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Published online: 15 Mar 2016.

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ABSTRACT

Seeds of *Panicum miliaceum, Panicum sumatrense, Setaria glauca,* and *Setaria italica* were raised in polyvinylchloride tubes filled with soil to determine interspecific differences in waterlogging tolerance and the effect of pre- and post-heading waterlogging on growth and grain yield. Four treatments were conducted including control (no-waterlogging stress during growth). Pre-heading waterlogging treatment was initiated 17 days after sowing to heading (TC). Post-heading waterlogging treatment was initiated heading till harvest (CT). Waterlogging treatment was initiated 17 days after sowing to harvesting (TT). The grain yield of *P. miliaceum, S. glauca,* and *S. italica* decreased 16, 18, and 4%, while that of *P. sumatrense* increased 210% under TT treatment and this showed *P. sumatrense* had most waterlogging tolerance. The grain yield was more affected under TC treatment in *S. italica and P. miliaceum.* However, there was not significant differences the grain yield between TC and CT treatment in *P. sumatrense* and *S. glauca*. Total dry weight, total root dry weight, number of crown root, and the proportion of lysigenous aerenchyma of *P. sumatrense* were significantly higher than those of other millets at harvesting. Plant growth rate, total root dry weight, number of crown root, and the proportion of lysigenous aerenchyma of *P. sumatrense* were significantly higher than those of other millets at heading. These results suggest that *P. sumatrense* exhibits waterlogging tolerance by enhancing root growth characterized by a high proportion of lysigenous aerenchyma in the crown root.

Abbreviations: LA: leaf area; MLA: mean leaf area; NAR: net assimilation rate; OA: osmotic adjustment; PGR: plant growth rate; PVC: polyvinyl chloride

Waterlogging is used to describe flooding of the root system and submergence is used to describe a situation when most or all aerial tissues is under water (Bailey-Serres, Lee, & Brinton, 2012). Waterlogging is a widespread problem in field crop production. In the United States, losses in crop production due to flooding were second to drought in many of the past 12 years, with these two abiotic stresses accounting for more than 70% of the reduction in harvests in 2011 (Bailey-Serres et al., 2012). In 2011, the land area affected by flooding in Queensland, Australia, was equivalent to the country areas of Germany and France (Perata, Armstrong, & Voesenek, 2011). In Japan, a concentrated heavy rain often seriously affects field crop production because half of the paddy field area is used for field crop production.

Much research has been done on the metabolic and morphological responses of plants to flooding, energy metabolism, fermentation pathway and toxicity of end products, transportation of photosynthate, linking hormones, water and nutrient uptake, root development, aerenchyma formation, signal transduction from root to shoot, and modified gene expression (For reviews; Irfan, Hayat, & Hayat, 2010; Setter & Waters, 2003; Subbaiah & Sachs, 2003; Visser & Voesenek, 2004; Yamauchi, Shimamura, Nakazono, & Mochizuki, 2013). Before research at the cellular level, the function of roots should be investigated since waterlogging changes the root environment directly and reduces root growth, shoot growth, and final grain yield for several crops (Cannell, Belford, Blackwell, Govi, & Thomson, 1985; Setter & Waters, 2003; Trought & Drew, 1980). It is also well established that flooding imposes water stress in the shoot since water absorption is decreased in plants with smaller root system and/or physiologically injured roots (Hayashi et al., 2013; Polacik & Maricle, 2013). Maintenance of root growth is considered an important adaptive response to excess water in several crops (Daugherty & Musgrave,

1994; Erdmann & Wiedenroth, 1986; Huang, Johnson, Box, & NeSmith, 1997; Huang, Johnson, NeSmith, & Bridges, 1994). Aerenchyma formation is a feature that enables roots to grow in waterlogged soils, by allowing more internal oxygen diffusion from shoots to the root tip and to rhizosphere (Jackson & Armstrong, 1999; Thomson, Armstrong, Waters, & Greenway, 1990; Visser, Blom, & Voesenek, 1996).

Millet is defined as gramineous crops which have small grains and are cultivated mainly in a summer in semiarid, tropic, or sub-tropic regions and also in temperature monsoon regions (Sakamoto, 1993). Millets have been cultivated as a food and a feed for long time (Austin, 2006) because millets have a number of merits, for example, they can grow under unfavorable conditions, many methods of cooking have been established, and local landraces are still cultivated in many areas of the world (Sakamoto, 1993). Nowadays, millet is one of the important genetic resources as they are able to maintain leaf photosynthesis under low stomatal conductance due to C_4 photosynthesis function (Lopes, Araus, van Heerden, & Foyer, 2011). Berg, de Noblet-Ducoudre, Sultan, Lengaigne, and Guimberteau (2013), using a newly developed agro-DVGM, reported that millets showed the potential productivity as the most important staple crop in Africa and India. *Setaria italica*, foxtail millet, is an annual crop originated from *S. viridis* (L.) P. Beauv (Kihara & Kishimoto, 1942) in Central Asia between Turkestan and the northwestern Indian subcontinent. This millet has been cultivated through Eurasia since about 5,000 BC. (Sakamoto, 1987). In Japan, *S. italica* might have been cultivated for as long as rice. The mean grain yield of *S. italica* from 1921 to 1970 is around 150 kg $10a^{-1}$ and is mainly cultured on hilly and mountainous areas in Japan. *Setaria glauca*, yellow foxtail millet, is an annual weed that grows along the roadside and elsewhere in Japan, but it is an important food crop often cultivated with *Panicum sumatrense*, little millet, in south India (Kimata, Ashok, & Seetharam, 2000). Farmers in India believe that *S. glauca* provides a reasonable harvest, while *P. sumatrense* might fail completely in severe drought (Kimata et al., 2000). Matsuura, Tsuji, An, Inanaga, and Murata (2012) reported that *S. glauca* showed more drought tolerance than *P. sumatrense* because of vigorous root growth. *Panicum miliaceum*, common millet, is one of the early maturing among these species and is well known for drought tolerance. The mean yield of *P. miliaceum* from 1921 to 1970 is around 120 kg $10a^{-1}$ and is mainly cultivated on hilly and mountainous areas in Japan; it is susceptible to damage by birds. Kono et al. (1987) reported that the waterlogging tolerance of *P. miliaceum* was higher than *S. italica,* however, the mechanisms of interspecific differences in waterlogging tolerance of millets are not clear. In this study, we determined interspecific differences in waterlogging tolerance and the effect of pre- and post-heading waterlogging on growth and grain yield among four millets.

