

## Method for Studying Root System

### V. Analysis on the Root System Structure of Soybean (*Glycine max* Merr.) by Using Pipe Model

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#### Summary

In this study the complicated structure of a soybean root system was analyzed to see how root structure of this plant fits the "ideal pipe" model. The methods used for investigating this model was to measure the total volume of the root system and the root volume per root diameter class. For simplicity the root system was separated into two distinct groups: thick roots, conforming to the pipe model, and thin roots, non conforming to the model. These two groups maintained their quantitative balance. The progress in the root nodule formation, also shown in a graphic form, fit with the "ideal pipe" model. It was found that thin roots have more nodules than large root. But large root nodules (with higher nitrogen-fixing activity) were found to be uniformly distributed across all root diameter classes. In conclusion, the "ideal pipe" model can be applied to study not only the structure and the quantity of a root system but the distribution of root nodules in the root system as well.

**Key words:** Pipe model theory, Root nodule, Root system, Soybean.

#### Introduction

Shinozaki<sup>1)</sup> and Yoda<sup>2)</sup> have advocated the "pipe model theory", which analyzes the quantitative relationship between leaves and stems of a tree, as a bundle of uniform pipes each crowned with a certain volume of leaves. This theory has made possible to estimate the volume of wood from two measurements, the breast diameter and the height of a tree. We intended to apply the "pipe model theory" to a root system of the annual plant, soybean, which has a tree-like ramification, in order to analyze its quantity and structure<sup>3)</sup>. The results suggested that the theory was applicable to thickening part of the root system, but not to the part of thin roots<sup>4)</sup>. In addition, interested in the relationship between the thickness of roots and their internal structure, we investigated the proportion of the central cylinder in the cross section of the roots. The result was that the proportion was greater at a root diameter of 1 mm or more, and smaller at less than 1 mm, indicating thick roots play a role in the conducting function and thin roots in the absorbing function, bordering at 1 mm in root diameter<sup>5)</sup>.

The present study analyzed the changes in structure of a soybean root system with

growth by using "ideal pipe" devised based on the pipe model theory. Additionally, we also observed the formation of root nodules progressing with root growth to investigate the relationship between the structure of the root system and the trend in the root nodule formation.

## Materials and methods

### 1. Construction of "ideal pipe" based on the pipe model theory

To make ideal pipe, the root system is divided into segments of uniform length (Fig.1). The root segments are classified by diameter into root diameter classes (Fig.1:A-C). For each root diameter class, a disk is made whose volume is equal to the root volume of the corresponding class, and whose height is the range of root diameter for each class(D). The root disks are piled from the disk for the thinnest roots in order. The pile of root disks is supposed to take a cylindrical form with a uniform thickness over the range of root diameter that conforms to the pipe model theory. The ideal pipe was indicated in half vertical section, with the center line as y-axis and the radius as x-axis(E).

In the actual operation, all roots of the root system were cut into segments of approximately 1 cm long. Five thousand of them were randomly selected, the diameter was measured, and the frequency distribution obtained. Root length per diameter class was estimated by converting the root length per unit weight, and, based on these values, disks for root volume were assumed and piled in order.

### 2. Investigation of root nodule formation

① In counting the number of root nodules per root diameter class, they were divided into two types by size; those 3 mm or more in diameter, and those less than 3 mm, since the activity of nitrogen fixation in root nodules varies with their size. The number of root nodules per root diameter class was shown in a graphic form and overlaid on the ideal pipe. ② The volume of root nodules per root diameter class was calculated from the number and the diameter of the nodules found in each class. Also in this case, nodules were divided into two types by size. For each root diameter class, a disk of root nodules was assumed whose volume was equal to the volume of root nodules found in the corresponding root class, and

