Water-Extraction by Split-Roots of Sesbania and Pigeon Pea Exposed to Spatially Heterogeneous Distribution of Soil Water

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Abstract: Previous studies have suggested that the deep roots of sesbania (Sesbania sesban) function less efficiently in water acquisition than those of pigeon pea (Cajanus cajan) despite similar rooting depths. To investigate this phenomenon, both species were grown in a vertically split-root system. The top soil was watered at two-day intervals and the bottom soil was kept wet. Fifty-seven days after sowing, the watering to the top soil was withheld and the water uptake was monitored in both the layers. At any given rate of transpiration, the water influx rate per unit root surface (WIR/RS) was higher in the top soil than in the bottom soil in sesbania, despite the greater availability of water in the latter. By contrast, in pigeon pea, the WIR/RS was higher in the bottom soil than in the top soil. In sesbania, aerenchyma tissue was observed only in the cortex of the roots in the bottom soil. On the other hand, aerenchyma tissue was scarce observed in pigeon pea roots, suggesting that the presence of aerenchyma tissue led to the reduced WIR/RS of sesbania roots in the bottom soil. Thermal image analysis showed that the stomata of sesbania leaves did not respond to water shortage. Instead, the sesbania leaves were shed in order to avoid desiccation, further reducing the potential to extract water. We therefore conclude that the water-extraction ability of deep roots was lower in sesbania than in pigeon pea as a result of aerenchyma formation and leaf shedding.

Key words: ABA, Deep root, Drought stress, Groundwater, Root distribution, Specific root length, Stomatal behaviour, Water logging.

The development of deep rooting systems in plants is thought to be a drought avoidance strategy (Kramer and Boyer, 1995; Huang, 2000). In natural environments, soil water potential tends to decrease downwards in the soil profile. Although rainfall events temporarily disrupt the soil water potential gradient, it recovers as a consequence of gravimetric water flow, runoff and evapotranspiration. Deep rooting systems might therefore allow plants that are experiencing drought conditions to utilize water sources deep in the soil layers, which are not available to those with shallow rooting systems. Indeed, several studies have shown that genotypes with deeper rooting systems have greater resistance to water deficit in species such as peanut (Arachis hypogaea L.) (Ketring and Reid, 1993), soybean (Glycine max (L.) Merr.) (Hirasawa et al., 1994) and cowpea (Vigna unguiculata (L.) Walp.) (Timsina et al., 1994).

However, gaining access to water sources through the root system does not always improve the water status of a plant. For instance, transpiration rates in rice (Oryza sativa L.) (Ishihara and Saito, 1987), maize (Zea mays L.) (Hirasawa and Hsiao, 1999) and soybean (Huck et al., 1985) are reduced by hydraulic resistance in their root systems even when water is available. The presence of a partial dry region within a single root system can also cause a reduction of stomatal conductance, even when the rest of the roots are kept wet (Gowing et al., 1990; Davies et al., 2002). The mechanism of this process involves abscisic acid (ABA)-based chemical signals that are produced in the roots in the dry region (Wilkinson and Davies, 2002). This means that deep rooting systems might not be able to fully utilize a water source if such a partial drying effect induces the substantial stomatal closure.

Previously, we found that deep roots were not always successful in water acquisition (Sekiya and Yano, 2002). We attempted to collect xylem sap exudation from sesbania (Sesbania sesban L.) and pigeon pea (Cajanus cajan Druce) grown in semi-arid Zambia during the dry season. Although both species had root systems that were able to reach groundwater at a depth of approximately 2 m, only pigeon pea exuded xylem sap. Furthermore, hydrogen stable isotope analysis revealed that pigeon pea obtained groundwater and supplied it to neighboring maize plants through hydraulic lift. However, there was no evidence of this process in sesbania (Sekiya and Yano, 2004). On the basis of these results, we suppose that the deep rooting systems in the two species differ in their water acquisition...
The present study was designed to test this hypothesis. Sesbania and pigeon pea plants were grown in a vertically-split root system, in which the top compartment was dried and the bottom compartment was kept wet to imitate the field environment. The rate of water influx into the roots was measured in both compartments. Furthermore, thermal image analysis of canopies was carried out to determine the stomatal response of the two species to this heterogeneous soil water environment. The main aim of the study was to determine the water-extracting ability of roots in two soil layers with different moisture levels. In addition, we also investigated the potential causes of interspecific differences in water acquisition.

