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Nature of oxidizing power of rice roots

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Summary The oxidizing power of rice roots comprises two components, *i.e.*, oxygen release and enzymatic oxidation as measured by α-naphthylamine oxidation. Microscopic examination of roots shows a columnar arrangement of cells having structural intercellular spaces that may serve as air passage pipes in the tip region of the root. Both oxygen release and α-naphthylamine oxidation were studied as function of atmospheric oxygen concentration, light, temperature of root environment, respiratory inhibitors, and nutritional status of the plant. The results led to the concept that α-naphthylamine oxidation is a part of the respiration, and that the oxygen which is not consumed by respiration is diffused into the surrounding environment. Both processes are governed by molecular oxygen supply from shoots. At 25°C, the oxygen release of 3-week-old seedlings of IR36 was about 9 times greater than the amount of oxygen needed to account for α-naphthylamine oxidation rate.

Introduction

Rice (*Oryza sativa* L.) roots can oxidize various compounds on the root surface and in the close vicinity of the roots. Submerged soils produce several toxic substances which may be a major factor causing poor rice growth. These two facts indicate that the oxidizing power of rice roots functions as a defense mechanism against toxic substances such as ferrous iron and hydrogen sulfide.

Armstrong[3] determined varietal differences in oxygen diffusion from rice roots and related the results to resistance to Akagare disease. He suggested that the polarographic technique may be useful in any effort to build up a high oxidizing power into new plant types proposed for the tropics.

Several attempts have been made to correlate root oxidizing power with plant tolerance for the physiological disorders, but these attempts are not always successful. Joshi *et al.*[9] reported that the varietal differences in tolerance for straighthead and mild sulfide disease could be attributed to differences in root oxidizing power (O_2 release rate). On the other hand, Jayawardena *et al.*[5] could not find any correlation between root oxidizing power (α-naphthylamine oxidation) and resistance to iron toxicity.

The oxidizing power of rice roots may be composed of two distinctly different

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processes: enzymatic oxidation on the root surface and release of molecular oxygen into the rhizosphere. The nature of these two processes and relative importance of each process in the defense against toxic substances may be quite different. This paper attempts to describe microscopic observations of root air space along with studies of enzymatic oxidation and the release of molecular oxygen from rice roots.

Materials and methods

Plant material

Seeds of rice (CV IR36) were sterilized by soaking in 0.1% $HgCl_2$ for one minute, rinsed with distilled water, and allowed to germinate for 2 days in a beaker containing a small amount of water. The germinated seeds were sown on a nylon screen framed with styrofoam board placed on a 7-liter plastic tray containing nutrient solution. The nutrient solution contained 40 ppm N (NH_4NO_3), 10 ppm P ($NaH_2PO_4 \cdot H_2O$), 40 ppm K (K_2SO_4), 40 ppm Ca ($CaCl_2 \cdot 2H_2O$), 40 ppm Mg ($MgSO_4 \cdot 7H_2O$) and traces of Mn, Mo, B, Zn, Cu and Fe, as described by Yoshida *et al.*[21]

The plants were grown in the glasshouse of the phytotron at 29°/21°C (day/night). During the first week after germination one-half strength of the nutrient solution was adjusted to pH 4.8 once daily. One week after germination, 15 or 30 plants were transplanted to a 7.5-liter tray and given the full strength of the nutrient solution. The nutrient solution was adjusted to pH 5.0 once daily and renewed once a week. The oxidizing power of 2- to 3-week-old rice seedlings was measured. The details of growth conditions were described in each experiment.

Microscopic observations

Cross sections or longitudinal sections of fresh rice roots were observed using the Olympus system microscope Model BH.

Measurement of oxygen concentration

A YSI Model 53 Biological Oxygen Monitor (Yellow Springs Instrument Co., Inc., Ohio, USA) was used for measuring oxygen release from rice roots and oxygen uptake by roots. The oxygen probe was covered with a standard thickness of teflon membrane and placed in a 50-ml plastic vial. The sample vial contained a magnet and 50 ml of distilled water. A plant was fixed in 11-mm-diameter hole with a split neoprene sponge. The sample vial was connected to oxygen-free water in a 100-ml Erlenmeyer flask through a capillary tube so as to avoid the negative pressure caused by transpiration during measurement.

