J. Agric. Sci., Tokyo Univ. Agric., 66 (2), 25-35 (2021)
東京農大農学集報，66（2），25-35（2021）

論　文
Articles

Strategies Employed by Two Floating-leaved Wetland Plants to Address Oxygen Demands in Anaerobic Conditions

By

Motoka NAKAMURA*†

(Received January 7, 2021/Accepted March 9, 2021)

Summary：Supplying O_2 to roots by convective gas flow from the shoot and O_2 consumption via respiration and radial O_2 loss (ROL) from roots are key to anaerobic tolerance in wetland plants. I aimed to evaluate the anaerobic tolerance of floating-leaved plants, *Nymphoides peltata* and *N. indica* grow in a similar anaerobic habitat with a convective gas flow system capable of supplying O_2 to roots and consumption by roots when O_2 is available. I measured the internal resistance (R), pressurization efficiency (e), and specific convective efficiency (E) as main indicators of convective gas flow in shoots, O_2 concentration in the shoot-root junction, rate of root respiration, and ROL rate in roots, are measured under both light and dark conditions. There were no differences in E and O_2 concentrations at the shoot-root junction between the two species. Both species also use the same amount of O_2 in the roots for respiration and ROL. However, *N. peltata* had enhanced pressurization efficiency in the shoot, while *N. indica* had variable respiration responses to O_2 decrease in the rhizosphere. These traits highlight their specific adaptation strategies to anaerobic conditions, and enhance survivability in such habitats.

Key words：aerenchyma, convective gas flow, oxygen supply from the shoot, root respiration, radial oxygen loss (ROL)

Introduction

Flooded soils where wetland plants grow are typically anaerobic in nature, as reducing toxic substances Mn^{2+}, Fe^{2+} and H_2S are eluted[1]. Wetland plants can supply O_2 from shoot to roots through the developed aerenchyma. The supplied O_2 is consumed by the roots for respiration and radial O_2 loss (ROL) to maintain activities in the roots[2, 3]. Since the ROL from the roots into the anaerobic soil can lead to formation of an oxide layer around the root surface, roots are protected from toxic substances[1, 4, 5, 6]. Additionally, in many wetland species, ROL barrier from the base of the shoot to the root tips formed in the outer cell layers and developed aerenchyma are beneficial features that enable roots to grow in waterlogged soils by allowing more internal O_2 diffusion to the root tips[5, 7, 8, 9, 10]. Movement of O_2 towards the root tip through the aerenchyma in shoots and roots is usually by a diffusion mechanism due to O_2 concentration differences. Interestingly, in some wetland plants, floating-leaved and helophyte species, internal pressurization and convective gas flow in shoots transport O_2 more efficiently than by simple diffusion[2, 4, 11]. This is because air including the high O_2 concentration flows through the shoot aerenchyma of relatively young leaves or leaf blade with high internal pressurization mechanism to transport O_2 to the underground tissue (i.e., rhizome) and release O_2 into the atmosphere from the old leaves and leaf blade with low internal pressurization. Thus, high O_2 gas flux by diffusion into roots aerenchyma occurs due to air containing high levels of O_2 in the shoot-root junction[12, 13, 14]. The mechanism of convective gas flow is accounted by the differences in water vapor pressure and temperature between the aerenchyma and the external atmosphere (humidity-induced pressurization, and thermal transpiration). This induces pressurization of shoot aerenchyma through the porous partition within the plant tissue, ideally with a pore size in the Knudsen regime (i.e., less than the mean

* Department of Biology, Graduate School of Science and Technology, Chiba Univ.
(Department of Bio-Production, Faculty of Bio-Industry, Tokyo Univ. of Agriculture)
† Corresponding author（E-mail : m5nakamu@nodai.ac.jp）

free diffusive path length of the gas molecules (<0.1 μm))[2, 12, 15]. A stomata with an appropriate opening and the parenchyma boundaries between the spongy and palisade tissues with intercellular space correspond to such porous partitions[4, 15, 16]. Thus, inner pressurization is governed by the environmental factors, atmospheric humidity, temperature and light.

Species with convective gas flow system support root respiration and ROL due to the diffusion of O_2 from the shoot-root junction[13, 14]. Species inhabiting deep-water and strictly anaerobic soil conditions need the ability to supply O_2 by depending on the convective gas flow system[14]. However, internal pressurization and resistance within the tissue to gas flow varies among the species growing in similar habitats. The actual convective gas flow rate in their shoot aerenchymas are very different between these species[11, 17, 18]. Moreover, seasonal and diurnal variations of convective flow rate in these species are large, since gas containing high O_2 concentration in the shoot does not always flow at maximum speed due to reduced internal pressurization from changes in temperature and humidity around the shoots[15, 18]. These suggest that tolerance strategies to anaerobic soils in wetland plants don't only depend on enhanced O_2 supply in shoots but also response of optimal O_2 demand for root respiration and ROL. Root respiration varies depending on the species that often appear in similar habitats with strict anaerobic soils despite possessing completely different O_2 supply systems (i.e., *Phragmites australis* with high aeration capacity by convective gas flow, and *Zizania latifolia* with low aeration capacity by diffusion)[19, 20, 21]. Therefore, analysis from both O_2 supply potential in the shoot and consumption rate in the roots are needed to understand the anaerobic tolerance strategy of wetland plants. Although there are few reports focusing on hypoxic responses in both shoot and roots in a plant to evaluate the adaptation strategies to anaerobic soils of wetland plants, the finding that reveals differences in O_2 supply capability in the shoot and O_2 demand for respiration in different species inhabiting similar environments could offer insights into the adaptation strategies of wetland plants to survive in anaerobic soils.

I examined two floating plants, *Nymphoides peltata* and *N. indica,* which employ gas flow by internal pressurization in the leaves[22]. Both species are perennial plants and commonly inhabit anaerobic soils with a depth of about 1.5 m in the temperate zone. Both species often form communities in the same lake, and their habitats can replace each other. They have similarities and differences in their strategies to adapt to such strict anaerobic conditions. In this study, I examined the responses from shoot and roots on the basis of convective gas flow in the shoot, such as the pressurization efficiency (e), inner resistance (R), specific convective efficiency (E), and O_2 demand in the roots, which comprised root respiration rate and ROL rate along with changes in O_2 availability in their rhizosphere. Additionally, I measured the O_2 concentration in the shoot-root junction and used tissue porosity as an indicator of aerenchyma development.

