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Research article

Effects of Dissolved O₂ and Fe Availability on Growth, Morphology, Aerenchyma Formation and Radial Oxygen Loss of *Canna indica* L. and *Heliconia psittacorum* L.f.

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Abstract In constructed wetlands (CWs), plants are usually affected by low O₂ levels. Under such conditions, most soluble iron is reduced to ferrous (Fe²⁺) which is highly soluble, and toxic to plants as well. As a consequence of excessive ferrous iron with low O₂ supply, plant growth is reduced, leading to declining nutrient removal efficiency. This study was conducted to determine the effects of different dissolved oxygen levels (normoxia and hypoxia) with Fe supplied on growth, morphology, and root anatomy of two wetland plants (*Canna indica* and *Heliconia psittacorum*). The plants were grown on a nutrient solution modified from Smart and Barko (1985) under normoxic and hypoxic conditions. All plants were grown in greenhouse conditions for 42 days. Plant growth rates and biomass accumulation were drastically reduced under hypoxia while leaf number was not affected. Under hypoxia, root diameter and root porosity also increased in *C. indica*, whereas *H. psittacorum* had greater aerenchyma formation. Moreover, *C. indica* showed adaptive traits to cope with hypoxia and Fe stress by increasing radial oxygen loss (ROL), releasing O₂ to the rhizosphere to resist toxic effects of ferrous iron under hypoxia. In contrast, *H. psittacorum* had no ROL under hypoxia. Moreover, the plants showed leaf chlorosis, leaf roll, and root rotting. Hence, it is suggested that *C. indica* could have better performance than *H. psittacorum* to treat wastewater in CWs as this species can adapt to hypoxic conditions and releases O₂ into rhizosphere which improves dissolved oxygen (DO) in the wastewater.

Keywords: Aerenchyma, Dissolved oxygen, Iron, Root porosity, Wetland emergent plant



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INTRODUCTION

Water pollution is a widespread global problem which is continuously increasing with increasing human population. The wastewater mostly has low dissolved oxygen which is a result of organic matter degradation by microorganisms. In addition, eutrophication from excessive nutrients (nitrogen and phosphorous) promotes the growth of phytoplankton and macrophytes. Subsequently, dissolved oxygen in water is consumed drastically. Moreover, domestic and agricultural wastewater may contain iron from household products, detergents, fertilizers, mine water drainage, and groundwater irrigation. Iron in water has an unfavorable impact on humans, microorganisms, and plants (Lesley et al., 2008; Tjandraatmadja et al., 2008; Jia et al., 2018; Marin-Rivera et al., 2019).

In an effort to reduce pollutants in the wastewater run into water bodies, many treatment systems have been developed such as physical aeration, iron exchange, reverse osmosis, chemical neutralization, and chemical precipitation (Lesley et al., 2008). Most wastewater treatment methods are expensive and require electrical power and are complicated to manage. Constructed wetlands (CWs) are an economical, eco-friendly, effective, and aesthetic method to improve water quality (Inamori et al., 2008; Marin-Rivera et al., 2019). In CWs, plants have essential roles in nutrient absorption and turning nutrients from the wastewater into plant biomass. Besides plant roots perform biofiltration by promoting suspended solid precipitation. The roots of plants provide areas for microbial growth and also release oxygen into the rhizosphere, facilitating nutrient removal by microbial degradation and plant uptake. Moreover, the secretion of root exudates, which are organic carbon compounds stimulates microbial activity and promotes the denitrification process (Sandoval et al., 2019; Zhai et al., 2013). However, plants in CWs tend to suffer from oxygen insufficiency, especially when high-strength wastewater is drained into CWs. As a result, plants decrease growth and lower their nutrient requirement, with consequent impact on water treatment efficiency. Moreover, under hypoxic conditions, most ferric iron (Fe^{3+}) in wastewater is reduced into ferrous iron (Fe^{2+}), which is more soluble and is easily taken up by plant roots. According to Sidek et al. (2020), when using *Eleocharis dulcis* (Burm.f) Trin. ex Hensch to treat Fe-contaminated wastewater ($\text{Fe} = 2.20 \text{ mg L}^{-1}$), the plants expressed leaf chlorosis and some plants were dead after 14 days. Treating acid mine drainage with *Phragmites australis* (Cav.) Trin. ex Steud., it was found that iron concentration higher than 75 mg L^{-1} can affect plant growth, and then decrease water treating efficiency (Wu et al., 2019).