The sample vial was placed in a water bath. The water temperature was controlled with ± 0.05 C precision by Taiyo BCL 19 cooling unit. The oxygen monitor was calibrated by introducing air into the sample vial at a specified water temperature.

Before the oxygen release rate was measured, nitrogen gas was introduced into the sample vial through the auxiliary relief hole to drive off traces of air until the oxygen concentration of the water was below 3% air saturation. A 2- or 3-week-old seedling was held with its roots in a sample vial through the hole of a plastic stopper. The hole was made airtight by use of soft rubber ring. Oxygen-free water was forced into the sample vial by N_2 gas pressure to remove the entrapped gas bubbles, if necessary. The auxiliary relief hole was then closed. Changes in the oxygen concentration were recorded by a Toshiba RSD recorder for 10 minutes after needle stabilization.

For measurement of oxygen uptake rate, water in the sample vial was saturated by air-bubbling. The decrease in the oxygen concentration recorded for 10 minutes after the vial was set up in the same manner as in the oxygen release measurement.

α-Naphthylamine oxidation

The method proposed by Sakai and Yoshida[16] was modified as follows: intact rice roots (about 1 g of fresh weight) were immersed in 50 ml of 20 ppm α-naphthylamine (α-NA) test solution for 10 minutes to exclude initial rapid absorption of α-NA by roots. The intact roots were transferred to another 50 ml of 20 ppm α-NA test solution which was maintained at 25° ± 1°C, and incubated up to 4 hours. After 2 and 4 hours of incubation, 2 ml of the α-NA sample solution was pipetted out and reacted with 10 ml of 0.1% sulfanilic acid (in 3% acetic acid) and then with 2 ml of 50 ppm $NaNO_2$. After the solution was diluted to 25 ml with distilled water, the absorbance of the colored solution was determined at 530 nm. The decrease in α-NA during incubation was calculated as amount of α-NA oxidized by the roots in a given time.

Results and discussion

1. Microscopic observations on air passage system

Although the presence of the air passage system of the rice plant is well documented[22], clear evidence that the intercellular spaces in the elongation zone of roots are filled with air is not available.

The following observations are evidence that the intercellular spaces in the elongation zone of roots function as air pipes connecting meristematic tissue to the lysigenous intercellular spaces commonly present at the base of rice roots:

(1) In the cross section of fresh nodal root of a 4-week-old IR36 plant, intercellular spaces are recognized in the cortex of the elongation zone (Plate 1). An intercellular space is surrounded by four cortical cells. Such arrangement of cortical cells is termed "columnar arrangement"*, and is characteristic of marshy plants. The columnar arrangement of cortical cells provides 2 to 3 times greater air space than an oblique arrangement[20].

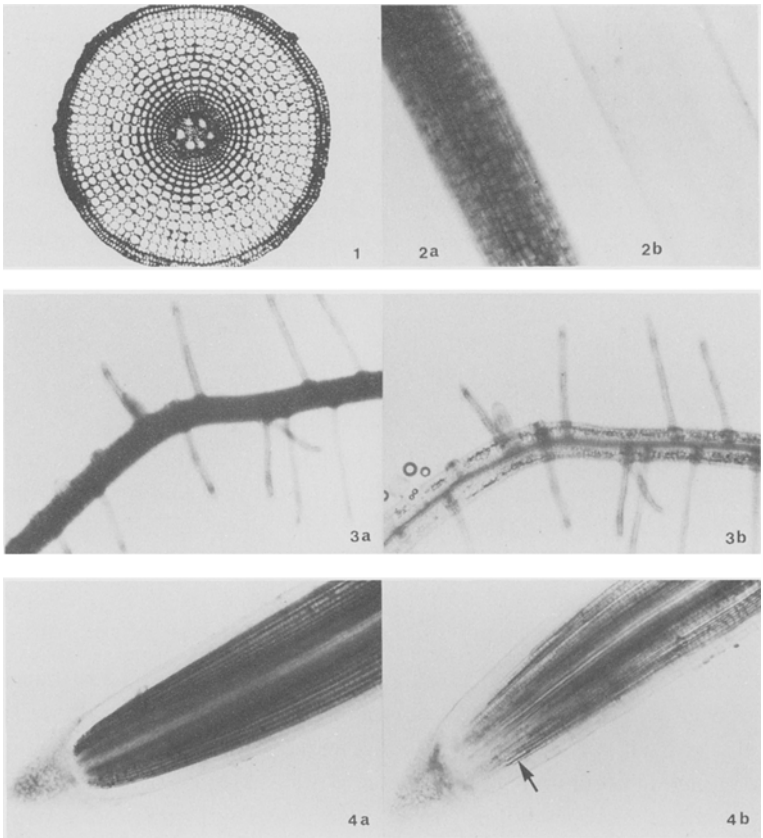
(2) A fresh, thin, nodal root looked opaque and dark under an optical microscope (Plate 2a). The same material became translucent (Plate 2b) after it was placed in a vacuum and presumably because air in the intercellular spaces was driven off and water entered the air spaces.