1. Materials and methods

1.1. Plant materials and culture

Common millet (*P. miliaceum* L. cv. 65), foxtail millet (*S. italica* (L.) P. Beauv. cv. 84-6-14-2), little millet (*P. sumatrense* Roth. cv. 97-4-12-2-1), and yellow foxtail millet (*S. glauca* (L.) P.Beauv. cv. 97-4-12-2-2) were used. Seeds of each species were sown in soil (Andosol) contained in plastic polyvinyl chloride (PVC) tubes (7.5 cm in inner diameter and 40 cm in height) in a greenhouse at Tokai University, Kumamoto, Japan on 1 June 2010. For half of plants, treatment (T) was initiated by completely submerging the tubes above 10 cm of the surface of a Hoagland and Arnon's nutrient solution into large containers (432 mm × 614 mm and 315 mm height) at 17 days after sowing (Figure 1). The solution contained twice the Fe content of the original Hoagland and Arnon's solution (EDTA-Fe: 45.0 mg l^{-1}). As a control (C), tubes were also placed into large containers and water level maintained 5 cm from bottom of the tubes.

At the onset of heading (*S. italica*; 67 days after the treatment (DAT) in control, 69 DAT in flooding treatment, *S. glauca*; 61 DAT in both treatments, *P. miliaceum*; 42 DAT in control, 46 DAT in flooding treatment, *P. sumatrense*; 74 DAT in both treatments), half of the plants under control were subjected to waterlogging treatment (CT) and the other half of the plants continued growth under the control condition (CC) till harvest. Also, half of the plants under the waterlogging treatment were subjected to control condition (TC) and the other half of the plants continued growth under waterlogging treatment (TT).

1.2. Dry matter production and grain yield

Plant was sampled in three tubes of each treatment a day before imposing treatment, at heading, and at harvest. Leaf area and leaf number were measured before leaf blade, leaf sheath, and stem samples were cured at 110 °C for 30 min, followed by drying at 65 °C for 48 h and weighing. Roots were sampled at 0–10 cm and 10–40 cm depths. The root samples were washed free of soil and dried as the shoot for the measurement of dry weight. Plant growth rate (PGR), net assimilation rate (NAR), and mean leaf area (MLA) were estimated by the following equations:

$$\text{PGR (g day}^{-1}) = \frac{W_2 - W_1}{T_2 - T_1} \tag{1}$$

$$\text{NAR (g m}^{-2}\text{ day}^{-1}) = PGR \times \frac{\log_e LA_2 - \log_e LA_1}{LA_2 - LA_1} \times 10^4 \tag{2}$$
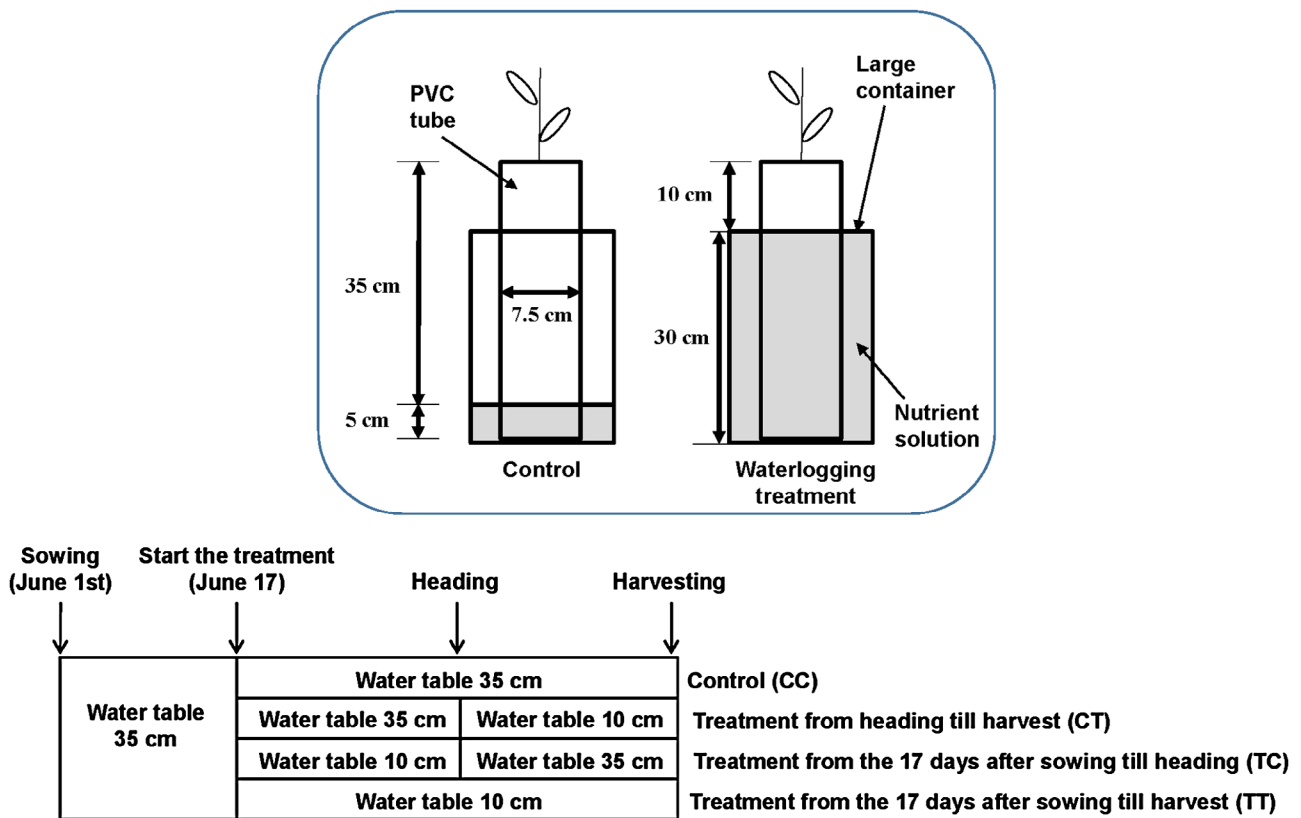
Figure 1. Culture system for millets used to waterlogging plants in nutrient solution and control and a design of four treatments.

$$MLA\ (cm^2) \times \frac{LA_2 - LA_1}{\log_e LA_2 - \log_e LA_1} \quad (3)$$

Where W_1 and W_2 were the dry weight of whole plant one day before the start of treatment (T_1) and the day when heading was started (T_2), respectively; LA_1 and LA_2 were the total leaf area per plant one day before the start of treatment (T_1) and the day when heading was started (T_2), respectively.