Materials and Methods

1. Experiment 1
   (1) Culture system and growth condition
   A split-root culture system was used in this experiment (Fig. 1). Polyvinyl chloride tubes (15 cm height, 5 cm diameter) were prepared so that their bottom ends were covered with 0.15 mm nylon mesh in order to prevent soil erosion while allowing root penetration. Each tube was filled with 400 g of loamy sand. Two tubes were then joined together using masking tape to tightly seal the junction between them. There was an air gap of approximately 8 mm between the two soils, which prevented the movement of water except through evaporation. The connected tubes were placed in a 1.0 L semi-transparent container along with 0.5 L of water. Measurements of water uptake and leaf area were carried out on four different dates, with four replications of each, so a total of 16 individual plants were required for each species. All plants were grown in a green house at Nogoya University, Japan, until the measurements were made.

Sesbania (Sesbania sesban L, provided by the International Center for Research in Agroforestry: ICRAF) and pigeon pea (Cajanus cajan Druce, purchased from Snow Brand Seed Co. Ltd.) were used. Two germinated seeds were sown in the top soil of each culture system on 22 August, 2001, and the seedlings were thinned to one stand when the third leaf emerged. The soil in the top tube of each culture system received 80 mL of tap water at two-day intervals and 100 mL of 1/500 Hyponex solution (5 : 10 : 5) at four-day intervals. It was estimated that the soil water content in the top tube fluctuated from 8.5 to 30% in pigeon pea and from 12 to 30% in sesbania throughout the experimental period. The soil in the bottom tube of each culture system was kept wet by maintaining the initial volume of water in the semi-transparent container. Consequently, all the roots developed in this tube were uniformly exposed to approximately 31% of soil water content and hence assumed to experience the similar oxygen levels.

(2) Measurement of water influx and leaf area
Fifty-seven days after sowing (17 October 2001), the top soil of each culture system was supplied with 100 mL of water and the semi-transparent container at the bottom was filled with water to maintain the initial volume. The surfaces of the top tube and the semi-transparent container were then wrapped with plastic sheets to prevent all moisture loss other than through transpiration. Twenty-four hours after watering, the volumetric soil water content in the top tube was measured using a soil moisture meter (HydroSense, Campbell Scientific, Australia). The soil water content in the bottom tube was measured by tracing the water surface level in the semi-transparent container. These measurements determined the initial soil water content in each tube. On the same day, all of the plants were transferred into a growth chamber, which was illuminated with natural light (30/25°C; 70% RH; 14/10 h day/night).

After 24 h (19 October 2001), the first measurements of water uptake and leaf area were made. The shoots
were cut at their bases. The leaves were then removed from the stems and spread on a transparent sheet, without overlap. Digitised images were taken using a scanner with a resolution of 200 dpi and an output format of 256 grey-scales. Leaf areas were calculated from the images using the NIH Image version 1.60 image-analysis software.

Immediately after removal of the shoots, the water content of the soil in each tube was measured using the methods described above. The amount of water that was lost from the soil through transpiration was calculated for each tube by subtracting the soil water content from the initial values. Each of these values was then divided by the number of days after withholding water, in order to determine the water influx rate into split-roots in the soil in each tube. The measurements of water uptake and leaf area were repeated every two days in order to assess the water influx rate into split-roots, the amount of transpiration per plant, leaf area and transpiration rate per unit leaf area.

(3) Measurement of root length, root surface area, root weight per unit root volume and water influx rate

Root length and width were measured for the plants sampled on the final day of the experiment. The roots were obtained separately from the top and bottom tubes by removing the soils on a sieve (212 µm). Each root sample was divided into two sub-samples of similar fresh weight. Then one was preserved in FAA (formalin: acetic acid: 70% ethanol = 1 : 1 : 18 by volume) until the morphological measurements and the other was dried at 80°C for 48 hours to weigh the dry matter. Each root sample in FAA was then rinsed with water and spread on a transparent sheet, without overlap. The digitised images were produced using the methods described above. Root length was determined by diameter class, using a macro-program developed by Kimura et al. (1999) on the NIH Image version 1.60 software. Root surface area and root volume were estimated from the root length by diameter, assuming that the roots for a given diameter were cylindrical. Root weight per unit root volume (RW/RV) was then calculated as the root dry weight divided by the estimated root volume. The root surface area was divided by 65 days (the period between sowing and the final day) in order to determine the increase rate of root surface area, and the root surface area on each sampling date was estimated from the increase rate. Water influx rate per unit root surface (WIR/RS) was then calculated as the water influx rate into split-roots divided by the root surface area for each tube.