Research methods

(1) Pressurization mechanisms

Internal pressurization in aerial organs can occur from two physical processes : thermal transpiration and humidity-induced pressurization through the porous partition within the plant tissue[22]. The thermal transpiration and the humidity-induced pressurization have been described by Brix *et al.* (1992) and Bendix *et al.* (1994). The former describes the movement of gas through a porous partition when there is a gradient in temperature across the porous partition in which pressure is high on the warmer side[11, 15]. The latter report is related to the pressure differentials induced by differences in the water-vapor pressure across the porous partition, in which total pressure is likely higher on the more humid side[15]. Two pressure differences induced by thermal transpiration (ΔP_t, Pa) related to the absolute temperature of the ambient air, T_a (K) and the leaf internal temperature, T_i (K), as well as by humidity-induced pressurization (ΔP_w, Pa which set nominally to 101.325 kPa) due to the difference in water-vapor pressure between the inside (P_{wi}) and outside (P_{wa}) the porous partition of plant can be described as follows :

$$\Delta P_t = P_a (T_i^{0.5} T_a^{-0.5} - 1) \quad \cdots\cdots(1)$$

$$\Delta P_w = P_{wi} - P_{wa} \quad \cdots\cdots(2)$$

where, P_a is the ambient pressure (Pa), and ΔP_w corresponds to the difference in water-vapor pressure between the leaf lacunae (P_{wi}) and the atmosphere (P_{wa}) under steady state conditions. The two pressurization scenarios operate simultaneously and independently. The potential static pressure differential (ΔP_{pot}, Pa) is the sum of the two gradients :

$$\Delta P_{pot} = \Delta P_t + \Delta P_w \quad \cdots\cdots(3)$$

where, ΔP_{pot} is calculated theoretically from temperature and humidity in the ambient air and the plant tissue. Water vapor pressure is measured within the leaf lacunae and calculated from Ti assuming water-saturated conditions[12, 23].

The pressurization efficiency (e, Pa P^{-1}), which is defined as the parameter to evaluate the pressurization ability is calculated based on the slope of the regression between the actual static pressure difference (ΔP_c, Pa)

and ΔP_{pot}, because ΔP_c has smaller than ΔP_{pot}[15], is shown as :

$$e = \Delta P_c \ \Delta P_{pot}^{-1} \qquad 0 \leqq e \leqq 1 \qquad \cdots\cdots(4)$$

Additionally, the specific convective efficiency (E, μmol air m^{-2} s^{-1} Pa^{-1}) is used for evaluating pressurization ability, which is defined as the convective gas flow rate per unit leaf surface area (m^2) (F, μmol air s^{-1} m^{-2}) and per ΔP_{pot}, calculated based on the slope of the regression between F and ΔP_{pot} :

$$E = F \ \Delta P_{pot}^{-1} \qquad \cdots\cdots(5)$$

The internal resistance inside the plant tissue (R, Pa s m^2 μmol^{-1} air) and the specific internal resistance (r, Pa s mm μmol^{-1} air) are evaluation parameters used to determine the effect of the tissue structure on gas flow, in such areas as the leaf blade and stem. Especially, the r can be used for a direct comparison of the relative tissue resistances for different species from different cross-sectional areas. They are calculated from the following expression :

$$R = \Delta P \ F^{-1} \qquad \cdots\cdots(6)$$

$$r = \Delta P \ A \ (F \ L)^{-1} \qquad \cdots\cdots(7)$$

where, P (Pa) is the static pressure or the applied pressure by using air pump when the measured tissue lacks inner pressurization mechanisms such as a stem. A and L are cross-sectional areas (mm^2) and the length of the tissue (mm) respectively. Further, the specific convective efficiency E is represented with the pressurization efficiency and the internal resistance R :

$$E = e \ R^{-1} \qquad \cdots\cdots(8)$$

(2) Plant material

Nymphoides peltata (Gmel.) O. kuntze and *N. indica* (L.) O. kuntze are perennial plants of menyanthaceae with rhizomes often growing in lakes. Although both species produce seeds, *N. peltata* and *N. indica* are mostly reproduced from the rhizome and turion, respectively. In this study, horizonal rhizomes of *N. peltata* growing wild in Kasumigaura lake, Ibaraki, Japan and the turions of *N. indica* that dominate Ojyaga lake, Chiba, Japan were collected and transplanted in experimental ponds. Rhizomes and turions were planted in individual pots (vertical × horizonal × soil depth : 11 × 9 × 10 cm) with soil collected from Inbanuma lake in Chiba, Japan. Thereafter, they were transferred to the experimental ponds (vertical × horizonal × water depth : 180 × 90 × 60 cm), and were grown for a month under natural light.

(3) Measurement of pressurization and convective flow

For measurements of internal pressurization and gas flow from cut leaf blade, each mature leaf blade about 10 days after foliation was cut at 10 cm from the base of the stem. The stem was connected to a silicon tube, which was completely filled with silicone rubber and Vaseline. A wet filter paper was placed on the back of the leaf to prevent drying. Measurement was performed in a chamber (height : 30 cm ; wide : 30 cm ; depth : 25 cm) connected to a transpiration measuring device, and the chamber was covered with a box pasted with a light-shielding cloth. The temperature and humidity in the chamber were maintained at constant levels (25℃, 37% relative humidity, RH) using an air pump (rate 2.7–3.0 L min^{-1}). The photosynthetic photon flux density (PPFD) under light condition (500 μmol s^{-1} m^{-2}) in which it was sufficient for photosynthesis and darkness condition (0 μmol s^{-1} m^{-2}) were controlled using a 150 W halogen lamp (Philips GCD100V150WM). The lamp was placed on the box so that light could enter from the ceiling of the chamber, and PPFD on the surface of the leaf was always inspected by installing a photon flux density sensor (KOITO IKS-25) next to the leaf. Small fans were put inside the chamber and the box to prevent the heat of the halogen lamp from affecting the humidity and temperature inside the chamber. Humidity and water-vapor pressure of gas from the air pump were measured with a humidity sensor (Eto Electric, THP2119A) at the entrance of the chamber. The water vapor pressure of the exhaust gas from the chamber was measured using dew point meter (EG & G Dew Prime I). RH in the leaf lacunae was assumed to be 100%. Temperature of the air inside the chamber (T_a) and leaf surface (T_i) were measured using a thermocouple data-logger. The actual static pressure differential (ΔP_c) and the convective gas flow rate (F_c) in the leaf blade were measured using an electronic pressure transducer (Japan Data Instruments, XCA-4WC, capacity -996.3-996.3 Pa) and a high-sensitivity gas flowmeter (KOFLOC, model 3810, capacity 20 ml min^{-1}) connected to the end of the silicon tube with the cut leaf blade. Each parameter was measured by switching the solenoid valve connected between the gas flowmeter and the leaf blade every 4 minutes. All measurements were recorded in a data-logger every 2 seconds, and the average value for each minute was recorded on a computer. After measurements, the leaf area was also measured using a graphic tablet with computer (DEGITIZER, GRAPHTEC, KW3320-RS M-3585).