Plants in four replicate tubes of each treatment were harvested at maturity for grain yield. Yield components, i.e. number of panicles per plant, number of grains per panicle, and 1,000 grain weight were determined on the harvested plants. Harvest index was calculated as the ratio of grain yield to total dry weight of the whole plant.

1.3. Water potential, osmotic potential, and gas exchange rate of leaves at heading

The water potential of the second fully expanded leaf was measured on three leaves per treatment by pressure chamber at midday, and put into sealed vinyl bag containing a small amount of distilled water on 41 days after the waterlogging treatment. Leaves became turgid in four hours

at 10 °C and were wrapped with an aluminum foil, then submerged in liquid nitrogen. Frozen leaves were retained in a freezer at −80 °C until they were used for measuring osmotic potential with a thermocouple psychrometer (Decagon Devices Inc., Tru Psi model SC10X). Osmotic adjustment (OA) of leaves was calculated as the difference in osmotic potential at full turgor between control and treatment (Pantuwan, Fukai, Cooper, Rajatasereekul, & O'Toole, 2002).

The photosynthetic (Pn) and transpiration (Tr) rates, stomatal conductance (Gs), and intercellular CO_2 concentration (Ci) in the fully expanded leaves of three plants each from the control and treatment were measured on 43 days after the treatment was started using a portable photosynthesis system (Li-6,400, Li-COR, Inc., Lincoln, NE, USA). During the measurement, photosynthetic photon flux density was set at 1,000 µmol m^{-2} s^{-1}, CO_2 concentration at 370 ppm, and air temperature at 30 °C. Relative humidity at sample chamber was 51.5 ± 6.0% (mean ± standard deviation).

1.4. Aerenchyma and lignin observation of crown root

Three samples of 10 mm length were taken from the crown root (10 mm below the root–shoot junction) of three

Table 1. Influence of waterlogging on plant growth, gas exchange rate, and water relations of four millets at heading.

		Heading date	PGR (g plant^{-1} day^{-1})	NAR (g m^{-2} day^{-1})	MLA (cm^2)	Pn (μmol m^{-2} s^{-1})	Gs (μmol m^{-2} s^{-1})	Ci (ppm)	Tr (m mol m^{-2} s^{-1})	ΨL (MPa)	OA (MPa)
P. sumatrense	C	Aug. 24	0.262	12.1	217	24.6	0.2	136	5.5	−0.68	0.02
	T	Aug. 24	0.277^{NS}	9.4**	297**	20.9*	0.18^{NS}	150^{NS}	5.1^{NS}	−0.80^{NS}	
P. miliaceum	C	28-Jul	0.116	7.1	164	25.7	0.24	153	5.5	−0.75	−0.08
	T	28-Jul	0.042**	4.2**	99**	17.1**	0.14**	133^{NS}	3.7**	−0.68^{NS}	
S. glauca	C	Aug. 17	0.101	10.2	99	24.3	0.18	109	4.9	−0.73	−0.01
	T	Aug. 17	0.045**	8.8^{NS}	52**	23.5^{NS}	0.18^{NS}	117^{NS}	5.0^{NS}	−0.78^{NS}	
S. italica	C	Aug. 13	0.062	7.8	79	23.5	0.28	208	6.1	−0.83	0.02
	T	Aug. 17	0.016**	5.2**	32**	13.5**	0.13**	212^{NS}	3.3**	−1.07*	

Notes: C; Control, T; Waterlogging treatment from 17 days after sowing till heading. ** and * indicate that C and T are significantly different at $p = 1$ and 5%; NS not significant by *F* test. PGR; Plant growth rate, NAR; Net assimilation rate and MLA; Mean leaf area were calculated based on the difference of leaf area and total dry weight of whole plant from the start of waterlogging treatment (17 days after sowing) till heading. Pn; Photosynthetic rate, Gs; Stomatal conductance, Ci; intercellular CO_2 concentration, and Tr; Transpiration rate were measured on 43 days after treatment. ΨL; leaf xylem water potential and OA; Osmotic adjustment were measured on 41 days after treatment.

plants per treatment and fixed in FAA (formalin: acetic acid: ethanol: distilled water = 1:1:9:9) solution. Transverse 6-μm-thick sections were obtained using plant microtome adjusted 6 μm thick (MT-3 Nippon Medical & Chemical Instruments, Osaka, Japan) stained with 0.01% toluidine blue or saturated phloroglucinol in 30% hydrochloric acid (Jensen, 1962) and photographed with a light microscope (BX53 Olympus Optical, Tokyo, Japan). The percentage of aerenchyma or cortex were calculated as the area of aerenchyma or cortex to the cross-sectional area of the crown root, respectively.

1.5. Statistical analysis

All of data at heading were analyzed using the *F* test to explore significant difference between control and the treatment. All of data were analyzed using two-way analysis of variance (ANOVA) at harvesting. At heading and harvesting, means were compared using Tukey–Kramer multiple range test to clear the significant difference among four millets or four treatments. A regression analysis was also performed to explore the relationship between grain yield and each yield component.

2. Results

2.1. Plant growth, leaf water potential, and photosynthetic rate at heading

The shortest period (41 days) from onset of the treatment to heading occurred in *P. miliaceum* while the longest period (68 days) was in *P. sumatrense* (Table 1). Waterlogging did not affect the duration to heading except for *S. italica*. The PGR of *P. sumatrense* was little affected by waterlogging treatment but those of the other millets decreased significantly. Waterlogging also decreased the MLA rather than NAR in *Setaria* species. The PGR of *P. miliaceum* decreased because of depletion in NAR as much as MLA. The NAR of *P. sumatrense* decreased but the MLA was increased

by waterlogging. Waterlogging had little effect on Pn of *S. glauca* but decreased those of the other millets (Table 1). This depletion was caused by lower Gs. The leaf water potential was decreased by waterlogging in *S. italica* but not in the other millets (Table 1). There was no interspecific difference in osmotic adjustment of the leaf.