(4) Observation of root cross sections

Anatomical examinations were carried out on cross sections of the root samples preserved in FAA. First-order lateral roots were randomly collected from the top and bottom soil, and cross sections were prepared by hand under a stereomicroscope. The sections were then observed under an inverted microscope (IX 70, Olympus, Japan) equipped with a digital camera, and the images were produced using image-editing software (Cool SNAP, Roper Scientific, USA).

(5) Statistical analyses

The data were analysed using analysis of variance (ANOVA). The mean separation between treatments was then determined using Fisher’s protected least-significant difference method (PLSD) for all analyses. In addition, simple regression analysis was performed between WIR/RS and transpiration rate per plant.

2. Experiment 2

(1) Culture system and growth condition

The same polyvinyl chloride tubes as those used in Experiment 1 were prepared so that their bottom ends were sealed with polyvinyl chloride disks. Each tube was filled with 350 g of loamy sand. Two water regimes (wet treatment and dry treatment) were tested with five replications of each, so a total of ten individual plants were required for each species. All plants were grown under controlled conditions in a growth chamber (30/25°C; 65% RH; 12/12 h day/night; light intensity 300 µmol m⁻² s⁻¹). Two germinated seeds were sown in each tube, and were thinned to one stand when the third leaf emerged. Each tube was weighed and watered daily to maintain 20% soil water content, and 1/500 Hyponex solution (5 : 10 : 5) was applied at

|                          | RL (m)     | RS (cm²)     | RW/RV (g cm⁻³) |
|--------------------------|------------|--------------|----------------|
| Sesonbia                 | Top 13.1±1.1 c | 193.7±17.7 c | 78.9±7.6 b     |
|                          | Bottom 47.4±4.9 a | 640.5±44.7 a | 27.8±1.1 d     |
| Pigeon pea               | Top 23.8±2.2 bc | 334.7±33.3 bc | 129.6±3.7 a    |
|                          | Bottom 35.8±6.4 ab | 493.9±77.6 b | 42.7±1.4 c     |

Values are mean±S.E. (n=4). The lower-case letters indicate significant differences within each parameter. (Fisher’s PLSD, P=0.05)
(2) Water regime and thermal image analysis

Thirty-nine days after sowing, watering was withheld from one half of the tubes of each species (dry treatment); the remaining tubes received water daily to maintain 20% soil water content (wet treatment).

To compare leaf temperatures between treatments, thermal images of the leaves from both treatment groups were captured using a thermal image analyser (TVS-200, Avionics, USA) immediately before the wet-treatment group received water. Emissivity was set at 1, as an absolute measurement of leaf temperature was not required. Images were analysed using thermographic software (PicEd AVIO, METZGER EDV, Germany).

Results

1. Experiment 1

(1) Root length, root surface area and root weight per unit root volume

The mean length, surface area and weight per unit volume of sesbania and pigeon pea roots in the top and bottom soils are shown in Table 1. Sesbania developed significantly longer roots with a larger root surface area in the bottom soil compared with those in the top soil. By contrast, the length and surface area of pigeon pea roots did not significantly differ between the two soils. In the bottom soil, the surface area of the sesbania roots was significantly larger than that of the pigeon pea roots although there was no statistically significant difference in root length between the two species.

Root weight per unit root volume (RW/RV) varied with the species and the soils. In each species, the roots in the bottom soil had significantly lower RW/RV than those in the top soil. In the bottom soil, the RW/RV of sesbania roots was significantly lower than that of pigeon pea roots.

(2) Changes over time in leaf area and transpiration rate per unit leaf area and per plant

Transpiration rate per plant is a function of the leaf area and the transpiration rate per unit leaf area. The values of these three parameters throughout the time period during which water was withheld are shown in Fig. 2.

In sesbania, the leaf area remained relatively stable after withholding water until day 3 (Fig. 2A). It had significantly decreased at day 5, owing to falling leaves. This trend continued until day 7, by which time the leaf area had been reduced to approximately 56% of the values recorded on day 1. In pigeon pea, after withholding water, some older leaves were lost between day 1 and day 3, which resulted in a significant reduction in leaf area (Fig. 2A). However, new leaves subsequently emerged and leaf area had significantly
increased by day 5, reaching a stable level by day 7 (Fig. 2A). Leaf area on day 7 was approximately 79% of that recorded on day 1 in pigeon pea.

