

# Morphological and Architectural Properties in Root Systems of Six Grain Legumes: A Fractal Approach

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**Abstract :** The morphology of root systems of 14 day-old seedlings of six legume species grown in root boxes was quantified through fractal methods. Kidney bean and groundnut developed root systems with higher fractal dimension ( $D$ ) than blackgram, cowpea, pigeonpea and soybean. These suggest that the seedlings of kidney bean and groundnut tend to construct a root system more intricately distributed structure as compared with blackgram, cowpea and pigeonpea. The tested legume species could be categorized into three groups based on the root system size (meristem number and root length),  $D$  and topology. The  $D$  was estimated with two different methods; i.e. box-counting method and mass-radius method. The  $D$  determined with mass-radius method ( $D_{\text{mass}}$ ) was less closely correlated with system size parameters than the  $D$  with box-counting method ( $D_{\text{box}}$ ), although both  $D$ s are closely correlated with topology, suggesting that  $D_{\text{mass}}$  is less sensitive to the parameters relating to system size as compared with  $D_{\text{box}}$ . The  $D_{\text{mass}}$  would be a suitable index alternative to  $D_{\text{box}}$ , when we evaluate root systems focusing on branching pattern rather than system size parameters.

**Keywords :** fractal dimension, legume, root branching, root system, topology

## Introduction

Legumes are characterized with a taproot system. The architecture of taproot systems shows variations among species (Mia et al., 1996; Coble, 1976; Weaver and Bruner, 1927). The root system structure and its relation to stress tolerance of various crop species, i. e., cereals and legumes have been reviewed in details by Yamauchi et al. (1996). They also reported that a crop root system consists of roots of different nature and the phenotypic plasticity of the root system structure plays a key role in plant expression for stress tolerance. Sangakkara et al. (1996) and Sponchiado et al. (1989) reported the extensive root system by *Phaseolous vulgaris* L. seedlings under dry soil moisture condi-

tions as a drought avoidance mechanism. Pigeonpea and groundnut have shown a relative advantage in the acquisition of available nutrients due to the large proliferation of thin higher order lateral roots (Rao and Ito, 1998). Under water stress cowpea cultivars developed deeper roots to improve the ability of water accumulation (Costa et al., 1997).

However, research information on genotypic characteristics in rooting procedures in legume species is very limited. Characterization of the morphology and architecture of root systems will assist crop improvement research in understanding the functional difference and growth strategy of root systems among the species as well as the screening for genetic improvement of the

legumes.

There have been many reports in which root systems were evaluated quantitatively with root weight and root length (Böhm, 1979; Harper et al., 1991). However, these parameters give few insights on the morphology and architecture of root systems. There have been several attempts to describe root system morphology or architecture quantitatively. Fitter (1986; 1988) introduced a geometric model involving topological aspects and described the root system architecture of dicotyledons and grasses.

Recently fractal geometry has been applied to the analysis and evaluation of morphology of root systems (Akasaka et al., 1998; Berntson, 1994; Eghball et al., 1993; Nielsen et al., 1997). Intricacy of the morphology of root systems could be characterized quantitatively by fractal dimensions ( $D$ ) (Tatsumi et al., 1989). The  $D$  is closely related to topological indices that reflect branching properties of root systems (Tatsumi, 1996). However, the information available on the genotypic variation of fractal dimension of root systems is very limited (Fitter and Stickland, 1992; Tatsumi et al., 1989).

In the other paper we analyzed the multifractal property of legume species using mass-radius method (Ketipearachchi and Tatsumi, 2000) and indicated that the mass fractal dimension was applicable for evaluating root morphology together with the fractal dimension determined with box-counting method. In the present experiment fractal dimension was determined with two different methods, i.e., the box-counting method and the mass-radius method. The root systems of six legume species were characterized with these dimensions in relation to system size and root topology, and tried to assess the variability of root system morphology and architecture among legume species.

### Materials and Methods

The seedlings of six species of grain legumes, blackgram (*Vigna mungo* Hepper cv. MI-1), cowpea (*Vigna sinensis* Endl. cv. MI-35), groundnut (*Arachis hypogaea* L. cv. MI-1), kidney bean (*Phaseolus vulgaris* L. cv.