(3) Similarly, an opaque, dark thin nodal root (Plate 3a) became translucent (Plate 3b) after it was lightly pressed; some air bubbles were observed under a microscope.

(4) In the longitudinal section of a fresh nodal root, dark and tubular structures (in Plate 4b), corresponding to intercellular air spaces in the cross section, developed parallel with the central cylinder and terminated before the root cap (Plate 4a).

(5) No "intercellular air spaces" were found in the epidermis, exodermis, and sclerenchyma.

* *Columnar arrangement*: An arrangement of many circles when each circle has four points of contact with neighboring circles. The centers of each circle are in the same horizontal and vertical line. *Oblique arrangement*: An arrangement of circles in which the centers are aligned on only one axis. In an axis 90° to the one on which the centers are aligned, the centers of the circles are aligned with the points of contact rather than with centers of neighboring circles.



Plates 1–4

1. Cross-section of the elongating zone of fresh nodal root of rice, showing intercellular spaces surrounded by four cortical cells.
2. Fresh rice root before (a) and after (b) it was placed in vacuum.
3. Fresh rice root before (a) and after (b) it was lightly pressed.
4. Central (a) and side (b) part of longitudinal section of fresh roots.

The above observations suggest that intercellular spaces are mostly, if not totally, filled with air. Thus, air transported from the shoot enters large lysigenous intercellular spaces in the basal part of a root, then moves in tubular intercellular spaces, and finally diffuses into surrounding medium at the terminal of the tubular structures; a portion of air may dissolve in water of cell walls, moves laterally, and finally diffuses into surrounding medium. The above conclusion is in agreement with Armstrong's results[1] that oxygen diffusion was highest at the root apex and diminished sharply toward the root base.

2. Oxidizing power of rice roots

To characterize the oxidizing power of rice roots, the effects of some environmental factors on oxygen release rate and enzymatic oxidation rate were

studied. Three-week-old IR36 seedlings were used for the series of experiments. The measurements were done at 25°C under room light conditions unless otherwise stated.

Effects of atmospheric oxygen supply and light on oxygen release from roots

When rice roots were placed in water of low oxygen concentration, the oxygen concentration of the water increased with time and levelled off within several hours (Fig. 1). The oxygen concentration of the water was maintained at a plateau value for several hours. This would imply that the rates of oxygen release and of oxygen uptake were equal at the plateau oxygen concentration of the water.

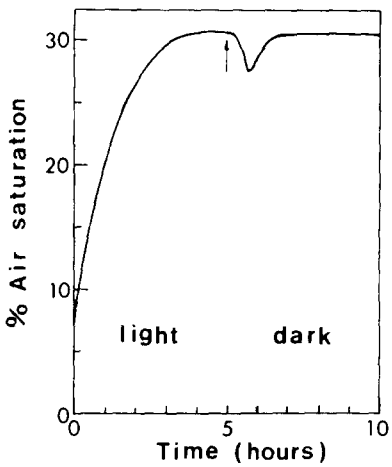


Fig. 1. Effect of light on oxygen release from rice roots.

Atmospheric oxygen supply The shoots of the 3-week-old IR36 seedlings were contained in a 30-cm-long glass tube. The atmosphere in the tube was changed from air to nitrogen gas. The change in the oxygen concentration of the vial containing the roots is shown in Fig. 2. Within 5 minutes after replacing air by nitrogen gas, the oxygen concentration of the root environment decreased rapidly. Five minutes after nitrogen gas was replaced by air, the oxygen concentration rapidly increased.

A similar rapid decrease in the oxygen concentration of the root environment also occurred when the shoot was removed and the cut-end of the shoot was sealed with grease. The decrease started 5 minutes after sealing.

