Evaluation of Root Traits at the Seeding Stage Using Rhizobox System

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Abstract. Studying and observing the distribution of roots as the ‘hidden half’ is very complicated because it requires an appropriate method and an understanding of the significant influences of the environment on the root growth. The root system is recognized as having a relationship with the crop productivity, thus can be optimized to increase crop productivity, particularly for growth under low input condition involving unevenly distributed water and nutrient surrounding the rhizosphere. The aim of this study is to evaluate the root growth in the seeding stage and QTL analysis using the Rhizobox system. In this study, ninety-three recombinant inbred lines originated from a cross between Peking and Tamahomare (PT-RILs) were used to identify for the QTL analysis. The seed of the soybean is planted in germination paper in the Rhizobox system for six days. Root parameters are the number of roots, root length, root growth angle (measured from the vertical axis by using software Image J), root width, and root depth. The results showed that for all traits, the PT RILs population exhibited a broad and continuous distribution of values ranging between the values of the parental cultivar. A total of nine putative QTLs were identified for the eight of the nine tested traits. No significant QTLs for root growth angle was detected. The phenotypic variation explained by each QTL range 14–21 %. In terms of root surface area, two major QTL was located on Chr.9 and Chr.19 for root width and root depth. Meanwhile QTLs for root length was located on Chr.8 and Chr.11

Key words : root traits, rhizobox, soybean, quantitative trait locus.

1. Introduction
Research on root development was initiated in the field by interests in studying root growth under various soil conditions. The measurement of root traits and specific traits employs a wide range of equipments and techniques, with several are dependent on crop growth conditions, whether in the field or specific containers. Furthermore, each method has its own advantages and disadvantages, thus the best method is chosen based upon the following considerations. First, at what growth stage (seedling, vegetative, or generative stage) the plant is evaluated. Second, the seedling type of plant: whether hypogaeal or epigeal. Third, the ease to observe the root growth and to reproduce the observation. Fourth, whether or not the method damages the root. Lastly, the ease to obtain high quality pictures of the root for documentation purposes.

The study of root traits in seedling using a germination paper was first developed for a study on Phaseolus vulgaris [1], with basal root as the focus of study. Later on, Liao et al. [7], expanded the technique to allow measurement of the root length. In 2009, it was reported that this technique was successfully employed for a study on maize seedling Hund et al., [5]. QTL analysis based upon high density genetic maps has allowed a better understanding of genetic control of root traits. The number...
of studies identifying QTLs in seedling root traits in wheat Hamada et al.; Christopher et al., [3,4]. The aim of the study is to apply rhizobox system for the QTL analysis of root traits.

2. Methodology

Plant Materials. Ninety-three recombinant inbred lines originated from a cross between Peking and Tamahomare (PT RILs) were used to identify for the QTL analysis.

Experimental procedures. Seeds were surface sterilized by rinsing in 30% hydrogen peroxide ($H_2O_2$) for two minutes and washing several times with sterile water. Sterilized seeds were placed in the middle of a moistened paper towel for 12 hours at room temperature 25 °C. Then, seeds were transferred to a moistened germination paper (Whatman) crease-side down in a rhizobox system to complete the germination. The plexiglass rhizobox system was based on the design developed for soybean. Each box measured length 34 cm x width 1.5 cm x height 27.5 cm. Chromatography paper (3 mm Chr) was folded in half and seeds were sandwiched in between. Papers were set in the rhizobox and the rhizoboxes were filled with vermiculite. The vermiculite was sterilized with oven at 200 °C for 8 hours and put in the rhizobox. Six hundred ml water was poured and kept for 2 hours, after paper was moistened the seed was placed inside the paper. The rhizobox was then covered with a black polythene film and placed inside the controlled environment room at 25 °C with 16-hours photoperiod. After six days treatment, seedlings were collected. All treatments were performed in two replicates. Root parameters, which consists of the number of roots, root length, root growth angle, and root width and root depth were observed (Figure 1.). The root growth angle was measured from vertical axis by using software Image J.

![Figure 1. Evaluating root traits in rhizobox system](image-url)
3. Results and Discussion

Values of the ranges, means and coefficient variations for five traits in PT-RILs are summarized in Table 1. The mean values of all traits PT-RILs population revealed a wide range of variation. The range of the root traits of PT-RILs generally exceed, those of the parents. The frequency distribution of the five traits in the PT-RILs are given in Figure 2. For all traits, the PT-RILs population exhibited a broad and continuous distribution of values ranging between the values of the parental cultivar.

A total of nine putative QTLs were identified for the eight of the nine observed traits (Table 3). No significant QTLs for root growth angle was detected. Putative QTL for the root number was located on Chr.10 named qRN1. The phenotypic variation explained by each QTL range 14-21%. QTLs for root length was located on Chr.8 and Chr.11, named qRL1 and qRL2, respectively. QTLs for the root width was located on Chr.9 and Chr.19 named qRW1 and qRW2 respectively. QTLs for root depth named qRD1, qRD2, qRD3 and qRD4 was located on Chr.9,10 and 19. The QTLs for root width (qRW1, qRW2) and Root depth (qRD1, qRD4) were located in the same position on Chr. 9 and Chr.19.

