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Distribution and Quantity of Root Systems of Field-Grown *Erianthus* and Napier Grass

Nobuhito Sekiya[1*], Fumitaka Shiotsu[2], Jun Abe[1], Shigenori Morita[1]

[1]Graduate School of Agricultural and Life Sciences, The University of Tokyo, Tokyo, Japan; [2]College of Agriculture, Ibaraki University, Ibaraki, Japan.
Email: *kapinivilage@yahoo.co.jp

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ABSTRACT

Cellulosic bioethanol produced from non-edible plants reduces potential food-fuel competition and, as such, is receiving increasing attention. In the raw material production of cellulosic bioethanol, the aboveground biomass of plants is entirely harvested; consequently, the plant roots represent the major source of organic matter incorporated into the soil. We selected *Erianthus* and Napier grass as the raw materials for cultivation in Asia. However, information about whether these 2 species provide sufficient root volume to sustain soil fertility is limited. Therefore, we examined the spatial distribution of the roots of these 2 plants, and quantified root mass and length. *Erianthus* and Napier grass were either grown in fields or greenhouses in Tokyo (Japan) and Lampung (Indonesia), and then their roots were exposed from adjacent soil profiles. Both species developed large, deep roots, penetrating 2.0 - 2.6 m deep into the soil. Root depth indexes showed that the roots of both species penetrated much deeper into the soil compared to monocot crop species, being more comparable to dicot species. *Erianthus* developed a root mass and length of 384 - 850 $g \cdot m^{-2}$ and 28.8 - 35.8 $km \cdot m^{-2}$, while the values for Napier grass were 183 - 448 $g \cdot m^{-2}$ and 15.6 - 43.6 $km \cdot m^{-2}$, respectively. These values exceeded the maximum values previously recorded for common crop species. Our study confirmed that *Erianthus* and Napier grass develop deep root systems, with substantially large biomass; hence, we suggest that both plants supply root biomass in large quantities, representing possible major sources of soil organic matter.

Keywords: *Erianthus arundinaceus*; *Pennisetum purpureum*; Profile-Wall Method; Root Depth Index; Root Length Density; Root Weight Density

1. Introduction

Although bioethanol is a promising alternative fuel that is viewed as a potential countermeasure to global warming, there are concerns that the rapid increase in global bioethanol production is leading to possible competition with food production. In comparison, cellulosic bioethanol produced from non-edible plants reduces potential food-fuel competition and, as such, is receiving increasing attention; hence, if such plants are grown on marginal non-arable lands, farmland soils are not required [1]. However, questions have been raised regarding the sustainability of material plant production [2]. In food crop production, plant residues are incorporated into soils after harvesting. In contrast, with cellulosic bioethanol production, the entire aboveground biomass is harvested. This practice might reduce soil fertility, and eventually threaten the sustainability of the bioethanol industry.

A solution to this problem might lie in the "hidden half" of material plants, namely the roots. Within the framework of a project to select suitable bioenergy plants for cultivation in tropical and sub-tropical regions of Asia, our research group selected *Erianthus arundinaceus* (*Erianthus*) and *Pennisetum purpureum* (Napier grass) as raw materials for cellulosic bioethanol on the basis of their large shoot biomass and high tolerance to biotic and abiotic stresses [1,3,4]. However, published information about the root system of these 2 species is limited. These plants are expected to develop extensive root systems that mechanically support their large, heavy shoots, and exploit water and nutrients for vigorous growth. In general, roots exude organic substances, leading to the substantial accumulation of carbon in soils [5,6]. Moreover, root biomass produced during the plant growing period is expected to be far greater compared to

*Corresponding author.

standing roots for which dead roots are included in the same period [7,8]. Thus, the root biomass of plants, including dead roots and associated exudates, might provide sufficient organic matter for sustaining soil fertility.