These results indicate that oxygen release from the roots depends on atmospheric oxygen and that oxygen in the atmosphere moves downward and reaches the root environment within 5 minutes.

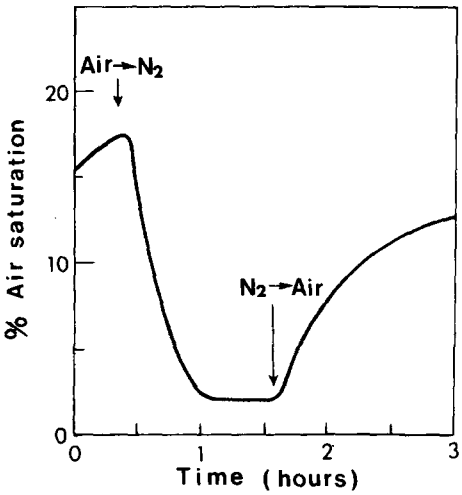


Fig. 2. Effect of atmospheric oxygen supply on oxygen release from rice roots.

Light After the oxygen concentration of the water reached the equilibrium level, the light (20 klx at the upper leaf level) was turned off (Fig. 1). The oxygen concentration of the water started to decrease slightly 15 minutes after the light was off, but returned to the equilibrium level within 2 hours.

Several authors have considered the possibility that the photo-synthetically-produced oxygen is transported to the roots. Cannon[4] reported that oxygen consumption by the root of Helianthus and Salix was greater in darkness than in light and postulated that oxygen is supplied also through the shoot in light. On the other hand, van Raalte[18] was not able to find any difference in oxygen concentration in the roots of rice whether the shoot was placed in light or in darkness. The oxygen produced in photosynthesis may play on a limited role in the oxygen transport from shoot to root and oxygen release from roots to the surrounding environment.

Effects of oxygen supply and light on α-NA oxidation

Oxygen supply To determine the extent to which α-NA oxidation by rice roots depends on atmospheric oxygen and dissolved oxygen, intact or excised roots of 2-week-old IR36 plants were placed in an α-NA solution bubbled with air or with N_2 (Fig. 3). In these measurements, presoaking in a α-NA test solution was omitted.

The rate of α-NA oxidation by intact roots was not affected by the presence or absence of oxygen in the rooting medium, indicating that oxygen transported from the shoot was sufficient to maintain the normal rate of α-NA oxidation. The rate of α-NA oxidation by excised roots was negligible when the α-NA solution was bubbled with N_2 gas; it was significantly higher when air was supplied to the

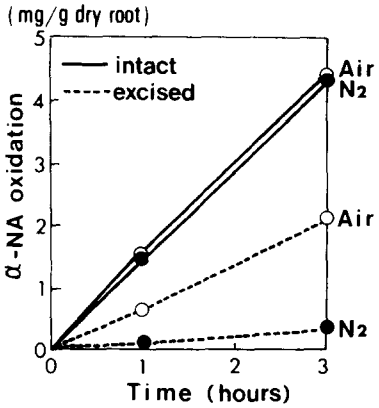


Fig. 3. Effect of oxygen supply on α-naphthylamine oxidation by intact or excised rice roots.

roots. That indicates that the α-NA oxidation by rice roots can utilize both atmospheric oxygen transported from shoot and oxygen dissolved in the rooting medium.

Light The effects of light on α-NA oxidation and evapotranspiration are shown in Fig. 4. There was a large difference in the evapotranspiration rate between light and dark; however, the α-NA oxidation rate was essentially the same under both light and dark. This means that light has no effect on α-NA oxidation process in a short period.

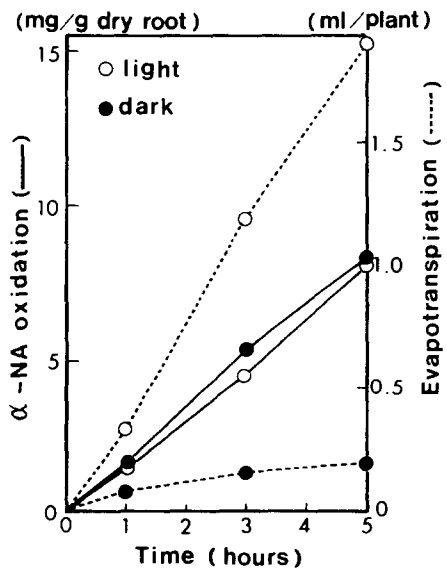


Fig. 4. Effect of light on α-naphthylamine oxidation and evapotranspiration by rice plants.