To evaluate the effect of the structure of gas transport pathways on convective flow, the internal resistance of the stem between the leaf base and shoot-root junction was studied. About 15 cm of the stem without leaf was

connected to an air pump in parallel with an electronic pressure transducer attached at one end. The other end of the stem was connected to a gas flowmeter. Pressure applied from the leaf base to roots by the air pump (50–2000 Pa) was regulated to achieve a flow rate of 2.7–3 min^{-1}. The joints were sealed airtight to eliminate gas leaks with silicone grease and Vaseline. Positive correlation exists between the applied pressure and the actual flow rate in the stem. So, the air pump was adjusted in a two-step process for this measurement. After completing the pressure measurements, the total length and cross section of the stem were measured to calculate the internal resistance of the whole stem (R, Pa s m^2 μmol^{-1} air) and the specific internal resistance (r, Pa s mm μmol^{-1} air)[11]. These data were also used for the calculation of the specific convective efficiency from the leaf blade to the root base (E*, μmol air m^{-2} s^{-1} Pa^{-1}), which was the value based on the resistance by the stem in addition to the leaf blade from the gas flow calculations of the entire shoot (about 30 cm).

(4) Porosity of stem and roots

In this study, porosity was used as an indicator of development of aerenchyma for gas transportation in the tissue. Three or four parts of the leaf, stem, rhizome, and roots were cut to about 0.5 g for porosity measurement (%, internal gas volume). Porosity for each tissue was determined using the pycnometer method[24], and this equation :

$$\text{Porosity } (\%) = 100\ (W_{pwo} - W_{wo})/(W_{pw} + W_o - W_{wo}) \quad \cdots\cdots(9)$$

where, W_{pwo} was the weight (g) of a pycnometer containing pure water and ground tissue, W_{wo} was the weight of pycnometer containing pure water and tissue before grinding, W_{pw} was the weight of water and pycnometer, and W_o was fresh weight of cut tissue. First, the fresh weight of tissue was measured. Then, the tissue was placed in a pycnometer filled with pure water to measure the weight of the pycnometer containing pure water and the sample. Next, the tissue collected from the pycnometer was ground to a paste using a mortar. The paste was carefully placed back in the pycnometer so as not to leave the sample in the mortar and to measure the weight of the sum of pure water and paste sample. Data of the three or four parts in each tissue were used to obtain the average value.

(5) O_2 concentration in shoot-root junction

I measured the O_2 concentration in the shoot-root junction to determine the actual amounts of O_2 diffusion to the root aerenchyma. Samples grown in pots were transferred to the experimental tank (3 L), filled with distilled water at 23℃ so that leaves floated on the water surface. At this stage, a gas-tight syringe was inserted in the shoot-root junction and was immersed in the tank while still remaining fixed to the stand. The site where the gas-tight syringe was inserted was tightly sealed with silicone grease and Vaseline to prevent water intrusion. Two treatments, in dark (PPFD ; 0 $\mu mol\ m^{-2}\ s^{-1}$, RH over 90%) and light (PPFD ; 500 $\mu mol\ m^{-2}\ s^{-1}$, RH below 50%) were established to assess the available O_2 concentration in the roots with high aeration by active convective gas flow or low aeration, mainly depending on the diffusion types that were designed. After placing the tank in a chamber covered with light shielding sheet for 16 hours at 23℃, 0.5 ml of gas was carefully collected using an air tight syringe from the dark treatment. The light shielding sheet was removed from the chamber to collect gas from the light treatment, as a 150 W halogen lamp was placed above the chamber and air gas was flowed from the compressed air cylinder to maintain the air in a constant condition in the chamber (RH below 50%, temperature 23℃) for 3 hours. O_2 concentration in the collected gas from the air tight syringes were analyzed using a gas chromatography-thermal conductivity detector (GC-TCD ; Shimadzu GC-8A column ; and 5A molecular sieve) within 3 hours to preventing mixing with atmospheric air.

(6) ROL and root respiration

Plants were taken out from their pots and carefully washed with distilled water to remove soil particles. The shoot-root junction was wrapped with silicone rubber and filled with silicon grease and Vaseline to avoid openings. Plant roots and the O_2 electrode (Central Science, Ultra DO meter) were placed in the glass chamber (300 ml) designed as a closed system and the glass chamber was further submerged in a tank (3 L) filled with distilled water. A stirrer was placed below the glass chamber to allow mixing of the experimental solution buffered with 20 mM HEPES at pH 6.5. O_2 concentration of the experimental solution in the glass chamber was maintained below 10 $\mu mol\ l^{-1}$ O_2 by circulating the experimental solution (23℃) that connected to the water tank in which it was gassed with N_2 to minimize dissolved O_2. During the measurement, the tube attached to the water tank was closed to convert the glass chamber into a closed system. The increase in O_2 concentration of the experimental solution in the glass chamber due to ROL from the roots was measured using an O_2 electrode at 1 minute intervals for 30 minutes under dark (PPFD ; 0 $\mu mol\ m^{-2}\ s^{-1}$, RH over 90%) and light (PPFD ; 500 $\mu mol\ m^{-2}\ s^{-1}$, RH below 50%) conditions, which resembled the conditions used in the O_2 measurement at the shoot-root junction. After measurement, the plants were divided into roots

and shoot, and the root portion was used to measure rate of root respiration.

Whole root detached from the shoot was placed in O_2 saturated experimental solution with 20 mM HEPES at pH 6.5 for 1 hour at 23℃ under dark condition for pre-processing. Respiration of whole root was then determined as the decrease in O_2 concentration in the airtight glass vessel (100 ml) containing the O_2 saturated-experimental solution until O_2 was completely consumed by the root respiration process. Measurements were performed under dark by covering the air-tight glass vessel with an aluminum foil. Roots after measurement were dried for 48 h at 80℃ to determine the dry weight.