2.2. Growth and morphology of root at heading

The number of crown roots of *P. sumatrense* increased but that of *P. miliaceum* decreased and those of *Setaria* species were not changed by the treatment (Table 2). Root dry weight in the upper soil layer (0–10 cm depth) of *P. sumatrense* was increased but those of the other millets were decreased by the treatment. Root dry weight in the lower soil layer (10–40 cm depth) decreased in all millets due to waterlogging. Total root dry weight of *P. sumatrense* was not changed and those of the other millets were decreased by the treatment.

Lysigenous aerenchyma markedly developed near the base of the crown root of *P. sumatrense* in both treatments (Figure 2). Lysigenous aerenchyma also developed on the crown root of *S. glauca* but was not well developed in *S. italica* and *P. miliaceum* under waterlogging. Lignin was observed in the sclerenchyma of crown roots in all millets, but was least observed in *P. sumatrense*. Waterlogging treatment did not affect the ratio of cross-sectional area of cortex to the crown root of all the millets (Table 2). The ratio of cross-sectional area of lysigenous aerenchyma to the crown root decreased in *S. italica* and increased in the other millets.

### 2.3. Grain yield and yield components

Days from start of stress treatment to harvest were longest (145 days) in *P. sumatrense* and were shortest (76 days) in *P. milliaceum* (Table 3). Waterlogging did not affect the duration from start of stress treatment to harvest, except TC treatment in *S. italica*. The grain yield of *P. miliaceum*,

Table 2. Influence of waterlogging on number of crown root, root dry weight, and proportion of cortex and lysigenous aerenchyma in crown roots of four millets at heading.

| | | Number of crown root | Root dry weight (g) | | Total root dry weight | Cortex[a] | Lysigenous aerenchyma[a] |
			0–10cm	20–40cm	(g)	%	%
P. sumatrense	C	102	2	1.8	3.9	66.5	31.1
	T	206**	2.8**	0.9**	3.7[NS]	74.2[NS]	39.3*
P. miliaceum	C	45	0.6	0.2	0.8	59.1	16.5
	T	23*	0.3**	0.0**	0.3**	58.2[NS]	31.4**
S. glauca	C	34	0.7	0.7	1.3	50.6	23
	T	27[NS]	0.6[NS]	0.1**	0.7**	63.7[NS]	28.5*
S. italica	C	22	1.4	0.6	1.4	50.9	14.3
	T	18[NS]	0.3**	0.1**	0.3**	58.7[NS]	10.2*

Notes: C; Control, T; Waterlogging treatment from 17 days after sowing till heading. ** and * indicate that C and T are significantly different at p = 1% and 5%; NS not significant by *F* test.
[a]Data were calculated from cross sections of crown roots by microscope observation as showed in Figure 2.
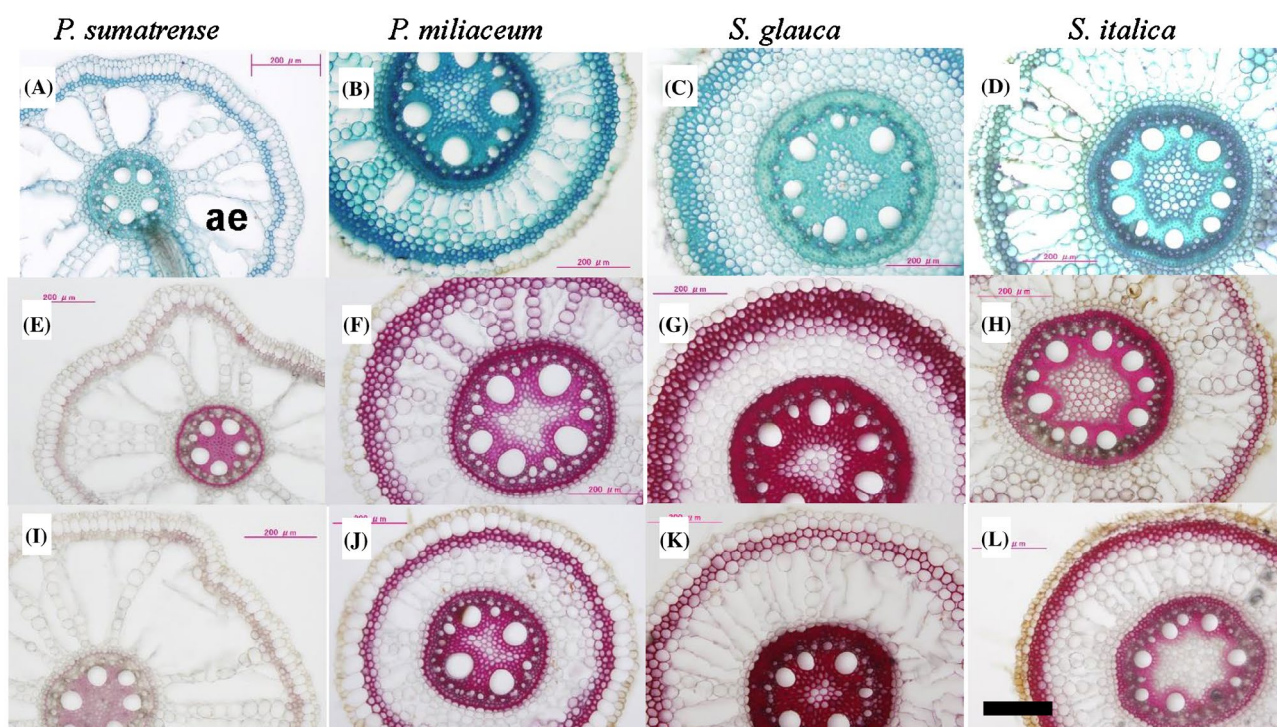
Figure 2. Cross section taken 10 mm below the root–shoot junction of crown root at heading. A, B, C, and D showed control plants, stained with 0.01% toluidine blue, of *P. sumatrense*, *P. miliaceum*, *S. glauca,* and *S. italica*, respectively. E, F, G, and H showed also control plants, stained with saturated phloroglucinol in 30% hydrochloric acid, of *P. sumatrense*, *P. miliaceum*, *S. glauca,* and *S. italica*, respectively. I, J, K, and L showed treatment, stained with saturated phloroglucinol in 30% hydrochloric acid, of *P. sumatrense*, *P. miliaceum*, *S. glauca*, and *S. italica*, respectively. ae = lysigenous aerenchyma. Bars = 200 μm.
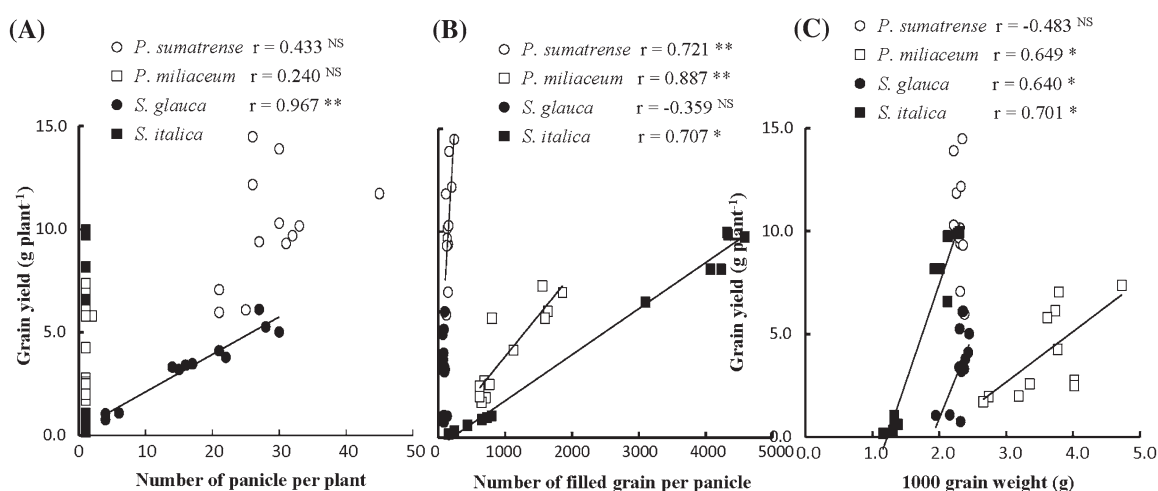
S. glauca, and *S. italica* decreased to 28, 18, and 4% under waterlogging; that of *P. sumatrense* increased to 211% (Table 3). When the waterlogging was imposed before heading, the grain yield of *P. miliaceum* and *S. italica* decreased more severely. Waterlogging before heading and after heading affected the grain yield of *S. glauca* and *P. sumatrense* similarly. Waterlogging reduced the number of panicle of *S. glauca* and number of filled grain per panicle of *S. italica* and *P. miliaceum* but increased those of *P. sumatrense*. It decreased the 1,000 grain weight and harvest index of *P. miliaceum* and *S. italica* but did not change those of *P. sumatrense* and *S. glauca*. Waterlogging decreased the total dry weight of all millets except *P. sumatrense*. There was a significant correlation between number of panicle per plant and grain yield in *S. glauca* (Figure 3(A)). There was also significant correlation between number of filled grain per panicle and grain yield of all millets except *S. glauca* (Figure 3(B)). There was a significant correlation