Many wetland plants have strategies to cope with low O_2 availability and high external ferric iron. Some species, for example, *Cyperus flabelliformis* Rottb., *Myriophyllum spicatum* L., *Vallisneria spiralis* L. and *Juncus effusus* L. produce aerenchyma for air preservation and release oxygen from roots into the rhizosphere. Therefore, hazardous dissolved substances (Fe^{2+} , Mn^{2+} , NH_4^+) turn into less toxic, insoluble, or unabsorbed forms (Fe^{3+} , FeOOH , Mn^{3+} , NO_3^-) (Lemonie et al., 2012; Liu et al., 2004; Deng et al., 2009).

Nowadays, a great number of wetland plant species have potential for use in treating many kinds of wastewater due to their fast growth, high nutrient uptake, robustness to wastewater stress conditions, and ease of management (Latune et al., 2017). Previous studies showed that the efficiency of CWs varied with different plant species. Wetland plants in tropical areas, that are exposed to higher temperatures and sunlight, tend to be more efficient than those in sub-tropical areas (Tran et al., 2019). Not only should the plants have good potential performance in CWs, but they should be easily available in their regions and have resistance to environmental conditions and diseases. Thus, we focused on naturalized species as a good choice for wastewater treatment in CWs. Among a large number of plant species, *Canna indica* L. and *Heliconia psittacorum* L.f. were selected. *C. indica* has high-efficiency nitrogen and phosphorous removal in CWs, and immense tolerance to high nutrient concentrations, chemicals, and metal ions. Its high stem and broad leaves block sunlight from penetrating the water column, limiting the growth of phytoplankton (Huang et al., 2017; Jiang et al., 2020). While *H. psittacorum* is tolerant of high nutrients and

drought, and maintains normal growth when CW's are drained (Latune et al., 2017). Besides, *Canna* and *Heliconia* are ornamental plants, bear long-lasting large colorful raceme inflorescences, attract pollinators, and increase esthetics of CWs (Konnerup et al., 2009). Even though, these two species have been considered for use in CWs, there was little known on their ability to grow and cope with iron particularly at low O₂ availability. Hence, this study aims to assess plant growth, morphology, aerenchyma formation, root porosity, and radial oxygen loss of *C. indica* and *H. psittacorum* when the plants grow under low O₂ with an iron supply. The new insights will provide useful information for plant selection in CWs.

MATERIALS AND METHODS

Plant preparation

C. indica were collected from water-saturated clay in Chiang Mai Province and *H. psittacorum* were collected from well-drained sandy soil in Lamphun Province, Thailand. Approximately 100 mm rhizome sections with mature plants were washed by hand to remove clay and soil, then grown in water-saturated sand until new plants emerged. Afterward the new plants (approximately 300 mm shoot length) were selected and gently cleaned by hand to remove sand. Then the plants were placed in 5 L buckets containing nutrient solution modified from Smart and Barko (1985) to which 0.25 mM NH₄-N and 0.25 mM NO₃-N prepared from (NH₄)₂SO₄ and KNO₃ and a plant micronutrient solution (Tropica, Denmark) were added. The pH of the nutrient solution was 6.8 ± 0.2 adjusted using 1.0 M NaOH and HCl. The plants were acclimatized under greenhouse conditions at the Department of Biology, Chiang Mai University for one week.