Top Crop), pigeonpea (*Cajanus cajan* Millsp. cv. ICPL 87), and soybean (*Glycine max* Merr. cv. Okuharawase) were used for the experiment. The growth condition and plant culture were reported elsewhere (Ketipearachchi and Tatsumi, in press). In shortly, seedlings were grown in narrow root-boxes (300W, 420H, 20D) filled with Akadama soil mixed with chemical fertilizer and calcium silicate. The seedlings in root boxes were well supplied with water and maintained near field capacity (containing around 30g water per 100g soil) under natural conditions in a green house in summer. Three root boxes of each species were sampled at two weeks after sowing.

Above ground vegetative parts of the seedlings were cut apart at the soil surface and these shoots with stems and leaves were dried at 70°C for about 48 hours to take the dry weight. Root systems were extracted from the soil following the needle board method (Kono et al., 1987), then root images were digitized at 144 dpi resolution (Tatsumi, 1995).

Fractal dimensions of root systems were estimated with the box-counting method as well as the mass-radius method, using NIH Image (v.1.55) software running on an Apple computer (Quadra 800). In the box-counting method fractal dimension was estimated as follows: The root image was first covered

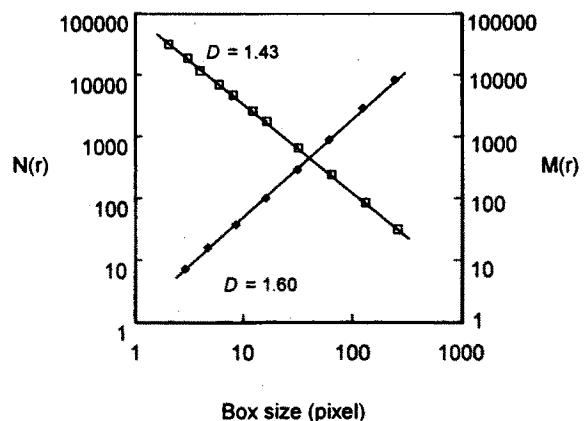


Fig. 1 An example of log-log relationship between box size and number of box counted ( $N(r)$ ) with box-counting method (empty square), and between box size and number of pixel counted ( $M(r)$ ) with mass-radius method (filled square) in blackgram root system.

with a frame. Then the frame was divided into boxes of side length  $r$ , and side length was varied over wide range. The number  $N(r)$  of boxes that intersect the root image was counted, and  $N(r)$  was plotted against  $r$  on a log-log scale (Fig. 1). The number of boxes,  $\log N(r)$  increases with decreasing  $\log r$  (Tatsumi et al., 1989). If the root system is fractal, we find that

$$N(r) = Kr^{-D} \quad (1)$$

where  $K$  is a constant and  $D$  is a fractal dimension defined by box-counting method ( $D_{box}$ ). In the present experiment box size ( $r$ ) was varied from 2 to 256 pixels (0.35~45.16mm) and the regression coefficient of  $r \cdot N(r)$  relationship of all the root images was over 0.999.

Mass-oriented fractal dimension was calculated by following equation (Smith et al. 1996).

$$M(r) = Fr^D \quad (2)$$

where  $M(r)$  is the mass (number of pixels) found in a box or disc of various size  $r$  centered on the image,  $F$  is the pre-factor and  $D$  is the mass fractal dimension ( $D_{mass}$ ). In this experiment we estimated  $D_{mass}$  as an average of  $D_s$  with boxes centered on 500 different points on the edge of the image, varying box size from 3 to 257 pixels (0.53~45.33mm). Figure 1 shows an example of the relationship between  $r$  and  $M(r)$ .

The total root length (TRL) was estimated with a computer by the method of Lebowitz (1988) with some modification (Tanaka et al., 1995). Topological data which had been acquired in the other experiment (Ketipearach-

chi and Tatsumi, 2000) with the same root sample were adopted.