Table 3. Influence of waterlogging on grain yield and yield components of four millets.

		Days from start of stress treatment to head-ing	Days from heading to har-vesting	Days from start of stress treatment to harvest	Grain yield (g plant^{-1})	Number of Panicle (plant^{-1})	Number of filled grain (panicle^{-1})	1000 grain weight (g)	Total dry weight (g plant^{-1})	Harvest index
P. sumatrense	CC	68	77	145	6.4 c	22 b	123 b	2.3 a	46.8 b	0.14 a
	CT	68	77	145	10.5 b	32 a	145 b	2.3 a	74.5 a	0.14 a
	TC	68	77	145	9.8 b	31 a	138 b	2.3 a	67.0 a	0.15 a
	TT	68	77	145	13.5 a	27 ab	217 a	2.3 a	83.7 a	0.16 a
P. miliaceum	CC	41	35	76	6.8 a	1 a	1684 a	4.1 a	18.2 a	0.38 a
	CT	41	35	76	5.3 b	1 a	1175 ab	3.7 ab	16.2 a	0.33 ab
	TC	41	35	76	2.6 c	1 a	691 b	3.8 ab	8.7 b	0.30 ab
	TT	41	35	76	1.9 c	1 a	659 b	2.9 b	8.5 b	0.22 b
S. glauca	CC	61	71	132	5.5 a	28 a	82 a	2.4 a	26.5 a	0.21 a
	CT	61	71	132	3.3 b	15 b	94 a	2.3 a	17.3 b	0.19 a
	TC	61	71	132	3.8 b	20 b	80 a	2.4 a	18.7 b	0.20 a
	TT	61	71	132	1.0 c	5 c	100 a	2.1 a	5.4 c	0.18 a
S. italica	CC	57	38	95	9.9 a	1 a	4414 a	2.2 a	30.6 a	0.32 a
	CT	57	38	95	7.6 b	1 a	3801 a	2.0 a	26.6 a	0.29 a
	TC	61	47	108	1.0 c	1 a	723 b	1.3 b	8.4 b	0.11 b
	TT	61	34	95	0.4 c	1 a	277 b	1.3 b	4.5 b	0.09 b
ANOVA										
Crop					**	**	**	**	**	**
Treatment					**	**	**	**	**	**
Interaction					**	**	**	**	**	**

Notes: CC = Control, TT = Waterlogging treatment from the 17 days after sowing till harvest, CT = Waterlogging treatment from heading till harvest, TC = Water-logging treatment from the 17 days after sowing till heading. Means with the same letter are not significantly different according to Tukey–Kramer multiple range test (5%).
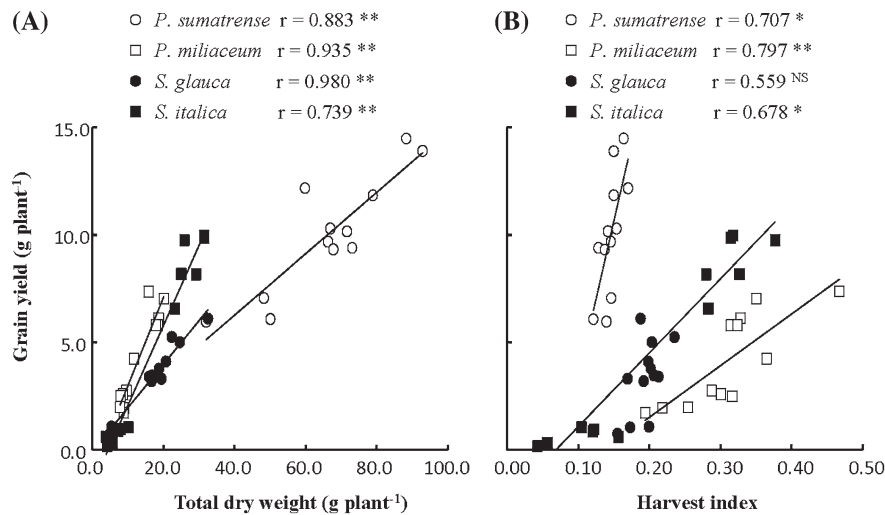

