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Screening of Submergence-Tolerant Rice Varieties and their Physiological Characteristics

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ABSTRACT: This study is to identify the physiological traits of submergence-tolerant varieties of rice plants (*Oryza sativa* L.) in Yeongnam area, southeastern part of Korea, where the reduction of rice yield due to submergence is remarkably severe. In the present study, two tolerant varieties of rice plants were selected from over 30 rice varieties grown in under a 10-day period. The tolerant varieties selected from a submerged paddy field. As a control, one intolerant variety of rice plant was chosen. Of the tolerant variety Samgangbyeo, rather than Haepyungbyeo, had a lower dissolved oxygen consumption and maintained a higher dry weight than the intolerant variety. The leaf photosynthetic rates (LPS) of the two tolerant varieties were significantly higher than that of the intolerant-variety after four days of submergence treatment. These results indicate that lower dissolved oxygen consumption in a limited pool is prevented by ethylene formation in the tolerant varieties, which may be a mechanism of submergence tolerance.

***Keywords*:** dissolved oxygen, *Oryza sativa* L., photosynthesis, rice, screening, submergence.

Submergence is stressful for higher plants because it inhibits the entry of atmospheric oxygen to plant leaves and roots due to the very slow rate of the diffusion of gases in water. Submergence had a deleterious effect on the ruderal population, which is evidenced by leaf decay and blackening of the root tissue (Lynn & Waldren, 2003). Despite this submergence, plants adapt to their changing environment in many ways, leading to a wealth of growth forms of varying complexity. All rice cultivars are damaged when completely submerged for several days. FR13A, as it is well known, has been found to be relatively more resistant to submergence (Jackson & Ram, 2003). The availability of lowland species that avoid submergence by a delimited shoot elongation in combination with submergence tolerance could be advantageous in agriculture, therefore, the excessive elongation of lowland rice would, however, be an undesirable trait, since tall plants might lodge when the water recedes (Vriezen *et al.*, 2003). In fact, the growth response is mediated by at least three different hormones, ethylene, GA and ABA. By recent reports, the proposed sequence of events upon submergence is that decreased gas diffusion and low oxygen concentration lead to an increase in ethylene concentration which causes a decrease in the ABA level, an increase of GA_1 concentration, and an increase of responsiveness to GA, with enhanced internodal elongation as a result (Raskin & Kende, 1984; Kende *et al.*, 1998). Submergence-tolerant types are also remarkably tolerant of anaerobiosis provided that they have first experienced some hours with partial O_2 shortage as whole plants (Ellis & Setter, 1999).

In Yeongnam area, which is located in the southeastern part of Korea, submergence is a serious problem; hence, a rice yield could not be expected in this region without cultivating of the submergence tolerant variety. Here, we conducted to identify types of submergence-tolerant rice under a submerged paddy field among cultivars to find the mechanisms that limit dissolved oxygen, photosynthesis ability and morphological changes of the root in different genotypes of rice.

MATERIALS AND METHODS

Screening of submergence-tolerant variety of rice

A screening experiment to obtain submergence tolerant types of rice was conducted using thirty varieties of rice (*Oryza sativa* L.) in a paddy field of Milyang submerged from 9 August 2002 until drainage occurred naturally (18 August 2002). Compound fertilizer was applied at the rate of 11-4.5-5.7 kg ha^{-1} (N-P-K) as a basal dressing. Twenty one-day old seedlings were transplanted on 10 June, measurements of 30×15 cm provided the planting density under

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the paddy field. The visual observation of the surviving plants was noted 5 days after drainage.

Morphological and physiological characters of submergence-tolerant varieties

Samgangbyeo, Haepyungbyeo, and IR50 were used in this study. Samgangbyeo and Haepyungbyeo are strong submerging tolerant varieties and IR50 is submergence intolerant variety. Germinated seeds were placed on a plastic vessel (ϕ105 mm, height 65 mm) and filled with 300 g of bed soil for three weeks, from 1 August to 21 August in 2003. The germinated seeds were housed in a glasshouse at Kyungpook National University. Seedlings were put into a PVC pot (ϕ125 mm, height 500 mm) filled with 6.5 of clean water for submergence treatment. The plants grew during 2, 4 and 8 day periods in an air-grown environment and were placed in completely submerged conditions in a glasshouse.