## Results

### 1. System size

In all species root nodules were not observed by eyes, probably due to the application of nitrogen fertilizer. Total root length (TRL) varied considerably (Table 1). Kidney bean was the largest in TRL, followed by soybean and groundnut in this order. Pigeonpea showed the lowest TRL. These differences in TRL, except groundnut, may be mainly due to the variation in lateral root development since the taproot length (Table 1) was not significantly differed among species other than groundnut. A positive relationship was found between the mean TRL and shoot dry weight in six species ( $r=0.913$ ). As compared with pigeonpea, soybean showed 2.9 and 7.0 times larger TRL and shoot dry weight, respectively. The system magnitude (number of meristems) also showed wide variation and the trend was very similar to TRL. The TRL and system magnitude are considered as the parameters directly reflecting the root system size. Considering basically on the system size, tested species can be categorized roughly into two groups, i.e., small and large. Blackgram, cowpea and pigeonpea are the former group while groundnut, kidney bean and soybean are the latter. Mia et al., (1996) reported that cowpea seedlings grown for 10 days using growth pouch (seed bag) had larger root systems as compared with blackgram in terms

**Table 1** Taproot length, shoot weight, system magnitude (number of meristems) and total root length of legume species at 14 DAS.

Crop	Taproot length (m plant <sup>-1</sup> )	Shoot dry weight (g plant <sup>-1</sup> )	m* (number plant <sup>-1</sup> )	TRL* (m plant <sup>-1</sup> )
Blackgram	0.52±0.02b	0.034±0.002a	318.0±19.9a	6.58±0.33a
Cowpea	0.46±0.02ab	0.038±0.003a	439.3±25.3a	7.88±0.33ab
Groundnut	0.35±0.02a	0.104±0.019b	1060.5±44.5bc	12.07±2.31ab
Kidneybean	0.49±0.01b	0.130±0.015b	1667.5±412.0c	15.59±4.77b
Pigeonpea	0.42±0.01ab	0.032±0.006a	377.3±36.1a	4.64±0.46a
Soybean	0.50±0.01b	0.223±0.028c	950.5±112.0abc	13.39±0.40b

Data are shown as mean ± standard error.

\*  $m$ : system magnitude, TRL: total root length.

\*\* Means within a column followed by the same letter are not significantly different at 5% level with Turkey-Kramer test.

of total and lateral root lengths, lateral root number and branching density, although there was large variation among cultivars. As shown in Table 1, TRL and system magnitude were slightly higher in cowpea than blackgram, however, the differences were not significant in this experiment.

## 2. Fractal dimensions

Table 2 shows fractal dimensions determined with box-counting method and mass-radius method, respectively. In all species  $D_{box}$  showed smaller values than  $D_{mass}$ . It was pointed out that for natural fractals the two  $D$ s were often different, with the mass fractal  $D$  value usually being the larger (Smith et al., 1996). Our result for root systems was in good agreement with this, although there was a close relationship between two  $D$ s (Fig. 2). Both  $D$ s were significantly larger in groundnut, kidney bean and soybean than blackgram, cowpea and pigeonpea. Thus, species can be categorized into two groups with higher  $D$ s (groundnut, kidney bean and soybean) and with lower  $D$ s (blackgram, cowpea and pigeonpea). Crops with higher  $D$  indicate that spatial distribution or morphology of root system is more intricate than that with lower  $D$  (Tatsumi, 1995).

## 3. Topology

Table 2 also shows topological data being acquired in the other experiment (Ketipearachchi and Tatsumi, in press). Topological model is a link-based system which describes the branching system (Fitter, 1988). Links are root axes between two branching points (interior links) or those ending in a meristem

(exterior links). The topological parameters are denoted as follows. Altitude ( $a$ ); the number of links in the longest path from any exterior link to the base of the system, total exterior pathlength ( $Pe$ ); sum of number of links in the path from all the meristems to the root base.