Figure 3. Correlations between grain yield and number of panicle per plant (A), number of filled grain per panicle (B) and 1,000 grain weight (C) of four millets under waterlogging. * and ** indicates significance at $p = 5$ and 1%, respectively. NS indicates not significant.

between 1,000 grain weight and grain yield in all millets except *P. sumatrense* (Figure 3(C)). However, there was no significant difference in the 1,000 grain weight of S. glauca among the four treatments (Table 3). For all millets, there was a significant correlation between total dry weight and grain yield (Figure 4(A)). There was a significant correlation between harvest index and grain yield in all millets except S. glauca (Figure 4(B)). However, there was no significant difference in the harvest index of *P. sumatrense* among the four treatments (Table 3).

2.4. Growth and morphology of root at harvest

The number of crown roots of *S. glauca* and *S. italica* decreased. By contrast, that of *P. sumatrense* increased, and that of *P. miliaceum* was unchanged by waterlogging (Table 4). Root dry weight within the 0–10 cm depth of *S. italica* was decreased by the treatment, but was not affected in other millets. Root dry weight at the 10–40 cm depth as well as total root dry weight of *P. sumatrense* was increased and those of the other millets decreased by waterlogging.

Figure 4. Correlations between grain yield and total dry weight (A) or harvest index (B) of four millets under waterlogging. * and ** indicates significance at p = 5 and 1%, respectively. NS indicates not significant.

Table 4. Influence of waterlogging on number of crown root, root dry weight, and proportion of cortex and lysigenous aerenchyma in crown roots of four millets at harvest.

			Root dry weight (g)		Total root dry weight	Cortex[a]	Aerenchyma[a]
		Number of crown root	0–10 cm	10–40 cm	(g)	%	%
P. sumatrense	CC	114 c	3.5 b	1.8 c	5.3 c	83.4 a	39.8 a
	CT	137 bc	6.7 a	1.9 c	8.6 bc	82.4 a	40.5 a
	TC	184 a	5.7 ab	8.3 a	13.7 a	87.0 a	37.9 a
	TT	167 ab	4.9 ab	6.0 b	10.9 ab	88.6 a	38.4 a
P. miliaceum	CC	32 a	0.9 a	1.0 a	1.9 a	76.8 a	18.9 a
	CT	49 a	1.5 a	0.7 ab	2.2 a	73.6 a	9.8 b
	TC	31 a	1.1 a	0.4 b	1.4 b	77.2 a	19.0 a
	TT	35 a	0.9 a	0.3 b	1.2 b	80.7 a	13.5 ab
S. glauca	CC	50 a	1.0 a	3.4 a	4.4 a	69.5 a	14.2 b
	CT	56 a	0.9 a	2.4 ab	3.3 ab	73.9 a	18.3 b
	TC	52 a	1.2 a	1.3 ab	2.5 ab	71.9 a	30.8 a
	TT	25 b	0.7 a	0.3 b	1.0 b	70.4 a	31.4 a
S. italica	CC	42 a	1.7 a	0.9 a	2.5 a	58.2 a	2.3 b
	CT	46 a	1.4 a	0.6 b	1.9 b	67.3 a	7.3 b
	TC	24 b	0.2 b	0.2 c	0.4 c	66.4 a	7.2 b
	TT	19 b	0.2 b	0.0 c	0.2 c	60.8 a	17.3 a
ANOVA							
Crop		**	**	**	**	**	**
Treatment		*	**	**	**	NS	**
Interaction		**	**	**	**	NS	**

Notes: CC = Control, TT = Waterlogging treatment from the 17 days after sowing till harvest, CT = Waterlogging treatment from heading till harvest, TC = Water-logging treatment from the 17 days after sowing till heading. Means with the same letter are not significantly different according to Tukey–Kramer multiple range test (5%).
[a]Data were calculated from cross sections of crown roots by microscope observation as showed in Figure 5.

Lysigenous aerenchyma markedly developed near the base of crown root of *P. sumatrense* in all treatments (Figure 5(A, E, I, M, Q)). It also developed more at the crown root of *S. glauca* than in *S. italica* and *P. miliaceum* under waterlogging (Figure 5(R, S, T)). Percentage of cross-sectional area of lysigenous aerenchyma did not change in *Panicum* species but increased in *Setaria* species as compared TT treatment with control (Table 4). Lignin was observed more clearly on the crown root of *P. miliaceum* and *S. italica* in all treatment (Figure 5). Waterlogging decreased the accumulation

of lignin at the crown root of *P. sumatrense* and *S. glauca* (Figure 5(Q, S)).

3. Discussion

3.1. Plant growth and leaf water potential

Waterlogging increased the dry weight of *P. sumatrense* and decreased those of the other millets and there was significant correlation between total dry weight and grain yield

Figure 5. Cross section taken 10 mm below the root–shoot junction of the crown root at harvesting. A, B, C, and D showed control plants, stained with 0.01% toluidine blue, of *P. sumatrense*, *P. miliaceum*, *S. glauca,* and *S. italica*, respectively. E, F, G, and H showed also control plants (CC), stained with saturated phloroglucinol in 30% hydrochloric acid, of *P. sumatrense*, *P. sumatrense*, *P. miliaceum*, *S. glauca,* and *S. italica*, respectively. I, J, K, and L showed treatment from heading till harvest (CT), M, N, O, and P showed treatment from the 17 days after sowing till heading (TC), Q, R, S, and T showed treatment from the 17 days after sowing till harvest (TT), stained with saturated phloroglucinol in 30% hydrochloric acid, of *P. sumatrense*, *P. miliaceum*, *S. glauca,* and *S. italica*, respectively. ae = lysigenous aerenchyma. Bar = 200 μm.

(Table 3, Figure 4). Result of multiple range test showed that total dry weight and PGR of *P. sumatrense* were significantly higher than those of the other three millets under waterlogging treatment (Tables 5 and 6). The interspecific difference of PGR may be attributable to differences in MLA rather than NAR (Table 1). The interspecific difference of NAR was consistent with that of Pn. Their reduction by waterlogging was partly attributable to depletion in leaf water potential, especially for the most susceptible millet, *S. italica* as reported previously for susceptible wheat

(Hayashi et al., 2013). Our results suggest that sustained plant growth may be important for grain formation during vegetative growth under waterlogging.