This study aimed to collect basic information about the root systems of these 2 species, as a first step towards evaluating their contributions to soil fertility. Specifically, we aimed to 1) examine the root distributions, particularly rooting depth; and 2) quantify root mass and length parameters of *Erianthus* and Napier grass. We believe that the result of this study will contribute information about the mechanisms facilitating the vigorous growth of these 2 species under water and nutrient deficient conditions.

2. Material and Methods

Field and greenhouse experiments were conducted in the Institute for Sustainable Agro-ecosystem Services (ISAS), Tokyo, Japan (35˚43′N, 139˚32′E) from June 2008 to October 2012. The soil at this site is volcanic ash of the Kanto loam type (Humic Andosol). A parallel experiment was conducted in a field belonging to the Toyota Bio Indonesia (TBI), Lampung, Indonesia (5˚23′S, 105˚22′E) from May 2010 to August 2012. This location was selected based on the results of a previous study [9]. The soil here is red-yellowish podzolic type. Details about the plant growth management used in each experiment are shown in **Table 1**.

The shoots of selected plants were harvested after measuring plant height (*Height*) and stem number (*Stem*). The shoots were then dried at 80˚C for 72 h, and weighed (*SW*). A soil profile (1 - 2 m long × 2 m deep) was exposed 0.1 m away from a selected plant by digging a trench (1 - 2 m long × 1 m wide × 2 m deep) mechanically or manually at a right angle or in parallel to a row (**Figures 1(a), (b)**). Soil cores (50 mm diameter × 51 mm long) were sampled from a profile by inserting stainless steel cylinders at 0.1 m or 0.2 m spacing (**Figure 1(c)**). A larger soil core (50 mm diameter × 600 mm long) was collected from the bottom of the trench, by inserting a liner sampler DIK-110C (Daiki Rika Kogyo, Japan) at a right angle to the trench bottom (**Figure 1(d)**). Thereafter, roots were gently washed under running water until free of soil using a 0.5-mm sieve. The cleaned roots were preserved in 70% ethanol. Then, the roots were spread on a transparent tray, without overlapping, and digitized images were obtained using a scanner with 300 dpi and 256 grey-scales. Root length (*RL*) was determined by using WinRHIZO Basic LA 2400 (Regent Instrument, Canada). After scanning, roots were dried at 80˚C for 48 h, and weighed. Root length and weight density (*RLD* and *RWD*) were calculated by dividing each parameter with a volume of soil core. The root depth index (*RDI*) indicates the mean depth of roots [10], and calculated by first summing *RL* for each soil layer. Then, the percentage *RL*

Table 1. Overview of the field and greenhouse experiments carried out in Tokyo and Lampung.

Site	Plant	Nursery plant	Plot	Spacing	Planting date	Cutting date	Fertilizer	Measurements
Field (Tokyo)	Er	0.9 - 1.1 m height 4 - 6 stems 2 - 3 leaves stem^{-1}	3 m × 4 m (1 plot)	3 rows (2 hills row^{-1}) 2.0 m between rows 3.0 m between hills	Jun 6, 2008	Apr. 2, 2009 Mar. 31, 2010 Mar. 30, 2011	N: 7.2 g·m^{-2} P_2O_5: 10.8 g·m^{-2} K_2O: 9.6 g·m^{-2} Before planting and after each cutting	2 hills in the middle row (Apr. 2011)
	Er	0.4 - 0.5 m height 30 - 40 stems 3 - 5 leaves stem^{-1}	6 m × 2 m (1 plot)	3 rows (6 hills row^{-1}) 1.0 m between rows 1.0 m between hills	May 31, 2010	Jun. 20, 2011		2 hills in the middle row (Jun. 2011)
	Na	0.5 - 0.7 m height 1 stem 3 - 5 leaves stem^{-1}	6 m × 2 m (1 plot)	3 rows (13 hills row^{-1}) 1.0 m between rows 0.5 m between hills		Jun. 21, 2011		
	Su	0.2 - 0.3 m height 1 stem 2 - 3 leaves stem^{-1}	6 m × 2 m (1 plot)			Jun. 23, 2011		
Green house (Tokyo)	Er	0.2 - 0.4 m height 2 - 4 stems 4 - 6 leaves stem^{-1}	6 m × 8 m (3 plots)	8 rows (4 hills row^{-1}) 1.0 m between rows 2.0 m between hills	Jun 9, 2010	Mar. 14, 2011 Sep. 29, 2011 Oct. 15, 2012		1 hill in each plot (Oct. 2012)
	Na	0.4 - 0.5 m height 1 - 2 stems 6 - 8 leaves stem^{-1}	7 m × 6 m (3 plots)	7 rows (15 hills row^{-1}) 1.0 m between rows 0.5 m between hills	May 30, 2011	Sep. 22, 2011 Oct. 5, 2012		
Field (Lampung)	Na	0.1 - 0.2 m stem cuttings with 1 - 2 buds	30 m × 50 m (1 plot)	1.0 m between rows 0.5 m between hills	May 30, 2010	Oct. 25, 2010 Oct. 24, 2011 Mar. 1, 2012 Aug. 27, 2012	N: 30 kg·ha^{-1} P_2O_5: 15 kg·ha^{-1} K_2O: 15 kg·ha^{-1} 0, 1.5 and 3 months after planting and cutting	3 hills in the plot (Aug. 2012)