Table 1. Distribution of lateral root traits in the parental cultivars and the RIL population (Rhizobox)

| Traits                  | Parents         | RILs            | Range    | Mean  | CV(%) |
|-------------------------|-----------------|-----------------|----------|-------|-------|
| Number of root          | 15.50           | 27.00           | 7.50 – 37.50 | 21.38 | 28.95 |
| Root length             | 17.25           | 17.25           | 7.75 – 23.00 | 16.49 | 20.25 |
| Root growth angle       | 127.46          | 101.77          | 92.12 – 152.02 | 128.88 | 9.50  |
| Root width              | 3.50            | 2.50            | 1.25 – 8.50  | 3.55  | 36.52 |
| Root depth              | 1.50            | 2.00            | 0.80 – 4.75  | 2.15  | 39.27 |

Table 2. QTLs for root architecture traits of PT-RILs grown in Rhizobox

| Trait               | QTL    | Interval         | Chromosome (linkage group) | Peak Position | LOD  | Additive effect | Variance (%) |
|---------------------|--------|------------------|-----------------------------|---------------|------|-----------------|--------------|
| No. of root         | qRN1   | BE801128-Satt592 | 10 (O)                     | 69            | 2.95 | -1.95           | 15.8%        |
| Root length         | qRL1   | Satt215-Sat_129  | 8 (A2)                      | 59            | 3.55 | 0.95            | 18.7%        |
|                     | qRL2   | Sat_272-Satt509  | 11 (B1)                     | 27            | 2.61 | 0.85            | 14.1%        |
| Root growth angle   | Not detected |                   |                             |               |      |                 |              |
| Root width          | qRW1   | Sat_167-Sat_126  | 9 (K)                       | 128           | 3.11 | -0.95           | 16.6%        |
|                     | qRW2   | Sat_113-Sat_184  | 19 (L)                      | 101           | 2.67 | -0.35           | 14.4%        |
| Root depth          | qRD1   | Sat_167-Sat_126  | 9 (K)                       | 126           | 4.09 | -0.57           | 21.2%        |
|                     | qRD2   | Satt358-Satt445  | 10 (O)                      | 0             | 2.76 | -0.54           | 14.8%        |
|                     | qRD3   | Satt592-Satt243  | 10 (O)                      | 97            | 3.60 | -0.24           | 18.9%        |
|                     | qRD4   | Sat_150-Sat_113  | 19 (L)                      | 91            | 2.97 | -0.28           | 15.9%        |

aQTL position from the first marker.

A positive value indicates that “Peking”-type allele increases phenotypic value and a negative value indicates that the “Tamahomare”-type allele increases phenotypic value.
Discussion

Many researchers who are working on root system architecture of crops exploited the seedling traits of the crops to save cost and time. In evaluating root traits at the seedling stage in this study, a rhizobox

Figure 2. Frequency distribution RILs Rhizobox ( ▼ Peking, ▽ Tamahomare)
system combined with germination paper was used. Moistened vermiculite was used to supply water to the germination paper. Vermiculite can keep water, so as to supply moisture to the paper but water drained down in six days making the moisture content of upper part germination paper become lower than that of the lower part of the paper. This gradient of the moisture content very much affect the direction of roots elongation. When upper part of rhizobox was covered with polyethylene wrap, the growth angle of seedlings become large due to the high moisture content of the upper part of the germination paper (Figure 3.)

Mapping QTL is becoming increasingly essential in modern breeding programs through marker-assisted selection (MAS). In terms root surface area, two major QTL was located on Chr. 9 (Sat_167-Sat_126) and Chr.19 (Sat_113-Sat_184) for root width and root depth. Hwang et al. [6], detected a QTL of root nodule weight was located on Chr.19. Meanwhile the QTL number of root and root depth on Chr.10. Liang et al. [8], detected QTL for maximum root length and identified on Chr.11, Chr.19, Chr. 3, and Chr. 10. The QTL of root length was located on Chr.8. Brensha et al. [2], detected several QTLs of root traits of soybean. Among them, a QTL of lateral root length and basal root thickness on Chr.18, and the QTL of root fresh/dry weight on Chr.3 and Chr.8. Among them I found the QTL of the root length at the overlapping region of the QTL of root fresh weight on Chr.8.

![Diagram showing Trial experiment in rhizobox by using wrapping in upper part](image)

**Figure 3.** The phenotyping of root growth angle due to the different moisture content in upper part

4. Conclusion

Soybean root traits are defined by the primary root, the lateral roots, their densities, and their distributions. The primary root is established as a framework to explore the soil for nutrients and water and is formed at the basal pole of the embryo. Lateral roots, which are responsible for increasing water and nutrient intake. In this study, we evaluate the root system at the seeding stage for six days using Recombinant Inbred Lines for QTL analysis of root traits. We are not able to detect QTLs for root
growth angle, but we can detect root depth, root width, root length, and the number of roots. Root
distribution in rhizobox method was difficult to evaluate due to the uneven moisture distribution of the
germination paper.

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