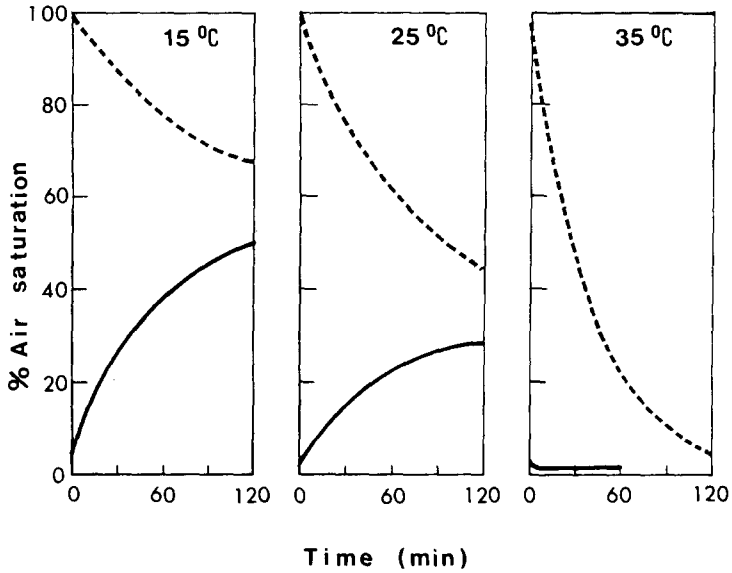


Fig. 5. Effect of temperature on oxygen release (*solid* line) and uptake (*dotted* line) by intact rice roots.

Effects of temperature on oxygen release and α-NA oxidation

Effects of water temperature on oxygen release and uptake by intact rice roots are shown in Fig. 5. The rate of oxygen release increased with decreasing temperature; the oxygen uptake rate decreased with decreasing temperature. These results can be understood in terms of temperature dependence of respiration. When oxygen moves downward from the basal part of the root to the root tip, a certain amount of oxygen may leak out laterally or may be consumed by tissue respiration along the pathway. At low temperature, the amount of oxygen consumed by respiration is small. As a consequence, more oxygen diffuses through the terminal of tubular structures of inter-cellular spaces or diffuses laterally. At higher temperature, the oxygen consumption by respiration is higher and less oxygen diffuses into the surrounding medium. On the other hand, oxygen uptake rate is higher at higher temperatures because of increased respiration rates.

The oxygen uptake by intact and by excised roots at 25°C and 35°C are shown in Fig. 6. The oxygen uptake by the excised rice roots proceeded almost linearly, was higher at 35°C, and was faster than that by the intact roots. The differences in the oxygen uptake rate between the intact and the excised roots can be attributed to presence or absence of oxygen supplied from the shoot.

The effect of water temperature on α-NA oxidation by rice roots is shown in Fig. 7. The rate of α-NA oxidation increased almost linearly with increasing temperature up to 30°C, leveled to 35°C, and declined sharply at 40°C. The temperature quotient is greater than 3 within the range of 15°C to 30°C. Such

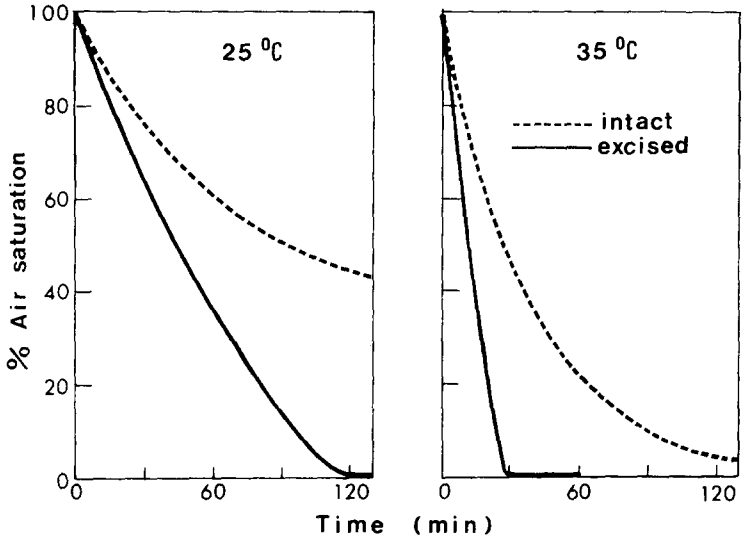


Fig. 6. Oxygen uptake by intact and excised rice roots.