Er: *Erianthus*, Na: Napier grass, Su: sugarcane.

Figure 1. Root measurement method. A trench (1 - 2 m long × 1 m wide × 2 m deep) was excavated mechanically (a) or manually (b). Soil cores (50 mm in diameter × 51 mm in length) were sampled from a profile (1 - 2 m long × 2 m deep) by inserting stainless steel cylinders at 0.1 m or 0.2 m spacing (c). A larger soil core (50 mm diameter × 600 mm long) was sampled from the trench bottom by using a liner sampler to investigate depths of 2.0 - 2.6 m depth (d).

in a layer compared to the whole 2-m sample depth was calculated (*e.g.*, *a*, *b*, *c*···, *t*% for 0 - 0.1, 0.1 - 0.2, 0.2 - 0.3···, 1.9 - 2.0 m, respectively). These percentage values were then multiplied by the depth of the mid-position of the corresponding layer (*e.g.*, 0.05*a*, 0.15*b*, 0.25*c*···, 1.95*t*), summed (*e.g.*, 0.05*a* + 0.15*b* + 0.25*c* + ··· + 1.95*t*), and divided by 100.

3. Results and Discussion

Erianthus and Napier grass developed large, deep root systems that penetrated the soil to a depth of 2.0-m in all experimental plots (**Table 2**). Some roots were even found at 2.6 m deep. Previously, *Erianthus* has been mainly studied as a source of new genetic variation for sugarcane breeding [11]. To date, studies about the root system of *Erianthus* remain limited, except for a study by Tiwari (1986) [12] who measured root biomass in soil monoliths (0.5 m long × 0.5 m wide × 0.6 m deep), which were collected from a Himalayan grassland dominated by 3 grass species, including *Erianthus rufipilus*. The author estimated the belowground net primary production without differentiating *Erianthus* from the other 2 species. Thus, the current study is the first to describe the deep rooting characteristics of *Erianthus*. In contrast, Napier grass has been extensively studied for fodder and other uses [13]. However, only a limited number of studies are available about its roots. Thus, some reports describe Napier grass as a shallow rooting species based on the visual observation of its extensive surface root system [14,15], while others consider it a deep rooting species, based again on visual observations [16]. Recently, Ma *et al.* (2012) [17] reported that more than 80% of the root mass of hybrid giant Napier grass was found in soil depths of 0 - 0.25 m when evaluating soil to a depth of 1 m, leading to the assumption that this plant is a shallow rooting species. In contrast, Knoll *et al.* (2012) [18] detected Napier grass roots at soil depths of 0.91 - 1.07 m. The authors suspected that high rates of nutrient removal by Napier grass might be ascribed to its capacity to extract nutrient reserves in soils deeper than 1.0-m. However, the authors also stated that they were unable to examine how deep Napier grass roots extended, due to the sampling difficulty. The present study provides evidence supporting the hypothesis of Knoll *et al.* (2012) [18].