A portable dissolved oxygen system (model 842, Orion) was used to determine dissolved oxygen content of water in the submerged pots. The concentration of dissolved oxygen was measured at 2, 4, and 8 days after initiating submergence treatment. A portable leaf carbon dioxide analyzer (LCA-4) and a Parkinson chamber (PLC-4, ADC system, UK) were used to determine the net photosynthetic rate (μmolCO_2 m^{-2} s^{-1}) per unit leaf area on the fully expanded leaves of the three individual plants at 24 h after drainage. The leaf net assimilation rate was determined at a CO_2 concentration rate of 350 ppm and under natural light intensity, at approximately 1,600 μmol m^{-2} s^{-1}, in a glasshouse.

The roots, about 2 mm in length from the tip, were fixed with FAA (formalin : acetic acid : 50% ethanol, 0.5 : 0.5 : 9 v/v) for 2 hours. After fixation, the roots were washed with distilled water and embedded in a 4% agar gel. The roots in agar were sliced with a cuter in 100 μm, in thickness, to get a hand-cross section. The sliced root sections were stained with 0.05% Toluidine blue O for 2 min at room temperature (Nakamura *et al.*, 2002). Then, each sample was washed well in distilled water. The sample was observed at 40 magnification under a reversal microscope (IMT2-21, Olympus Ltd.).

RESULTS AND DISCUSSION

We identified a submergence-tolerant variety from thirty different types of rice using submerged paddy field conditions (Table 1). Out of 30 hills in each variety, twenty-nine hills in Samgangbyeo and 26 hills in Haepyungbyeo were survived. In terms of visual observation at the 5-day period after drainage, Samgangbyeo represented a more stronger variety of rice (degree 3), followed by Haepyungbyeo (degree 4) and Hwabongbyeo (degree 4) in terms of submerging tolerance. Five days after drainage, we selected Samgangbyeo and Haepyungbyeo as the submergence-tolerant variety for the next physiological study. These types of rice were selected with the results of their high survival ratio

Table 1. Survival ratio and degree of observation of 30 rice varieties (*Oryzxa sativa* L.) after drainage under paddy field conditions.

Variety	Type	No. of surviving plants (30 $plants^{-1}$)	Degree of observation*	Variety	Type	No. of surviving plants (30 $plants^{-1}$)	Degree of observation*
Sangjubyeo	Japonica	3	9	Tamjinbyeo	Japonica	0	9
Junghwabyeo	Japonica	1	9	Chucheongbyeo	Japonica	0	9
Sangmibyeo	Japonica	6	9	Sampyungbyeo	Japonica	2	8
Yeonghaebyeo	Japonica	14	6	Junambyeo	Japonica	3	8
Hwaanbyeo	Japonica	10	5	Sobibyeo	Japonica	7	7
Naepungbyeo	Japonica	2	7	Nampyeongbyeo	Japonica	3	9
Haepyungbyeo	Japonica	26	4	Sangnambatbyeo	Japonica	0	9
Ilpumbyeo	Japonica	18	7	Shindongjinbyeo	Japonica	1	8
Ilmibyeo	Japonica	6	7	IR50	Indica	0	9
Dongjinbyeo	Japonica	8	5	Namcheonbyeo	Tongil	1	5
Hwanambyeo	Japonica	0	9	Dasanbyeo	Tongil	1	9
Namgangbyeo	Japonica	13	7	Andabyeo	Tongil	8	6
Hwabongbyeo	Japonica	17	4	Areumbyeo	Tongil	6	7
Nagdongbyeo	Japonica	8	8	Samgangbyeo	Tongil	29	3
Hwayeongbyeo	Japonica	8	7	Milyang 181	Tongil	5	6

*The visual observation of the surviving plants was noted 5 days after drainage. The number of degree means 1: strong, 9: weak to the submergence.

results and the degree of visual observation. Recently, a total of 13 doubled haploid lines of rice and their parents (submergence tolerant FR13A and submergence intolerant CT6241) were assessed using 2-week-old seedlings. Seven lines were determined to be submergence tolerant, and six relatively intolerant. They suggested that the difference in susceptibility to submergence between the FR13A and CT6241 and their progeny was not due to the outcome of differential susceptibility in terms of oxygen deficiency at the roots (Mohanty & Ong, 2003).

From the results of the screening experiment, using rice varieties Samgangbyeo and Haepyungbyeo as submergence tolerant, and IR50 as submergence intolerant, we investigated their morphological and physiological characteristics in order to determine their tolerance levels.