(7) Statistical analyses

In each parameter for evaluation of convective gas flow and O_2 concentration in shoot-root junctions, significant differences between species and treatments were determined by two-way analysis of variance (ANOVA). In the r of stem and porosity (%) of leaf, stem and roots, significant difference between species were compared using t-test. Interspecific differences in continuous changes in the root respiration rate between both species were examined by two-way repeated-measures ANOVA along with the dissolved O_2 (DO) concentration in the experimental solution. Moreover, significant differences in each of their relative root respiration rate (%) among the two species in some DO concentrations were also examined by t-test. Differences between the measurement in treatments in the two species with regard to ROL rate and O_2 demand rate in the roots were examined by three-way repeated-measures ANOVA along with DO concentration in the experimental solution. Additionally, for each treatment, differences between the two species with regard to ROL rate and O_2 demand rate in roots were also examined by two-way repeated-measures ANOVA along with the DO concentration in the experimental solution. The % values of porosity and relative root respiration were analyzed after performing the arc-sine conversion. The analyses were performed using STATISTICA for Windows (Version 5.1 ; StatSoft Inc., Tulsa, OK, USA).

Results

(1) Convective gas flow ability, tissue porosity, and O_2 concentration in the shoot-root junction

F, ΔP_c, and ΔP_{pot} were the measurement data used to calculate R, e, and E (Table 1). In both species, R values of leaf blades treated in dark were higher than those treated with light. Also, the values of *N. peltata* were higher than that observed for *N. indica* in both the treatments, but the difference was statistically insignificant (Table 1). The r value for the stem of *N. indica* was sig-

Table 1 Means (±SEs) in the parameters related to convective gas flow in shoots and O_2 concentration in the shoot-root junction

	N. peltata		*N. indica*	
	Dark	Light	Dark	Light
Convective gas flow rates of leaf blade (F; μmol air s^{-1} m^{-2})	360 ±199	408 ±277	272 ±132	116 ±50
Static internal pressure differentials of leaf blade (ΔP_c; Pa)	335 ±173	178 ±125	109 ±83	28 ±30
Potential steady state pressure differentials of leaf blade (ΔP_{pot}; mPa)	1168 ±553	1204 ±672	717 ±255	661 ±249
Internal resistance of leaf blades (R; Pa s m^2 μmol^{-1} air)	1.47 ±1.66	0.5 ±0.4	0.4 ±0.1	0.2 ±0.1
Internal resistance of leaf (R_l; Pa s m^2 μmol^{-1} air)	1.28 ±1.66	0.33 ±0.35	0.26 ±0.14	0.09 ±0.11
Internal resistance of stem (R_s; Pa s μmol^{-1} air)	0.32 ± 0.15		0.27 ± 0.07	
Specific internal resistance of stem (r; Pa s mm μmol^{-1} air)	0.71 ± 0.26		1.13 ± 0.69**	
Pressurization efficiencies of leaf blade (e; mPa Pa^{-1})	316 ±144[a]	164 ±87[b]	192 ±180[ab]	61 ±79[b]
Specific convective efficiencies of leaf blade (E; μmol air m^{-2} s^{-1} Pa^{-1})	0.40 ±0.43[a]	0.38 ±0.25[a]	0.47 ±0.31[a]	0.24 ±0.18[a]
Specific convective efficiencies of shoot (E*; μmol air m^{-2} s^{-1} Pa^{-1})	0.23 ±0.08[ab]	0.20 ±0.07[ab]	0.31 ±0.24[a]	0.19 ±0.13[b]
O_2 concentration in the shoot-root junction (mmol l^{-1})	5.84 ±1.21[b]	7.69 ±0.96[a]	6.46 ±1.16[b]	7.90 ±1.13[a]

The parameters related to convective gas flow in the shoots: *N. peltata* (n = 9), *N. indica* (n = 5)

O_2 concentration in the shoot-root junction: *N. peltata* (n = 4), *N. indica* (n = 5)

asterisks (**) indicates a significant difference between species (t-test, $p < 0.01$)

Different letters indicate statistically significant differences between species and treatment (Tukey's test after testing for two-way ANOVA, $p < 0.05$)

nificantly higher than that seen for *N. peltata* ($p<0.01$) (Tables 1, 2). *N. peltata* exhibited significantly higher values for e in the leaf blade than that for *N. indica* ($p<0.05$). Both species had high values in dark treatments compared to that when treated with light ($P<0.05$) (Tables 1, 2). For the E value in the leaf blade, no significant differences between the treatments or between species were observed. On the other hand, the E* value for the whole shoot for both species in dark conditions were significantly higher than those subjected to light treatments ($p<0.05$) (Tables 1, 2). These high E* values could be attributed to high e values of the leaf blade and resistance to gas flow in the stem. Values of R_l and R_s used to calculate E* were shown in Table 1.

The values of leaf and root porosity in *N. peltata* were similar to those in *N. indica* (Table 3). On the other hand, stem porosity of *N. peltata* was higher than that of *N. indica* ($p<0.05$) (*N. peltata* has favorable characteristics for O_2 supply to roots), and resembled r value for stem (Tables 1, 3).

O_2 concentration in the shoot-root junction in both species under light treatments were higher than those observed under dark treatments ($P<0.01$) (Tables 1, 2). These findings indicate that both species are capable of maintaining high O_2 concentration for diffusion to roots tips.

(2) Root respiration, ROL, and O_2 demand in root

Root respiration rate per unit root dry weight of both species decreased along with decrease in DO concentration of the experimental solution (Fig. 1a). There were no significant differences in the root respiration rates along with the DO concentration of experimental solution between the two species. As the respiration rates at each concentration were expressed relatively (%) to the maximum rate of respiration (O_2 saturation) of the experimental solution as 100 %, the values in *N. peltata* were significantly higher than those in *N. indica* (two-way repeated-measures ANOVA, $P<0.05$), and changes in root respiration rate along with the decrease of DO concentration of experimental solution were significantly different between the two species (t-test, $P<0.05$ and <0.01) (Fig. 1b). Change in root respiration rate in *N. peltata* remained constant up to 70 μmol O_2 l^{-1} in the experimental solution after saturation with O_2, but decreased sharply with additional decrease in O_2 concentration. The root respiration rate in *N. indica* decreased gradually with decreasing O_2 concentration near the roots.

In both species, ROL rate per unit root dry weight along with the increase in DO concentration of experimental solution under light treatment tend to be higher than those under dark treatments, but the difference was insignificant (Fig. 2a, b). Moreover, for each treatment, the values in both species were similar (two-way repeated-measures ANOVA, $P>0.05$).

The O_2 demand rate per root dry weight along with the change in DO concentration of experimental solution was calculated from the sum of root respiration rate and ROL rate. The O_2 demand rate in *N. peltata* was similar to that seen in *N. indica* for both treatments (Fig. 3a, b) ; there were no significant differences in the O_2 demand rates between treatments or species based on the statis-

Table 2 Results of t-test or two-way ANOVA for the variable of each parameter related to convective gas flow in the comparison between species or between treatments.

