



Fractal analysis on root systems of rice plants in response to drought stress

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ABSTRACT

A fractal analytical method was used to examine the developmental responses of root systems in upland rice genotype CT9993-5-10-1-M (*japonica*) and lowland genotype IR62266-42-6-2 (*indica*) (abbreviated as CT9993 and IR62266, respectively) to soil water stress. The root systems were grown for one month in root boxes with 25 cm in length, 2 cm in width and 40 cm in depth, which were filled with soil. The root systems were sampled by following the needle-pinboard method, and then spread on the transparent plastic films with nets after carefully washing out the soils. The two-dimensional images of root systems were digitized by using a scanner. The digitized images were used for analysis based on fractal geometry with the box-counting method. The reductions in shoot dry weight, photosynthesis rate and transpiration rate of IR62266 by soil drought were greater than those of CT9993. The change of fractal parameters in response to soil moisture conditions differed between the two rice genotypes. The values of fractal abundance (FA) and fractal dimension (FD) in well-watered IR62266 plants were larger than in CT9993. The value of FA of IR62266 was decreased more by drought stress than that of CT9993, indicating that the volume of soils explored by the whole root systems of CT9993 was maintained or less decreased under drought stress in comparison to IR62266. Moreover, the values of FD tended to increase in CT9993 while it tended to decrease in IR62266 in response to drought. These root responses detected by the fractal analysis in CT9993 may be advantageous for its extracting more water from drying soils, which explains its better growth under drought-stressed condition.

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1. Introduction

Rice, the world's most important food crop, can experience soil drought at several different growth stages (Price and Courtois, 1999). It is estimated, about one third of world rice area is rainfed lowlands and mostly prone to drought (David, 1991; MacLean et al., 2002). Even for deep water rice, accounting for 11% of world rice area (David, 1991), its productivity is threatened by the scarcity of water in the seedling stage before floods (Evenson et al., 1996). Moreover, upland rice, which is grown under aerobic conditions and rainfed fields without standing water and accounts for 13% of the world rice area (David, 1991), easily encounters drought stress (Tran, 1986; Ahmadi, 2004).

Root growth and development is considered very important for rice to adapt to soil water deficit stress (O'Toole, 1982; Fukai and Cooper, 1995; Price and Courtois, 1999; Price et al., 2002; Wade et

al., 1999, 2000; Kato et al., 2006; Wang and Yamauchi, 2006). The development of a deep and extensive root system in upland rice is one of drought-adaptation strategies, which enables the rice plants to access water at soil depth under upland conditions (O'Toole, 1982; Kondo et al., 2003). Rainfed lowland rice is grown in banded fields without irrigation where soil conditions may fluctuate from flooded and anaerobic to droughted and aerobic (Wade et al., 1998; Suralta and Yamauchi, 2008). Some rice genotypes showed a high adaptation to such cycles with the ability of the roots to proliferate quickly prior to and during the early stages of drought (Bañoc et al., 2000; Kamoshita et al., 2000; Suralta et al., 2008). Despite having fewer roots in deeper layers, rainfed lowland rice can extract water from soil depth below 15-cm in subsequent drought periods (Wade et al., 1999). Enhanced capacity of the root system to penetrate the hardpan is considered another key factor for drought adaptation of rice plants grown under rainfed lowland conditions (O'Toole, 1982; Fukai and Cooper, 1995; Wade et al., 1999).

Two rice genotypes, upland adapted *japonica* CT9993 and lowland adapted *indica* IR62266 were reported to show contrasting root morphology and adapting mechanisms in response to

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different water conditions. CT9993 shows low osmotic adjustment and is well adapted to rainfed lowland conditions with deep and thicker root system and strong root penetration ability (Wade et al., 2000; Azhiri-Sigari et al., 2000; Samson et al., 2002). IR62266 has a higher osmotic adjustment capacity (Lilley and Ludlow, 1996) and a shallow root system (Azhiri-Sigari et al., 2000; Wade et al., 2000; Nguyen et al., 2004). A population of doubled-haploid lines (DHLs) derived from a cross of CT9993 and IR62266 were used to identify some QTLs related to root traits, such as root biomass, root thickness, root length, and root penetration (Zhang et al., 2001; Kamoshita et al., 2002a,b; Nguyen et al., 2004), and the plasticity in lateral root development (Wang et al., 2005). Siopongco et al. (2005, 2006) used this population to identify the root and shoot developmental and physiological traits that are mainly responsible for adaptation to various soil water conditions. However, it still remains to be unclear how the entire root systems of CT9993 and IR62266 respond to water stress.

