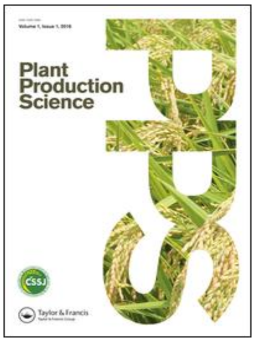

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Root-shoot relationships in four strains of field-grown *Erianthus arundinaceus* at seedling stage

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ABSTRACT
The production of cellulosic bioethanol from non-edible plants is a potential countermeasure against global warming. *Erianthus* species provide cellulosic raw material for bioethanol because they have high biomass productivity and high tolerance to environmental stress, associated with their large, deep root systems. However, it is difficult to select *Erianthus* species for breeding by direct observation of their root systems because the roots are widely dispersed in the soil. Instead, we examined shoot morphological traits that could be closely related to root morphology to find effective reference indices for selection. The potential to evaluate root structure and function in *Erianthus* according to bleeding rate was also examined. An analysis of root–shoot relationships in seedlings indicated that root number and mean length were closely related to stem number and diameter, respectively. These results suggest that root–shoot relationships may provide useful criteria for selective breeding of root systems in *Erianthus*.

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Bleeding rate; Erianthus arundinaceus; plant type; root–shoot relationship

Erianthus arundinaceus is a perennial C4 grass that produces numerous tillers (Hattori et al., 2010). It is anticipated that *E. arundinaceus* will be grown as a raw material for cellulosic bioethanol because of its large shoot biomass (Amalraj et al., 2008). Although a few strains (cultivars) have been released for practical use (Uwatoko & Gau, 2013), additional strains with higher biomass production and stress tolerance are required to improve bioethanol production.

Erianthus species have high biomass production and high tolerance to environmental stresses probably because of their large, deep root systems (Matsuo et al., 2002; Sekiya et al., 2013). Thus, breeding *Erianthus* cultivars according to structural and functional characteristics of the root system should improve biomass yield in poor and problem soils. However, direct observation and measurement of *Erianthus* roots for breeding purposes is difficult because the root system is extensive and deep. If shoot traits, which are easy to measure, correspond closely to the structural and functional characteristics of the root system, they could provide effective reference indices for selecting plants to accelerate breeding new cultivars.

Rice (*Oryza sativa*) is classified into two morphological types: panicle-number and panicle-weight types. Panicle-number types, including IR8, form many small heads and thin tillers, whereas panicle-weight types such as Takanari and Lemont have fewer thick tillers with large panicles (Imbe et al., 2004; Morita et al., 1995; Sakai et al., 2009). Each of these plant types has a characteristic root system. Panicle-number types have more nodal roots and a relatively shallow root system, and panicle-weight types have a deep root system with long and thick nodal roots (Abe et al., 1998; Morita et al., 1995; Kato et al., 2007). These differences suggest that panicle-number and panicle-weight plant types may have different strategies for developing root biomass, which could be determined by the combination of nodal root number and mean root weight or length.

Although *E. arundinaceus* strains vary from stem-number type (analogous to panicle-number type in rice) to stem-weight type (analogous to panicle-weight type in rice) (Matsunami et al., 2014), there is little information on root–shoot relationships among strains of this species. In the present study, root–shoot relationships were examined in four *E. arundinaceus* strains of different plant types, and these relationships are discussed with reference to root physiological activity examined by bleeding rate. Although *Erianthus* are perennial plants, we investigated the morphology of three-month-old seedlings because it is feasible to perform measurements on seedlings, which

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Table 1. Morphological traits of shoots and roots in four Erianthus arundinaceus strains at 91 days after planting.

