Morphological Difference of Rice Seedling Grown under Different Dissolved Oxygen Conditions

Jong Gun Won*†, Jang Soo Choi*, Deck Jong Ahn*, Seung Phil Lee*, Sang Chul Lee, and Tomohiko Yoshida*****

**Gyeongbuk Agicultural Technology Administration, Taegu 702-708, Korea,*
***Department of Agronomy, Kyungpook National University, Daegu 702-701, Korea*
****Faculty of Agriculture, Utsunomiya University, Utsunomiya 321-8505, Japan*

ABSTRACT : The response of dissolved oxygen (DO) concentrations caused significant change in root number, root length, coleoptile length, shoot length and leaf age of seedlings. The genotypic difference in the effect of DO also highly significant ($P<0.01$) for all of the seedling traits. The number and length of root were extremely inhibited at the condition of 0.39 ± 0.09 DO concentration. While the coleoptile elongated markedly in the lowest DO concentrations, the shoot did not develop. The root growth was improved slightly at the 1.39 ± 0.27 mg L^{-1}, however, there were no difference among genotypes at these two low DO concentrations. As the DO concentration become higher, the growth of root and shoot was improved remarkably. Root number, root length and shoot length was significantly different between 20 and 30°C in DO rich and normal conditions, the development of those traits were apparently accelerated in high water temperature, however those traits of seedlings in DO deficiency were not different between the two temperatures except for shoot length. On the other hand the coleoptile length was not affected by the stagnant water temperature; it was stimulated by the low DO concentration. The competition of DO was greater as the seedling density was increased in the stagnant water, therefore the seedlings grown under high density have long and white coleoptiles, and the growth of roots and shoots was retarded severely.

Keywords: dissolved oxygen, rice, seedling growth, water-seeding

The basic methods of the direct-seeding cultivation of rice are divided into dry-seeding and water-seeding based on the physical condition of the seedbed and seed In the dry-seeding technique, the dry seeds are sown into the not irrigated paddy field and allowed to develop to about five-leaf stage and then flooded continuously to the practice. In water-seeding, the pre-germinated seeds are sown in a flooded field and then the flooded water may be drained temporarily for a few days to facilitate seedling anchorage to the soil (De Datta, 1986; Westcott *et al*, 1986; Lee, 1995). The seeds sown into the flooded water will not usually emerge through both flooded water and soil because of the lack of O_2 (Jones, 1933; Dore & Thevan, 1959, Kordan, 1974). The deficiency of O_2 is caused by various factors like water depth, position of seed in the flooded soil profile (Jones, 1933; Park *et al.*, 1995), temperature of water which influences dissolved O_2 (Chapman & Peterson, 1962), seeding rate (Chapman & Mikkelsen, 1963), and source and amount of organic matter in soil (Patrick & Sturgis, 1955)

In general, there is no influence of O_2 level in the rate or percentage of rice germination itself and the coleoptile can be developed and elongated by the reduced partial pressure of oxygen. However, no or poor root growth occurs and the growth of the primary leaf is barely detectable in an anaerobic condition (Sasaki, 1926; Turner *et al.*, 1981; Park *et al*, 1993).

The coleoptile length of rice was longer with deep water while for the radicle length shorter As water depth was increased, the percentage of seedling stand was decreased slightly in rice, while sharply increased in barnyard grass. Plant height of rice with increasing water depth was longer, whereas that of barnyard grass reduced significantly with weaker (Park *et al.*, 1993).

It is very important to understand the differences of rice seedlings which are grown under water-seeded rice. Therefore, this study was carried out to determine the effect of dissolved oxygen and temperature on morphology of rice seedlings, to identify the difference of genotypes on endurance to low dissolved oxygen concentration and the competition for dissolved oxygen under different seedling populations.