3.2. Grain yield

Grain yield per plant (or per land area) is first priority in cereal farming. When the waterlogging tolerance is determined, investigation should be based on the yield in the target environment (Zou, Hu, Zeng, et al., 2014). Based

Table 5. Multiple range test of growth and root aerenchyma under control (C) and waterlogging treatment (T) of four millets at heading.

		PGR (g day^{-1})	Total root dry weight (g plant^{-1})	Number of crown root (plant^{-1})	Cortex[a] (%)	Aerenchyma[a] (%)
C	*P. sumatrense*	0.262 a	3.9 a	102 a	66.5 a	31.1 a
	P. miliaceum	0.116 b	0.8 b	45 b	59.1 a	16.5 bc
	S. glauca	0.101 bc	1.3 b	34 b	50.6 a	23.0 b
	S. italica	0.062 c	1.4 b	22 b	50.9 a	14.3 c
T	*P. sumatrense*	0.277 a	3.7 a	206 a	74.2 a	40.5 a
	P. miliaceum	0.042 b	0.3 b	23 b	58.2 b	33.3 b
	S. glauca	0.045 b	0.7 b	27 b	63.7 ab	28.5 b
	S. italica	0.016 b	0.3 b	18 b	58.7 b	10.2 c

Notes: C; Control, T; Waterlogging treatment from 17 days after sowing till heading. Means with the same letter are not significantly different according to Tukey–Kramer multiple range test (5%).
[a]Data were calculated from cross sections of crown roots by microscope observation as showed in Figure 2.

Table 6. Multiple range test of grain yield and yield components under control (CC) and waterlogging treatment (TT) of four millets at harvest.

		Grain yield (g plant^{-1})	Total dry weight (g plant^{-1})	Harvest index	Total root dry weight (g plant^{-1})	Number of crown root (plant^{-1})	Cortex[a](%)	Aerenchyma[a] (%)
CC	*P. sumatrense*	6.4 b	46.8 a	0.14 b	5.3 a	114 a	83.4 a	39.8 a
	P. miliaceum	6.8 b	18.2 c	0.38 a	1.9 b	32 b	76.8 ab	25.6 b
	S. glauca	5.5 b	26.5 bc	0.21 b	4.4 ab	50 b	69.5 b	14.2 c
	S. italica	9.9 a	30.6 b	0.32 a	2.5 b	42 b	58.2 c	2.3 d
TT	*P. sumatrense*	13.5 a	83.7 a	0.16 ab	10.9 a	167 a	88.6 a	38.4 a
	P. miliaceum	1.9 b	8.5 b	0.22 a	1.2 b	35 b	80.7 ab	13.5 c
	S. glauca	1.0 b	5.4 b	0.18 ab	1.0 b	25 b	70.4 bc	31.4 b
	S. italica	0.4 b	4.5 b	0.09 b	0.2 b	19 b	60.8 c	1.0 d

Notes: CC = Control, TT = Waterlogging treatment from the 17 days after sowing till harvest. Means with the same letter are not significantly different according to Tukey–Kramer multiple range test (5%).
[a]Data were calculated from cross sections of crown roots by microscope observation as showed in Figure 5.

on grain yield, we found that *P. sumatrense* showed a strong waterlogging tolerance, "apparent" tolerance like a rice (Vartapetian & Jackson, 1997), whereas *S. glauca, P. miliaceum,* and *S. italica* were susceptible (Table 6). In general, when performance under waterlogged conditions was considered in proportion to yield under no stress conditions, plants responded equally to the stress (Musgrave, 1994). There is some evidence that genotypic differences control tolerance to waterlogging in cereals (Davies & Hillman, 1988; Huang et al., 1994; Musgrave, 1994; Thomson, Colmer, Watkin, & Greenway, 1992). In this study, there is no significant correlation of grain yield between waterlogging and control ($r = 0.297^{NS}$ including all millets). Musgrave and Ding (1998) also showed that yields of waterlogged cultivars were not proportionally related to control yields ($r^2 = 0.14$), reflecting differential response by cultivars to stress.

Results of earlier investigations were grouped into three categories depending on whether the cereals showed (1) a maximum response and/or the only response to soil moisture conditions at specific growth stages, (2) a response at all stages of growth, (3) no response to soil moisture conditions at any stage; most previous reports came into category (1) (Salter & Goode, 1967). In this study, the grain yield of all millets decreased or increased at all stages of growth (Table 3) and this suggested that four millets came into category (2). When waterlogging was imposed

before heading, the grain yields of *P. miliaceum* and *S. italica* decreased more. Waterlogging stress reduced the grain yield of *S. glauca* similarly whether the treatment was imposed before or after heading. Vartapetian and Jackson (1997) considered that long-term survival of flooding and submergence by species well adapted to waterlogged is achieved in two principal ways, escape and tolerance of anoxia. In our study, it was suggested that *P. sumatrense* well adapted to waterlogging during longest stress period (145 days). *S. glauca* also showed longer duration (132 days) of waterlogging than *P. miliaceum* (76 days) and *S. italica* (95 days), however, it showed small waterlogging tolerance. These results showed that the duration of waterlogging was not coincidence of waterlogging tolerance. It was also previously reported that waterlogging stress during 10–15 days after sowing to 30 days after heading decreased the dry matter production of *P. miliaceum* and *S. italica* more than other gramineous crops (Kono et al., 1987). When waterlogging stress was imposed during vegetative growth, the grain yield of wheat was reduced by 60% and barley by 55% (Leyshon & Sheard, 1974; Collaku & Harrison, 2002). However, the period from the beginning of stem elongation to anthesis, in wheat and barley, were the most sensitive to waterlogging in terms of yield penalties (De San Celedonio, Abeledo, & Miralles, 2014). For rice, when waterlogging stress was imposed at tillering and milky ripening stage, the grain yield was reduced by 77 and

78% (Shao et al., 2014). These reductions were attributable to decreases in number of panicles, number of spikelets, percentage of filled grains at tillering, and grain weight at milky ripening stage. When waterlogging stress was imposed at vegetative growth, the grain yield was reduced by the reduction in total dry weight as for *P. miliaceum, S. glauca,* and *S. italica* in this experiment and for sorghum in previous reports (Promkhambut, Polthanee, Akkasaeng, & Younger, 2011). These results showed that vegetative growth was mainly important to maintaining grain yield. Our results showed that number of filled grains per panicle of *P. sumatrense*, number of filled grains per panicle, grain weight of *S. italica* and *P. miliaceum*, and number of panicles per plant of *S. glauca* determined grain yield under waterlogging, respectively (Table 3 and Figure 3).

