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Effects of Restricted Oxygen, Nitric oxide, and Mercuric Chloride on the Seed Germination and Early Elongation Growth of Rice

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ABSTRACT: Germination and early elongation of rice after germination were investigated in anoxic air treatment, nitric oxide gas treatment, and six concentrations of mercuric chloride solutions to determine the effects of limited oxygen environment, nitric oxide, and inhibited water flux through cell membrane in 17 °C. Anoxic air treatment affected germination of tested six varieties very little. However root elongation rates were severely inhibited while shoot growth was affected less. Reductions in shoot and root elongations demonstrated genotypic variations. Nitric oxide delayed the germination of rice even though it didn't affect the final percent germination. Elongations of root and shoot were inhibited in nitric oxide treatment. The inhibitor effect of nitric oxide on the shoot elongation of rice was less severe, while nitric oxide completely inhibited the root emergence of rice. Concentrations of $HgCl_2$ greater than 300 μM dramatically reduced the rate and percentage of germination when compared to distilled water treatment. The reduced percent germination showed the greatest variation among rice varieties in 500 μM solution of mercuric chloride. Ansanbyeo, Jinheung, and Odaebyeo were affected less by $HgCl_2$, Nonganbyeo and Sangmibyeo were intermediate, and the germination of Andabyeo was greatly reduced by $HgCl_2$. Root elongation of germinated rice seedlings was more sensitive to oxygen deficits, nitric oxide, and $HgCl_2$ treatments than germination and shoot elongation. In conclusion, poor seedling establishment of rice sown in flooded paddy soils, in which the oxygen supply to the seeds is restricted, appears to the result of limited root elongation rate.

***Keywords*:** rice, seed germination, oxygen, nitric oxide, mercuric chloride

Seedling establishment is diminished when rice seeds are subjected to flooded rice paddies especially when flooding levels are greater than a few cm above the paddy soil. Successful establishment of rice seedlings requires rapid germination and rapid elongation rates of both shoots and roots. Uniform distribution of broadcast seeds but uneven leveling of paddy soils results in heterogeneous anoxic conditions at the flooded water and soil surfaces. Many cereal seeds including wheat and barley are unable to germinate under anoxia, whereas rice continues amylolytic activity during anaerobic conditions (Guglielminetti *et al.*, 1995; Perata *et al.*, 1993). Kubota *et al.* (1994) used sodium dithionate that removes the oxygen dissolved in water to test germination and early growth of rice in limited oxygen. Seed coating by a calcium peroxide carrier "Calper" has been reported to alleviate some of these difficulties associated with seedling establishment in anaerobic environments (Ishimoto, 1982). Another approach for resolving these interactions would be to quantify differences in seed germination and seedling development responses among different rice varieties. These data could be used to determine varietal differences, which could be incorporated into plant-breeding programs designed to enhance the resistance of seeds to the varying anoxic conditions in paddy rice fields. Seed germination and subsequent seedling development in restricted oxygen environments of flooded paddy cultures are more important genetic qualities especially in water-seeded rice than in any other cultural methods. Early cell division and elongation of radicle, hypocotyl, and root tissues of germinating seeds are directly controlled by the aerobic/anaerobic conditions associated with seedling environments (Biswas & Yamauchi, 1997). Oxygen status also controls the endogenous regulators in cellular elongation, results in reduced growth of rice coleoptile (Horton, 1991).

Nitric oxide known to stimulate the seed germination and de-etiolation of lettuce was reported to inhibit the hypocotyl and internode elongations of the plants (Beligni & Lamattina, 2000). This phenomenon pointed out the involvement of nitric oxide as a stimulator molecule in photomorphogenesis or plant photoreceptor.

Mercuric chloride has been reported to inhibit the channel-mediated water flux through cell membranes in tomato root systems (Maggio & Joly, 1995), the roots of aspen seedlings (Wan & Zwiazek, 1999), wheat root cells (Zhang & Tyreman, 1999), maize (François *et al.*, 1998) and rice roots (Lu & Neumann, 1999). Recent discoveries in water perme-

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ation of plant vacuolar and plasma membranes have elucidated the presence of water channel protein named aquaporins. The effect of mercuric chloride on the inhibition of water flux across cell membranes is reported for many plants cells including tobacco suspension cultures (Maurel, 1997), even though some plant aquaporins are recognized as resistant to mercury action (Daniels *et al.*, 1994). The mechanism of water flux inhibition by mercury derivatives is thought to block water channels via oxidation of cystein residues proximal to aqueous pores of cells and subsequent occlusions of the pores by the large mercury ion (Maurel, 1997).

