

# Genotypic differences in root traits of rice (*Oryza sativa* L.) seedlings grown under different soil environments

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**Abstract:** We investigated mainly root traits of rice (*Oryza sativa* L.) seedlings grown under four soil conditions (i.e. anaerobic, aerobic, low and high soil density) in thin and long transparent polyvinyl chloride tubes for 21 days. Using 70 rice varieties from four agricultural ecotypes (i.e. *japonica* upland (JU), *japonica* lowland (JL), *indica* upland (IU), and *indica* lowland (IL)), we examined the effects of genotypes, environment and their interaction on seminal root length (SRL), seminal root thickness (SRT), number of crown roots (NCR), root and shoot dry weight (RDW and SDW) and root/shoot ratio (R/S ratio). The significant effects of genotype, environment and their interaction on all the traits were detected. Rice varieties could be clearly classified into their own ecotypes by a combined principle component analysis (PCA) for NCR, SRT, and SDW. Seventy rice varieties could be separated into upland and lowland varieties based on the scores of the first principle component (PC) and, among upland varieties, JU varieties could be separated from IU varieties based on the scores of the second PC. JU varieties had longer and thicker seminal roots than the other varieties in aerobic soil conditions, indicating that these varieties may be more suitable for aerobic soil conditions from the view point of the seedling establishment.

**Keywords:** G×E interaction, lowland rice, principle component analysis, root traits, upland rice

**Abbreviations:** DAS, days after sowing; G×E interaction, genotype × environment interaction; IL, *indica* lowland; IU, *indica* upland; JL, *japonica* lowland; JU, *japonica* upland; NCR, the number of

crown roots; PCA, principle component analysis; RDW, root dry weight; R/S ratio, root/shoot ratio; SDW, shoot dry weight; SRL, seminal root length; SRT, seminal root thickness

## Introduction

Rice is cultivated under four different land types in the world, and the distribution of land usage for rice cultivation as of 2004-2006 is as follows: irrigated, 57%; rainfed lowland, 31%; upland, 9%; deepwater, 3% (IRRI, 2009). Therefore, soil water conditions should largely differ among cultivation types; irrigated paddy condition is anaerobic, rainfed lowland, alternately anaerobic and aerobic, and upland, aerobic. As compared to the yield obtained under irrigated paddy conditions, that obtained under rainfed lowland and upland conditions is low and unstable. This is because the water supply largely depends on uncertain and uneven rainfall, resulting in large fluctuations of the soil moisture conditions. Drought stress is a major constraint to rice production and yield stability in rainfed regions (Evenson et al. 1996). It has been suggested that upland rice, which is usually cultivated under rainfed conditions, is more sensitive to water stress than other upland crops due to its shallow root system (Angus et al. 1983). Therefore, a deep root system is needed for acquiring water and nutrition from the relatively wet deep soil layer to obtain a stable yield under rainfed conditions. Upland rice varieties with deep root systems were noted to take up a considerable amount of water from the deeper soil layers during periods of water stress (Yoshida and Hasegawa 1982). Fukai and Cooper (1995) proposed that the genotypic variations in the root system in order to enhance the water-capture ability of the plant

could be an avenue for improving the drought tolerance of rice. Penetration ability of root system is one of the important traits to confer the deep root systems to rice plants (Nguyen et al. 1997). It is well known that soil mechanical impedance inhibits root growth in many plant species (Materchera et al. 1991). In rice, Nhan et al. (2006) assessed the root penetration ability of 72 rice varieties using a wax layer method and reported that upland varieties possessed higher penetration ability than lowland varieties. Further, they reported that *japonica* upland varieties had higher penetration ability than *indica* upland varieties. Other root traits, such as seminal and crown root developments, which were effective for rice plants to grow under drought and/or upland conditions, have been widely reported (Bañoc et al. 2000; Kondo et al. 2000; Zhang et al. 2001; Sharp, 2002).