The parameters related to convective gas flow	Analysed variables (t-test or two-way ANOVA)					
	Species (S)		Treatment (T)		S×T	
	F value	*P* value	*F* value	*P* value	*F* value	*P* value
Specific internal resistance of stem (r)	14.9	**0.00**				
Pressurization efficiencies of leaf blade (e)	5.21	**0.03**	8.08	**0.01**	0.05	0.83
Specific convective efficiencies of leaf blade (E)	0.05	0.82	0.89	0.36	0.66	0.43
Specific convective efficiencies of shoot (E*)	0.01	0.90	4.30	**0.05**	2.37	0.14
O_2 concentration in the shoot-root junction	2.87	0.09	45.00	**0.00**	0.67	0.41

Numbers in bold indicate $P < 0.05$

Table 3 Means (± SEs) of the porosity (%) in the leaf, stem, and roots of *N. peltata* and *N. indica*

	N. peltata	*N. indica*	Analysed variable	
			F value	*P* value
Leaf	38.7 ± 3.1 (n = 4)	44.4 ± 3.3 (n = 4)	4.63	0.10
Stem	48.9 ± 2.7 (n = 13)	43.9 ± 3.7 (n = 7)	9.12	**0.01**
Roots	31.3 ± 4.5 (n = 15)	31.1 ± 4.5 (n = 13)	**0.01**	0.91

F and *P* values for the variable obtained on conducing t-test.
Numbers in bold indicate $P < 0.05$.

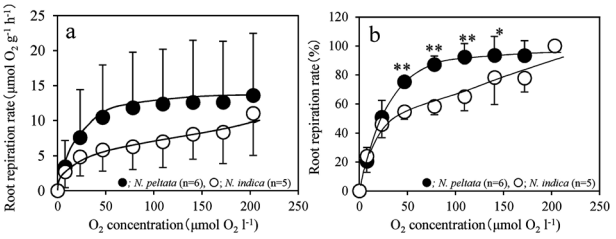

Fig. 1 Means (± SDs) of the root respiration rate per unit root dry weight along with the DO concentration of the experimental solution (a). The relative root respiration rate (%) expressed relative value to the maximum rate of the respiration (O_2 saturation) along with the DO concentration of the experimental solution (b). The relative root respiration rate of *N. peltata* was higher than that of *N. indica* (two-way repeated-measures ANOVA, $P<0.05$). Changes in the respiration rate to the decrease of DO concentration of the experimental solution are different between species (t-test, * ; $P<0.05$ and ** ; $P<0.01$).

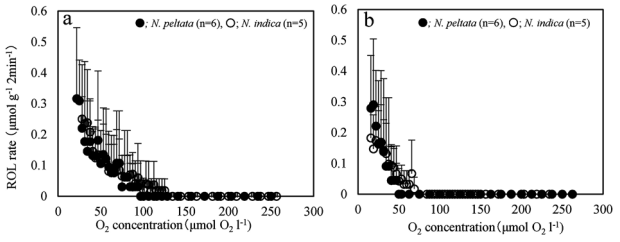

Fig. 2 Means (±SDs) of ROL rate from the roots per root dry weight along with the DO concentration of the experimental solution under light (a) and dark treatments (b). Both species have same amount of ROL from the roots under each treatment (two-way repeated-measures ANOVA, $P>0.05$). ROL falled 0 μmol g^{-1} $2min^{-1}$ around 50 μmol $O_2 l^{-1}$ (dark treatments) − 130 μmol $O_2 l^{-1}$ (light treatments) in the experimental solution in both species, because O_2 derived from ROL from the roots were consumed again by root respiration in the closed chamber.

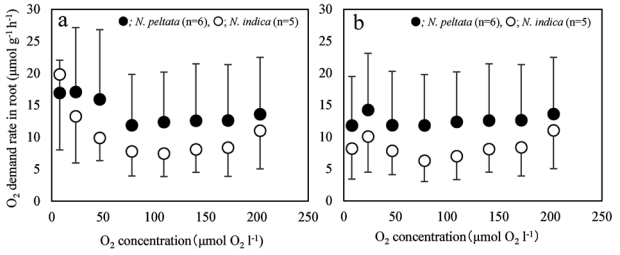

Fig. 3 Means (±SDs) of the O_2 demand rate in the roots calculated from the sum of the root respiration rate and the ROL rate per root dry weight along with the DO concentration of the experimental solution under light (a) and dark treatments (b). There were no significant differences in values of the O_2 demand rate along with the change in DO concentration of the experimental solution between treatments or species (three-way repeated-measures ANOVA, $P>0.05$).

tical results obtained for root respiration and ROL (three way repeated-measures ANOVA, $p>0.05$).

Discussion

(1) Differences in O_2 supply in shoot between the two species

There was significant difference in the convective gas flow in shoot as expressed by the E* value between treatments (Table 1). Given that the humidity and temperature in the vicinity of the pressurized parts in leaves were regularized in the experiments, the differences in the E* between species were likely due to the difference in e value in the leaf and resistance to gas flow in the stem. *N. indica* has a few beneficial traits enabling convective gas flow in the leaf due to low R_l and leaf porosity which tend to be higher than that of *N. peltata*, although the e value of the leaf blade was not as high as that of *N. peltate* (Tables 1, 3). Moreover, the r value in the stem of *N. indica* was higher than that of *N. peltata* (Table 1). We observed that *N. peltata* could possess beneficial traits, given the high e value for leaf blade and low r value for stem, although it is not advantaged by the tissue structure in the leaf that affects gas flow (tend to be higher values of R in leaf and lower leaf porosity than those of *N. indica*.) (Tables 1, 3). Enhancement of internal pressurization in the leaf of *N. peltata* contributes to even higher convective gas flow under optimal conditions. In other words, *N. peltata* has high capacity to supply O_2 to roots as an adaptative approach to survive in anaerobic environments.