We hypothesized that reduced oxygen has little effect upon the germination of rice, but low oxygen contents could severely reduce the development of root and shoot elongations. In addition, nitric oxide was hypothesized to influence on the seed germination and subsequent elongation of rice differently from the stimulator effects on the germination of lettuce that germinates under the presence of light. Furthermore, we suggested that $HgCl_2$ reduces the uptake and utilization of water affecting germination and elongation of rice seedlings and subsequent whole plant responses. This study was designed to identify differences in germination of the rice varieties to reduced oxygen conditions. Twenty-three rice varieties were tested for these studies by a germination and emergence pretest in the phytotron at 18 °C located at the National Institute of Crop Science, Seodundong 209, Suwon, 441-857, Republic of Korea. One hundred seeds of each variety were planted to 1cm depths of soil and flooded with 1cm of water. In these germination and emergence preliminary studies, three rice varieties: Ansanbyeo, Jinheung, and Odaebyeo were classified as exhibiting high emergence rates (74-78%) from soils in 20 days. Three other varieties: Andabyeo, Nonganbyeo, and Sangmibyeo exhibited low emergence rates (22-41%). Studies reported here were conducted in a temperature-controlled growth chamber to find out the effects of anoxic air treatment, nitric oxide, and inhibited water flux by $HgCl_2$ on the germination and subsequent early elongation of the selected six rice varieties described above.

MATERIALS AND METHODS

Germination and early elongation growth of rice varieties in three different oxygen concentrations

Initially germination and plant elongation experiments were completed under controlled oxygen levels separately in 0, 3, and 21% levels. Gas tight aluminum chambers (55×55×170cm: width×depth×height) were used in a walk-in temperature (17°C) controlled room. To test the germination of rice in different oxygen environment, 100% nitrogen gas (0% oxygen), compressed air (21% oxygen), and 7:1 mixture of nitrogen gas and compressed air (3% oxygen) were supplied separately by bubbling 1 L min^{-1} through distilled H_2O to maintain the humidity in the germination chambers. Flow rate meters (Gilmont Co.) installed between the gas valves and distilled water monitored the amount of gas supplied into chambers. The oxygen content in mixed gas was monitored by a pre-calibrated oxygen analyzer (Model-d, Arnold O. Beckman Inc.), to which gas mixtures flowed through peristaltic pump (Miniplus2, Gilson Co.) at 1000 rpm. Twenty-three rice varieties were germinated by evenly placing 100 seeds on standard blue blotter germination paper (8×19 inches, Anchor Paper Co. located at St. Paul, Minnesota, USA) and saturated by distilled water and covered by brown paper to prevent drying. The number of germinated seeds was counted at 3, 6, and 9 days after treatment initiation. In each chamber, the 23 rice varieties were treated by completely randomized block configuration with three replications. These studies were performed at 17°C, the average soil surface temperature during the seedling establishment period from May 10 to May 30 in Korea. Results of this germination test for rice variety sensitivity to O_2 environment are listed in Table 1. Based upon the percent emergence of rice varieties pretested, six varieties (Ansanbyeo, Jinheung, Odaebyeo, Andabyeo, Nonganbyeo, and Sangmibyeo) of rice were selected for the further investigations for germination, and root and shoot elongations of rice in strict oxygen deficits, nitric oxide treatment, and mercuric chloride solutions.

Further effects of restricted oxygen on rice seed germination were evaluated by placing 50 evenly distributed dry seeds, of six different varieties in polystyrene petri dishes (100×15 mm) having three layers of blue germination paper, wetted by 50 ml of degassed distilled water. Water was degassed by boiling for 30 minutes and cooled before using. Germination chambers (55×55×170cm; width×depth×height), around which edges were sealed with closed-cell foam rubber to prevent gas flux into the chamber, specially constructed were used to test germination and elongation of rice in different atmospheric oxygen conditions. To maintain a strict anaerobic gaseous environment in a chamber, humidified nitrogen gas was supplied continuously at the rate of 4 L min^{-1}, and small (approximately 3 mm) outlet was made at the top of the chamber to prevent the development of overpressure. Gas flow was monitored by flow rate-meter installed between gas valve and gas dispersion system during experiment. To determine the germination of rice in atmospheric oxygen concentration, 50 dry seeds were treated in another gas-tight aluminum seed germination chamber into which humidified compressed air was supplied at the rate of 4 L min^{-1} using the same supplying methods as

Table 1. Germination of 23 rice varieties in the three different oxygen levels, n=3.