Experimental design

After acclimation, 20 plants (each individual plant was approximately 350 mm height and 30 g fresh weight) were selected. Each individual plant was grown in a tall cylinder-shaped glass container (Ø = 10 cm and height 20 cm) containing 1 L nutrient solution modified from Smart and Barko (1985) supplied with 0.25 mM NH₄-N, 0.25 mM NO₃-N and 80 mg L⁻¹ Fe-EDDHA (Fe³⁺) (pH = 6.8). The plants were grown in the following treatments (n = 5): (i) normoxic condition (DO = 6-8 mg L⁻¹), the nutrient solution was continually supplied with air pumped by an aerator and (ii) hypoxic condition (DO = 2-3 mg L⁻¹), agar was added to the nutrient solution (0.5g L⁻¹) and the water surface was covered with a polyethylene sheet to prevent air from penetrating the water.

The plants were grown in greenhouse conditions at the Department of Biology, Chiang Mai University. The temperature and light regimes during the experiment were 33-36°C: 15-23°C day: night and approximately 13 h light/ 11 h dark. The nutrient solutions were changed every week to ensure adequate nutrient, iron concentration, pH, and dissolved oxygen levels. The treatments were placed in a randomized arrangement.

Growth and morphological study

After 42 days, plant growth and morphology were determined by measuring total plant height, root length, leaf number, lateral root density, and average leaf area. Then, the plants were harvested and separated into 3 parts: leaves, rhizomes, and roots. The relative growth rate (RGR, d⁻¹) was calculated according to Evans (1972) using the formula: $(\ln W_2 - \ln W_1) / (d_2 - d_1)$ where W₁ and W₂ are initial and final dry mass (g) and d₁ and d₂ are initial and final time (days). The shoot elongation rate (SER, mm d⁻¹) was calculated from the increase of plant height (mm) divided by number of days of experiment.

Root radial oxygen loss (ROL) and porosity

A day before harvest, the ROL from roots was observed according to Armstrong and Armstrong (1988). The plants (n = 5) were cleaned and placed in glass vials

containing 2 L agar solution. The solutions were prepared by dissolving methylene blue 0.012 g L^{-1} in agar-water medium (0.5 g L^{-1}) and O_2 was reduced by addition of 0.12 g L^{-1} sodium dithionite ($\text{Na}_2\text{S}_2\text{O}_4$). The solution is colorless after O_2 is reduced. Then, a blue area develops around roots as methylene blue reacts with O_2 released from the roots. Approximately 300 mg fresh mature roots were used for root porosity measurement by the pycnometer method according to Sojka (1988). The roots were cut, then dried with tissue paper. The fresh weight was recorded as W_r . Then, the pycnometer was filled with distilled water and weighed as W_w . The roots were put into the pycnometer containing water and weighed as W_{w+r} . To remove air collected in the roots, the roots were crushed using mortar and pestle and placed back into the pycnometer filled with water and weighed as W_h . The root porosity was calculated following by the formula:

$$\% \text{ root porosity} = 100 (W_h - W_{w+r}) / (W_w + W_r - W_{w+r})$$

Root anatomy

Three parts (basal, middle, and apical) of the mature roots ($n = 10$) were selected. Root cross-sections were made by free-hand section techniques (Lux et al., 2005). The sections were stained with 0.05% safranin O. Then, the sections were observed and photographed under a 40x light microscope (Olympus, Japan). Root diameters and cortical air spaces were calculated using the program Image J (National Institutes of Health, USA).

Statistical analysis

All results were analyzed using SPSS statistics, version 17.0 (SPSS Inc., USA). The results were tested for variance homogeneity using Levene's test. Then, one-way analysis of variance (ANOVA) was performed followed by Tukey's HSD test at a 5% significance level to determine treatment differences. All results were presented as means with standard errors (Mean \pm SE).

RESULTS

Effect of O_2 levels under Fe supplied on plant growth and morphology

Generally, *C. indica* had higher growth rates and total biomass than *H. psittacorum*. Under hypoxic condition, *C. indica* decreased relative growth rates and shoot elongation rates (Figure 1a, b). Total biomass, particularly leaf dry mass of each species, significantly decreased when the plants grew under hypoxia, resulting in low shoot:root ratios (Table 1).