Convective gas flow mechanisms in wetland plants depend on the pressurization parts in the leaf, such as stomata with an adequate opening, and the parenchyma border between the spongy and palisade tissues containing intercellular space[5, 16, 25]. In *Nuphar lutae* (L). Sm and *Typha angastifolia* L, inner pressurization are caused at the pores in the parenchyma boundaries, because the stomata opening is too large for pressurization (length × width of *Nuphar lutae* and *Typha angastifolia* ; 5.6 × 2.4 μm and 10 × 1.0 μm, respectively[15, 23]. Both experimentally and theoretically, it has been demonstrated that open stomatas with larger pore than the optimum could decrease e value, owing to pressure release in the leaf lacunae[16, 26]. Bendix *et al.* (1994) suggested that inner pressurization was strongly affected by the size of the stomata opening in response to light[15]. For instance, in *N. peltata* and *N. indica*, the e values for light treatments were lower than that of the dark treatments (Table. 1). These suggest that increased stomata opening under light treatments reduce the pressurization. The e value is also higher when the pore circumference increases. The R value decreases with increasing pore area surrounded by guard cells[26]. Thus, difference in the e values between the two species might be due to differences in the size of the opening, circumference of stomata, and the fine structure of parenchyma boundaries comprising intercellular space. The high density of stomata per leaf area might also contribute to increase in the e value because of the increase of porous partitions for internal pressurization. Stomatal density in the leaves of *N. peltata* and *N. indica* were 526 m^{-2} and 365 m^{-2}, respectively. This would support the high e values seen in *N. peltate* compared that that seen in *N. indica*.

Internal pressurization and subsequent convective gas flow in the leaf and stem are affected by aging[5, 17]. In the leaf, the size of stomata opening increases with age. Internal pressurization decreases with age due to tissue damage, and so, the pressurization ability in old leaf is

less than that of a young leaf[2, 22]. Aging of tissue in base parts of the leaf and roots result in strong resistance to gas flow. In the stem, aging causes pressure and gas leaks[17, 27]. As *N. indica* forms turion containing compact storage of substances as they mature, nodes with turions develop in the petiol near the leaf. The r value in the petiole with turion of a mature *N. indica* plant was about three times higher than that in stem (data not shown). Turions, therefore, could confer strong resistance to gas flow to the roots. We targeted relatively young plants with similar leaf age in both species (especially, selecting *N. indica* without turions). However, the species-specific reproduction trait and life cycle would negatively affect its O_2 supply to roots during vegetative growth period in the natural habitats.

Maintenance of high O_2 concentration in the shoot-root junction contributes to the high diffusion flux to roots tips, likely through the aerenchyma[4, 5]. Although the estimated E* values were high under dark conditions in both species, the actual O_2 concentration in the shoot-root junctions were high under light treatments in both species (Table 1). This is because both species are capable of photosynthesis under light conditions used in this study (PPFD ; 500 μmol m^{-2} s^{-1}). So, they are expected to diffuse high amounts of O_2 to the root under light condition compared to that in dark and low-light conditions (night, or rainy weather) in their natural habitats. Both species under dark conditions could support the convective gas flow but not at high levels because of temperature and humidity used in the chamber for convective gas flow. This suggests that both species are capable of maintaining relatively high O_2 concentrations in the shoot-root junction not only by diffusion but also convective gas flow even if placed in dark. Wetland plants do not normally support convective gas flow at high efficiencies, given the uncommon environment in their natural habitats. The actual O_2 concentration in the aerenchyma in the root may suggest differences among many species, particularly given the differences in their tissue structure (i.e., diameter and length of main and lateral roots, and root density per unit soil volume), and the developmental traits of root aerenchyma may add to the differences in their ability to diffuse O_2 at the root tips through the aerenchyma. It had been showed that the O_2 partial pressure in the root decrease from the cortex to stele, and they decrease steeply from the root base to root tips[28, 29]. The O_2 partial pressure within the 80 μm thick of epidermal/hypodermal cylinder at the 10 mm from the root apex of wetland plants, *P. australis* with high convective gas flow system, is below 2 kPa, and the O_2 diffusion within the root tissue is similar to water[28, 29, 30]. In this study, we observed similarity in the root porosities in *N. peltata* and *N. indica* (Table 3). Consequently, they are likely to develop similar amounts of aerenchyma in their roots. However, further studies are needed to clearly understand how these factors impact actual O_2 concentration gradient in the root aerenchyma.

(2) O_2 demand in roots for ROL and respiration

Large amounts of ROL from the roots are helpful not only for maintaining healthy roots activity with high O_2 content in the root aerenchyma but also to protect roots from the reduced toxic substances that are released in the anaerobic soils resulting from increased rhizosphere oxidation[5, 31]. *N. peltata* and *N. indica* have similar ROL values in all treatments (Fig. 2a, b), indicating equal consumption of O_2 to protect the roots. A relatively high ROL value under light treatment in both species would result from convective gas flow with gas containing high O_2 concentration due to active photosynthesis.

ROL was evaluated using a closed chamber in which the net O_2 efflux resulting from the whole root was detected by immersing the roots in deoxygenated experimental solution. It was detected using a Clark-type O_2 electrode sensor[32]. This method often underestimates, because Clark-type O_2 electrode is incapable of detecting slow increase in O_2 near the root. Also, O_2 released from the root may be reabsorbed by other parts of the root (e.g., aerobic respiration in roots)[6]. So, the calculation of ROL is unchallenging when the O_2 concentration of the experimental solution is below 25 μmol l^{-1} or higher than 50 μmol l^{-1} (dark treatments) or 130 μmol l^{-1}(light treatments). In particular, the reabsorption by root respiration increases as accumulation of O_2 in experimental solution seemed to be attributed to underestimation. For these problems, Matsui and Tsuchiya (2006) proposed using the open chamber method with anthraquinone as an O_2 detector instead of an O_2 electrode[33]. In the proposed method, maximum (light condition) and minimum (dark condition) ROL in *Typha latifolia* in strict anaerobic soils depositing organic matter were 1.18 μmol O_2 g^{-1} root DW h^{-1} and 0.64 μmol O_2 g^{-1} root DW h^{-1}, respectively. These were relatively closer to values recorded in our experiments. Moreover, ROL of *Juncus ingens* growing in waterlogged soils or shallows relying on frequent flooding were 0–1.56 μmol O_2 g^{-1} root DW h^{-1} when measured using a closed chamber with O_2 electrode[6]. Although these species were measured under different methods or treatment conditions than those used in our study, their ROL values were lower than those obtained for *N. peltata* and *N. indica*. It is assumed that *N. peltata* and *N. indica* have higher O_2 demands to protect their whole root and maintain normal activity than that of *T. latifolia* and *J. ingens*. These traits of O_2 demands for ROL would ascribe

to their habitats in which soils would be extremely anaerobic with frequently fluctuating water levels.