Variety	Germination of rice varieties in different oxygen levels (%)		
	0%	3%	21%
Hwaseongbyeo	97.0 ± 1.0†	97.3 ± 1.5	94.7 ± 1.5
Daeanbyeo	95.3 ± 2.9	93.3 ± 4.0	96.3 ± 1.2
Hoanbyeo	89.0 ± 1.0	87.7 ± 2.3	87.0 ± 2.6
Jinheung	94.7 ± 2.1	94.0 ± 1.7	92.3 ± 1.2
Andabyeo	97.3 ± 1.5	98.0 ± 1.7	96.3 ± 1.2
Wonhwangbyeo	90.7 ± 2.1	88.0 ± 3.0	91.3 ± 3.2
Chunhyangbyeo	98.0 ± 0.0	98.7 ± 0.6	96.0 ± 0.0
Nonghobyeo	96.0 ± 1.7	95.3 ± 2.1	95.7 ± 1.5
Nonganbyeo	87.7 ± 6.7	89.7 ± 3.5	87.0 ± 2.0
Kwanganbyeo	96.7 ± 2.1	97.3 ± 2.9	96.0 ± 1.7
Odaebyeo	94.7 ± 2.5	96.0 ± 1.7	92.0 ± 1.0
Ansanbyeo	92.0 ± 1.0	89.3 ± 1.5	91.3 ± 0.6
Sangmibyeo	73.7 ± 8.5	69.7 ± 4.2	72.3 ± 2.9
Hwabongbyeo	93.0 ± 3.6	89.3 ± 1.5	92.7 ± 4.0
Geurubyeo	95.3 ± 1.5	92.7 ± 1.5	94.3 ± 1.5
Dasanbyeo	93.7 ± 2.3	95.0 ± 2.0	94.3 ± 0.6
Ilpumbyeo	95.7 ± 2.5	94.0 ± 2.6	94.7 ± 0.6
S. 433	83.7 ± 4.9	79.0 ± 1.0	81.7 ± 1.2
S. 434	82.7 ± 4.0	80.0 ± 4.0	78.7 ± 2.5
S. 437	87.7 ± 3.8	79.7 ± 3.8	82.0 ± 2.0
S. 438	84.7 ± 2.9	90.0 ± 2.6	86.0 ± 3.5
S. 442	80.0 ± 1.7	80.7 ± 4.9	79.0 ± 2.6
S. 443	82.7 ± 2.9	82.0 ± 1.7	82.7 ± 3.1

†Data are means ± standard deviations of three replications.

described for the restricted oxygen treatment. Ten pre-germinated seeds of the six varieties, previously soaked in distilled water for five days resulting in coleoptile lengths of approximately 1 mm, were transferred to a petri dish (100×15 mm) as the same treatment methods with those of germination to determine the effect of anoxic air treatment on the elongation of rice after germination. Three plates, each of them having a set of six varieties for the test of germination and another three plates for the test of elongation of rice were placed into each chamber. Seed germination rates were determined by observing all seeds every two days. The final germination count was determined 10 days after initiation of treatment (DAT). All treatments were replicated three times. Experimental design for all studies was a completely randomized block configuration. The individual lengths of shoot and root were measured at 10 DAT and converted into elongation rates per day.

Germination and early elongation responses of rice seeds to different concentrations of $HgCl_2$ solution

Seeds for each of six rice varieties (Ansanbyeo, Jinheung, Odaebyeo, Andabyeo, Nonganbyeo, and Sangmibyeo) were subjected to 0, 10, 100, 300, 500, and 1000 μM solutions of $HgCl_2$ reported to limit water flux through cell membranes of rice roots (Lu & Neumann, 1999). Seeds were soaked for 15 days in 100 ml Erlenmeyer flasks containing 50 ml of their respective solutions, as listed above. Fifty dry seeds for each variety were soaked with three replications. The flasks were sealed with parafilm to minimize evaporation and to allow air permeation into them. The number of germinated seeds was counted every day and the final germination determined at 15 DAT. To identify the elongation of rice after germination as influenced by $HgCl_2$, ten pre-germinated seeds for each of the same six varieties were soaked in six different concentrations as those treatment methods in the germination treatment. Lengths of rice shoots and roots were measured at 10 DAT.