The transpiration rate per unit leaf area of sesbania did not significantly change throughout the period that water was withheld (Fig. 2B). In pigeon pea, the transpiration rate per unit leaf area decreased significantly between day 1 and day 5, then showed a slight increase by day 7 (Fig. 2B).

In sesbania, the transpiration rate per plant began to decrease on day 5 and was kept low until day 7 (Fig. 2C). This trend was similar to that observed in leaf area (Fig. 2A), but was the opposite of the non-significant trend observed in transpiration rate per unit leaf area. The transpiration rate per plant for pigeon pea was significantly reduced at day 3 (Fig. 2C) as a result of a reduction in both leaf area (Fig. 2A) and transpiration rate per unit leaf area (Fig. 2B). However, the transpiration rate per plant did not significantly increase until day 7 (Fig. 2C), despite the recovery of leaf area on day 5 (Fig. 2A). This might have been a result of the reduction in transpiration rate per unit leaf area on day 5 and its slight recovery on day 7 (Fig. 2A). It, therefore, seems likely that water efflux in sesbania is predominantly determined by leaf area, whereas water efflux in pigeon pea is controlled by a combination of both leaf area and transpiration rate.

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**Fig. 3.** Changes over time in water influx rate into roots in the top and bottom soils in sesbania and pigeon pea after withholding water to the top soils. Each data point represents the mean ± S.E. (n = 4). The lower-case letters indicate significant differences between values within species (Fisher’s PLSD, P = 0.05).

**Fig. 4.** Changes over time in water influx rate per unit root surface of sesbania and pigeon pea roots in the top and bottom soils after withholding water to the top soils. The root surface on each date was estimated from the increase rate of root surface. Each data point represents the mean ± S.E. (n = 4). The lower-case letters indicate significant differences between values within species (Fisher’s PLSD, P = 0.05).
Water influx rate into split-roots and water influx rate per unit root surface

The rate of water influx into split-roots of both species during the period that water was withheld is shown in Fig. 3. In sesbania, the water influx rate into split-roots in the top soil was significantly reduced on day 3 and subsequently remained at this level owing to the depletion of water (Fig. 3A). In the bottom soil, the water influx rate had significantly increased at day 3, however, it decreased on day 5 and had not recovered on day 7 probably as a result of the reduced leaf area (Fig. 2A).

In pigeon pea, the water influx rate into split-roots in the top soil continuously decreased until day 5 as a result of water depletion (Fig. 3B). In the bottom soil, the rate was reduced on day 3, but subsequently increased. This increase in water influx rate into split-roots in the bottom soil can be explained by concomitant increases in both leaf area (Fig. 2A) and transpiration rate per unit leaf area (Fig. 2B).

Fig. 4 shows the changes over time in WIR/RS in the top and bottom soils during the period that water was withheld. In sesbania, the water influx rate on day 1 was significantly higher in the top soil than in the bottom soil, however, this difference was not observed between day 3 and day 7 (Fig. 4A). In pigeon pea, also, a significant difference in the water influx rate of the two soils on day 1 became absent between day 3 and day 5, however, WIR/RS in the bottom soil was significantly increased on day 7 (Fig. 4B).

Fig. 5 shows the correlation between transpiration rate per plant and WIR/RS. In sesbania, at any given transpiration rate per plant, the WIR/RS was higher in the top soil than in the bottom soil. By contrast, in pigeon pea, at any given transpiration rate per plant,
the WIR/RS in the bottom soil was higher than that in the top soil.

We predicted that WIR/RS at a given transpiration rate per plant would be higher in the bottom soil than in the top soil, as water depletion progressed in the top soil and more water was available in the bottom soil. This was the case in pigeon pea, but not in sesbania. These results suggest that sesbania roots in the bottom soil had some resistance against water influx that was not present in those roots in the top soil.

(4) Root cross sections
To investigate the cause of the differences in water influx rate per unit root surface at a given transpiration rate per plant, root cross-sections were examined (Fig. 6). The most significant anatomical difference between the two species was the presence of aerenchyma in the cortex of sesbania roots from the bottom soil; these tissues were not observed in pigeon pea roots. The low RW/RV of sesbania roots in the bottom soil may be attributed to the presence of aerenchyma tissues in the cortex.