Topological indices were the ratios dividing  $\log a$  on  $\log E(a)$  (the ratio of  $a/E(a)$ ) or dividing  $\log Pe$  on  $\log E(Pe)$  (the ratio of  $Pe/E(Pe)$ ), respectively (Fitter, 1986 and 1988). The  $E(a)$  and  $E(Pe)$  are the expected values for  $a$  and  $Pe$ , respectively, assuming that the system branches randomly. Topological indices, the ratios of  $a/E(a)$  and  $Pe/E(Pe)$  range from maximum values for a strict herringbone branching pattern to minimums for a dichotomous branching pattern. Table 2 shows that the ratio of  $a/E(a)$  of groundnut and

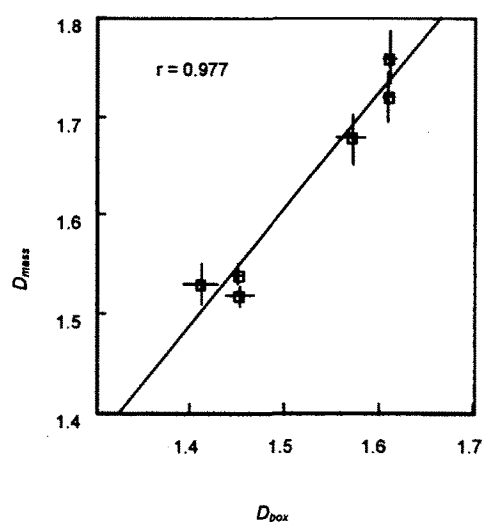


Fig. 2 Relationship between box dimension and mass dimension in root systems of six legume species. Bars in figure shows standard error of the mean.

Table 2 Fractal dimensions ( $D_{box}$  and  $D_{mass}$ ) and topological indices ( $a/E(a)$  and  $Pe/E(Pe)$ ) of legume root systems at 14 DAS.

Crop	$D_{box}$	$D_{mass}$	$a/E(a)$	$Pe/E(Pe)$
Blackgram	1.45±0.03a*	1.52±0.02a	1.26±0.01b	1.09±0.01a
Cowpea	1.45±0.01a	1.54±0.02a	1.24±0.02b	1.07±0.01a
Groundnut	1.61±0.01b	1.76±0.05b	1.10±0.01a	1.02±0.03a
Kidneybean	1.61±0.01b	1.72±0.05b	1.09±0.03a	1.02±0.01a
Pigeonpea	1.41±0.04a	1.53±0.04a	1.26±0.01b	1.08±0.01a
Soybean	1.57±0.03b	1.68±0.05b	1.22±0.02b	1.07±0.01a

Data are shown as mean ± standard error.

Data for topology were adopted from Ketipearachchi and Tatsumi (2000).

\* See Table 1.

kidney bean are significantly lower (1.10 and 1.09, respectively) than the other species, implying branching being near random pattern. In the other species, on the contrary, the ratio recorded in the range between 1.22 to 1.26, suggesting a herringbone-like branching. The  $Pe/E(Pe)$  of groundnut and kidney bean was lower than the other species, however, this difference was not significant and  $Pe/E(Pe)$  showed very small variation.

Based on these data, the tested species can be categorized into two groups in terms of branching pattern; i.e., herringbone-like branching (blackgram, cowpea, pigeonpea and soybean) and random-like branching (groundnut and kidney bean).

Table 3 shows summary of the score evaluated by the system size, fractal dimensions and topology for six species. Blackgram, cowpea and pigeonpea were classified into Type 1 in which the root system was characterized by its smaller system size/ less intricate morphology/ herringbone-like branching. In contrast groundnut and kidney bean were classified into Type 2 which was characterized by larger system size/ highly intricate morphology/ random-like branching. Soybean was the intermediate (Type 3) between Type 1 and Type 2, which had a large size and highly intricate morphology like Type 2 but

had a herringbone-like branching.

#### 4. Relationships among system size, $D$ and topology

There found negative correlations between fractal dimensions and topological indices (Table 4). This indicates that the root systems with higher  $D$  may possess the architecture more random-like branching pattern. This is in good agreement with the result found in kidney bean and pigeonpea grown under various light conditions (Tatsumi, 1996; Tatsumi and Takagai, 1997). Both  $D_{box}$  and  $D_{mass}$ , shows very similar correlation coefficients with topological indices. However, the correlation coefficients with system size parameters were considerably lower in  $D_{mass}$  than  $D_{box}$ , particularly it was lower with TRL. This suggests that  $D_{mass}$  is less sensitive to the parameter relating system size such as meristem number and root length as compared with  $D_{box}$ , although both  $D$ s are intimately associated with branching pattern.