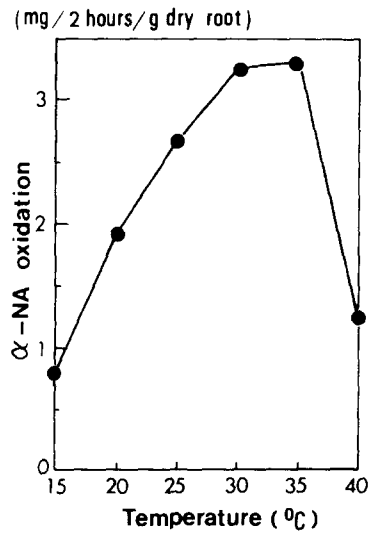


Fig. 7. Effect of temperature on α-naphthylamine oxidation by rice roots.

temperature dependence of α-NA oxidation is in good agreement with the fact that α-NA oxidation is catalyzed by peroxidase[11]. Thus, the rate of oxygen release and the α-NA oxidation rate are affected by temperature in opposite directions.

Effects of metabolic inhibitors on oxygen release and α-NA oxidation

Oxygen release from roots to the surrounding environment is usually considered a physical diffusion process[6]. Several lines of evidence presented in the

preceding sections also support a physical diffusion of oxygen in the rice plant, with atmosphere being the source and anaerobic root environment being the sink. Mitsui *et al.*[12,13], however, proposed a biochemical process by which oxygen could be generated. They suggested that hydrogen peroxide produced from a glycolic acid pathway in rice roots is degraded by catalase into molecular oxygen and water.

To test Mitsui's hypothesis, the effect of some metabolic inhibitors on oxygen release rate was examined. Since catalase is a hemoprotein and is easily inactivated by cyanide and azide, the enzyme-catalyzed oxygen release rate of the roots should be decreased by the cyanide or azide treatment. If the oxygen release from the roots is a physical diffusion, the oxygen release rate should be increased by the treatment of cyanide and azide. The increased rate of oxygen release should also be observed in the roots treated with 2,4-dinitrophenol (DNP) which is also a respiratory inhibitor.

The intact roots were pretreated with 10^{-5}, 10^{-4} or 10^{-3} *M* NaN_3, KCN and DNP for 3 hours. Immediately after the oxygen release rate of the intact plants was measured, the roots were excised and subjected to measurement of respiration rate (Fig. 8).

The pretreatment with 10^{-5}, 10^{-4} and 10^{-3} *M* NaN_3, 10^{-4} and 10^{-3} *M* KCN and 10^{-4} *M* DNP resulted in higher oxygen release rates and lower respiration rates. The results clearly indicate that the oxygen release from the roots is not operated by a biochemical process such as the glycolate pathway-catalase system proposed by Mituis *et al.*[12,13].

Both the oxygen release and respiration rate were decreased by 10^{-3} *M* DNP pretreatment. The roots lost their turgidity with time and appeared translucent after 3 hours of the DNP pretreatment. During the DNP pretreatment, some morphological changes in the air passage system of the roots might have occurred, resulting in decreased rate of oxygen transport and release.

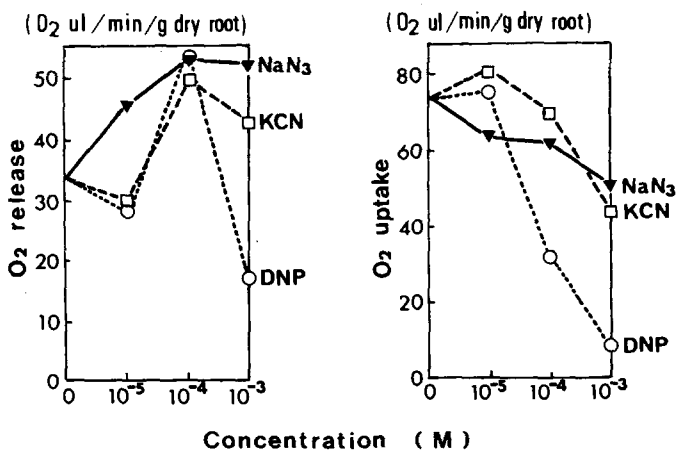


Fig. 8. Effects of metabolic inhibitors on oxygen release and uptake by rice roots.

