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Relative Contribution of the Coleoptile and the First Leaf Length to Seedling Establishment of Rice (*Oryza sativa* L.) as Affected by Anaerobic Seeding in Two Different Soils

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Abstract: The relative importance of coleoptile and first leaf length in seedling establishment under varying levels of oxygen depleted conditions due to flooding deserves clarification for the development of an anaerobic seeding technology for rice. Sprouted seeds of 14 rice genotypes were sown in the flooded soils collected from upland and lowland sources. The soils from upland and lowland sources appeared to be hypoxic and anoxic, respectively in nature after 24 h of submergence. The contribution of coleoptile length in seedling establishment was several times more than first leaf length in the soil from upland source. But in the soils from lowland source, the seedling establishment appeared to be equally dependent on both coleoptile and first leaf lengths.

Key words: Anaerobic seeding, coleoptile, first leaf, rice, seedlings establishment

Introduction

Unstable seedling establishment is a common feature in the directly seeded lowland conditions of the tropical as well as the temperate regions. Anaerobic seeding, an alternative method of direct seeding onto lowland paddy could be helpful to overcome the problem of poor seedling establishment (Yamauchi *et al.*, 1993). The sprouted seeds of anoxia-tolerant genotypes, are sown in the puddled soil, inundated with few centimeters of water to protect the seeds from the water stress and to provide better root anchorage (Yamauchi, 1995). As the cultivars are anoxia-tolerant, depleted oxygen environment should not be the problem of poor seedling establishment.

The practicing of anaerobic seeding with anoxia tolerant rice genotype is more convenient than the other methods of direct seeding (Yamauchi and Choung, 1995). But this kind of genotypes also vary their seedling establishment with the agro-ecolgical conditions (Yamauchi and Biswas, 1996; Yamauchi *et al.*, 2000). The variation in seedling establishment could be attributed to varying intensity of stresses imposed by different flooded soils and the mechanism of survival played by the genotype itself against the stresses. The prime role of the coleoptile and first leaf length in seedling establishment under oxygen depleted conditions has already been documented (Kordan, 1977; Yamauchi and Biswas, 1996; Biswas and Yamauchi, 1997). Further more, understanding the relative contribution of the coleoptile and first leaf length in seedling establishment under different levels of stress in the lowland conditions is important for the breeding programs related to the development of anaerobic seeding technology. Therefore, this study was conducted to identify the relative importance of the coleoptile and first leaf length in seedling establishment with respect to two different soils under lowland conditions.

Materials and Methods

Two experiments were conducted in the phytotron of the Faculty of Agriculture, Yamagata University, Japan in 1999. Except the soil source, materials and methods were the same for two experiments. Experiment 1 was conducted with soil from upland source (Inceptisols, pH 4.8, organic carbon 4.0%) and experiment 2, with lowland soils (Entosols, pH 5.0, organic carbon 3.8%). They were designated hereafter as "upland" and "lowland" soils respectively.

Four anoxia-tolerant genotypes from the International Rice Research Institute (IR41996-50-2-1-3, IR50363-61-1-2-2, RP1669-1529-4254 and BR736-20-3-1) and 10 (Oou 344, Oou 360, Fujisaka 5, Haenuki, Domanaka, Akitakimachi, Arroz da terra, Dunghun shali, Italica livorono and Calrose) from Shonai Agricultural Experimental Station, Yamagata Prefecture, Japan were used for the study. Germination percentage and germination rate (Krishnasamy and Seshu, 1989) of these genotypes were more than 98% and 0.98, respectively. The seeds were sterilized in 2% sodium hypochlorite solution for 15 minutes, washed thoroughly and soaked for 24 h at 30°C in the dark. Thereafter, the soaked seeds were again washed and kept for sprouting in the petri dish lined with moist filter paper for another 12h at 30°C in dark. Plastic pots (90 mm X 85 mm) were used as experimental units. The pots were filled with soils (mixed with 0.13% rice straw powder to enhance the soil reduction) up to the rim and soaked for overnight in tap water. Ten sprouted seeds were sown horizontally by forceps at 25 mm soil depth carefully. Then the pots were submerged in 1/5000a Wagner pot to maintain the lowland conditions. The water depth on the soil surface was maintained to 25 mm. The seedlings were grown for 10 d in a phytotron under natural light with a constant day and night temperature of 30°C. The experiments were laid out in 4-replicated randomized complete block design.

Seedling establishment (%), coleoptile length, first leaf length, plant height, seeding depth and dissolved oxygen (DO) were observed. Except seeding depth and DO, the procedure of measuring the seedling organs was mentioned in Biswas *et al.* (2001). The seeding depth was estimated from the length between the base of the sprouted seedling and the top margin of the white portion (white due to bury in the soil) of the seedling or coleoptile (in case of failure to produce first leaf). DO was measured at 24 hours after seeding by Hand held Dissolved Oxygen Temperature System (YSI model 55). A separate set up with test tubes containing submerged soil was arranged for DO measurement. The sensor tip of the instrument was dipped into the submerged soil (soil water ratio was 1:2) in test tubes. The soil-water of the test tube was mechanically stirred around the sensor tip to get the stabilized reading.