Strains	Stem number (per plant)	Root number (per plant) Main stem (per plant)	Whole plant# (per plant)	Stem diameter (mm)	Root diameter (mm)	Mean root length (m)	Total root length (m per plant)	Root dry weight (g per plant)	Specific root length (m g^{-1})
IK1	7.4 ± 0.4 a	14.7 ± 1.8 a	27.7 ± 2.5 a	8.8 ± 0.3 b	0.96 ± 0.07 a	1.36 ± 0.13 c	35.3 ± 2.6 c	0.38 ± 0.04 b	98.4 ± 7.1 a
IK2	3.0 ± 0.3 c	14.6 ± 0.7 a	17.1 ± 0.7 b	10.5 ± 0.4 a	1.06 ± 0.05 a	2.34 ± 0.15 b	38.7 ± 1.7 bc	0.57 ± 0.06 b	71.9 ± 4.4 bc
IK3	4.3 ± 0.3 b	13.6 ± 0.8 a	19.3 ± 0.8 b	10.1 ± 0.4 ab	1.09 ± 0.07 a	3.32 ± 0.26 a	63.3 ± 6.1 a	1.24 ± 0.16 a	54.4 ± 3.4 c
IK4	3.3 ± 0.4 bc	13.2 ± 1.0 a	17.4 ± 1.0 b	9.5 ± 0.6 ab	1.23 ± 0.09 a	3.03 ± 0.17 a	53.8 ± 4.9 ab	0.76 ± 0.10 b	75.3 ± 4.8 b

Note. Values are means and standard errors for 12 plants.
Different letters indicate significant differences at $p < .05$.
Whole plant: total number of nodal roots formed on main stem and tillers.

are easy to handle; this may enhance the selection process for future breeding.

Materials and methods

Pot experiment

Seeds of four strains of *E. arundinaceus* (IK1, IK2, IK3, and IK4) were sown in a nursery box (60 cm × 30 cm × 3.6 cm) filled with fertilized soil on March 15, 2010 and grown in a greenhouse at the National Agricultural Research Center for Kyushu Okinawa Region (Kumamoto, Japan). IK1 is a synthetic1 between two clones derived from strain JW630 (originally collected from Shizuoka Prefecture; Tsuruta et al., 2012) and one clone derived from KO2 (preserved in Kyushu National Agricultural Experiment Station). IK2 is a synthetic1 between two clones derived from JW4 (originally collected in Okinawa Prefecture; Tsuruta et al., 2012). IK3 is a selfed line of a clone derived from the second-generation selfed lines of JW4. IK4 is a selfed line of clone of JW630. Seedlings of each strain were transplanted to a 128-cell tray (cell size: 30 × 30 × 44 mm) using tweezers at 21 days after planting (DAP). The seedlings were transplanted to plastic pots (7.5 cm diameter × 9.0 cm height; 1 plant per pot) at 52–55 DAP.

At 91 DAP, the stem and root numbers of 12 plants per strain were recorded. The diameter of the main stem with leaf sheaths (average of the major and minor axes) was measured at the topmost root-emerging node, whereas the diameter of nodal roots (average of the major and minor axes) was measured at the base of individual nodal roots using a digital caliper. In addition, images of roots were scanned at 200 dpi using an image scanner (Epson Expression 10000XL) for the measurement of root length including laterals, which was performed using a WinRHIZO Regular (Regent Instruments Inc.). The total root length including laterals was divided by the number of roots (seminal and nodal roots) to calculate mean root length. Thereafter, the shoots and roots were dried at 80 °C for 3 days and weighed, and specific root length (ratio of total root length to total dry weight) was calculated.

Field experiment

A field experiment was conducted at the Institute for Sustainable Agro-ecosystem Services, University of Tokyo, Nishitokyo, Japan (lat 35°43′N, long 139°32′E). We aimed to investigate whether the root traits at 91 DAP (pot experiment) were related to root function after the roots were widely dispersed in the soil. The top 30 cm of the soil is Andosol, and the subsoil is Tachikawa loam (Yamagishi et al., 2003); both originated from volcanic ash. Before planting seedlings, the field was fertilized with chemical fertilizer at rates 72 kg ha^{-1} N, 108 kg ha^{-1} P_2O_5, and 96 kg ha^{-1} K_2O. The plot size was 4 m × 4 m with three replications for each strain (12 plots in total). Seedlings of the four strains (86 DAP), prepared as for the pot experiment, were transplanted at a density of one plant per m^2 (1 m × 1 m spacing) on June 9, 2010. Weeds were removed manually.