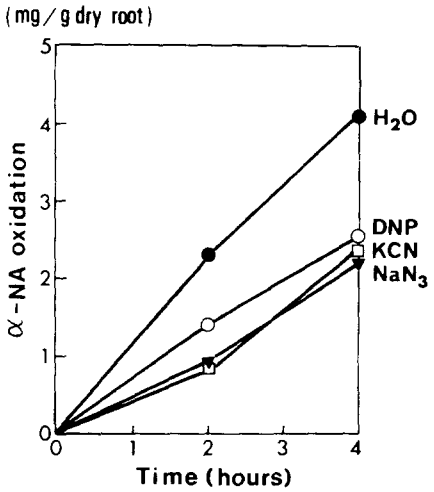


Fig. 9. Effects of metabolic inhibitors on α-naphthylamine oxidation by rice roots.

The effects of the metabolic inhibitors on α-NA oxidation by rice roots are shown in Fig. 9. The intact rice roots were pretreated with 10^{-3} *M* of NaN_3, KCN or DNP for 2 hours and then subjected to the α-NA oxidation. These metabolic inhibitors inhibited α-NA oxidation by the roots, indicating that the process of α-NA oxidation is closely related to the respiration process.

Effects of growth conditions on oxygen release and α-NA oxidation

Aerobic root environments Two-week-old IR36 seedlings were subjected to either anaerobic or aerobic medium conditions for 7 days. The anaerobic or aerobic medium conditions were provided by vigorously passing nitrogen gas or air into the nutrient solution, respectively.

The rice plants grown in aerobic conditions showed better growth and longer roots than anaerobically grown plants (Table 1). The beneficial effect of aerated culture solution on seedling growth of IR36 merits attention. Vlamis and Davis[19] reported that aeration had no effect on both shoot and root growth. Subsequently, Lin[10] using very young seedlings (incubated for 56 to 128 hours for germination) showed that oxygen supply improved both shoot and root growth, particularly root growth when seedlings were attached to the seeds, but it had no effect on seedling growth when seedlings were detached from the seeds. Recently, John *et al.*[7] reported that when culture solution was bubbled with nitrogen gas, shoot growth was unaffected but root dry weight decreased and root length became shorter. Thus root growth of rice seedlings appears to be more sensitive to external supply of oxygen than shoot growth does. Whether shoot growth is affected by external supply of oxygen may depend on the variety used and that requires further examination.

The aerated roots showed lower oxygen release rate and higher respiration rate

Table 1. Effect of aerobic and anaerobic conditions on growth and oxidizing power of 3-week-old rice plants*

Treatment	Shoot		Root	
	Height (cm)	Dry weight (mg/plant)	Length (cm)	Dry weight (mg/plant)
N_2	36.9	206	10.9	51.1
Air	41.5	282	18.2	73.9
F value	17.5**	26.2**	76.2**	20.9**

	O_2 (μl/plant/min)		α-NA oxidized
	Release	Uptake	(mg/g dry weight/hr)
N_2	0.90	2.63	1.51
Air	0.08	3.27	1.54
F value	31.41**	4.81	0.09

* Each value is the mean of 4 replicate plants.

** The difference between the two treatments is significant ($P < 0.01$).

Table 2. Effects of mineral nutrition on growth and oxidizing power of 2-week-old rice plants*

Treatment	Shoot		Root	
	Height (cm)	Dry weight (mg/plant)	Length (cm)	Dry weight (mg/plant)
Control	42.4^{ab}	308^{a}	12.1^{d}	61^{b}
No N	32.1^{c}	183^{c}	20.1^{a}	93^{a}
No P	45.8^{a}	237^{b}	15.5^{b}	64^{b}
No K	37.0^{bc}	245^{b}	10.8^{d}	45^{c}
No Ca	31.4^{c}	187^{c}	7.8^{e}	32^{c}
No Mg	41.4^{ab}	286^{ab}	12.3^{c}	65^{b}

	O_2 (μl/plant/min)		α-NA oxidized**
	Release	Uptake	(mg/g dry weight/hr)
Control	0.87^{a}	2.96^{a}	1.11^{b}
No N	0.39^{bc}	2.03^{bc}	0.80^{c}
No P	0.66^{ab}	2.48^{ab}	1.39^{a}
No K	1.02^{a}	2.43^{ab}	1.27^{ab}
No Ca	0.27^{c}	1.65^{c}	1.35^{a}
No Mg	0.93^{a}	2.87^{a}	1.09^{b}

* Each value is the mean of 4-replicate plants. For each column numbers followed by a different letter are significantly different ($P < 0.05$).