The ability of roots to change developmentally and functionally in response to the changing conditions was suggested to be one of the most important traits for adaptation (Ingram et al. 1994; Yamauchi et al. 1996). Therefore, in addition to a deep root system, root morphological change to a given environment may be an important factor under rainfed conditions where soil moisture conditions often fluctuate. Although there are some reports on the phenotypic change of root morphology in response to changing soil conditions in rice (Luquet et al. 2005), and barley and wheat (Bingham et al. 1997, 2003), limited reports are available for this aspect, possibly due to its complexity of this feature and the laborious and time-consuming nature of investigations for root traits. Consequently, limited information is available regarding the relative effects of genotype, environment and their interaction on variations in root morphology. These problems may hinder the progress of plant breeding in terms of root-trait manipulation. More convenient and effective methods, which can deal with the large number of genotype and environment, should be developed to facilitate the selection of rice varieties that can adapt to several environmental conditions. Although there are few reports on root traits of the large number of varieties, Laffite et al. (2001) investigated root traits of 136 rice varieties under an irrigated aerobic condition, and demonstrated that root traits were related with agricultural ecotypes. Moreover, they reported that *japonica* varieties had thicker roots with wider vessels and high root/shoot ratio (R/S ratio), while *indica* varieties had thin roots with narrow vessels and low root/shoot ratio. Kato et al. (1992) compared 28 rice varieties which included lowland and upland types in terms of seminal root elongation rate, lateral root development, nodal root development and their xylem vessel dimension, and found a large genotypic variation. They also showed that upland rice varieties had longer seminal

root and thicker root systems than lowland ones. Further, they classified 10 of 28 rice varieties by a principle component analysis (PCA) and relatively clearly separated upland varieties from lowland ones (Kato et al., 1992). In this way, classification of the varieties into some groups may be important to facilitate the selection of varieties for following studies or breeding. However, these studies were conducted under single environment. Therefore, it is unclear to what extent G×E interaction affects on root morphologies. Kondo et al. (2003) classified 11 rice varieties based on the behavior of root traits under three different sites. This approach should be useful to select varieties and traits toward specific environments, but few studies have focused on this aspect from the viewpoint of root traits.

In this study, we assessed root and shoot traits of 70 rice varieties with a simple and easy method under four soil environments (anaerobic, aerobic, and low- and high-soil density). Because it is difficult and time-consuming to investigate complicated traits, such as anatomical traits with a microscope, for many varieties, we investigated basic traits, i.e., seminal root length (SRL) and thickness (SRT), the number of crown roots (NCR), root and shoot dry weight (RDW and SDW) and R/S ratio. Then, we analyzed the effects of genotype, environment and G×E interaction on them. Further, we attempted to detect which environmental factors had the largest effects on the traits and to classify the 70 varieties with respect to each trait by the PCA.

## Materials and Methods

### *Plant material*

This study included 70 rice (*Oryza sativa* L.) varieties, namely, 16 *japonica* upland (JU), 30 *japonica* lowland (JL), 19 *indica* upland (IU) and five *indica* lowland (IL) varieties (Table 1).

### *Growth conditions*

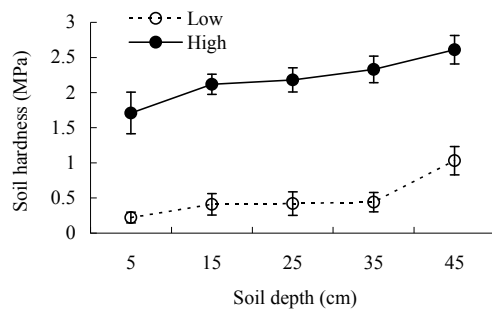
Four treatments were conducted to compare the effects of anaerobic and aerobic conditions (Anaerobic and Aerobic), and to compare the effects of soil hardness (low- and high-soil density, Low and High) on the root and shoot traits. The growth method was similar to that employed by Kato et al. (1991) with some modification. Transparent polyvinyl chloride (PVC) tubes (length 50 cm; inner diameter 1.6 cm) were sealed at the bottom with perforated packing tape and filled with vermiculite or clay loam soil (41.1% sand, 34.3% silt and 24.6% clay) with 0.18 g chemical fertilizer (4% N, 4% P<sub>2</sub>O<sub>5</sub> and 4% K<sub>2</sub>O). In the first two treatments (Anaerobic and Aerobic), PVC tubes