At 76 days after transplanting (162 DAP; August 24, 2010) bleeding rate was measured (Morita & Abe, 2002). Shoots were removed at approximately 15 cm above the soil surface for application of weighed cotton (ca. 20 g) at 10:00 h. The cotton was wrapped with polyethylene film and sealed with a vinyl band to prevent evaporative loss. The cotton was removed after 1 h to quantify xylem sap. The bleeding rate was measured for 3–6 plants per plot (a total of 10–13 plants per strain). Stem numbers were recorded and shoot weight was measured after drying at 80 °C for 3 days.

Differences in bleeding sap rates among the strains were examined with Turkey's multiple range tests. Because the *Erianthus* strains are not genetically pure and show considerable variation, data for individual plants were used for correlations.

Results and discussion

The morphological characteristics of shoots and roots in the pot experiment are shown in Table 1. Stem number, total root length, and root dry weight varied significantly among *E. arundinaceus* strains. The relationships between

Table 2. Shoot dry weight, number of stems, and bleeding rate at 76 days after transplanting for four Erianthus arundinaceus strains.

Strains	Shoot dry weight (g per plant)	Stem number (per plant)	Bleeding rate (g·h^{-1} per plant)
IK1	355 ± 40.2 a	64.5 ± 6.6 a	12.6 ± 2.3 b
IK2	323 ± 38.5 a	35.5 ± 3.4 b	15.1 ± 3.5 b
IK3	322 ± 55.7 a	32.5 ± 4.5 b	20.0 ± 3.5 b
IK4	395 ± 22.8 a	39.2 ± 2.4 b	23.5 ± 2.9 a

Note. Values are means and standard errors for 10–13 plants.
Different letters indicate significant differences at $p < .05$.