The architecture of the root system is also well known to be a major determinant of root functions in the acquisition of soil resources such as nutrients and water (Lynch, 1995; Yamauchi et al., 1996; Fitter, 2002; Wang et al., 2006). Fractal geometry is being widely applied to assess the root system architecture and the distribution of root systems in soils (Fitter and Stickland, 1992; Berntson et al., 1997; Lynch and van Beem, 1993; Tatsumi et al., 1989; Tatsumi, 1995, 2001; Masi and Maranville, 1998; Walk et al., 2004; Dannowski and Block, 2005). Fractal geometry is a system of geometry that is more suitable for the description of complex natural objects than standard Euclidian geometry (Mandelbrot, 1983). A fractal is an object having a non-integer dimension. Root systems also have self-similarity and are considered as the approximate fractal objects over a finite range of scales (Tatsumi et al., 1989). Fractal analysis in root biology often typically utilizes box-counting method and the equation: $N_{(r)} = Kr^{-D}$ is obtained (Tatsumi et al., 1989; Tatsumi, 2001; Walk et al., 2004), where r is the length of the box side, and $N_{(r)}$ is the number of boxes of size r needed to cover the object. In terms of fractal analysis, the equation: $N_{(r)} = Kr^{-D}$ is transformed to the regression of \log of $N_{(r)}$ intersected by roots vs. r levels. The slope (D) and intercept $\log K$ are computed. D is the fractal dimension (FD), and $\log K$ is associated with fractal abundance (FA). The FD is closely related with the branching pattern of roots, while the FA with the volume of space explored by roots (Tatsumi et al., 1989; Tatsumi, 2001; Walk et al., 2004).

The FD is found to be correlated with root topology (Fitter and Stickland, 1992) and root architecture (Nielsen et al., 1997). The variation of FD has been noted among four species of dicots and monocots (Fitter and Stickland, 1992), as well as among genotypes of sorghum, rice and common bean (Izumi et al., 1995; Masi and Maranville, 1998; Nielsen et al., 1998). Fitter (1994) showed that the increase of the volume of the soils explored by the roots, as a result of continuous branching, may reflect the plant's adaptive ability to make best use of unevenly distributed water and nutrients in soils. The analysis on the characterization of root system architecture would assist in better understanding the functional and growth strategy of root systems of rice plants when they are faced to insufficient supply of soil water (Ketiepearachchi and Tatsumi, 2000). Izumi et al. (1995, 1997) reported that soil moisture affected rice seminal root system development and its architecture. However, there have been only few studies about evaluation of the architecture of whole rice root systems in response to soil drought stress using the fractal analysis. In this study, we applied fractal analytical method to determine root system architecture of upland adapted *japonica* CT9993 and lowland adapted *indica* IR62266 under different soil water conditions, and compared the differences of fractal parameters for describing root architecture between these two rice cultivars in response to soil water deficit stress.

2. Materials and methods

2.1. Plant materials and culture

Two rice genotypes, upland adapted *japonica* CT9993 and lowland adapted *indica* IR62266, were selected. The seeds were soaked in water and incubated in seed germinator maintained at 28 °C for 24 h prior to sowing. Pre-germinated seed from each genotype was individually grown in root box with 25 cm in length, 2 cm in width and 40 cm in depth according to the methods developed by Kono et al. (1987).

After sieving in 2 mm mesh, 2.5 kg air-dried loamy sand soils mixed with 0.25 g of compound fertilizer (N 12%, P 16%, K 14%) were filled in each root box.

Root boxes were soaked in water pool until soils were saturated. Two soil moisture treatments were prepared in the experiment. In well-watered treatment, the soil was first submerged in the water for 24 h followed by draining to maintain 25–30% soil moisture content throughout the experimental period. The 25–30% soil moisture content is close to field capacity of the used loamy sand soil (26%). In drought-stressed condition, the soils were also first saturated as mentioned above, watering was then withheld from planting until the sampling. As a result, soil moisture content gradually reduced to 10% at two weeks after planting, and maintained to 8% for the final two weeks. The 8–10% soil moisture content was equivalent to 30–38% of the field capacity. The soil moisture content was estimated by using gravimetric method. The reduced amount of soil water was added to maintain the soil moisture for each treatment. All the root boxes were placed under a vinyl house to grow plants. The experiment was set up in a randomized complete design with 3 replicates.

2.2. Sampling and measurements

The rice plants were grown in root boxes for four weeks. At harvest, the shoots were detached from the roots and sampled. The shoot dry weight was then measured after drying at 70 °C for 72 h.

The intact whole root systems were obtained following the needle-pinboard methods and the transparent plastic sheets with nets were used to fix and keep root samples *in situ*. This method enables us to sample the entire root system with nearly original orientation and distribution in soil with minimum damage and disturbance (Kono et al., 1987). Root samples were stored in FAA solution (70% ethanol:formalin:acetic acid = 18:1:1 parts by volume) prior to measurements. The root systems were then stained with 0.25% Coomassie Brilliant Blue R aqueous solution to get clear contrast for computer images, which were digitized with the EPSON ES2200 scanner at 300 dpi resolution for further analysis. The total root length was measured with image analysis method proposed by Kimura et al. (1999) and Kimura and Yamasaki (2003).

After removing the plastic sheets, the lengths of seminal and nodal root axes were directly measured by using ruler. Lateral root length was estimated as a difference between the total root length and the sum of the length of seminal root and nodal root axes. Total nodal root number was counted. After such measurements, root dry weight was determined after drying at 70 °C for 72 h.

2.3. Fractal analysis

The digitized root images were used for fractal analysis following the box-counting method described by Tatsumi et al. (1989) and Ketiepearachchi and Tatsumi (2000), who developed the macro program for fractal analysis within NIH 1.61 software (developed at the US National Institutes of Health, <http://rsb.info.nih.gov>). The root image was first covered with the frame and the frame was

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