** Each value is the mean of duplicate plants.

than the roots grown in anaerobic conditions but there was not much difference in α-NA oxidation between these treatments (Table 1). The higher rates of oxygen release in anaerobically grown plants may be considered on adaptation to the root environment. Adaptation of rice seedlings to the anaerobic environment has been examined in terms of nutrient uptake, respiration, and ethanol formation[7,8].

Mineral nutrition The nutritional status of the rice plants has been found to affect the oxidizing power of the plants. Okajima[15] reported that nitrogen-deficient rice plants have a lower ability to oxidize esculin.

The 2-week-old IR36 seedlings grown in complete nutrient solutions were grown without either nitrogen, phosphorus, potassium, calcium or magnesium for 1 week. The results are shown in Table 2. After 1 week of the treatment, typical nitrogen, calcium and magnesium deficiency symptoms were observed, but potassium and phosphorus deficiency symptoms were not obvious. The root length and weight were characteristically changed by these treatments.

The oxygen release rates were lower in nitrogen- and calcium-deficient plants. The oxygen release rate is probably affected by several factors such as entry of oxygen to the plant, transport of oxygen within the plant, and consumption of oxygen by the tissue along the pathway of transport. Size, structure, and metabolic activity of shoot and root may affect each of the above processes. For instance, a longer root may have a lower oxygen release rate because more oxygen is consumed by the tissue along the pathway of transport[6]. The same explanation may apply to a lower oxygen release rate of the nitrogen-deficient plants. It, however, fails to account for a low oxygen release rate of the calcium-deficient plant. The rate of α-NA oxidation was least in the nitrogen-deficient plant roots. This is in good agreement with Okajima's finding[15].

Some reports indicate that potassium-deficient plants were more susceptible to iron toxicity[14,17]. Our results indicate that both oxygen release rate and α-NA oxidizing power of potassium-deficient plants were even higher than those of the control plants. Thus, the susceptibility of potassium-deficient plants to iron toxicity can probably not be accounted for by either oxygen release rate or α-NA oxidizing power.

Nature of oxidizing power of rice roots

The foregoing experiments lead to the following points:

(1) Atmospheric oxygen transported from shoots is the main source of oxygen for both processes of molecular oxygen release and α-NA oxidation.

(2) Light condition during the measurement hardly affects the rate of both processes.

(3) With decreasing temperature of the root environment, the rate of oxygen release increases, but that of α-NA oxidation decreases.

(4) Metabolic inhibitors such as DNP, NaN_3 and KCN increase the oxygen release rate, but decrease the rate of α-NA oxidation.

(5) An aerobic root environment decreases the oxygen release rate, but does not affect the rate of α-NA oxidation.

(6) The oxygen release rate is low in nitrogen- and calcium-deficient plants and the rate of α-NA oxidation is low in nitrogen-deficient plants.

These observations suggest that the α-NA oxidation is part of the respiration which consumes oxygen in the roots, and that oxygen which is not consumed by respiration diffuses into the surrounding environment. These processes are governed by molecular oxygen supply from the shoots.

Matsunaka[11] reported that the peroxidatic oxidation of α-NA needed one mole of H_2O_2 per mole of α-NA. Because 2 moles of H_2O_2 are equivalent to one of mole of O_2 (22.4 ℓ at standard conditions), the α-NA oxidizing power can be converted into oxygen equivalent. For the control plant in Table 2, the amount of O_2 consumed in α-NA oxidation was 0.098 O_2 $\mu\ell$/plant/min. This rate is 11.3% of O_2 release rate and 3.3% of respiration rate.

Armstrong[2] stated that the oxidizing activity of roots in two bog species was nine times greater than could be accounted for by oxygen diffusing from roots. Rice is totally different in this respect. At 25°C, IR36 released about 9 times more oxygen than was needed to account for the α-NA oxidation. Such processes may be very sensitive to temperature, however, so caution is needed in generalizing these observations.

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