C. indica had no symptoms on leaves under low O_2 supply, while *H. psittacorum* showed leaf chlorosis, leaf curling (epinasty), withered leaves and defoliation under hypoxia.

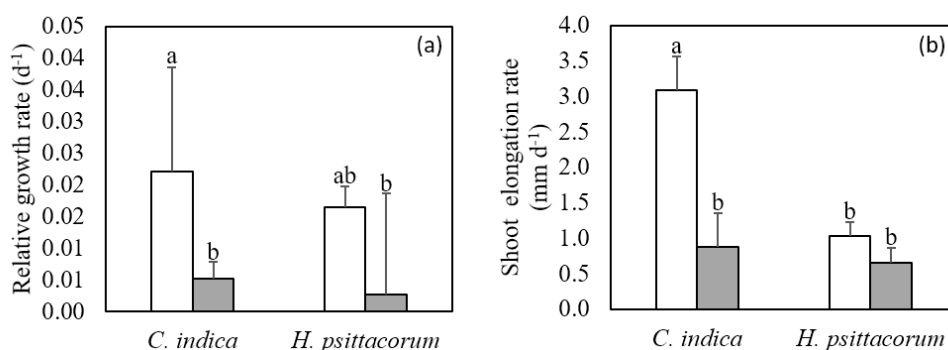


Figure 1. Relative growth rate (a) and shoot elongation rate (b) of *C. indica* and *H. psittacorum* with different dissolved oxygen levels (□ normoxia, ■ hypoxia) under Fe supplied. Different letters above columns indicate significant differences between treatments.

O₂ levels did not affect root biomass, but they affected root morphology in both species. Under hypoxia, *H. psittacorum* root length significantly increased. New roots of *C. indica* had a slightly red-brown color. In *H. psittacorum* under hypoxia, it had longer but fewer number of roots. Moreover, it had decreased apical lateral root density (Table 1, Figure 3).

Table 1. Biomass, growth and root parameters of *C. indica* and *H. psittacorum* (mean \pm SE) grown at different dissolved oxygen levels under Fe supplied. Different letters designate significant differences between treatments ($P < 0.05$).