Statistical analysis: Combine Analysis of Variance (ANOVA) of seedling establishment and coleoptile length across the two soil sources was done to compare the performance between the two soils and the genotypes as well. Multiple linear regression was performed to estimate the contribution of first leaf length, coleoptile length and plant height in seedling establishment, (Gomez and Gomez, 1984; IRRI, 1992).

Results and Discussion

Arroz da terra produced the highest percentage of seedling establishment in the upland soil (Table 2). Haenuki, Italica livorono, Dunghun shali, Calrose, Akitakomachi, Oou 360 demonstrated the similar seedling establishment but significantly lower than Arroz da terra in the same soil. In lowland soil, the significantly highest seedling establishment was observed in Haenuki. Haenuki established 80% seedling. The second highest seedling establishment was observed in Arroz da terra (72%) which was

Table 1: Soil type, organic carbon, pH and dissolved oxygen (DO) of the soils used for the study

Soil	Soil type	Organic carbon (%)	pH[a]	DO[b] (mg L^{-1})
Upland	Inceptisol	4.0	4.8	4.4
Lowland	Entisol	3.8	5.0	0.5

[a] pH (soil : water = 1.5) was measured by TOA pH meter (model HM 20S)

[b] Dissolved oxygen was measured by Hand held Dissolved Oxygen Temperature System (YSI model 55) at 24 h after the soil submerged (soil:water = 1:2). The sensor tip of the instrument was dipped into the submerged soil. The soil-water was mechanically stirred around the sensor tip to obtain the stabilized readings.

Table 2: Seedling establishment and seedling parameters of different genotypes as affected by anaerobic seeding in the upland and lowland soils.

	Seedling establishment (%)			Coleoptile length (mm)		
	Soil			Soil		
Genotypes	U	L	D	U	L	D
Haenuki	77.5ab	80.0	ns	34.3ab	28.6a	ns
Oou344	52.5b	52.5a-e	ns	28.2abc	20.8a-d	ns
Fujisaka	65.0b	25.0e	*	23.6c	12.5d	*
Akitakomachi	82.5ab	60.2a-e	ns	34.7ab	27.2ab	ns
Oou360	87.5aab	55.0a-e	*	36.2a	24.6abc	*
Arroz da terra	95.0a	72.5ab	ns	33.0abc	28.9a	ns
Dunghun shali	82.5ab	70.0abc	ns	33.0abc	23.9abc	ns
Italica livorono	80.0ab	37.0b-e	*	32.2abc	15.2cd	**
Calrose	90.0ab	57.5a-e	*	34.8ab	24.4abc	*
IR41996-50-2-1-3	60.0b	60.0a-e	ns	30.6abc	24.6abc	ns
RP1669-1529-4254	62.5b	35.0cde	ns	28.0abc	16.0cd	*
IR50363-61-1-1-2-2	70.0b	40.0b-e	ns	29.0abc	21.8abc	ns
BR736-20-3-1	60.0b	27.5de	ns	28.5abc	18.8bcd	*
Domanaka	57.5b	70.0abc	ns	26.0bc	29.6a	ns
Mean	73.0	53.0	**	31.0	29.6	ns
CV%		a			21.5	

[a] arcsine transformation ** = significant at 1% level by LSD, * = significant at 5% by LSD, ns = not significant by LSD at 5% level

In a column, means followed by a common letter are not significantly different at 5% level by DMRT

U = Upland soil, L = Lowland soil, D = Difference

Table 3: Dependence of seedling establishment on coleoptile, first leaf length and plant height

Soil source	Equation	R^2
Upland	Y = -12.03+ 0.33X1+ 0.05X2+ 2.27X3**	0.72**
Lowland	Y = -6.93+ 1.36X1+ 0.06X2+ 1.36X3*	0.91**

Y = Seedling establishment X1 = First leaf length, X2 = Plant height and X3 = Coleoptile length *= Significant at 5% level, ** = Significant at 1% level R^2 = Coefficient of determination

followed by Dunghun shali and Domanaka (70%) in the same soil. The establishment of the IRRI-designated anoxia-tolerant genotypes was not satisfactory both in the upland as well in the lowland soils. Fujisaka 5, Oou 360, Italica livorono and Calrose performed significantly lower seedling establishment in the lowland than upland soil. The over all performance of seedling establishment in the upland soil was significantly better than the lowland soil.