MATERIALS AND METHODS

To determine the effect of dissolved oxygen on morphology of rice seedlings, six genotypes, two Japonica type cultivars (Koshihikari and Sasanishiki), two medium grain

†Corresponding author (Phone) +82-53-320-0271 (E-mail) jgwon67@empal.com <Received March 12, 2004>

cultivars from America (M302 and Magnolia) and two Indica type cultivars (IR661 and Lemont) were used. Rice seeds were sterilized with 70% ethanol for 30 second and 2% sodium hypochloride for 15 minutes successively. The seeds were incubated for 2 days at 25°C for uniform pre-sprouting. Ten presprouted seeds per each genotype were placed into the seedbeds which were made of transparent acrylic plate with 6×12×7 cm depth and 12 partitions per one seedbed. Then, these seedbeds were submerged into oxygen-controlled water in boxes of 7 cm in depth. The water boxes containing the seedbed were placed in a growth chamber, which provided 25 air temperature under 400 mol m^{-2} s^{-1} light for 12 hours per day.

Five DO concentrations were adjusted using olive oil (0.39 ± 0.09 mg L^{-1}), N_2 gas (1.39 ± 0.27 and 2.24 ± 0.35) and air pump (4.59 ± 0.33 and 6.92 ± 0.08). To obtain the 0.39 ± 0.09 mg L^{-1} DO concentration, first the DO of the sterilized water was adjusted to 0.45 mg L^{-1} using N_2 gas (99.995%), which was bubbled through distilled water and then overlaid with a 0.7cm thick layer of olive oil to prevent contacting with O_2 in air (Kordan, 1974). To obtain the 1.39 and 2.24 mg L^{-1} DO levels, the DO concentration was controlled every day within the respective level using N_2 gas. We used one air pump continuously for maintaining the 4.59 DO level and two air pumps for the 6.92 DO level. A model ODT-101 O_2/DO meter of SIBATA Company was used to continuously monitor the DO level.

To determine the effect of temperature and DO concentration, two temperature, 20 and 30°C, and three DO concentrations, 0.5 ~ 1.0 (Deficiency), 2.0 ~ 2.5 (Normal) and 4.5 ~ 5.0 (Rich) mg L^{-1} were adjusted as described above. To clarify the competition of using DO, four seed amounts, 10, 20, 30, 40 seeds, were also seeded in the seedbed in 2.5 mg L^{-1} concentration. In this experiment, the water was left stagnant and the DO concentration was not maintained during the rice plants were growing.

The root number (longer than 0.5 cm), length of longest root, length of coleoptile, length of shoot were measured for 5 randomly selected seedlings from each cultivar in eight days after placing the seed in the seedbed. These measurements were made for each of the five DO levels. Analysis of variance (ANOVA) of this study was done using the IRRISTAT program.

RESULTS AND DISCUSSION

Dissolved oxygen (DO) concentrations changed significantly ($P<0.01$) root number, root length, coleoptile length, shoot length and leaf age of seedlings, indicating wide range of variation among the DO concentrations studied (Table 1) The genotypic variation in the effect of DO also highly significant ($P<0.01$) for all of the seedling traits, suggesting that there was a genotypic difference in the tolerance to low DO levels during the seedling stage. The interactions of DO x Genotype were detected on the all of the seedling traits except root number.

Fig. 1 shows the morphological difference of seedlings grown under different DO concentrations. As the DO concentrations became lower, the coleoptiles grew longer. This result was similar to that reported by Alpi & Beevers (1983). They reported that the coleoptiles in N_2 grow at a steady rate between days 3 and 7 and considerably exceeded the length achieved in air. Tuner *et al.* (1981) also suggested that the coleoptile length was the longest at 0 % O_2. Previous studies (Kordan, 1974; Turner *et al.*, 1981) suggested that the elongation of the coleoptile at low O_2 concentration was an adaptive response of the seed for acquiring O_2 for seedling development. The elongated coleoptile could possibly extend to an aerobic zone and allow O_2 necessary for the shoot and root. There were significantly different in coleoptile length among the genotypes at low DO concentrations. However, it was not different at high DO concentrations. The coleoptiles grown under low DO concentration were not greened, thin and not shooted despite its greater length, being in agreement with the result reported by Alpi & Beevers (1983). They said that the anaerobic coleoptile is quite thin and fragile and its fresh weight was less than that in air. The number and length of root were extremely inhib-

Table 1. Mean squares of analysis of variance for root number, length, coleoptile length, and shoot length for dissolved oxygen levels and genotypes