	<i>C. indica</i>		<i>H. psittacorum</i>		F-ratio
	normoxia	hypoxia	normoxia	hypoxia	
Total leaf dry mass (g)	3.6 \pm 0.2 ^a	2.3 \pm 0.2 ^b	2.4 \pm 0.2 ^b	1.2 \pm 0.4 ^c	14.4 ^{***}
Total rhizome dry mass(g)	1.8 \pm 0.5 ^a	1.1 \pm 0.1 ^{ab}	0.7 \pm 0.1 ^{ab}	0.8 \pm 0.2 ^b	3.6 [*]
Total root dry mass (g)	0.8 \pm 0.2	0.9 \pm 0.1	0.6 \pm 0.1	0.5 \pm 0.1	2.7
Total dry biomass (g)	6.3 \pm 0.7 ^a	4.3 \pm 0.1 ^b	3.6 \pm 0.4 ^{bc}	2.4 \pm 0.4 ^c	13.1 ^{***}
Shoot:root ratio	3.9 \pm 0.7	2.7 \pm 0.2	3.9 \pm 0.9	2.5 \pm 0.6	1.8
Plant height (mm)	427.7 \pm 58.3	404.6 \pm 16.2	394.0 \pm 15.5	403.0 \pm 14.8	0.2
Average leaf area (cm ²)	82.80 \pm 8.41 ^a	58.46 \pm 3.15 ^{ab}	56.04 \pm 10.00 ^{ab}	48.58 \pm 2.09 ^b	4.7 [*]
Number of leaves	8.2 \pm 0.4 ^a	8.8 \pm 0.9 ^a	5.4 \pm 0.07 ^b	2.8 \pm 0.7 ^b	16.3 ^{***}
Root length (mm)	160.4 \pm 12.0 ^{ab}	127.6 \pm 13.8 ^b	135.6 \pm 13.8 ^b	199.2 \pm 6.0 ^a	5.9 ^{**}
Lateral root density (LRs/cm)					
Basal	7.5 \pm 0.7	9.0 \pm 1.2	8.8 \pm 1.3	5.2 \pm 1.1	2.6
Middle	12.8 \pm 1.0 ^{ab}	17.8 \pm 3.8 ^a	8.9 \pm 1.3 ^b	10.8 \pm 2.8 ^b	8.3 ^{**}
Apical	12.4 \pm 1.6 ^{ab}	17.8 \pm 3.9 ^a	6.9 \pm 2.6 ^{bc}	4.7 \pm 2.2 ^c	15.2 ^{***}
Root diameter (μ m)					
Basal	2,324.3 \pm 130.2 ^a	2,390.8 \pm 161.8 ^a	594.6 \pm 48.1 ^b	546.8 \pm 43.9 ^b	90.0 ^{***}
Middle	2,003.0 \pm 56.9 ^b	2,441.9 \pm 121.9 ^a	452.4 \pm 35.2 ^c	476.2 \pm 24.9 ^c	212.9 ^{***}
Apical	1,533.6 \pm 71.5 ^{ab}	1,717.8 \pm 141.4 ^a	401.8 \pm 23.8 ^{bc}	361.8 \pm 40.1 ^c	76.5 ^{***}
Aerenchyma formation (%)					
Basal	0.5 \pm 0.2 ^b	2.8 \pm 1.2 ^{ab}	3.8 \pm 1.6 ^{ab}	6.1 \pm 1.0 ^a	4.5 [*]
Middle	2.7 \pm 0.8 ^b	3.2 \pm 0.8 ^b	7.0 \pm 1.8 ^{ab}	12.9 \pm 2.2 ^a	8.5 ^{**}
Apical	1.8 \pm 0.6 ^b	1.4 \pm 0.3 ^b	3.6 \pm 1.8 ^b	10.0 \pm 2.0 ^a	6.6 ^{**}
Internal air space (μ m ²)					
Basal	25,790.0 \pm 3,800.5 ^b	87,254.4 \pm 10,572.5 ^a	25,492.2 \pm 10,008.2 ^b	22,055.4 \pm 2,415.8 ^b	16.2 [*]
Middle	63,235.3 \pm 10,729.4 ^b	108,903.4 \pm 19,035.0 ^a	21,022.6 \pm 3,595.6 ^b	24,522.5 \pm 6,122.9 ^b	12.4 ^{**}
Apical	23,164.7 \pm 6,035.0	28,735.6 \pm 10,740.5	6,830.1 \pm 2,966.0	15,238.5 \pm 838.9	2.0

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

Effect of O₂ levels under Fe supplied on root anatomy, aerenchyma formation, root porosity and radial oxygen loss

Under hypoxia, *C. indica* significantly increased root diameter and internal air space in the basal and middle parts of the roots. While *H. psittacorum* tended to increase internal air space under hypoxia but there was no significantly different from the normoxic conditions. Similarly, both species tended to increase aerenchyma under hypoxia. However, significantly increased in aerenchyma formation in apical root zone of *H. psittacorum* was observed (Table 1, Figure 4).

Under hypoxia, *C. indica* significantly increased in root porosity while there was no significant change in *H. psittacorum* (Figure 2a). Moreover, both species had slightly increased in root angle, and the roots tended to rise to the water surface when grown under low O₂ supply. (Figure 2b).

Under normoxic conditions, the pattern of ROL was similar in both species, as the basal and the middle part of the roots stained light blue. After an hour, the lateral roots tended to blue-stain faster than the main roots, which took about twelve hours

to appear blue stained. Interestingly, there were different ROL patterns between two plant species under hypoxia. *C. indica* had more O₂ leaked from all parts of the roots, whereas *H. psittacorum* had no O₂ leaked from the whole roots (Figure 3).