Under flooded conditions seedling establishment depends not only on cultivars but also on different soil types and other environmental factors (Yamauchi *et al.*, 1993; Yamauchi and Biswas, 1996). The over all performance of seedling establishment was significantly high in the upland soil. This may be due to the existence of high dissolved oxygen (DO) of the submerged flooded soil at 24 h after seeding (Table 1). The DO concentration as observed in the *in-vitro* study for the upland and lowland soils at 24 h after submergence were 4.4 and 0.5 mg l^{-1} respectively. Assuming the similar trend of DO in the pot experiments we could consider that at 24 h after flooding the upland soil remained to be hypoxic and the lowland soil almost to be anoxic in nature. The seeds were sown at 25 mm seeding depth. But after sampling it was observed that the actual seeding depth for both the soils was 19 mm (data not shown). The average coleoptile lengths were more than the seeding depth in both upland and lowland soils (Table 2). The coleoptile length equivalent to seeding depth was required to reach its tip to the oxidized soil-water transition layer. The snorkel mechanism, a mechanism of transporting oxygen to the growing region established through coleoptile (Kordan, 1976). So, elongated coleoptile supposed to help superior seedling establishment. As the DO in submerged upland soil was considerably higher than the submerged lowland soil, it appeared that the genotypes in the upland soil had to suffer quite less than the lowland soil from the oxygen tension.

The contribution of coleoptile, first leaf length and plant height in the variation of seedling establishment was 72% for upland and 91% for lowland soil (Table 3), indicating that the stress imposed by the lowland soil was higher than the upland soil. In the lowland soil some genotypes like Fujisaka 5, Italica livorono, RP1669 had the coleoptile length not enough to approach the oxygenated soil-water transition layer (Table 2). Obviously that was one of the reasons why these genotypes showed poor seedling establishment in this soil. In contrast Oou 344, Akitako machi, Oou 360, Calrose, IR41996, IR50363 had enough coleoptile length to approach the oxidized soil-water transition layer in the lowland soil. Even though these genotypes showed poor seedling establishment in this soil. The longer coleoptile was supposed to transport oxygen in the growing region of the seedlings of these genotypes to initiate first leaf growth.

It seemed that the growth of first leaf length was affected by the accumulation of anaerobic toxic products from the highly reduced lowland soil. The partial regression coefficient of first leaf length was only 0.33 as compared with 2.27 of the coleoptile length in the upland soil. The partial regression coefficient of coleoptile length was also significant but at 5% level. However the coefficient values for the first leaf and coleoptile lengths were equal (1.36) in the lowland soil. So it could be said that, in highly reduced soil both coleoptile and leaf contributed equally to the seedling establishment. In other word, the elongated coleoptile must supply oxygen to the growing region of the seedling to initiate first leaf growth. But first leaf should have the ability to sustain the anaerobic toxins. So it is assumed that both the mechanisms of avoidance (by elongated coleoptile) and tolerance (by first leaf) are required to establish seedling in highly reduced soil. As in the upland soil the stress appeared to be relatively low, coleoptile alone was enough for taking care and support the first leaf formation through oxygen transportation to the growing region i.e. avoidance mechanism. As the upland soil was not highly reduced the accumulated toxins should be lower as compared with that of the highly reduced lowland soil. That might be the reason why first leaf growth affected little in the upland soil. From these results it is appeared that the mechanism of tolerance of rice seedlings might be different in two soils.

Varapetian *et al.* (1977) demonstrated that high degree of flooding resistance in rice plant as a whole is due partly, to avoidance, rather than tolerance to oxygen deficit. Kordan (1977) also demonstrated this. Rice coleoptile can sufficiently elongate in anoxia. Under anoxia, if the injury is due to toxins accumulation, stress tolerance can conceivably be due to the prevention of toxin accumulation (strain avoidance), or tolerance of toxin accumulation (stress tolerance) (Levitt, 1972). So, despite the longer coleoptile length, the genotypes unable to establish seedling may either be lacking the mechanism of strain avoidance or stress tolerance or both for the first leaf in the highly reduced lowland soil. But in the upland soil the survival of seedling could be easily explained by the simple avoidance mechanism of elongated coleoptile.

Plant height showed a little influence on the variation of seedling establishment (Table 3). We did not consider root length because seedlings were grown for only 10 d and their growth was severely restricted in lowland soil. More so, it has no direct effect on seedling establishment at the earlier phase of the seedling establishment (Biswas and Yamauchi, 1997). But indirectly, it helped in seedling establishment under oxygen depleted conditions by suppressing its growth to conserve energy until the formation of shoot (Cobb and Kennedy, 1987). However the root growth can not be ignored at the later stage of seedling growth as it helps in anchoring the soil.

Most of the IRRI designated anoxia-tolerant genotypes produced longer coleoptile. It is rather interesting to note that these anoxia-tolerant genotypes could not do well even in the submerged upland soil. Though they are collected from different sources but their germination rate and percent germination were almost perfect. We observed that RP1669 had quite good adaptability to acetic, propionic and butyric acid at pH 5 and 7 (Biswas *et al.*, 2001). But this genotype also could not perform well in this study. This phenomenon could be attributed to some of the inherent properties of the soil that influence the accumulation of ferrous iron. During screening of the genotypes adapted to lowland condition, very little attention was given on the kinetics of ferrous iron of the soil. But there is a significant interaction effect of ferrous iron and predominantly occurring acetic acid (unpublished data). So it is important to study the genotypic difference of the interaction effect of these factors on seedling growth as wel.

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