**Table 1.** Plant materials.

Ecotype	Variety name	History	Ecotype	Variety name	History
<i>japonica</i> upland	Rikuto Norin 6	Improved	<i>japonica</i> lowland	Koshihikari	Improved
	Rikuto Norin 12	Improved		Nishihomare	Improved
	Rikuto Norin 22	Improved		Nipponbare	Improved
	Rikuto Norin 24	Improved		Yumetsukushi	Improved
	Rikuto Norin Mochi 1	Improved		Shinriki	Traditional
	Rikuto Norin Mochi 8	Improved		Hinohikari	Improved
	Rikuto Norin Mochi 18	Improved		Norin 18	Improved
	Rikuto Norin Mochi 20	Improved		Reiho	Traditional
	Rikuto Norin Mochi 25	Improved		Shirobeniya	Traditional
	Hataminori Mochi	Improved		Asahi	Traditional
	Minamihata Mochi	Improved		Akenohoshi	Improved
	Owarihata Mochi	Improved		Akebono	Improved
	IRAT 109	Improved		Chiyonishiki	Improved
	IRAT117	Improved		Akamoro	Traditional
	Azucena	Traditional		Toyonishiki	Improved
	Sensho	Traditional		Domannaka	Improved
	<i>indica</i> upland	Beo dien		Traditional	Haenuki
Beo san van		Traditional	Miyazakizairai	Traditional	
CT6510-24-1-2		Improved	Aikoku	Traditional	
Ep hat dai		Traditional	Lemont	Improved	
Ep hat den		Traditional	Shiranui	Improved	
Ep hat to		Traditional	Tsukushiwase	Improved	
Ep hat tron		Traditional	Sengoku	Improved	
IR47686-30-3-2		Improved	Akihikari	Improved	
IR57920		Improved	Kamenoo	Traditional	
IR71525-19-1-1		Improved	Nekken 2	Improved	
Khau kai		Traditional	Zuiho	Improved	
Khau ken		Traditional	Taichung 65	Improved	
Khau linh		Traditional	Kinmaze	Improved	
LC-90-12		Traditional	Asominori	Improved	
Mo do		Traditional	<i>indica</i> lowland	IR 36	Improved
Nep bao lac		Traditional		DV 85	Improved
Nep khau non		Traditional		HA 2	Traditional
Te rau	Traditional	IR 24		Improved	
UPLRi-7	Improved	Bhadua		Traditional	

were filled up to 3.5 cm from the top with approximately 25 g of vermiculite (bulk density, 0.25 g cm<sup>-3</sup>) that had been passed through a 4 mm sieve mesh. Only for Aerobic, 30 mL water was added to wet all the vermiculite in the tubes. For both treatments, clay loam soil with a gravimetric moisture content of approximately 13 % was added over the vermiculite up to 2.0 cm from the top in order to sow seeds. In the next two treatments (Low and High), the clay loam

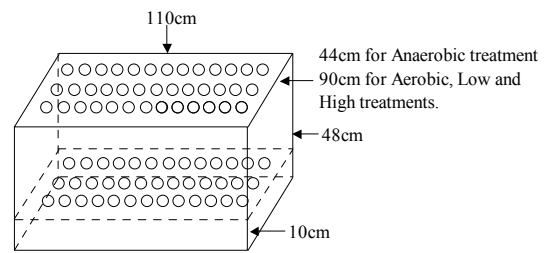
soil used was sieved through a 4 mm mesh screen and dried in an oven. Gravimetric moisture content of the soil was adjusted at 13 % before use. The PVC tubes were filled with this soil up to 2.0 cm from the top. The bulk density was set at 1.10 g cm<sup>-3</sup> and 1.34 g cm<sup>-3</sup> for Low and High treatment, respectively. On commencement of the experiment, the water potential of the soil was -0.43 MPa (measured with WP4; Decagon Devices Inc., Pullman, WA, USA). The soil



**Fig. 1.** Soil hardness along the soil depth. The open circles and dotted line indicate the Low treatment and closed circles and solid line indicate the High treatment. Data are presented as the mean  $\pm$  S.D. (n = 5).

hardness was measured along the depth using a soil hardness meter (DIK-5561, Daiki Rika Kogyo, Saitama, Japan), and the values obtained are shown in Fig. 1.