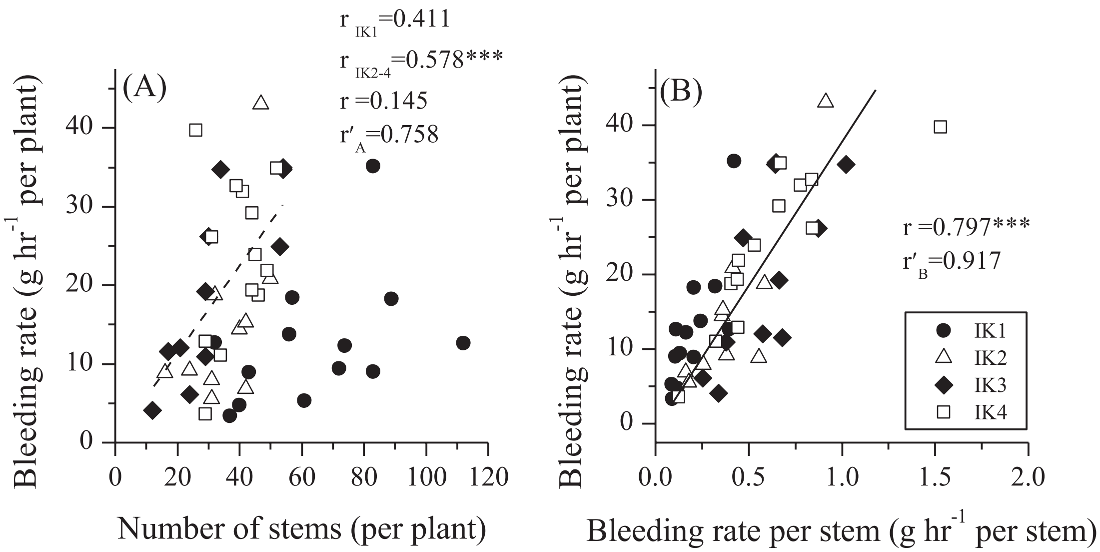

Figure 1. Relationships between the number of stems and bleeding rate per plant (A), and between bleeding rate per stem and bleeding rate per plant (B) in *Erianthus arundinaceus*. The broken line in A indicates the linear regression among IK2, IK3, and IK4. The solid line in B indicates the linear regression among all the four strains. *r*: correlation coefficient among all the four strains. r_{IK1}: correlation coefficient in IK1. r_{IK2-4}: correlation coefficient among IK2, IK3, and IK4.
Notes. ***Correlation is significant at $p < .001$. r'_A: Partial correlation coefficient eliminating the influence of bleeding rate of each stem. r'_B: Partial correlation coefficient eliminating the influence of the total number of stems.

shoot and root traits in these strains were similar to those reported for rice (Abe et al., 1998; Morita et al., 1995). Root number was closely correlated with stem number ($r = .722$, $n = 48$; $p < .001$) when all plants of all strains were analyzed together. Mean root length was correlated with stem diameter ($r = .385$, $n = 48$; $p < .01$). IK2, IK3, and IK4 were stem-weight type that had a small number of stems and a large diameter of main stems, consistent with the panicle-weight type in rice. Those strains, especially IK3 and IK4, had a large mean root length, resulting in a large total root length (Table 1), like Lemont in rice (Morita et al., 1995).

IK1, like panicle-number rice types, formed approximately twice as many thin stems and short roots than did the other three strains. Stem thickness could relate to root thickness and growth direction in rice (Abe & Morita, 1994; Kato et al., 2006; Morita et al., 1997), and panicle-weight types with thick stems tend to develop large, deep root systems. IK1 had the lowest total root length despite its large number of stems and roots. These results suggest that IK3 and IK4, with fewer but thicker stems (similar to panicle-weight type cultivars in rice), presumably have the advantage of developing large root systems, but further studies with more strains are required.

Shoot growth and bleeding rate of the four strains in the field experiment are summarized in Table 2. Plant height at 156 DAP (August 18) was 215 ± 3.7 (IK1), 226 ± 4.6 (IK2), 223 ± 8.8 (IK3), and 250 ± 3.6 cm (IK4), respectively. Significant differences were observed among the strains in the number of stems and bleeding rate despite similar shoot dry weight. IK4 had a significantly greater bleeding rate per plant, whereas IK1 had the smallest bleeding rate despite the presence of many stems. Bleeding rate per plant can be explained by the product of the number of stems and the bleeding rate per stem (Morita & Abe, 1999). Here, bleeding rate per stem contributed more than stem number to the bleeding rate per plant (see partial correlation coefficients in Figure 1), which suggests an advantage of strains with thick stems (similar to panicle-weight types in rice) in terms of physiological activity of the root system in *E. arundinaceus*. The results of the two experiments suggest the relationship between root morphology at the seedling stage and root function at a later stage. For instance, IK3 and IK4 had a large total root length due to a large mean root length at the seedling stage (Table 1) and had high bleeding rates at later growth stages (Table 2), whereas IK1, which had a higher number of small stems, had numerous small roots at the seedling stage and showed a lower bleeding rate in the field. These results suggest that strains with large stems at the seedling stage (stem-weight type), rather than strains with numerous tillers (stem-number type), can form large root systems with high viability in the field, which is advantageous for biomass production under stressful conditions. Upland rice cultivars with high drought tolerance are often panicle-weight types with thick stems and deep root systems (Kato et al., 2006). IK3 and IK4, strains with a small number of thick stems, might have deep root systems that are tolerant to drought (Uga, 2013), although root systems in the field were not investigated in this study. The shoot characteristics of IK3 and IK4 may relate to the depth of the root system and the total quantity of roots.

This analysis of root system morphology with reference to root physiological activity, including its possible relationship to shoot morphological traits, provides useful data for breeding new, better performing cultivars of *Erianthus*.

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