Nitric oxide treatment

To determine the effect of nitric oxide on the germination of rice, 50 dry seeds of Jinheung that showed the highest germination among tested six varieties in mercuric chloride solution experiment were subjected in 250 ml of Erlenmeyer extraction flasks, each of which contains 200 ml of distilled water. Four Erlenmeyer extraction flasks were connected in turn from each of nitric oxide and compressed air cylinder by tygon tubing and glass tube inserted through the flask stopper. Last flasks maintained open system. Air and nitric oxide was supplied through bubblers soaked in water at the rate of 8.0 L h^{-1} at inlet. Seed germination was observed every two days and final germination was determined at 10 DAT. Shoot and root lengths of germinated plants, pH of solutions by pH meter (Model – pH meter 130, Corning Co.) were measured at 12 DAT. Air in the flasks was sampled by syringe and needle, and injected in 10 ml of vacutainer (Becon Dikinson Co.) after closing the open system for 2 hours at 12 DAT. Infrared CO_2 analyzer (Model LI-6252, LI-COR) determined the CO_2 concentrations by the injection of 1 ml of sampled gas. CO_2 concentration was calculated based upon the calibration curve using eight concentrations (1, 0.8, 0.6, 0.4, 0.2, 0.1, 0.05, and 0.03%) of CO_2.

Calculations and statistical evaluations

V_{max} $t_{1/2}$ of germination was calculated as follows.

V_{max} $t_{1/2}$ of germination (day)=$(G_H-G_A)/\{(G_B-G_A)/D\}+D_{GA}$,

where; G_H is half of the final germination percent. G_A is the percent germination observed at the first measurement immediately before G_H. G_B is the percent germination at the measured day immediately following G_H. It should be noted that G_B and G_H could be the same day. D represents the day interval between G_A and G_B. D_{GA} is the number of days between the onset of the treatment and G_A. Collected data including average values and statistical evaluations of the means were analyzed by SAS statistical software package.

RESULTS AND DISCUSSION

Germination and early elongation growth of rice varieties in three different oxygen concentrations

Seed germination of rice showed significantly different varietal responses to oxygen treatment (Table 2). In the two varieties, Nonganbyeo and Sangmibyeo, which were significantly different (by the $LSD_{0.05}$) in germination from other varieties of rice, especially the germination of Sangmibyeo was lower considerably (Fig. 1A). This lower germination in Sangmibyeo compared to those in other varieties is estimated as a genetic property that it has. Restricted oxygen condition, however, had no influence on the germinations in six varieties of rice tested.

V_{max} $t_{1/2}$ of germination (V_{max} $t_{1/2}$), that represents the days after treatment initiation on which germination rate reaches half of the final germination, showed significant differences among the rice varieties (Fig. 1B). Based on significant difference among varieties in 5% of LSD, it is estimated that Jinheung and Sangmibyeo as showing rapid germination, Ansanbyeo as an intermediate, Odaebyeo and Andabyeo as slow, and Nonganbyeo as the slowest germination, respectively. Rapid germination was observed in Jinheung that showed high percentage of germination and in Sangmibyeo that had poor germination rate, respectively.

This non-inhibited germination of rice in restricted oxygen is explained by its ability to degrade starch granules in anaerobic condition (Guglielminetti *et al.*, 1995) by the concerted action of the complete set of amylolytic enzymes in the seed of rice, which catalyze starch into sugar under anoxia (Perata *et al.*, 1997), resulted in substrate production required to produce energy for germination. In addition, the subsequent alcoholic fermentation of rice in limited oxygen environment contributes to the energy production (Bertani *et al.*, 1997). This energy production by alcoholic fermenta-

Table 2. Significances in analysis of variance (ANOVA) for germination and elongation of rice in two levels of oxygen and six rice varieties.

Variable	Oxygen	Variety	Oxygen×Variety
Germination (%)	ns†	***	ns
V_{max} $t_{1/2}$ of germination (day)	ns	***	ns
Shoot elongation rate (mm/d)	**	***	ns
Root elongation rate (mm/d)	***	***	***

†Each of **, ***means significant difference at 1 and 0.1 % of probability, respectively, ns represents no significance.

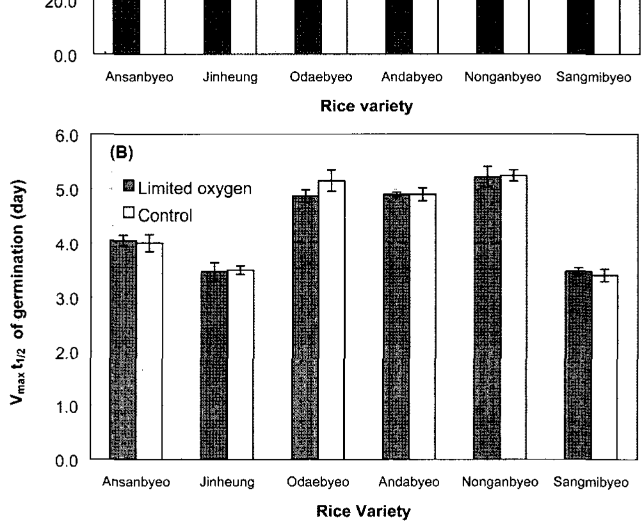

Fig. 1. Germination (A) and V_{max} $t_{1/2}$ of germination (B) of six rice varieties in limited oxygen and control (21% O_2) conditions. Error bars are standard deviations of three replications.

tion, however, has merely lower energy conversion efficiency than aerobic respiration, requiring more substrate to produce equivalent energy. Even though limited oxygen conditions didn't affect the germination of rice, energy cost for germination might be much higher and more nutrients reserved in the endosperm of rice for the subsequent root and shoot growth during the growth stage dependant upon endosperm would be consumed by germination in alcoholic fermentation.