Sources	df	Mean Squares			
		Root number[2)]	Root length (mm)	Coleoptile length (mm)	Shoot length (mm)
Replication	1	0.003	5.4	0.28	3.0
Treatment	29	4.466**	1137.9**	116.09**	672.5**
DO [1)](O)	4	29.663**	7254.5**	601.07**	4308.6**
Genotype (G)	5	1.159**	444.8**	137.05**	218.5**
O × G	20	0.253	87.8**	13.86*	58.7**
Error	29	0.030	21.8	6.35	14.2

[1)]Dissolved oxygen concentration, [2)]Root number longer than 5mm

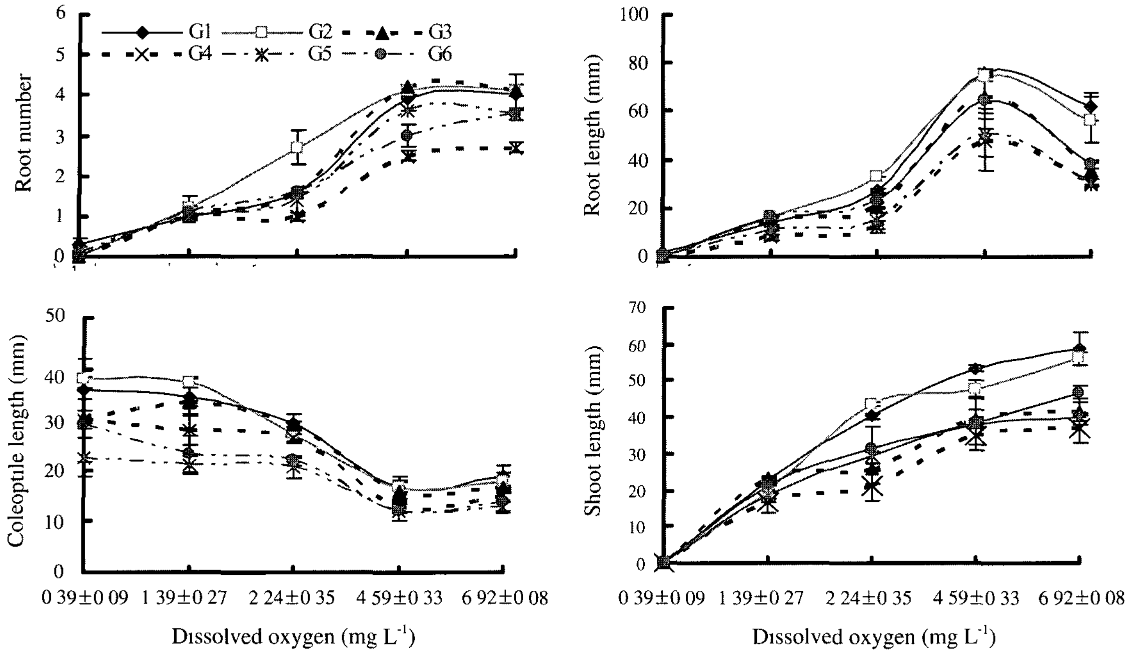

Fig. 1. Morphological differences of 8-day-old rice seedlings grown in different dissolved oxygen concentrations (G1. Koshihikari, G2. Sasanishiki, G3: M302, G4: Magnolia, G5 IR661, G6. Lemont)

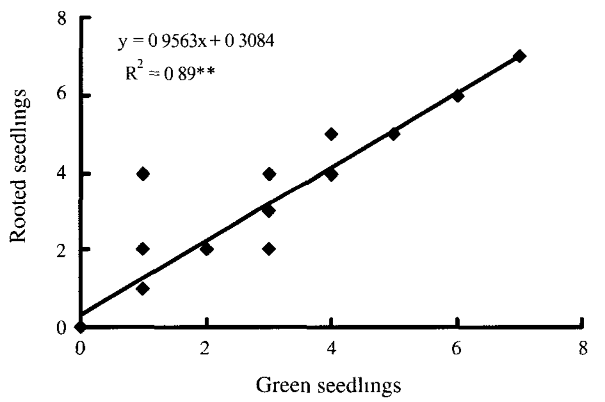

Fig. 2. Relationship between green seedlings (shooted seedlings) and rooted seedlings