Although the root respiration rate decreased along with the DO concentration in the experimental solution in both species, there were differences in traits observed (Fig. 1a, b). Since the root respiration rate of *N. peltata* under relatively low DO concentration in the experimental solution (~70 μmol $O_2 l^{-1}$) was maintained similar to that under O_2 saturation, it is expected that they would consume more O_2 under strict O_2 deficient conditions where convective gas flow does not function well. *N. indica* can maintain high root activity without wasting O_2 to root respiration, because they can modulate their root respiration depending on the O_2 concentration in the rhizosphere. This trait in *N. indica* seems to be a more promising strategy for adaptation to anaerobic soil conditions[34]. Similar results were reported for root respiration in *Z. latifolia* grown in hypoxic hydroponic culture with DO concentration in the culture solution at 20 μmol $O_2 l^{-1}$ [20]. The O_2 suppling ability of *Z. latifolia* is not high because it depends only on diffusion. It could co-exist with *P. australis*, which possesses the convective gas flow mechanism under strict anaerobic conditions and with relatively higher water levels in the habitat. Thus, the traits for root respiration of *Z. latifolia* are considered as one of the adaptation strategies to minimize aeration from the shoot to the root, and manage sudden changes in O_2 availability in the aerenchyma in roots.

Given that the rate of root respiration increases as the accumulation of O_2 by ROL in the experimental solution near the roots in our studies (done in a closed chamber), it is likely that O_2 demand may be underestimated. Thus, this method may lead to inaccurate O_2 demand calculations, although the diffusion of O_2 from the roots to the rhizosphere by ROL does not occur under high O_2 situations. In this study, O_2 demand rates of both the species under light treatments were similar to those under the dark treatments (Fig. 3a, b). This was because O_2 demand under both treatments were calculated using the same root respiration data. Meanwhile, actual root respiration rates in both species are likely to be low at night or during precipitation, because of reduced respiration caused by temperature drop[35]. It has been assumed that O_2 demand in the roots of both species fluctuate widely depending on the soil temperature in their habitats, and that they might suffer from O_2 deprivation when their O_2 demand increases from high root respiration. *N. peltata* has relatively high O_2 demand in the roots due to high root respiration rate compared to that in *N. indica* (Figs. 2, 3). This seems to be a disadvantage for efficient survival in O_2-deficient soils. However, *N. peltata* overcomes such situations by compensating with the high pressurization ability of the shoot. Therefore, balancing O_2 supply in the shoot and demand in the roots might play a much greater role in the survival of wetland plants than previously thought.

Conclusions

This study has demonstrated the differences between *N. peltata* and *N. indica* in their ability to supply O_2 to roots and how they respond to root respiration needs in anaerobic conditions. *N. peltata* enhances the inner pressurization in leaf to increase O_2 supply to roots, while *N. indica* is able to respond flexibly to root respiration by decreasing DO concentration in the rhizosphere. These features of shoot and roots in both species allow their existence in habitats with strict anaerobic conditions and very high water levels. These results indicate that the adaptation strategies of wetland plants to survive in anaerobic soils seems to be characterized not only by the ability to supply O_2 but also O_2 consumption in the roots. Further studies are needed to understand energy requirements and efficiency of O_2 consumption.

Acknowledgments

I am grateful to Prof. Takayoshi Tsuchiya and laboratory members of plant ecophysiology, faculty of science, Chiba University for advice and encouragement. I also thank Naoko Yokoyama for support and cooperation in the analysis of convective gas flow.

References

1) Setter T, Waters I (2003) Review of prospects for germplasm improvement for waterlogging tolerance in wheat, barley and oats. *Plant. Soil.* **253** : 1–34.
2) Grosse W, Bauch C (1991) Gas transfer in floating-leaved plants. *Vegetatio.* **97** : 185–192.
3) Kotula L, Ranathunge K, Schreiber L, Steudle E (2009) Functional and chemical comparison of apoplastic barriers to radial oxygen loss in roots of rice (*Oryza sativa* L.) grown in aerated or deoxygenated solution. *J. Exp. Bot.* **60** : 2155–2167.
4) Armstrong J, Armstrong W (1990) Light-enhanced convective throughflow increases oxygenation in rhizomes and rhizosphere of *Phragmites australis* (Cav.) Trin. ex Steud. *New. Phytol.* **114** : 121–128.
5) Colmer T (2003) Long-distance transport of gases in plants : a perspective on internal aeration and radial oxygen loss from roots. *Plant. Cell. Environ.* **26** : 17–36.
6) Sorrell B, Armstrong W (1994) On the difficulties of measuring oxygen release by root systems of wetland plants. *J. Ecol.* **82** : 177–183.
7) Abiko T, Kotula L, Shiono K, Malik A I, Colmer T D, Nakazono M (2012) Enhanced formation of aerenchyma and induction of a barrier to radial oxygen loss in adventitious roots of Zea nicaraguensis contribute to its waterlogging tolerance as compared with maize (*Zea mays* ssp. mays). *Plant. Cell. Environ.* **35** : 1618–1630.