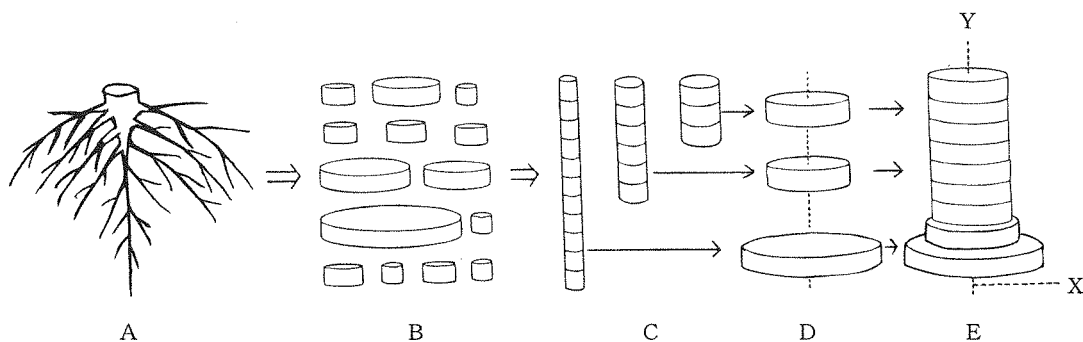


Fig. 1 Making and indication of "Ideal pipe" of a root system.

A : soybean root system, B : root segment of unit length, C : classification according to their diameter, D : ideal disks; E : "Ideal pipe", X : horizontal axis, Y : vertical axis.

whose height, or the range of root diameter of each class. The root nodule disks were piled in order, from the disk of nodules formed in the thinnest roots. The volume of root nodules per root diameter class was shown in a graphic form in half vertical section and overlaid on the ideal pipe, with the center line as y-axis and the radius as x-axis.

### 3. Cultivation and collection of materials

The variety tested in this study was Murayutaka, and the soil and containers used were clay loam and 1/2000a Wagner pots. For fertilization, nitrogen, phosphorus and potassium were used as basal dressing of 3, 3 and 4 kg/10a, respectively, but no top dressing was applied. Pots were set in the field of the Agriculture Department. Three seeds were sown in the center of each pot on June 28, 1994. After emergence, one exhibiting good growth was selected from every three plants and singled. From day 10 of seeding to day 60, 3 plants were collected at 10-day intervals, freed of adhering soils, and investigated for the formation of root nodules and the structure of the root system according to the above-mentioned procedures. Flowering and podding began about day 50.

## Results

### 1. Growth of underground parts

#### 1. 1. Root length, root dry weight and root volume

Root length markedly increased between days 20 and 30 and between days 40 and 50, and finally reached 1550 m on day 60. Root dry weight increased at a rate of approximately 0.15 g/day from day 20 to day 40 and at a rate of approximately 0.28 g/day thereafter, and finally reached a total weight of 9.1 g on day 60. The total volume of the roots, like their length, rapidly increased between days 20 and 30 and between days 40 and 50, and finally reached approximately 110 cm<sup>3</sup> (Table 1).

#### 1. 2. Root growth expressed in the form of ideal pipe

In the ideal pipe (Fig.2), every radius of root diameter class uniformly increased in the part of thick roots, at a root diameter of 1.5 mm or more. For the part of thin roots, at a root diameter of less than 1.5 mm, the maximum radius of the ideal pipe (hereafter, referred to as peak) was 0.6-0.7 mm on day 10, and 0.2-0.3 mm on and after day 20, showing marked increase with the peak. The root volume for the thin-root part rapidly increased

Table 1. Growth characteristics of a root system.