Comparing shoot elongation rates (SER) of rice, both oxygen condition and variety have influenced on them (Table 2). The effects of restricted oxygen environments differed according to rice varieties. Jinheung, Odaebyeo, and Nonganbyeo showed inhibited SERs, whereas Ansanbyeo, Andabyeo, and Sangmibyeo were not significantly different in SER in either oxygen condition (Fig. 2), based on the probabilities of least squares means. Root elongation rates (RER), however, decreased by anoxic air treatment in all tested six varieties of rice (Fig. 2). Oxygen condition and variety influenced on the root elongation of rice, and both of them interacted each other for RER (Table 2). The decreased extend of elongation in root exhibited varietal differences. In fully aerated condition, the elongation rate of root was the greatest in Jinheung followed by Odaebyeo, Nonganbyeo and Sangmibyeo, Ansanbyeo, and Andabyeo showed the poorest elongation. In restricted oxygen condition, however, RERs in Jinheung, Odaebyeo, and Ansanbyeo were similarly higher than in subsequent Nonganbyeo=Sangmibyeo, and Andabyeo. Especially the RER in Andabyeo, the hybrid variety between Indica and Japonica, showed great restriction by limited oxygen than in any other varieties. When we compare the RER to SER in the sense of overall responses of six varieties, the slope of RER to SER declined in limited oxygen condition (Fig. 3). This declined slope in restricted oxygen environment would result in the greater difference of shoot and root length as the shoot elongates further.

Inhibitions of SER and RER of rice in anaerobic condition suppose the possibility of low energy efficiency resulted from alcoholic fermentation mentioned by Bertani *et al.* (1997). Furthermore, since the ratio of RER in restricted oxygen to control was not correlated with the ratio of SER in limited oxygen to control (correlation coefficient = -0.19^{ns}), it is suggested that the energy, produced by the activities of amylolytic enzymes and subsequent energy production mechanisms, might be transferred to sink tissues (root and shoot) by different control mechanisms.

Severely inhibited root elongation would negatively influence seedling establishment of rice in flooded paddy by both delay and poor anchorage. In addition, considerably enhanced root elongation of rice in higher oxygen concentration implies the importance of aeration for promoting the growth of root, which has the functions of anchorage and nutrient absorption. On the other hand, soil aeration responses would be emphasized differently according to varieties, since the root elongations of rice in the varieties such as Jinheung, which showed rapid elongation under aerated conditions and severe inhibition of root elongation in limited oxygen, are expected to be stimulated considerably.

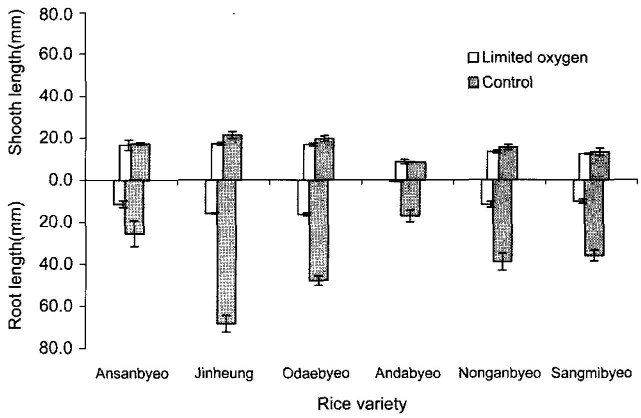

Fig. 2. Shoot and root lengths of rice varieties at 10 DAT as affected by the limited oxygen condition. Error bars are standard deviations of three replications, each of which with 10 seedlings.