8) KOTULA L, RANATHUNGE K, STEUDLE E (2009) Apoplastic barriers effectively block oxygen permeability across outer cell layers of rice roots under deoxygenated conditions : roles of apoplastic pores and of respiration. *New. Phytol.* **184** : 909–917.
9) SHIONO K, OGAWA S, YAMAZAKI S, ISODA H, FUJIMURA T, NAKAZONO M, COLMER T D (2010) Contrasting dynamics of radial O_2-loss barrier induction and aerenchyma formation in rice roots of two lengths. *Ann. Bot.* **107** : 89–99.
10) SOUKUP A, ARMSTRONG W, SCHREIBER L, FRANKE R, VOTRUBOVÁ O (2007) Apoplastic barriers to radial oxygen loss and solute penetration : a chemical and functional comparison of the exodermis of two wetland species, *Phragmites australis* and *Glyceria maxima*. *New. Phytol.* **173** : 264–278.
11) BRIX H, SORRELL B K, ORR P T (1992) Internal pressurization and convective gas flow in some emergent freshwater macrophytes. *Limnol. Oceanogr.* **37** : 1420–1433.
12) DACEY J W (1981) Pressurized ventilation in the yellow waterlily. *Ecol.* **62** : 1137–1147.
13) SORRELL B K, HAWES I (2009) Convective gas flow development and the maximum depths achieved by helophyte vegetation in lakes. *Ann. Bot.* **105** : 165–174.
14) STRAND V V (2002) The influence of ventilation systems on water depth penetration of emergent macrophytes. *Freshw. Biol.* **47** : 1097–1105.
15) BENDIX M, TORNBJERG T, BRIX H (1994) Internal gas transport in *Typha latifolia* L. and *Typha angustifolia* L. 1. Humidity-induced pressurization and convective throughflow. *Aquat. Bot.* **49** : 75–89.
16) ARMSTRONG W, ARMSTRONG J, BECKETT P (1996) Pressurised ventilation in emergent macrophytes : the mechanism and mathematical modelling of humidity-induced convection. *Aquat. Bot.* **54** : 121–135.
17) ARMSTRONG J, ARMSTRONG W (2009) Record rates of pressurized gas-flow in the great horsetail, *Equisetum telmateia*. Were *Carboniferous Calamites* similarly aerated? *New. Phytol.* **184** : 202–215.
18) KONNERUP D, SORRELL B K, BRIX H (2011) Do tropical wetland plants possess convective gas flow mechanisms? *New. Phytol.* **190** : 379–386.
19) ASAEDA T, FUJINO T, MANATUNGE J (2005) Morphological adaptations of emergent plants to water flow : a case study with *Typha angustifolia*, *Zizania latifolia* and *Phragmites australis*. *Freshw. Biol.* **50** : 1991–2001.
20) NAKAMURA M, NAKAMURA T, TSUCHIYA T, NOGUCHI K (2013) Functional linkage between N acquisition strategies and aeration capacities of hydrophytes for efficient oxygen consumption in roots. *Physiol. Plant.* **147** : 135–146.
21) YAMASAKI S (1987) Oxygen demand and supply in *Zizania latifolia* and *Phragmites australis*. *Aquat. Bot.* **29** : 205–215.
22) GROSSE W, BÜCHEL H B, TIEBEL H (1991) Pressurized ventilation in wetland plants. *Aquat. Bot.* **39** : 89–98.
23) SCHRÖDER P, GROSSE W, WOERMANN D (1986) Localization of thermo-osmotically active partitions in young leaves of *Nuphar lutea*. *J. Exp. Bot.* **37** : 1450–1461.
24) JENSEN C, LUXMOORE R, VANGUNDY S, STOLZY L (1969) Root air measurements by a pycnometer method 1. *Agronomy. J.* **61** : 474–475.
25) AFREEN F, ZOBAYED S, ARMSTRONG J, ARMSTRONG W (2007) Pressure gradients along whole culms and leaf sheaths, and other aspects of humidity-induced gas transport in *Phragmites australis*. *J. Exp. Bot.* **58** : 1651–1662.
26) WHITE S D, GANF G G (2000) Influence of stomatal conductance on the efficiency of internal pressurisation in *Typha domingensis*. *Aquat. Bot.* **67** : 1–11.
27) ENGLONER A I (2009) Structure, growth dynamics and biomass of reed (*Phragmites australis*)—A review. *Flora-Morphol. Distrib. Funct. Ecol. Plants.* **204** : 331–346.
28) ARMSTRONG W, WEBB T, DARWENT M, BECKETT P M (2008) Measuring and interpreting respiratory critical oxygen pressures in roots. *Ann. Bot.* **103** : 281–293.
29) ARMSTRONG W, BECKETT P M (2011) Experimental and modelling data contradict the idea of respiratory down-regulation in plant tissues at an internal O_2 substantially above the critical oxygen pressure for cytochrome oxidase. *New. Phytol.* **190** : 431–441.
30) ARMSTRONG W, COUSINS D, ARMSTRONG J, TURNER D, BECKETT P (2000) Oxygen distribution in wetland plant roots and permeability barriers to gas-exchange with the rhizosphere : a microelectrode and modelling study with *Phragmites australis*. *Ann. Bot.* **86** : 687–703.
31) WU C, YE Z, LI H, WU S, DENG D, ZHU Y, WONG M (2012) Do radial oxygen loss and external aeration affect iron plaque formation and arsenic accumulation and speciation in rice? *J. Exp. Bot.* **63** : 2961–2970.
32) SORRELL B K, DROMGOOLE F (1987) Oxygen transport in the submerged freshwater macrophyte Egeria densa Planch. I. Oxygen production, storage and release. *Aquat. Bot.* **28** : 63–80.
33) MATSUI T, TSUCHIYA T (2006) A method to estimate practical radial oxygen loss of wetland plant roots. *Plant. Soil.* **279** : 119–128.
34) LAAN P, TOSSERAMS M, BLOM C, VEEN B (1990) Internal oxygen transport in *Rumex* species and its significance for respiration under hypoxic conditions. *Plant. Soil.* **122** : 39–46.
35) ATKIN O K, BRUHN D, HURRY V M, TJOELKER M G (2005) Evans Review No. 2 : The hot and the cold : unravelling the variable response of plant respiration to temperature. *Funct. Plant. Biol.* **32** : 87–105.

浮葉植物２種における嫌気環境下での酸素要求性に対する応答戦略

中　村　元　香*†

（令和３年１月７日受付/令和３年３月９日受理）

要約：水生植物の嫌気土壌（湛水土壌）耐性についての生理生態学的研究は，これまでシュートを介した大気からの根への酸素供給（給気）や根の呼吸による酸素消費，根からの放射状酸素放出（ROL）がそれぞれ評価されてきた。しかし，これらの特性は同所的に生育する種間でもそれぞれ大きく異なるため，個々の特性の評価だけでは嫌気土壌耐性を十分に説明できていない。そこで，本研究は，水生植物の嫌気土壌耐性をシュートの給気能力と根の酸素要求性の両面から総合的に評価することを目的とした。同様の嫌気土壌環境に分布する浮葉植物２種（*Nymphoides peltata* および *N.indica*）を対象に，自生地を再現した土壌環境で栽培した。そして，明暗２条件下で，給気能力としてシュート内の対流ガス流（換気）とシュートと根の接合部分の酸素濃度，組織の空隙率を測定するとともに，根の酸素要求性として根の呼吸速度と ROL 速度を調べた。換気能力の指標には，葉身と葉柄の内部抵抗（R），葉身の加圧効率（e），比対流効率（E）を用いた。その結果，E とシュートと根の接合部の酸素濃度は種間で差がなく，また，根の呼吸速度と ROL 速度から求めた根の酸素要求速度も同程度だった。このことは，根の酸素需要とそれに応じた給気量が両種間で同程度であることを意味する。一方で，シュートの給気特性と根の呼吸特性には両種間で違いがみられ，*N. peltata* は空気を送り込む能力である e が高く，また，*N. indica* は根の周りの酸素濃度の減少に対して可変的な呼吸応答を示した。これらは，各種の嫌気土壌への適応戦略を特徴づけるものであり，同様の嫌気土壌環境下での生存を可能とする重要な特性であると示唆された。

キーワード：通気組織，対流ガス流（換気），酸素供給，根の呼吸，放射状酸素放出（ROL）

* 東京農業大学生物産業学部北方圏農学科
（生態系保全学研究室　博士研究員）
† Corresponding author（E-mail : m5nakamu@nodai.ac.jp）