Day after sowing	Total length (m)	Dry weight (g)	Total roots volume (cm <sup>3</sup> )	Thickening roots <sup>1)</sup> volume (cm <sup>3</sup> )	Fine roots <sup>2)</sup> volume (cm <sup>3</sup> )	Fine/Total (v/v %)
10	30	0.2	1.5	0.3	1.2	80.0
20	80	0.7	8.9	1.9	7.0	78.7
30	550	2.0	38.6	2.2	36.4	94.3
40	740	3.6	49.5	6.3	43.2	87.3
50	1300	6.8	82.5	9.4	73.1	88.6
60	1550	9.1	109.9	10.3	99.6	90.6

1) : root diameter  $\geq 1.0$ mm, 2) : root diameter  $< 1.0$ mm.

between days 20 and 30 and between days 40 and 50. At the flowering/podding stage, the ideal pipe took a cylindrical shape with uniform thickness over the range of root diameter at 1.5 mm or more, while in the range less than about 1.5 mm, a great quantity of thin roots was indicated with the peak class.

## 2. Formation of root nodules

### 2. 1. The number and the volume of root nodules

The formation of root nodules was found from day 10 onward. The number of root nodules rapidly increased between days 30 and 40, and reached a total number of 1419 on day 50, but slightly reduced by day 60. For the large nodules sized 3 mm or more in diameter, in which nitrogen fixation is more activated, their number was 0, 12, 81, 101, 128 and 172 at 10-day intervals, respectively. This indicates that the increase in the number of root nodules on and after day 40 was owing to the small nodules less than 3 mm in diameter (Table 2). The volume of root nodules rapidly increased between days 20 and 30, and continued gradual increase thereafter. The increase in the volume of root nodules from day 30 to day 50 was owing to the small nodules less than 3 mm in diameter, while the increase from day 50 to day 60 was owing to the large nodules 3 mm or more in diameter.

### 2. 2. Relationship between root diameter and root nodule formation

Root nodules were sporadically distributed to all root diameter classes until day 20. The distribution, however, began to concentrate on the thin root part thereafter; in the range of root diameter at less than 1 mm by day 30, and around the peak by day 40, showing a similar distribution profile to the ideal pipe (Fig. 2).

The large root nodules with higher nitrogen-fixing activity, sized 3 mm or more in diameter, were found from day 20 onward. Many were found in the root diameter class from 0.3 to 0.8 mm on day 30, and root nodules of this type were also seen in the range of root diameter at less than 0.3 mm on day 40. The root diameter class showing the greatest number of large root nodules on day 60 was 0.4 to 0.5 mm.

### 2. 3. Relationship between root diameter and the volume of root nodules

The distribution of the volume of root nodules was shown in Fig.2, overlaid on the ideal pipe. The volume of root nodules was uniformly distributed in the range of root diameter from 0.1 mm to 3 mm, and did not concentrated in certain classes. However, since aged

Table 2. Changes in number and volume of root nodules per root system.

Days after sowing	Total number	No.of nodules in thickening roots	No.of nodules in fine roots	Fine/Total % ※	Root nodule volume(cm <sup>3</sup> )	
					* ~3mm	3mm~
10	5( 0)	4( 0)	1( 0)	20.0( 0 )	-	-
20	46( 12)	6( 2)	40( 10)	86.9(83.3)	0.3	0.3
30	164( 81)	22(19)	142( 62)	86.6(76.5)	4.0	0.6
40	1072(101)	36(24)	1036( 75)	96.6(74.3)	4.1	1.6
50	1419(128)	48(31)	1371( 97)	96.6(75.8)	4.5	2.8
60	1257(172)	25(16)	1232(156)	98.0(90.7)	5.5	2.3

Numeral in a parenthesis represents a large root nodule(diameter  $\geq 3$  mm).

※ : percentage of nodule number in fine roots. \* : diameter of root nodule.