Seeds were soaked in water at 30 °C for 72 h. One seed was sown in each tube at 1.0 cm below the soil surface. For Anaerobic, the PVC tubes were placed in a steel tank (110 × 44 × 48 cm, Fig. 2), while for the other three treatments, the PVC tubes were placed in another steel tank (110 × 90 × 48 cm). The water level was maintained at the position of 5 mm above the soil surface and at 5 cm up from the bottom of the tubes in the former and latter tanks, respectively. Small holes (2.0 cm in inner diameter), the distance between the holes was 2 cm, were made toward the top and bottom of the tank to keep the tubes in place at an inclination of approximately 10 degrees. For Aerobic, Low and



**Fig. 2.** Simplified diagram of the experimental tank used in this study.

High, the top of the tube was covered with paraffin paper to prevent excessive evaporation from the soil surface. On seed germination, a small hole was punctured in the paraffin paper to allow the shoots to elongate outside the tube. The plants were grown in a climate-controlled chamber (day, 12 h at 30 °C; night, 12 h at 24 °C; light intensity, approximately 180  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ). For Anaerobic, tubes were placed randomly, and for the other three treatments, a randomized complete block design was used. Three replications were performed for each treatment.

#### Measurements

Shoots were cut at the soil surface at 21 days after sowing (DAS), and roots were removed from PVC tubes and washed carefully with tap water. Then, SRL was measured and NCR was counted. SRT was measured at 1.0 cm from the root tip by using a

**Table 2.** Mean values of seminal root length (SRL), number of crown roots (NCR), seminal root thickness (SRT), root dry weight (RDW), shoot dry weight (SDW) and root/shoot ratio (R/S ratio) in each treatment and results of two-way ANOVA in each trait.

	SRL (cm)	NCR	SRT (mm)	RDW (mg)	SDW (mg)	R/S ratio
Anaerobic	187 c <sup>a</sup>	14.5 a	0.19 c	13.5 a	92.0 a	0.15 b
Aerobic	364 a	2.1 b	0.30 a	8.2 b	45.0 c	0.22 a
Low	358 a	1.0 b	0.27 b	7.0 bc	41.8 c	0.20 a
High	323 b	1.0 b	0.30 ab	5.7 c	51.8 b	0.12 b
Genotype	6.02 *** <sup>b</sup>	12.24 ***	7.19 ***	8.74 ***	5.47 ***	3.63 ***
Environment	574.10 ***	3333.00 ***	211.78 ***	767.72 ***	296.31 ***	89.97 ***
G×E	2.66 ***	7.40 ***	3.38 ***	5.31 ***	3.66 ***	3.80 ***

Factors influencing these traits are also expressed as F-values.

<sup>a</sup> Means followed by the same letters were not significantly different among treatments at  $P < 0.05$  by Tukey's test (n=70).

<sup>b</sup> \*\*\* indicates significance at  $P < 0.001$ .

microscope. Thereafter, samples were dried at 70 °C for at least 72 h, and SDW and RDW were determined. R/S ratio was calculated by dividing RDW by SDW.

#### Data analyses

Data were analyzed by a two-way analysis of variance (ANOVA). Then, Tukey's test was performed to separate means among treatments for each trait. Each variety was separated into its own agricultural ecotypes and Scheffe's F-test was conducted to separate means among agricultural ecotypes in each treatment and trait. Genotype means were used for the PCA with respect to each trait. According to the factor loadings of the first and second principal components (PCs), the environment factors which explained for the first and second PCs were analyzed for each trait. Then, rice varieties were plotted on a scatter diagram with respect to each trait, based on the scores of the first and second PC. Finally, 70 rice varieties were plotted on a scatter diagram based on a combined PCA operated for phenotypic data of the selected traits. Each data analysis was conducted using UNISTAT Ver. 5.6 (UNISTAT Ltd., London, UK).