First, we examined the effects of submergence treatment on dissolved oxygen (DO) in a pot with three rice varieties after being submerged in a glasshouse. After the 2 day-submergence treatment, the DO concentration in the submerged pots was significantly decreased (Fig. 1). After a 4 day-submergence treatment, the DO concentration further decreased and the amount of DO that was contained in a pot of the submergence-tolerant variety Samgangbyeo and Haepyungbyeo was only 86% and 83% respectively. The amount of DO that was contained in a pot with the submergence-intolerant IR50 variety was near 81% compared with the control (without plants) pots. After 8 day of submergence treatments, we found that the amounts of DO did not decrease in any pots compared with the 4 day-submergence treatments. Oxygen starvation in submerged-soils arises from an imbalance between the slow diffusion of gases in water compared with air and the rate that oxygen is consumed by microorganisms and plant roots. The outcome is that the flooded soil quickly becomes devoid of oxygen at depths below a few millimeters (Visser *et al.*, 2003). In our results, the high DO concentration in the pots with Samgangbyeo, rather than Haepyungbyeo, remained in the pots even after 4-days of submerged conditions (Fig. 1, open circle), indicating that oxygen is slowly consumed in limited space. On the contrary the pots, in which the submergence-intolerant IR50 plants were grown, the amounts of dissolved oxygen were lower than in the other two pots. In submergence conditions, the concentration of dissolved O_2 was 3 ppm on the first day, but decreased to 0.38 ppm by 48 h after germination, confirming that submergence was associated with a low level of O_2, and cell death of the rice seedlings occurred in a restricted cell position in the parenchyma (Kawai & Uchimiya, 2000). Jackson & Ram (2003) also reported that O_2 respiratory demand is satisfied by a mass flow of dissolved O_2 to the tissue surface rather than by diffusion in a submerged situation.

Second, in order to investigate whether the effects of submergence tolerance were related to photosynthesis ability, we examined the leaf photosynthetic rate (LPS) in leaves of three types of rice. Photosynthesis by submerged leaves severe limited CO_2 uptakes because of the small concentrations involved compared with O_2 and the slow diffusion rates through the inevitable boundary layer (Setter *et al.*, 1989). From these reasons, we calculated the leaf photosynthetic rates 24 hours after desubmergence. Fig. 2 shows

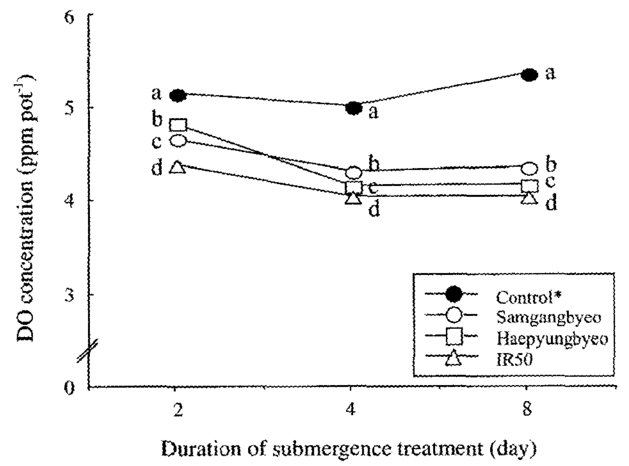

Fig. 1. Time course change of dissolved oxygen (DO) concentration in each submerged pot conditions. Each value is the mean ±SE (n=3 individual pots). If not shown, error bars are smaller than the symbol size. Bars with the same letter within each investigation time are not different at the 5% level of significance, as determined by LSD test. *Control means pots without plants.

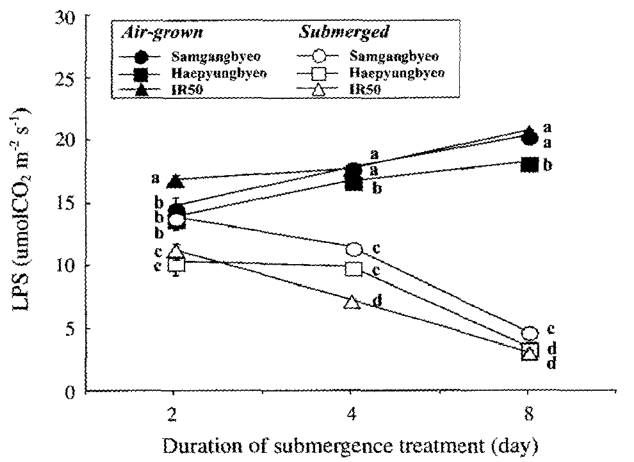

Fig. 2. Time course change of the leaf photosynthesis of three rice varieties in submergence treatment. Each value is the mean±SE (n=3 individual pots). If not shown, error bars are smaller than the symbol size. Bars with the same letter within each investigation time are not different at the 5% level of significance, as determined by LSD test.