3.3. Growth and aerenchyma formation of root

Root adaptation is considered a most important characteristic for growth and grain production in millets because deficiency of oxygen around the root room is the main cause of damage under waterlogging stress. Hayashi et al. (2013) reported a correlation between root length density and waterlogging tolerance in wheat. In wetland species, the ability to form adventitious roots under flooded soil conditions was very closely related to the flooding frequency of their natural habitat (Visser et al., 1996). In this experiment, *P. sumatrense* which showed the best waterlogging tolerance among the four millets increased its number of crown roots and root biomass under waterlogging (Table 4). Multiple range test showed that total root dry weight and number of crown root of *P. sumatrense* were significant higher than the other millets (Tables 5 and 6). Thus, the waterlogging tolerance of *P. sumatrense* was partly explained by its vigorous root growth under waterlogging.

There are two types of aerenchyma – schizogenous and lysigenous, first mentioned in Sachs (1882), named by De Bary, Bower, & Scott (1884) (schizogenetic spaces and lysigenetic spaces) and Arber (1920) (schizogenous and lysigenous); "aerenchyma (aerenchym)" was first used by Schenk (1889). Lysigenous aerenchyma is a beneficial morphological adaptive trait found in many crops, including rice, wheat, barley, and oats (Setter & Waters, 2003), maize and teosinte (Abiko et al., 2012), sorghum and wetland plants. The lysigenous aerenchyma of the four millets was categorized as "radial lysigeny", a type prevalent among Alismatales, Poales, Ranunculales, Myrtales, and Malpighiales (Seago et al., 2005). We investigated the aerenchyma formation at 10 mm below the root–shoot junction of crown roots to determine whether plants have an entrance for oxygen flow from shoots. Furthermore, a high proportion of aerenchyma near the base may be one of good trait to waterlogging stress because waterlogging-tolerant species (*Oryza sativa*, *Avena sativa,* and

Sorghum bicolor) showed higher development of aerenchyma near the tip (McDonald, Galwey, & Colmer, 2002). *P. sumatrense* had the most developed lysigenous aerenchyma compared with other millets whether under waterlogging or not (Tables 5 and 6), and its ratio of aerenchyma to root cross-sectional area was not significantly different between waterlogging and control treatments at harvest (Table 4), although it showed a significant increase at heading (Table 2). This result was consistent with that reported for four tropical forage grasses (Baruch & Merida, 1995) or rice and barley (Peason & Havill, 1988). Peason and Havill (1988) considered that there was no correlation between the rates of growth and the volume of root aerenchyma in the species studied. It has been shown that flood-intolerant species (*Agropyron pungens* and *Hordeum vulgare*) and a wetland plant (*Aster tripolium)* exhibited an increased percentage of root aerenchyma, whereas *Oryza sativa* did not change (Peason & Havill, 1988). However, the percentage of aerenchyma in roots in two wetland plants was larger than flood-intolerant species under unaerated condition. It is also well known that wetland plants developed lysigenous aerenchyma under both waterlogged and aerated conditions (Cardoso, Rincon, Jimenez, Noguera, & Rao, 2013). These reports and our results suggested that the most important tolerance feature under waterlogging may be the percentage of aerenchyma rather than the increase in percentage of aerenchyma by waterlogging treatment.

Another beneficial feature was observed from the percentage of cortex area of *P. sumatrense* (Tables 2, 4, 5, and 6) which was not affected by waterlogging and was always higher than the other millets. This observation was consistent with that of McDonald et al. (2002). Cardoso et al. (2013) showed that the proportion of stele of tolerant *Brachiaria humidicola* accessions was decreased by waterlogging. Armstrong, Cousins, Armstrong, Turner, and Beckett (2000) showed that the oxygen partial pressure of cortex was higher than stele of *Phragmites australis* and concluded that the stele used more oxygen than cortex. McDonald et al. (2002) showed that the proportion of aerenchyma in root cross section of *Oryza sativa* (35.0%) was higher than *Avena sativa* (32.4%) and *Sorghum bicolor* (13.9%) and the proportion of stele in root cross section of *O. sativa* (4.2%) was lower than *A. sativa* (15.1%) and *S. bicolor* (26.4%) under stagnant solution. These results suggests that smaller stele is beneficial for increasing the area of aerenchyma and decreasing oxygen consumption as reported in Cardoso et al. (2013).

Lignin is known as an apoplastic barrier to radial oxygen loss (ROL) in sclerenchyma and as suberin in exodermis. Kotula, Ranathunge, Schreiber, and Steudle (2009) showed that the total amount of lignin increased more in basal part of roots, while the total amount of aromatic suberin

increased more in the apical part of roots when seedlings of upland rice were grown in stagnant solution and the total amount of lignin was higher than that of suberin by around tenfold. We observed an accumulation of lignin in the sclerenchyma or exodermis of all millets regardless of treatment (Figures 2 and 5). It seemed there was less accumulation of lignin in the basal part of the crown root of *P. sumatrense* and *S. glauca*. In lowland rice, Shiono et al. (2011) showed that barrier induction occurred prior to histochemically detectable changes in suberin and lignin after 5 days of stagnant solution treatment. In this study, the millets were subjected to longer periods (76–203 days) of waterlogging and lignin may play a role as a barrier to ROL in the basal part of crown root.

4. Conclusion

In this study, we determined interspecific differences in waterlogging tolerance among four millets. Based on grain yield, we found that *P. sumatrense* showed a strong waterlogging tolerance, whereas *S. glauca*, *P. miliaceum* and *S. italica* were susceptible. We also determined the effect of pre- and post-heading waterlogging on growth and grain yield among four millets. The grain yield of all millets changed at all stages of growth by waterlogging. *P. sumatrense* showed strong tolerance to waterlogging because larger root growth with higher proportion of lysigenous aerenchyma through the growing period. This root character is considered to help water and nutrient uptake maintaining oxygen supply under waterlogging condition.

Acknowledgments

Disclosure statement

Funding

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*In Japanese with German abstract.

**In German.