#### Results

##### Shoot and root growth

Two-way ANOVA indicated significant ( $P < 0.001$ ) effects of genotype and environment on all the traits (Table 2). Further, there was a significant ( $P < 0.001$ ) effect of  $G \times E$  interaction on all the traits. Mean values of SRL and SRT in Aerobic were the largest among four soil conditions and those in Anaerobic were significantly lower than those of the other three soil conditions. NCR, RDW and SDW in Anaerobic treatment were significantly higher than those in the other soil conditions. R/S ratio in Aerobic and Low were significantly higher than those in Anaerobic and High.

Table 3 shows the mean values of root and shoot traits with respect to each agricultural ecotype under each treatment. Among agricultural ecotypes, JU varieties tended to have longer SRL in aerobic conditions (i.e. Aerobic, Low and High) than the other ecotypes, but shorter in Anaerobic. Although IU varieties showed the largest SRT among ecotypes in Anaerobic, JU varieties tended to have larger SRT in aerobic conditions. For NCR, lowland varieties tended to have larger values than upland varieties in all the treatments. For RDW and SDW, however, consistency was not observed among agricultural ecotypes in all the treatments. Upland varieties tended to have higher R/S ratio than lowland varieties.

**Table 3.** Mean values of seminal root length (SRL), number of crown roots (NCR), seminal root thickness (SRT), root dry weight (RDW), shoot dry weight (SDW), and root/shoot ratio (R/S ratio) in each treatment and ecotype.

	Anaerobic	Aerobic	Low	High
<b>SRL (mm)</b>				
JU	155 b <sup>a</sup>	378 a	393 a	360 a
JL	202 a	348 a	358 b	329 ab
IU	194 a	370 a	339 ab	286 b
IL	168 ab	390 a	318 b	312 ab
<b>NCR (nr)</b>				
JU	13.6 b	0.5 a	0.1 c	0.3 b
JL	17.1 a	2.6 a	1.7 a	1.7 a
IU	9.8 c	2.9 a	0.5 bc	0.4 b
IL	19.4 a	1.4 a	1.3 ab	1.2 ab
<b>SRT (mm)</b>				
JU	0.19 b	0.32 a	0.34 a	0.37 a
JL	0.16 b	0.29 a	0.25 b	0.27 b
IU	0.23 a	0.30 a	0.26 b	0.30 b
IL	0.15 b	0.29 a	0.25 b	0.23 b
<b>RDW (mg)</b>				
JU	15.4 a	7.6 a	7.0 a	6.3 a
JL	13.4 a	7.6 a	6.9 a	5.4 a
IU	12.6 a	9.7 a	7.3 a	5.6 a
IL	11.2 a	7.8 a	6.5 a	5.3 a
<b>SDW (mg)</b>				
JU	96.6 ab	37.7 b	32.1 a	43.3 a
JL	96.7 a	38.6 b	43.0 a	55.0 a
IU	76.0 b	54.8 a	43.9 a	51.5 a
IL	116.5 a	46.5 ab	45.1 a	59.0 a
<b>R/S ratio</b>				
JU	0.15 a	0.22 a	0.25 a	0.16 a
JL	0.14 a	0.22 a	0.17 a	0.10 b
IU	0.16 a	0.23 a	0.24 a	0.12 b
IL	0.10 a	0.17 a	0.15 a	0.10 b

<sup>a</sup> Means followed by the same letters were not significantly different among ecotypes at  $P < 0.05$  by Scheffe's F-test ( $n=16$  for JU, 30 for JL, 19 for IU and 5 for IL).