ited at the 0.39 ± 0.09 mg L^{-1} DO concentration. Even though the coleoptile elongated markedly in the lowest DO concentrations, the shoot did not develop, meaning the primary leaf did not develop and not grow. As the concentration of DO became higher, the length of shoot also became longer and the age of shoot became more, suggesting that the growth of shoot was affected by the concentration of DO. The root growth was somewhat improved at the $1.39 \pm 0\ 27$ concentration, however, there were no difference among genotypes at these two low DO concentrations. As the DO concentration became higher, the growth of root was developed and improved remarkably, and there were significant differences among genotypes Fig. 2 shows the highly positive relation between shooted rice seedlings (green seedlings) and rooted rice seedlings ($r^2 = 0\ 89^{**}$). It means that even though the coleoptile can be elongated under low DO condition, the shoot and root are developed under DO existence and the rooting is very important to develop shoot of seedlings in water-seeded rice cultivation.

The effect of DO concentration under different temperature on rice seedlings was shown in Fig. 3. Root number, Root length and shoot length was significantly different between 20 and 30 °C in DO rich and normal conditions, the development of those traits were apparently accelerated in higher water temperature. Those traits of seedlings in DO deficiency were not different between the two temperatures except shoot length. On the other hand the coleoptile length was not affected by the stagnant water temperature, it was stimulated by the low DO concentration, instead. The number and length of root was inhibited in normal DO conditions compared to rich DO conditions at high water temperature in stagnant culture, but the length of shoot was not inhibited in normal DO condition In normal DO condition, the growth of root was not different at low temperature, however, that of root was inhibited at high temperature The results indicate that the DO in high temperature was consumed more rapidly than that of low temperature as the seedling was growing. This result was supported from the statement by Chapman Peterson (1962). They stated that growth was slowest at low temperature and biological oxygen demand would be smallest so that even when the DO

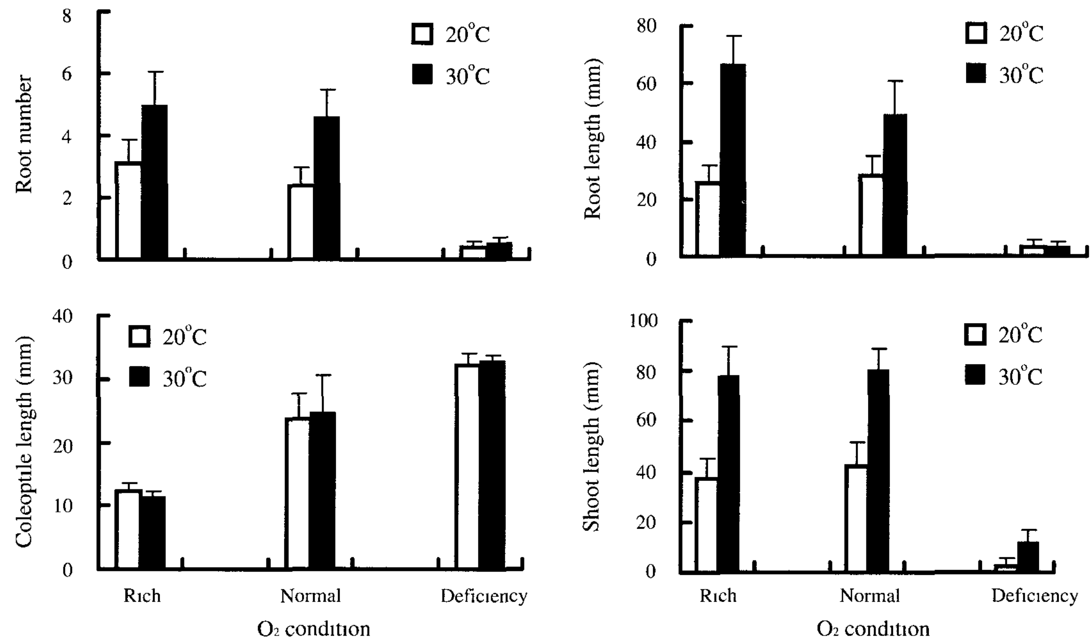

Fig. 3. Effect of dissolved oxygen concentrations under different water temperature on the growth of rice seedlings.