2. Experiment 2
Leaf temperature
Fig. 7 shows thermal images of the canopies of sesbania and pigeon pea plants from the wet and dry treatment groups. The soil water content for the dry treatment group corresponds to approximately – 170 kPa, according to the soil water retention curve calculated previously. Significant differences in leaf temperature between the two treatments were observed in pigeon pea, but not in sesbania. Differences in leaf temperature measured under a certain atmospheric conditions can be attributed to differences in transpiration rate (Hashimoto et al., 1984; Merlot et al., 2002). As plants from the dry treatment group experienced dry conditions only for two-day period, differences in leaf surface morphology were not expected between treatments. It is therefore possible that the differences in leaf temperature were caused by stomatal behaviour. These results suggest that pigeon pea reduced its stomatal apertures in response to the dry treatment, whereas the stomata of sesbania remained open even at approximately – 170 kPa of the soil water potential.

Discussion
The split-root system used in the present study allowed us to evaluate the water-extraction ability of roots in different soil layers without the need for isotope labels. As a result, we have revealed that the deep rooting system of sesbania is unable to fully exploit water sources.

We propose that the presence of aerenchyma tissues in the root cortex was the major cause of reduced WIR/RS in the deeper roots in sesbania (Fig. 6). Aerenchyma formation is often associated with dense cellular packing, suberin deposits and lignification in root cells, which reduces radial oxygen loss (Visser et al., 2000; McDonald et al., 2002; Colmer, 2003). All of these factors are also thought to reduce water influx

Fig. 7. Thermal images of sesbania and pigeon pea plants from the dry and wet treatment groups. The values above and below the images indicate the temperature and soil water content, respectively.
rate through increased hydraulic resistance in roots (Kramer and Boyer, 1995). In addition, Miyamoto et al. (2001) suggested that water shortage in well-watered rice was caused by the presence of barriers in the peripheral layers and the endodermis as a result of aerenchyma formation. These studies support our theory that aerenchyma formation in the deeper roots of sesbania increases the hydraulic resistance and lowers the WIR/RS.

Interspecific difference in the water-extraction ability of deeper roots was caused by stomatal behaviour also. Interestingly, the stomata of sesbania lacked the predicted response to water shortage (Fig. 7). This might have caused sesbania to shed its leaves in order to avoid desiccation (Fig. 2), as the water depletion progressed in the top soil and the water supply from the bottom soil remained stagnant probably due to the high hydraulic resistance in deeper roots (Fig 3). As a consequence, there was a decrease in the driving force of water acquisition, which lowered the WIR/RS in the bottom soil (Fig. 5).

It is well known that stomatal behaviour is regulated by ABA, which accumulates around the guard cells in response to drought stress (Assmann and Shimazaki, 1999; Schroeder et al., 2001). ABA is also known to be involved in leaf senescence, and so, by promoting senescence, it might indirectly increase the formation of ethylene, which stimulates leaf shedding (Taiz and Zeiger, 1998). In our study, the leaves of sesbania turned yellow in colour before they fell, which is a typical symptom of leaf senescence, suggesting the accumulation of ABA in the leaves. However, leaf temperature showed little response to drought stress in sesbania. Therefore, we suggest that the guard cells of sesbania might be insensitive to ABA signalling. Further research is required to clarify this issue.

Our study highlights that simple measurements of root length density are not always sufficient to estimate the water-extraction ability of roots, especially those in deeper soil layers (Table 1). Several previous authors have reported that root length density in the deeper soil layers is positively correlated with water relation parameters in plants under drought conditions, and have attributed this correlation to water acquisition by the deep roots (Ketting and Reid, 1993; Hirasawa et al., 1994; Timmsina et al., 1994). In addition, some investigators have attempted to compare the relative abilities of different species competing for soil water within plant community by measuring root length densities (Lehmann et al., 1998; Smith et al., 1999; Livesley et al., 2000). However, as shown in the present study, the water-extraction ability of the deeper roots might differ from that predicted on the basis of root length, as a result of structural differences both within and between species.

Aerenchyma formation is recognized as an adaptive response of plants to oxygen-deficiency in a wide range of wetland species (Armstrong and Drew, 2002). Rice plants have been shown to develop the aerenchyma tissue even under upland conditions. Several studies have suggested that deeper rooting systems should be targeted as an important primary trait for upland rice production under drought conditions (Fukai and Cooper, 1995; Kondo et al., 2003). However, our results suggest that improvements in the anatomical response to soil water environments should also be considered.

In conclusion, the deep roots of sesbania have a relatively low water-extraction ability compared with those of pigeon pea. We propose that aerenchyma formation is a primary factor for the low water-extraction ability in the deeper roots of sesbania. Furthermore, leaf shedding in response to the water shortage also reduces the water influx into the deep roots of sesbania. It is considered that the leaf shedding in this species is induced by the lack of a stomatal response to water shortage.

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