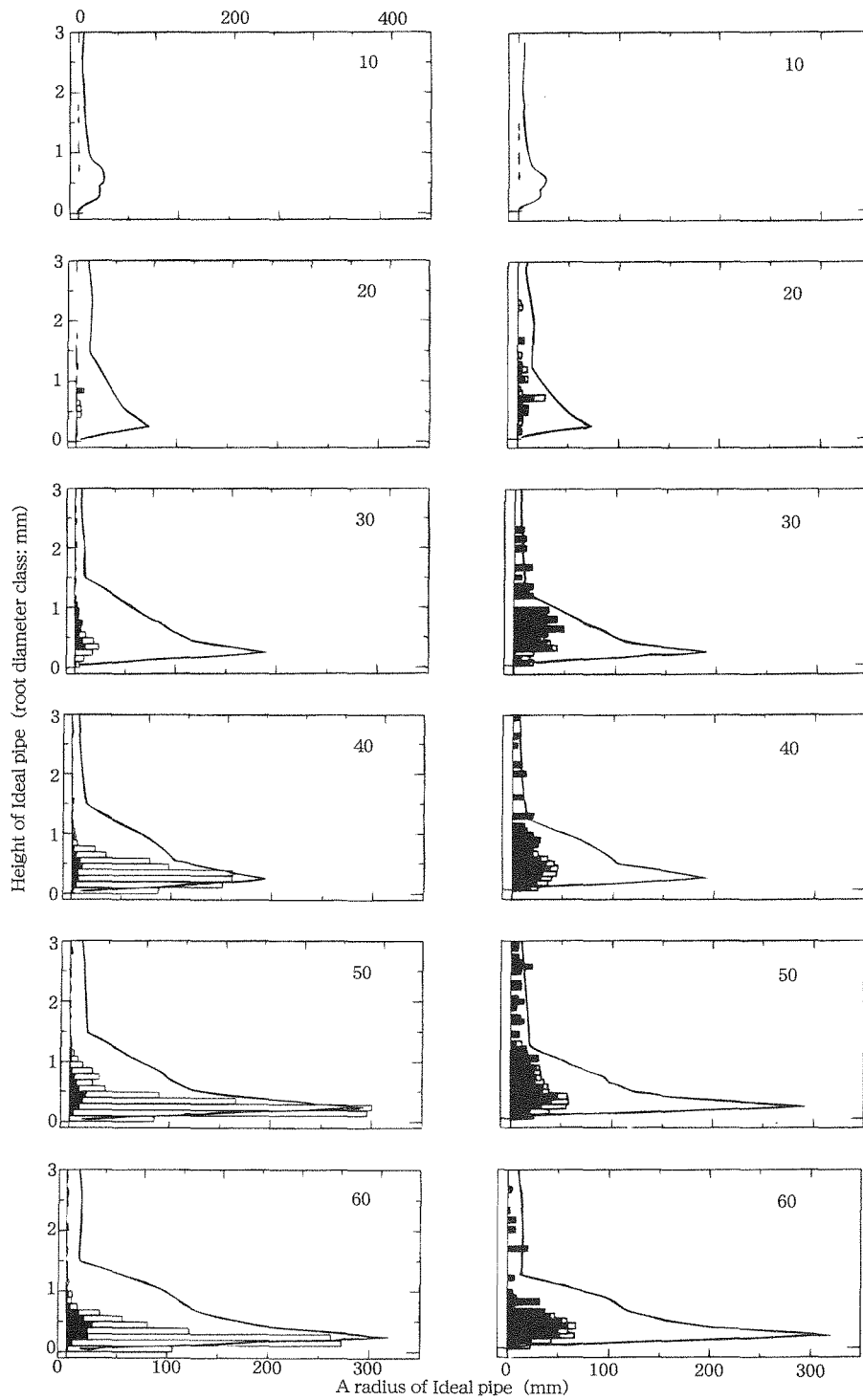


Fig. 2 Half vertical section of "Ideal pipe" and distributing form of root nodules in a Ideal pipe (the right line: volume of root nodule, the left line: number of root nodules). Polygonal line : cross section of Ideal pipe, □ : small root nodules (diameter < 3mm), ■ : large root nodules (> 3mm). Numerals in Figure are days after sowing.

root nodules were detached from roots on and after day 50, the volume of root nodules were reduced in the thick-root part, and distributed in the range of root diameter at less than 1 mm.