#### PCA

The PCA was performed for each root and shoot trait and the results are shown in Table 4. In all the traits, the first and second PCs accounted for approximately 70% of the total variation. Low and High treatments yielded almost identical values, with greater effects on the first component; this indicated that the difference of soil media (i.e. vermiculite vs. soil) caused different phenotypes among rice varieties. The main effects on the second component differed among the traits tested. The main effects on the second component were

**Table 4.** Eigenvalue, proportion, and eigenvector of the principle components for seminal root length (SRL), number of crown roots (NCR), seminal root thickness (SRT), root dry weight (RDW), shoot dry weight (SDW), and root/shoot ratio (R/S ratio)

Traits	Principle component	Eigenvalues	Proportion (%)	Eigenvector			
				Anaerobic	Aerobic	Low	High
SRL	1	1.848	46.201	0.208	0.316	0.651	0.658
	2	0.977	24.419	0.895	-0.439	0.008	-0.080
NCR	1	2.2865	57.162	0.356	0.410	0.594	0.594
	2	1.0679	26.696	0.738	-0.653	-0.115	0.123
SRT	1	1.7396	43.489	0.355	0.331	0.599	0.637
	2	1.0097	25.244	0.619	0.608	-0.418	-0.269
RDW	1	1.6917	42.292	0.397	0.250	0.601	0.647
	2	1.1416	28.541	0.540	0.707	-0.384	-0.247
SDW	1	1.8887	47.218	0.340	-0.086	0.661	0.664
	2	0.9972	24.929	0.107	0.991	0.082	-0.007
R/S ratio	1	2.0149	50.373	-0.423	-0.505	0.550	0.514
	2	1.1270	28.175	0.618	0.442	0.407	0.507

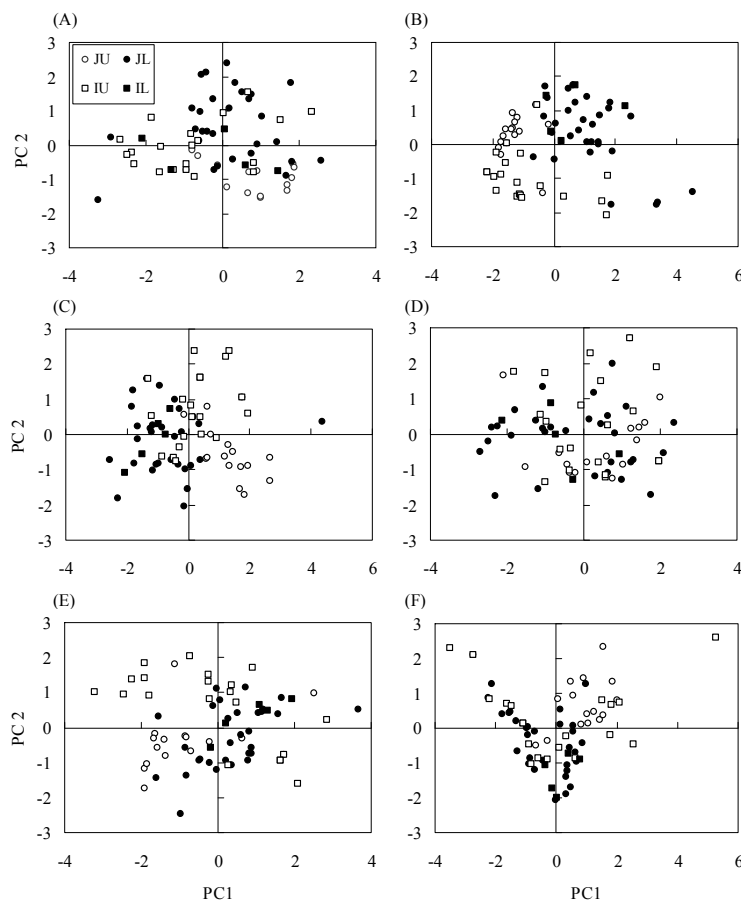
observed in Anaerobic for SRL and R/S ratio, both Anaerobic and Aerobic for NCR and SRT, Aerobic for RDW and SDW.