In this study, the depth of *Erianthus* and Napier grass root systems was confirmed with *RDI* values (**Table 2**). *RDI* is the weighted average of root depth in soils [10], which have been previously used to successfully screen deep rooting wheat cultivars in Japan [19]. This parameter has also been used to determine the root depths of different crop species. However, the *RDI* values obtained in these preceding studies were low, such as 0.06 - 0.12 for wheat [10,19], 0.08 - 0.14 for rice [20], 0.08 - 0.1 for soybean [21], and 0.16 - 0.27 for sugarcane [22]. In contrast, *Erianthus* and Napier grass had high *RDI* values, ranging from 0.34 to 0.79 and from 0.24 to 0.73, respectively. Because previous studies investigated root distributions in shallow soil layers of 0.16 - 0.27 m, the obtained *RDI* values might increase if root penetration into deeper soil layers was investigated [23]. Therefore, we estimated the *RDI* values of some crop species using the root distribution data from deeper soil layers. The estimated *RDI* values were 0.26 for wheat (1.8 m) [24], 0.27 - 0.29 for maize (1.5 m) [25], 0.19 for sorghum (1.4 m) [26], 0.17 for rice (0.8 m) [27], 0.16 - 0.36 for sugarcane (1.0 - 2.0 m) [28, 29], 0.34 for oilseed rape (1.8 m) [30], 0.75 for soybean (1.8 m) [31], and 0.66 - 1.2 for cotton (1.8 m) [32]. The values in parentheses indicate the soil depths investigated. The results indicate that monocots with fibrous root systems (*i.e.*, wheat, maize, sorghum, rice, and sugarcane) have low *RDI* values compared to dicots with primary root systems (*i.e.*, oilseed rape, soybean, and cotton). Thus, *Erianthus* and Napier grass, which develop fibrous root systems, should be considered deep rooting species among monocot crop species, with their root depths being comparable to those of dicot crop species, which tend to develop deep rooting systems.

Table 2. Various parameters that were measured for *Erianthus* (Er), Napier grass (Na), and sugarcane (Su) grown in the field and greenhouse experiments in Tokyo and Lampung. Root weight density (*RWD*, g·m^{-3}) and root length density (*RLD*, km·m^{-3}) were measured at each 0.4-m soil layer; root depth index (*RDI*, m) was measured using the whole root length found in soil depths of 0 - 2.0 m; root weight (*RW*, g·m^{-2}), and root length (*RL*, km·m^{-2}) were measured per square meter; shoot weight was measured per square meter (*SW*, g·m^{-2}); plant height (*Height*, m) and stem number were measured per hill (*Stem*, hill^{-1}).

