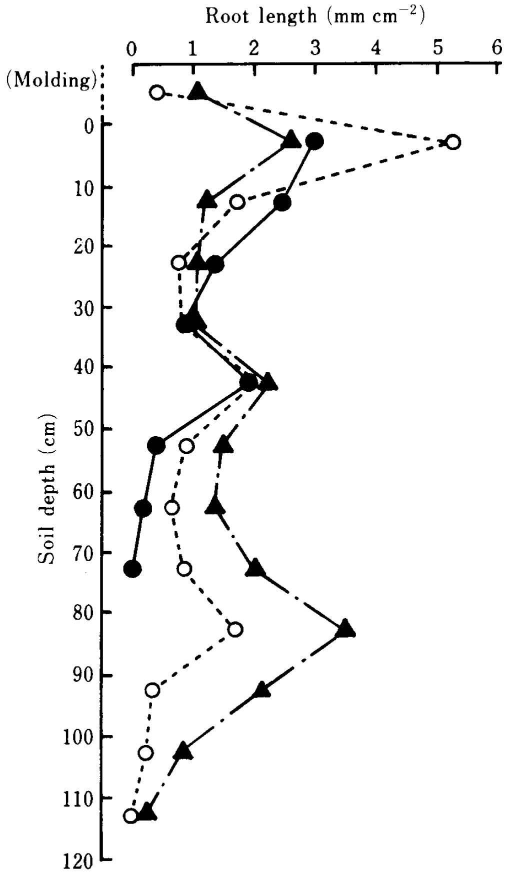

Fig. 4. Changes of vertical root distribution measured with the minirhizotron system in soybean plants growing in the field. Root length was measured on the upper half of the observation tube. Solid circles, open circles and solid triangles represent the measurements at the 6th leaf expanding stage, the flowering stage and the ripening stage, respectively. The plants were grown under deficient soil moisture conditions after flowering by withholding irrigation and intercepting rain-water.

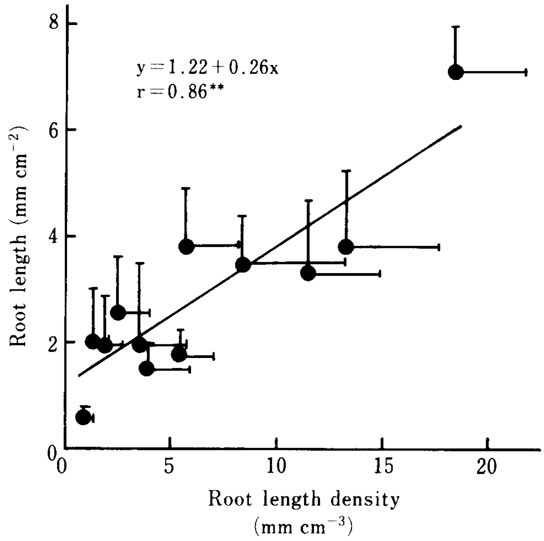

Fig. 5. Relationship between root length density measured by a soil-core sampling method* and root length measured with the minirhizotron system** in soybean plants growing in the field. Vertical, horizontal bars represent the standard deviations calculated from five measurements in root length and root length density, respectively. Measurements were taken at the different planting densities and soil depth from surface to the depth of 40 cm.

*Soil cores were taken every 20 cm in depth from the surface.

**Root length was measured at every 5 cm of soil depth on the upper half of the observation tube and the average of every 20 cm in depth was calculated.

of root length. These vertical profiles of root distribution indicate that the installation of observation tubes did not affect root system formation significantly. With the growth progress from the 6th leaf expanding to the flowering stages, roots penetrated to the deeper soil layers and root length increased in both the surface and the deeper soil layers. The plants were grown under deficient soil moisture conditions after flowering by withholding irrigation and intercepting rain-water. During grain filling, root length increased in the deeper soil layers and decreased slightly in the surface soil layers. Especially, the increase in root length with time was quite clear in layers deeper than 50 cm from the surface. These results coincided well with previous reports[11,12,22]. Thus, it was concluded that root growth of soybean in the field could be estimated adequately with the minirhizotron system.

To estimate a quantitative root system development of soybean plants growing in the field with the minirhizotron system, the root length density and the root length were measured by a soil-core sampling method, which is one of the conventional methods for quantitative measurements of rooting density in the field, and with the minirhizotron system, respectively. Before planting, surface soil to 40 cm in depth was taken out from the field, mixed up and then returned to the field. The observation tubes were equipped to 40 cm depth in a row as mentioned above. The soil cores were taken from the regions between individuals in the row. The larger the root length density, the larger was the root length.

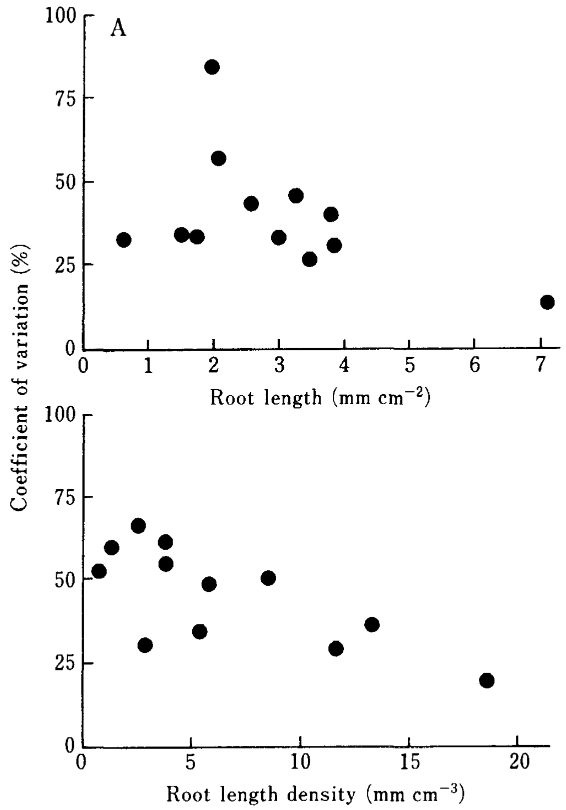

Fig. 6. Coefficient variation plotted against root length (A) measured by the minirhizotron system and root length density (B) measured by the core-sampling method.