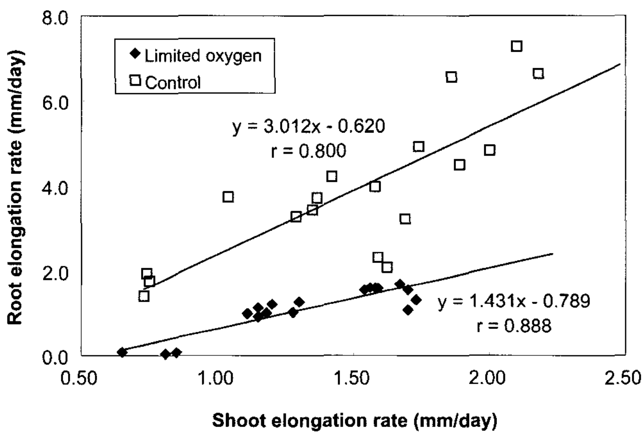

Fig. 3. Relationship between root and shoot elongation rates in limited oxygen and aerated (control) conditions. Data represent six varieties with three replications of 10 seedlings each.

Germination and early elongation responses of rice seeds to different concentrations of $HgCl_2$ solution

Germination, V_{max} $t_{1/2}$, SER and RER of all six varieties were influenced significantly by mercuric chloride concentrations (Table 3). Considering the overall responses in different concentrations of $HgCl_2$ solution, germination of rice began to decrease in 300 μM and 500 μM according to vari-

Table 3. Significances in analysis of variance (ANOVA) for germination and elongation of rice in six concentrations of mercuric chloride and six rice varieties.

Variable	$HgCl_2$	Variety	$HgCl_2$×Variety
Germination (%)	***†	***	***
V_{max} $t_{1/2}$ of germination (day)	***	***	***
Shoot elongation rate (mm/d)	***	***	***
Root elongation rate (mm/d)	***	***	***

†*** means significant difference at 0.1 % of probability.

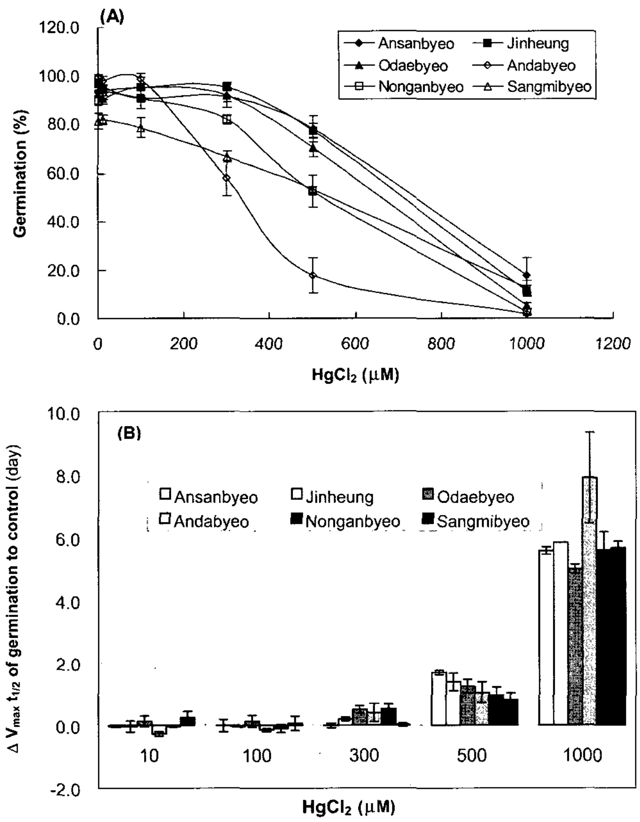

Fig. 4. Germination (A) and $\Delta V_{max}.t_{1/2}$ of germination to control (B) in different concentrations of $HgCl_2$ solutions. Error bars are standard deviations of three replications.

eties (Fig. 4A), whereas they showed no significant difference in low concentrations including 10 and 100 μM of mercuric chloride solution. The decreased germination in relatively higher concentrations of $HgCl_2$ showed varietal differences. In 300 μM, Ansanbyeo, Jinheung, and Odaebyeo demonstrated resistant responses of germination to $HgCl_2$ based upon the statistical mean difference probability. In the three other varieties, Andabyeo, Nonganbyeo, and Sangmibyeo, however, the germination in 300 μM exhibited decreased differences than in control (0 μM). Both decreased germination and varietal separation of sensitivity to mercuric chloride were initiated in 300 μM of solution. As the concentration of mercuric chloride increases above 300 μM, the germinations of rice were influenced negatively, and in 1000 μM of $HgCl_2$, the germinations in all the tested varieties were lower than 20%. In 500 μM, the germination affected by $HgCl_2$ showed the greatest variation among rice varieties. Especially, Andabyeo, the hybrid variety between Indica and Japonica ecotype, exhibited severely reduced germination (Fig. 4A).