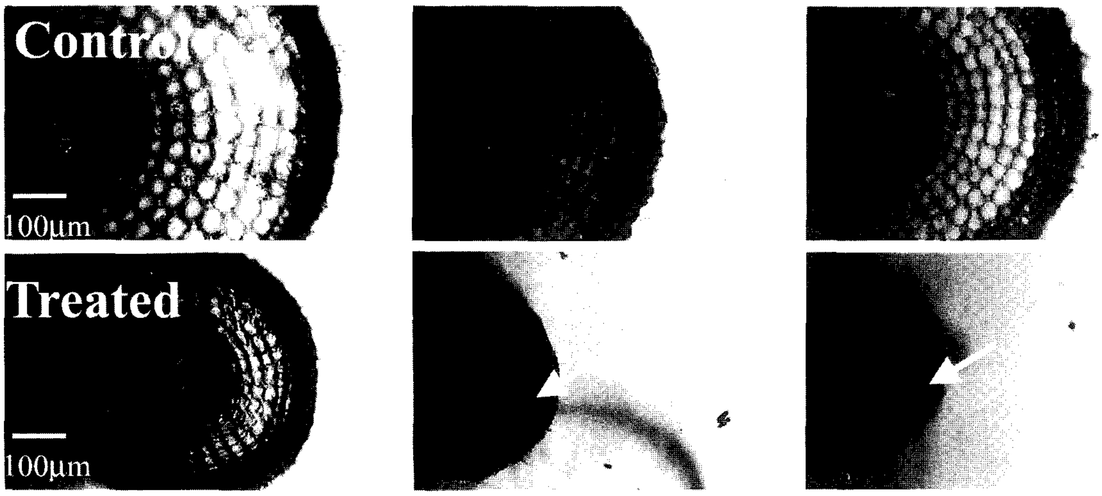

Fig. 3. Microscopic difference of root tips between varieties after 8 days of submergence. Arrows indicate accumulation of methyl-lignin.

changes in the photosynthetic rate of leaves over time in air-grown and submerged conditions. Two submergence-tolerant varieties seem to possess interesting physiological properties in this respect, which allow plants to tolerant high remaining LPS. The level of the LPS in Samgangbyeo did not change in the 2 day-submerged plants compared with the control plants, but levels of the other two rice variety plants rapidly decreased. The level of LPS in the Samgangbyeo and Haepyungbyeo rice varieties were higher than that of the intolerant variety IR50. The levels appeared at 4 days after submergence and then decreased by the 8 days after submergence. Between the tolerant rice varieties, the LPS of Samgangbyeo remained higher compared to the levels of the other tolerant variety Haepyungbyeo, in all submergence treatments.

Third, to elucidate the changes of the tissue structure in the root tip of submergence treatment, we examined the microscopic observation of the root tip with 8 day-submerging treated rice plants using Toluidine blue O (Fig. 3). With the results, the level of the submergence stress remarkably decreased in the root thickness and cell volume of plants with long-term (8 days) submergence. The thickness of the root tip in Samgangbyeo was approximately maintained at 63%, followed by Haepyungbyeo (53%), and then IR 50 (43%). These figures were compared with the root tip of each control plant, respectively. Mergemann & Sauter (2000) reported that submergence-induced growth of adventitious roots was preceded by the death of epidermal cells. It is this cell death that weakens and then perforates the epidermal cell layer and facilitates the emergence of the root in deep rice. By Toluidine blue O staining, methyl-lignin is stained dark green in the epidermis and the outer cortex in the ligneous tissues (Nakamura *et al.*, 2002). In our result, the methyl-lignin accumulation was found both in the epidermis and the outer cortex of the elongation zone in the intolerant rice variety IR50, whereas the accumulation was found only in the epidermal layer of Haepyungbyeo by submergence treatment. In Samgangbyeo, which is more tolerant than Haepyungbyeo on other physiological traits, methyl-lignin accumulation was found in a few of the epidermal layers of the 8 day-submerged rice plants. Other reports stated that adventitious root growth and epidermal cell death were therefore, linked to the ethylene signaling pathway which was activated in response to low oxygen conditions (Mergemann & Sauter, 2000; Colmer, 2003). From our results, we considered that submergence stress was a factor in prohibiting thickness growth, and this resulted in the accumulation of methyl-lignin.

Accordingly, these results indicate that low dissolved oxygen consumption in a limited pool is prevented by ethylene formation and a high maintenance of LPS in a tolerant variety, such as Samgangbyeo, which may be a mechanism of submergence tolerance.

Further genetic studies using Samgangbyeo, are necessary in order to identify genetic markers that are associated with the submergence tolerance in FR13A, which is a well-known submergence tolerant variety.

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