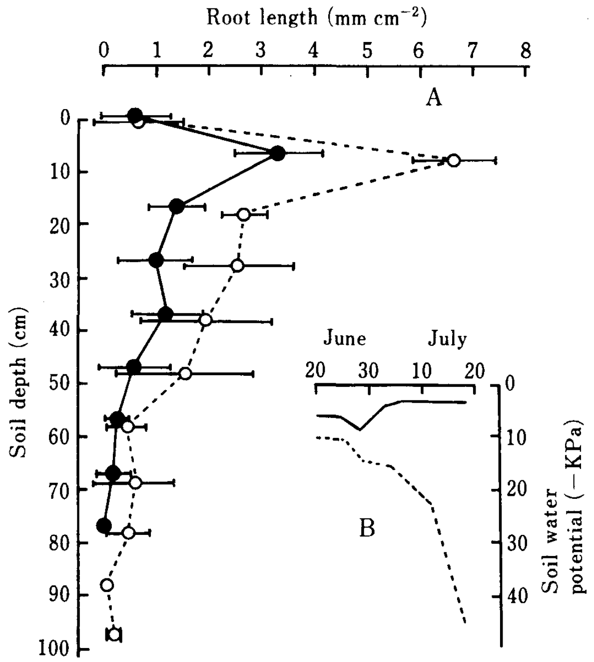

Fig. 7. (A) Difference in vertical root distribution of soybean plants under sufficient soil moisture conditions (solid circles) and under deficient soil moisture conditions (open circles). The length of roots was measured with the minirhizotron system at the 6th leaf expanding stage (July 17). Root length was measured on the upper half surface of the observation tube. Horizontal bars represent the standard deviation calculated from five measurements. (B) Changes in soil water potentials at 30 cm in depth in sufficient (solid line) and deficient (dotted line) soil moisture.

The root length, therefore, correlated linearly with the root length density (Fig. 5). This indicated that comparison in rooting density could be possible between soybean plants growing in the field using the minirhizotron system. Standard deviations were large in root length as well as root length density (Fig. 5), as had been previously reported[7,26]. The coefficient of variation in root length measured with the minirhizotron was about 50% at around 2 mm cm^{-2}. It decreased with the increase in the root length and reached 13.2% at 7.1 mm cm^{-2} of the root length (Fig. 6A). The coefficient of variation in root length density measured by the core-sampling method was about 50% at around 3 mm cm^{-3}. It decreased with the increase in the density and reached 19.8% at 18.5 mm cm^{-3} (Fig. 6B). These results mean that root distribution was extremely non-uniform in the field even at the same soil layers, and, therefore, that taking many repeat measurements was required for estimating root system development quantitatively in the field using the minirhizotron and soil-core sampling methods.

Quantitative comparison of rooting density was made in the field using the minirhizotron system. It is well known that plant root system develops well under deficient soil moisture conditions compared with sufficient soil moisture[9,10,11,12,22]. Root length of soybean plants was compared under different soil moisture conditions after emergence of the seedlings (Fig. 7). A maximum root length was observed at 5 cm of soil depth in both plants and root length decreased with the increase of soil depth in both plants. Roots penetrated to deeper regions of the soil under deficient soil moisture conditions than under sufficient soil moisture conditions. Mean root length was larger in all soil layers under the dry conditions than under wet conditions. The same results were obtained by the soil-core sampling method[10]. These results indicate that the difference in root density between soybean plants growing under various field conditions can be estimated quantitatively using the minirhizotron system.

In the soil-core sampling method, taking the soil cores and washing soil away for roots require a great deal of labor and time. Therefore, it is not easy to increase measurements. On the other hand, it took only a few days for one person to measure root length at every 5 cm of depth on 10 observation tubes installed at 120 cm of soil depth in this system. Therefore, the minirhizotron method allows many measurements without a remarkable increase in labor after the installation of the observation tubes in the soil and measurements can be repeated on same plants. We installed observation tubes into trenches in the field. In this case, disturbing the profiles of soil texture and density might affect the degree of root system development. It could not be known how disturbed profiles of soil texture and density affected the root system development. The observed pattern of root system development with this system was quite similar to the earlier measurements in the field taken without soil disturbance. This suggested that comparison of root growth and rooting density could be made between the plants growing under various field conditions. The *in situ* quantitative measurement of rooting density would be required to install the observation tubes without disturbing the soil conditions. However, even in an installation with an auger without disturbing the soil, voids between the soil-tube interface could be hardly avoided and might affect root growth and distribution. Significant compaction of soil adjacent to the observation tube might affect rooting density several months after installation[21], resulting in the reduction of rooting density at the tube-soil interface. Therefore, the effects of installing observation tubes on root system development should be always considered irrespective of the installation method of the tube. The minirhizotron system should be a useful and labor-saving technique for measuring the root system of soybean plants growing in the field.

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