Concentration of mercuric chloride and rice variety influenced significantly on the V_{max} $t_{1/2}$ (Table 3). When we consider ΔV_{max} $t_{1/2}$ to control, the delayed V_{max} $t_{1/2}$ initiated in 300 and 500 μM of $HgCl_2$ solutions resulted in the same trend with germination (Fig. 4B). In 300 μM of mercuric chloride solution, the increased V_{max} $t_{1/2}$ initiated showing varietal difference to $HgCl_2$. The V_{max} $t_{1/2}$ in Odaebyeo and Nonganbyeo initiated to increase in 300 μM of $HgCl_2$ solution, based on the mean difference probability, whereas in other four varieties they began to increase from 500 μM. As the mercuric chloride concentration increased, V_{max} $t_{1/2}$ was prolonged considerably. ΔV_{max} $t_{1/2}$ of Andabyeo in 1000 μM $HgCl_2$ was the highest. Considering the germination percents and rates in Fig. 5, it is estimated that 300 μM of $HgCl_2$ is the critical concentration to determine the susceptibility and resistance of rice germination to mercuric chloride.

In 500 μM of mercuric chloride that showed the highest variation of germination among rice varieties (Fig. 4A), it was estimated that Ansanbyeo, Jinheung, and Odaebyeo as being resistant, Nonganbyeo and Sangmibyeo as intermediate, and Andabyeo as sensitive to mercuric chloride (Fig. 5). Based on the V_{max} $t_{1/2}$, Sangmibyeo was evaluated as showing fast elongation, Jinheung as intermediate, and other four varieties as exhibiting slow germination, respectively.

The resulted effects of $HgCl_2$ on the inhibition of rice germination and delayed V_{max} $t_{1/2}$ imply the involvement of channel-mediated water flux through membrane by aquaporins in the water imbibition stage of rice. Furthermore, the different germination responses of rice varieties to $HgCl_2$ suggests the possibility of different membrane characteristics of rice varieties involved in water flux through it. The involvement of channel-mediated water flux into the cell and aquaporin in water imbibition stage of rice remains to be assessed.

Both $HgCl_2$ and rice variety contributed to the significant differences of SER (Table 3). The inhibitions of SERs were initiated in 10 μM of mercuric chloride solution, the lowest concentration treated, which didn't influence on the germination rate and V_{max} $t_{1/2}$ (Fig. 6). For the SER, the means in 0, 10, 100, and 300 μM of $HgCl_2$ solution showed significant differences each other by $LSD_{0.05}$, and those in 500 and 1000 μM were different from SERs in above four concen-

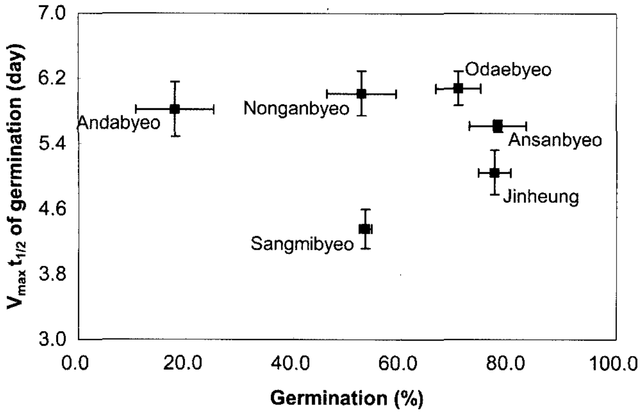

Fig. 5. Germination (%) and V_{max} $t_{1/2}$ of germination in 500 μM of mercuric chloride solution. Error bars are standard deviations of three replications.

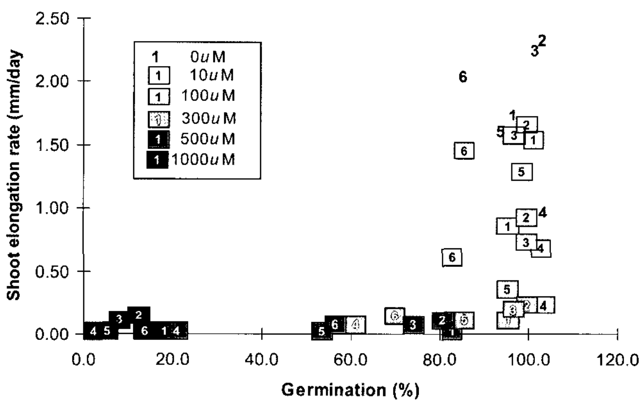

Fig. 6. Varietal distributions of germination and shoot elongation rate in six different concentrations of $HgCl_2$ solutions. †1: Ansanbyeo, 2: Jinheung, 3: Odaebyeo, 4: Andabyeo, 5: Nonganbyeo, 6: Sangmibyeo.

trations. In higher concentrations of $HgCl_2$, including 500 and 1000 μM, the SERs were ignorable. According to rice varieties, shoot elongation showed resistance to $HgCl_2$ in the order of Jinheung > Odaebyeo > Ansanbyeo and Sangmibyeo > Nonganbyeo > Andabyeo, based upon 5% of LSD.