Site	Plant (months after planting)	Root						Shoot		
		Density			*RDI* (m)	*RW* (g·m^{-2})	*RL* (km·m^{-2})	*SW* (g·m^{-2})	*Height* (m)	*Stem* (hill^{-1})
		Depth (m)	*RWD* (g·m^{-3})	*RLD* (km·m^{-3})						
Field (Tokyo)	Er (33)	0 - 0.4	694	34.0	0.79	657	35.8	n.a.	n.a.	224.3
		0.4 - 0.8	387	16.0						
		0.8 - 1.2	227	11.7						
		1.2 - 1.6	165	13.7						
		1.6 - 2.0	169	14.2						
		2.0 - 2.6	34	4.3						
	Er (13)	0 - 0.4	432	40.9	0.40	384	28.8	n.a.	n.a.	124.2
		0.4 - 0.8	243	11.1						
		0.8 - 1.2	175	8.9						
		1.2 - 1.6	55	5.2						
		1.6 - 2.0	56	6.1						
		2.0 - 2.6	n.a	n.a						
	Na (13)	0 - 0.4	264	37.1	0.47	183	29.3	n.a.	n.a.	12.5
		0.4 - 0.8	62	10.1						
		0.8 - 1.2	49	9.4						
		1.2 - 1.6	47	8.8						
		1.6 - 2.0	37	7.9						
		2.0 - 2.6	n.a	n.a						
	Su (13)	0 - 0.4	233	17.7	0.48	158	14.6	n.a.	n.a.	3.2
		0.4 - 0.8	88	6.7						
		0.8 - 1.2	23	4.0						
		1.2 - 1.6	35	4.3						
		1.6 - 2.0	15	3.7						
		2.0 - 2.6	n.a	n.a						
Green house (Tokyo)	Er (28)	0 - 0.4	1,121	60.8	0.34	850	31.6	1517	3.7	61.3
		0.4 - 0.8	440	8.5						
		0.8 - 1.2	284	3.8						
		1.2 - 1.6	160	3.7						
		1.6 - 2.0	120	2.2						
		2.0 - 2.6	64	1.8						
	Na (17)	0 - 0.4	566	93.6	0.24	334	43.6	2678	4.6	7.8
		0.4 - 0.8	86	6.0						
		0.8 - 1.2	69	3.2						
		1.2 - 1.6	53	3.6						
		1.6 - 2.0	60	2.7						
		2.0 - 2.6	62	2.9						

Continued

Field (Lampung)	Na (28)	0 - 0.4	460	19.9	0.73	448	15.6	1669	3.1	10.0
		0.4 - 0.8	176	5.3						
		0.8 - 1.2	169	4.9						
		1.2 - 1.6	153	4.0						
		1.6 - 2.0	163	4.8						
		2.0 - 2.6	34	0.9						

The extensiveness of the *Erianthus* and Napier grass root systems was confirmed from *RW* and *RL* values (**Table 2**). *Erianthus* had a root mass and root length of 384 - 850 $g \cdot m^{-2}$ and 28.8 - 35.8 $km \cdot m^{-2}$, respectively. In comparison, the values for Napier grass were 183 - 448 $g \cdot m^{-2}$ and 15.6 - 43.6 $km \cdot m^{-2}$, respectively. These values were much larger than those obtained for sugarcane, namely 158 g or 14.6 km, respectively. Gregory (2006) [33] reviewed previous studies and summarized the maximum *RW* and *RL* recorded for 18 common crop species. While wheat had an exceptionally large *RW* value of 350 $g \cdot m^{-2}$, the other crop species had low values, ranging from 25.5 to 166 $g \cdot m^{-2}$, which were well below the *RW* values of *Erianthus* and Napier grass. Some crop species recorded relatively high *RL* values, such as 26.5 $km\ m^{-2}$ in sorghum, 18.5 - 23.5 $km \cdot m^{-2}$ in wheat, 17.5 $km\ m^{-2}$ in subterranean clover, and 15.1 $km \cdot m^{-2}$ in maize, which are comparable to the *RL* values of Napier grass, but well below those of *Erianthus*.

Lemus and Lal (2005) [34] argue that the removal of aboveground biomass of energy plants might have little influence on soil organic carbon (SOC) due to their large belowground biomass. Indeed, Ma *et al.* (2000) [35] reported large *RW* values of 1,500 - 3,633 $g \cdot m^{-2}$ for switchgrass. The authors concluded in a different report that switchgrass increases SOC several years after establishment [36]. These previous reports indicate that *Erianthus* and Napier grass might also have limited influence on SOC, but that more time might be required for SOC to increase compared to switchgrass. This phenomenon requires clarification.

4. Conclusion

The current study confirmed that *Erianthus* and Napier grass develop deep root systems that penetrate to soil depths of 2.0 - 2.6 m. The root mass and length of both species are large compared to common crop species. This indicates that quantities of associated exudates and dead roots would also be produced. Therefore, it is necessary to quantify the amount of dead roots and exudates that are produced, to further evaluate the capacity of these 2 species in sustaining soil fertility.

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