The rice varieties used were plotted on scatter diagram based on the scores of first and second PCs for each trait and results are shown in Fig. 3. Among the traits investigated, rice varieties could be clearly classified into their own agricultural ecotypes for NCR, SRT and SDW (Fig.3 B, C, E). Rice varieties were located separately on the scatter diagram for the other traits. For NCR, varieties which had large values of X axis showed larger NCR in Low and High, and varieties which had large values of Y axis showed larger NCR in Anaerobic but smaller in Aerobic. For SRT, varieties which showed large values of X axis possessed larger SRT in Low and High, and varieties which showed large values of Y axis possessed larger SRT in Anaerobic and Aerobic. For SDW, varieties which showed large values of X axis indicated larger SDW in Low and High, and varieties which showed large values of Y axis indicated larger SDW in Aerobic.

We selected the NCR, SRT and SDW from the classification results of 70 rice varieties for each trait,

and operated the combined PCR including phenotypic data of these three traits under four soil conditions (Table 5). The cumulative contribution of the first three PCs was 65.4 %. The first PC had higher positive correlation with NCR in Low and High and negative correlation with SRT in Low and High. The second PC showed higher positive correlation with NCR and in Anaerobic and negative correlation with NCR and SDW in Aerobic, and SRT in Anaerobic. The third PC indicated higher positive correlation with SRT in Aerobic and SDW in Low and High.

Fig. 4 shows the scatter diagram of the scores of the first and second PCs obtained by the combined PCA. Varieties which had large values of X axis showed larger NCR in Low and High and smaller SRT in Low and High, and varieties which had large values of Y axis showed larger NCR in Anaerobic, smaller NCR in Aerobic, smaller SRT in Anaerobic, larger SDW in Anaerobic, and smaller SDW in Aerobic. Each variety could be clearly separated into its own agricultural ecotypes; JU varieties were located on second quadrante; IU varieties, third quadrante; JL and IL varieties, first and fourth quadrates.

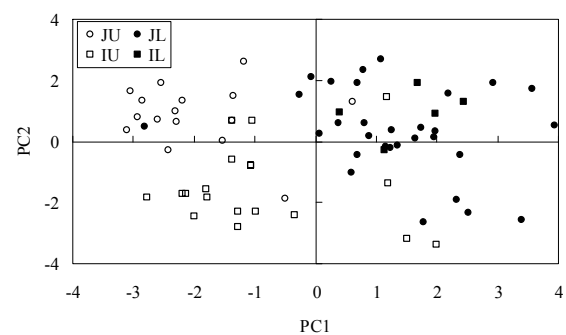


**Fig. 3.** Classification of 70 rice varieties based on the scores of the first and second PC for seminal root length (A), number of crown roots (B), seminal root thickness (C), root dry weight (D), shoot dry weight (E), and root/shoot ratio (F).

## Discussion

In the present study, two-way ANOVA revealed significant effects of genotype, environment and G×E interaction on root and shoot traits (Table 2). Further, it was found that the soil environment, especially the difference of soil media had the largest effect on all the traits tested (Table 4).

Lowland varieties could be separated from upland ones based on the scores of the first PC and upland varieties could be separated into *japonica* and *indica* varieties based on the scores of the second PC by the combined PCA (Fig. 4). Factor loadings of NCR and SDW were positive values and that of SRT was negative values for the first PC regardless of the treatments (Table 5). These results indicate that lowland varieties had larger NCR and SDW than upland ones, while thinner SRT regardless of soil environments. Although lowland varieties tended to have larger NCR than upland ones, RDW was not significantly different between lowland and upland varieties in all the treatments (Table 3), probably



**Fig. 4.** Classification of 70 rice varieties based on the scores of the first and second PC by the combined PCA for the selected traits.

because upland varieties had thicker root systems and larger number of lateral roots. Previous study has also demonstrated that upland varieties had thicker root systems and smaller NCR than lowland varieties in a hydroponic culture and aerobic conditions (Kondo et

**Table 5.** Eigenvalue, proportion, and eigenvector of the first, second and third principle components for the selected traits.