#### Discussion

As indicated in Table 3, Type 1 root system (small system size: blackgram, cowpea and pigeonpea) was characterized with the herringbone-like branching and less intricate morphology, while Type 2 (large system size: groundnut and kidneybean) was character-

**Table 3** System size, fractal and topological characteristics of root systems of six legume species and the classification of root systems based on these characteristics.

Crop	System size	$D_s$	Topology	Root type
Blackgram	Small	Low	H*	1
Cowpea	Small	Low	H	1
Groundnut	Large	High	R	2
Kidneybean	Large	High	R	2
Pigeonpea	Small	Low	H	1
Soybean	Large	High	H	3

\* H: Herringbone-like branching, R: Random-like branching.

**Table 4** Correlation coefficient among fractal dimensions and other root parameters in legume root systems.

Fractal dimension /system size	System size*		Topology	
	$m$	TRL	$a/E(a)$	$Pe/E(Pe)$
$D_{box}$	0.850	0.834	-0.893	-0.806
$D_{mass}$	0.723	0.621	-0.861	-0.813
$m$	-	-	-0.815	-0.715
TRL	-	-	-0.677	-0.525

\*  $m$ : system magnitude, TRL: total root length.

ized with the random-like branching and higher fractal dimension. A simple interpretation of the result is that the difference in branching pattern between these two types is mainly due to the difference in system size, since in general the branching proceeds as the root system develops. Fitter (1988) reported that topology was generally insensitive to changes in the supply of N and P but large differences between species were found: slow-growing perennials had efficient, herringbone systems, whereas annuals were at the opposite extreme. This feature, however, may not apply to soybean root system since it had a large and intricate morphology like Type 2 but had a herringbone-like branching.

The alternative interpretation of the result in Table 3 is that the topological characteristics found in this experiment are basically depend upon the branching habit being specific to plant species in seedling stage rather than the root system size. It has been often reported that cowpea have higher ability to avoid drought stresses owing to their deep root systems being supported by the strongly developed taproot (Costa et al., 1997; Mia et al., 1996). The herringbone-like root system is more adaptive for the construction of deeper root system as well as the efficient acquisition of mobile resources such as soil water than the dichotomous branching root system, although it requires higher cost for system growth (Fitter, 1988). The architecture of root systems can be altered greatly with growth conditions (Fitter, 1986 and 1988). The phenotypic plasticity was often greater than the genotypic variation (Izumi et al., 1995). There found a large intraspecific variation of root system structure in legume species (Mia et al., 1996). Further investigations are needed to make clear the genotypic variation of root system morphology and architecture among legume species.

As shown in Table 4 intimate correlations were found among  $D_s$  and topology. Similar relationships were reported for pigeonpea and kidneybean root systems in different ages and growth conditions (Tatsumi, 1996; Tatsumi and Takagai, 1997). It is likely that such relationships are a common feature

through the legume species tested, and  $D$  can be an universal index for estimating root topology of these species with similar plant age, although further data might be needed for the more precise estimation since there may be considerable modifications specific to plant species.

It was demonstrated that the correlation coefficients of system size parameters were considerably lower for  $D_{mass}$  than  $D_{box}$ , while the coefficients of topological indices for these  $D_s$  were both relatively high (Table 4). Smith et al. (1996) pointed out that  $D_{box}$  and  $D_{mass}$  are often different, since the definition of these two  $D_s$  are different that  $D_{box}$  is a length-relating measure, while  $D_{mass}$  is a mass-relating measure focusing on spatial distribution of the object. The present finding is in good agreement with above suggestion that  $D_{box}$  was more affected with root length as compared with  $D_{mass}$ . When we focus the analysis on the root branching pattern rather than system size, the  $D_{mass}$  would be a suitable index alternative to  $D_{box}$ .

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