Root elongation followed germination was not observed in all concentrations of mercuric chloride treated when pregerminated seeds having approximately 1 mm of coleoptile emerged out of hull were soaked in $HgCl_2$ solution. Only in distilled water, roots of six varieties elongated (data not shown). In other words, $HgCl_2$ inhibited root elongation completely even in low concentration of solution. When rice germinates soaked in water, coleoptile emerges ahead of root emergence out of hull due to the insufficient oxygen supply to seeds. Even though the seeds of rice placed in solution resulted in inhibited root elongation, complete inhibitor action of $HgCl_2$ upon root emergence should be

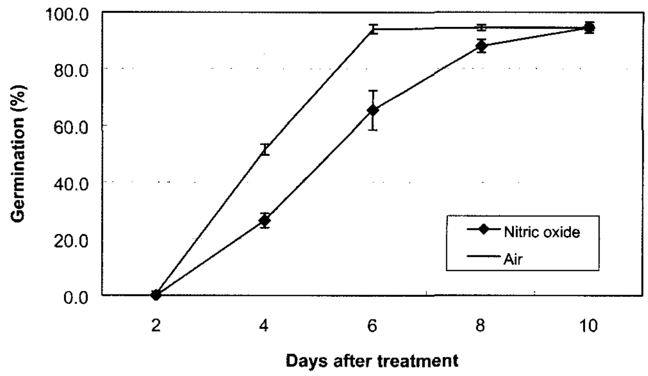

Fig. 7. Germination of rice seeds in nitric oxide and air treatment. Error bars are standard deviations of four replications.

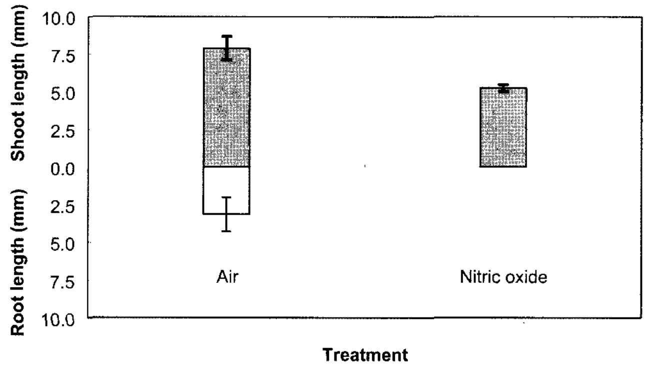

Fig. 8. Shoot and root lengths of rice plants measured at 12 days after treatment initiation in nitric oxide and air treatment. Error bars are standard errors of four replications.

assessed separately from inhibited root elongation by oxygen deficit. It is notable that elongation after germination was more sensitive than germination, and again root elongation was more susceptible than shoot elongation to $HgCl_2$.

Nitric oxide treatment

In air treatment, the final germination was determined approximately at 6 DAT. However, nitric oxide showed inhibitor effects on the germination rate of rice, even though it didn't influence on the final germination at 10 DAT (Fig. 7). This delayed seed germination of rice by nitric oxide was different from its stimulator effects on the seed germination reported in lettuce (Beligni & Lamattina, 2000). These different effects of nitric oxide on the rice seed germination suggest its different role from the functions involved in photomorphogenesis, because the seed germination of rice doesn't require light while it is essential to the germination of lettuce seed.

Nitric oxide treatment inhibited the shoot and root elongations of rice. Especially root emergence was completely inhibited by nitric oxide treatment, whereas the reduced shoot length was less strict (Fig. 8). The pH of solution in nitric oxide treatment (7.31±0.40, mean±standard deviation) was higher than in air treatment (6.03±0.16). In addition, the concentration of carbon dioxide in the air showed elevated trend in the nitric oxide treatment (1242±791 ppm) than in the air treatment (1052±470 ppm). The reduced growth of rice plant in nitric oxide treatment was possibly induced by direct nitric oxide effect, oxygen deficit (Fig. 2) due to the continuous NO supply, and/or higher pH that results in inhibition of seedling growth of rice (Hoshikawa, 1974). Nitric oxide had clear direct or indirect adverse effects on the germination rates and elongations of root and shoot of rice. The precise factors induced by nitric oxide treatment, which affected germination rates and elongations of root and shoot, remained further investigation.

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