	Principle component		
	1	2	3
Eigenvalue	3.57	2.46	1.81
Proportion	29.8	20.5	15.1
Parameter			
NCR Anaerobic	0.3007	0.4171	-0.1707
NCR Aerobic	0.2422	-0.4511	-0.1133
NCR Low	0.3879	-0.1210	-0.0621
NCR High	0.4512	0.0017	-0.1023
SRT Anaerobic	-0.2574	-0.3753	0.2131
SRT Aerobic	-0.0975	0.0088	0.5612
SRT Low	-0.3318	0.3120	0.0038
SRT High	-0.3492	0.1172	-0.0344
SDW Anaerobic	0.1453	0.4542	-0.1437
SDW Aerobic	0.0425	-0.3725	-0.2932
SDW Low	0.2847	-0.0064	0.4959
SDW High	0.2919	0.1039	0.4792

al. 2000, 2003). Upland rice varieties have been well known to be more resistant to drought stress than lowland ones. Thus, it may be concluded that the varieties which showed the negative scores of the first PC were more resistant to drought than those which showed the positive scores.

Upland varieties could be clearly separated into *japonica* and *indica* varieties based on the scores of the second PC. The second PC had higher positive correlation with NCR and SDW in Anaerobic, but negative with NCR and SDW in Aerobic (Table 5). Similarly, the second PC was positively correlated with SRT in Anaerobic, but negatively correlated with that in Low. These mean that JU varieties had larger NCR, thinner SRT and larger SDW than IU varieties in Anaerobic, while JU varieties had smaller NCR and larger SDW in Aerobic, and thicker SRT in Low than IU varieties, indicating the existence of G×E interaction on such traits. Significant difference of SRT was observed between JU and IU varieties in Low and High (Table 3). SRT of JU varieties in Low and High were much thicker than those in Anaerobic (0.19 in Anaerobic, 0.34 in Low and 0.37 mm in High), while SRT of IU varieties ranged 0.23 to 0.30 mm. It was reported that *japonica* upland rice varieties had thicker roots than *indica* ones under aerobic conditions (Kondo et al. 2000; Lafitte et al. 2001). In the present

study, JU varieties had larger SRT values than the other varieties in aerobic conditions (i.e. Aerobic, Low and High). Our result partly coincided with previous reports. Nhan et al (2006), who investigated root penetration ability of the same varieties as used in this study by using wax-layer methods, reported that the root penetration ability of JU varieties was significantly higher than that of IU varieties and the ability of root penetration was highly correlated with root thickness. Significant difference of root penetration ability between JU and IU varieties might cause the difference of SRL between JU and IU varieties (Table 3). Seminal root growth is important for the emergence and establishment of seedlings under upland conditions (Zhang et al. 2001; Sharp, 2002). Thus, it may be concluded that JU varieties are more adaptable to aerobic and hard soil conditions from the view point of initial plant establishment. However, the difference of SRL and SRT between JU and IU varieties could not explain the insignificant difference of RDW and SDW among varieties in Low and High. Therefore, future field experiments are needed to clarify the relationship between SRL and/or SRT and initial plant establishment under aerobic and hard soil conditions.

Because it is difficult to analyze root systems for many varieties under field conditions, approaches used in this study may be useful for the first selection of varieties. Further, to find interesting traits or varieties which are different from their own agricultural ecotypes may be also available for physiological studies or future breeding. For example, JL variety, “Lemont” was located in the second quadrante and was clearly separated from JL varieties even at seedling. This variety has been often used for study of drought resistance (Lilley and Fukai 1994a, 1994b, 1994c, Boonjung and Fukai 1996a, 1996b) and known to possess thicker root systems and larger root length density than the other lowland varieties (Lilley and Fukai 1994a, Ogata et al. 1996).

In conclusion, we detected the effects of genotype, environment and G×E interaction on root and shoot traits by using 70 rice seedlings. Also we found the difference of soil media had the largest effects on the traits. Moreover, we could clearly separate rice varieties into their own agricultural ecotypes based on the combined PCA for NCR, SRT and SDW. These approaches may lead to smooth selection of varieties for further studies or breeding. Future studies will be needed to investigate the root and shoot traits of plants grown in actual field conditions.

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