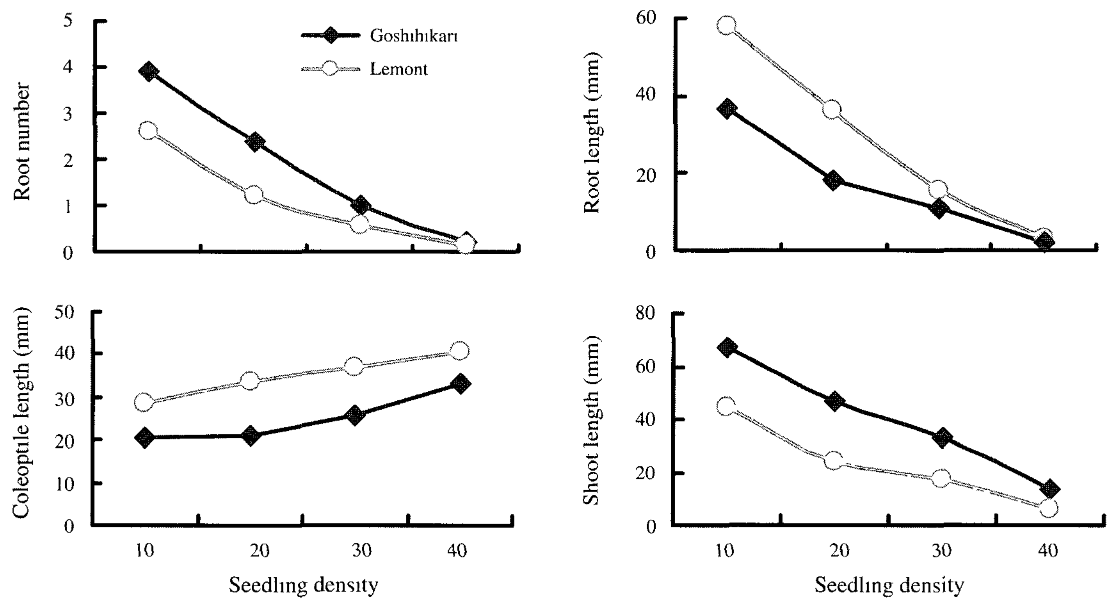

Fig. 4. Effect of seedling density on the growth of rice seedlings between two cultivars

was as low as 2.5 ppm for a considerable period of time seedling emergence will be possible.

It is apparent that as the plant densities were increased the development of both roots and shoots was considerably retarded (Fig. 4). As the previous results, the inhibition of root development was also greater than that of the shoot in this experiment At plant densities more than 30, there were little or no development of secondary roots and shoots. On the other hand the elongation of the coleoptile was stimulated by high plant densities The patterns of rice seedling growth as affected by different seedling densities were similar to those as affected by different DO concentrations. On the basis of the known effect of low oxygen partial pressure on the development of the rice shoot and root and the dissolved oxygen data obtained in these experiments, the effect of plant density probably can be interpreted mainly in terms of biological oxygen demand and supply. In the similar pot experiment with clay soil, Chapman & Peterson (1962) reported that as the seedling populations per pot were increased the DO concentrations were more sharply decreased and the DO concentration in the unplanted control pot was higher than in all other densities during the experimental period From these results it is clear that the competition of DO was greater as the seedling densities were increased in the stagnant water, therefore the seedlings grown under high densities have long and white coleoptiles, and the growth of roots and shoots was retarded severely.

In summary, there was a genotypic difference in the tolerance to low DO levels during the seedling stage. The number and length of root were extremely inhibited at the low DO concentration. Even though the coleoptile elongated markedly in the lowest DO concentrations, the shoot did not develop. As the DO concentration was higher, the growth of root and shoot was developed and improved remarkably, being significantly different among genotypes. Root number, root length and shoot length was significantly different between 20 and 30 °C in DO rich and normal conditions, the development of those traits were apparently accelerated in high water temperature. On the other hand the coleoptile length was not affected by the stagnant water temperature; it was stimulated by the low DO concentration, instead. The seedlings grown under high densities have long and white coleoptiles, and the growth of roots and shoots was retarded severely, suggesting that the competition of DO was greater as the seedling density was increased in the stagnant water.

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