## Discussion

### 1. Ideal pipe of the soybean roots and the trend in root nodule formation

The ideal pipe was separated into two distinct parts regardless of the growth stage; the part of thick roots at root diameter of 1.5 mm or more, conforming to the pipe model theory<sup>1,2)</sup>, and the part of a numerous number of thin roots, not conforming to the theory. When viewed in half vertical section, the ideal pipe protruded in the lower part. For both the "pipe" part and the thin-root part, the root volume changed with growth differently in each root diameter class; however, the basic trend unchanged in that the volume was larger for the thin-root part, and the peak was constantly observed in a certain class.

The percentage of the thin root part in the whole root mass was calculated (Table 1). Assuming that the thin-root part functions in water-absorption, and the pipe part in water-conduction, a thin root rate represents a quantitative proportion of these primary functions assigned to the root system. The thin root rate continuously increased until about day 30, once decreased afterward, and stabilized at 90% on and after day 40. This indicates that in a root system the water-absorbing part is first to grow, increasing until about 30 days after sowing; next comes a growth of the water-conducting part consisting of thick roots; and finally, the both absorbing and conducting parts develop in a constant proportion.

Root nodules were mostly formed in the roots at root diameter less than 1 mm, suggesting that the opportunity of infection with leguminous bacteria is high for thin roots, which occupy most of the surface area of the root system<sup>7-9)</sup>. The volume of root nodules, unlike their number, was distributed to all root diameter classes, ranging from very thin to thick roots up to about 3 mm in diameter, and was not concentrated in certain classes. The distribution profile of the volume of root nodules was almost similar to the "ideal pipe of central cylinders" prepared in a previous paper<sup>4)</sup> based on the diameter of the central cylinder. The cylindrical form derived from the volume of root nodule was more similar to the theoretical form of the pipe model theory. The close resemblance between the distribution profile of the root nodule volume and the "ideal pipe of central cylinders" is an indication of morphological necessity required to maintain the symbiotic relationship between root nodules and the root system.

### 2. Possibilities of the ideal pipe in diagnosing a root system

Root length, root weight and root surface area are the parameters generally measured for assessing the growth state and the structure of a root system<sup>10)</sup>. Recently, novel methods<sup>6,11-15)</sup> have been devised including the branching index and the fractal analysis<sup>16)</sup>, which can indicate the branching aspect of roots<sup>6)</sup>. However, none of these methods can provide a comprehensive information of the conditions of a root system. In diagnosing the development of a root system, our interests would be focused on both the quantitative

structure and the physiological activity, but simultaneous measurement is difficult. The root system is composed of roots with various thickness from fine roots to thick roots, and the roots vary their internal structure and physiological function according to their thickness, thus varying their roles in the root system. The ideal pipe can not only provide information about total root mass but reveal the root system structure from a functional viewpoint, on the assumption that thin roots and thick roots carry out their respective functions of absorption and conduction. The present study showed a root system only in a graphic form, but expression in a numerical formula is necessary for the comparative analyses of the total root mass, the root volume distribution by diameter, the ratio of thin roots to thick roots and the extent of conformity with the pipe model.

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## 作物根系の調査法に関する研究

## 第5報 パイプモデルを応用したダイズ根系構造の解析

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## 摘 要

複雑な形状のダイズ根系をパイプモデル理論に基づいて「仮想パイプ」として図示化し、根系の総量ならびに直径階級別根量を把握する方法について検討した。ダイズ根系は、パイプモデルに当てはまる太根部分と当てはまらない細根部分に分かれ、それぞれの部分が量的な均衡を示した。また、生育に伴う根粒の着生経過を仮想パイプに併せて図示した結果、大半の根粒は細根部に着生したが、窒素固定活性の高い大粒の根粒はいずれの直径階級の根にも均等に着生する様相を示した。以上のことから、仮想パイプは根系の形状と量だけでなく、根粒の着生状態を含めて根の諸量を一括して示す方法となりうることが示唆された。