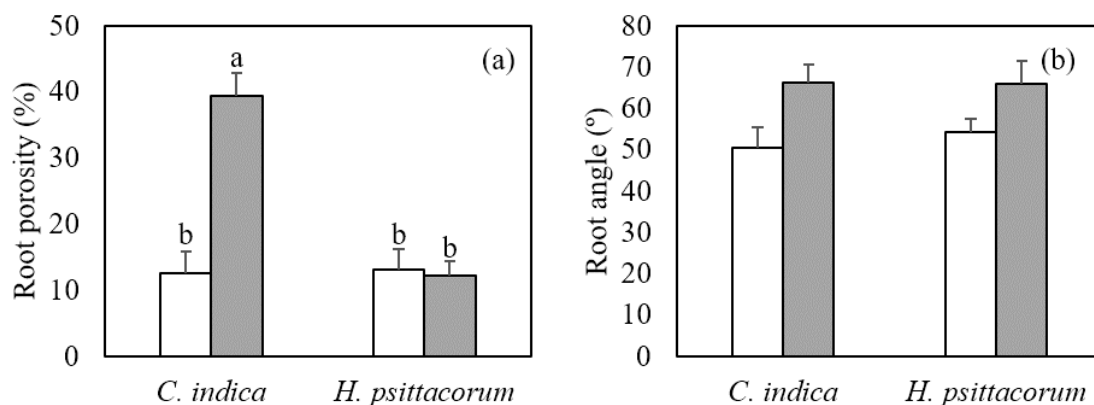


Figure 2. Root porosity (a) and root angle (b) of *C. indica* and *H. psittacorum* grown at different dissolved oxygen levels (□ normoxia, ■ hypoxia) under Fe supplied. Different letters above column indicate significant differences between treatments.

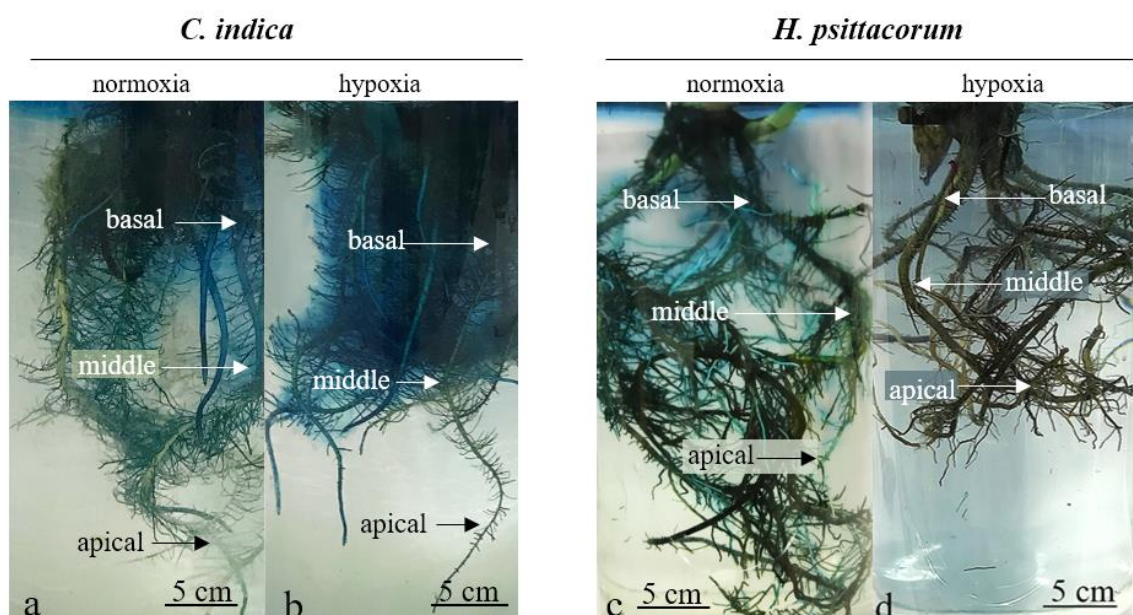


Figure 3. Oxygen released from roots of *C. indica* (a, b) and *H. psittacorum* (c, d) grown under normoxic and hypoxic conditions under Fe supplied. Blue color indicates zone of O₂ released from root to rhizosphere.

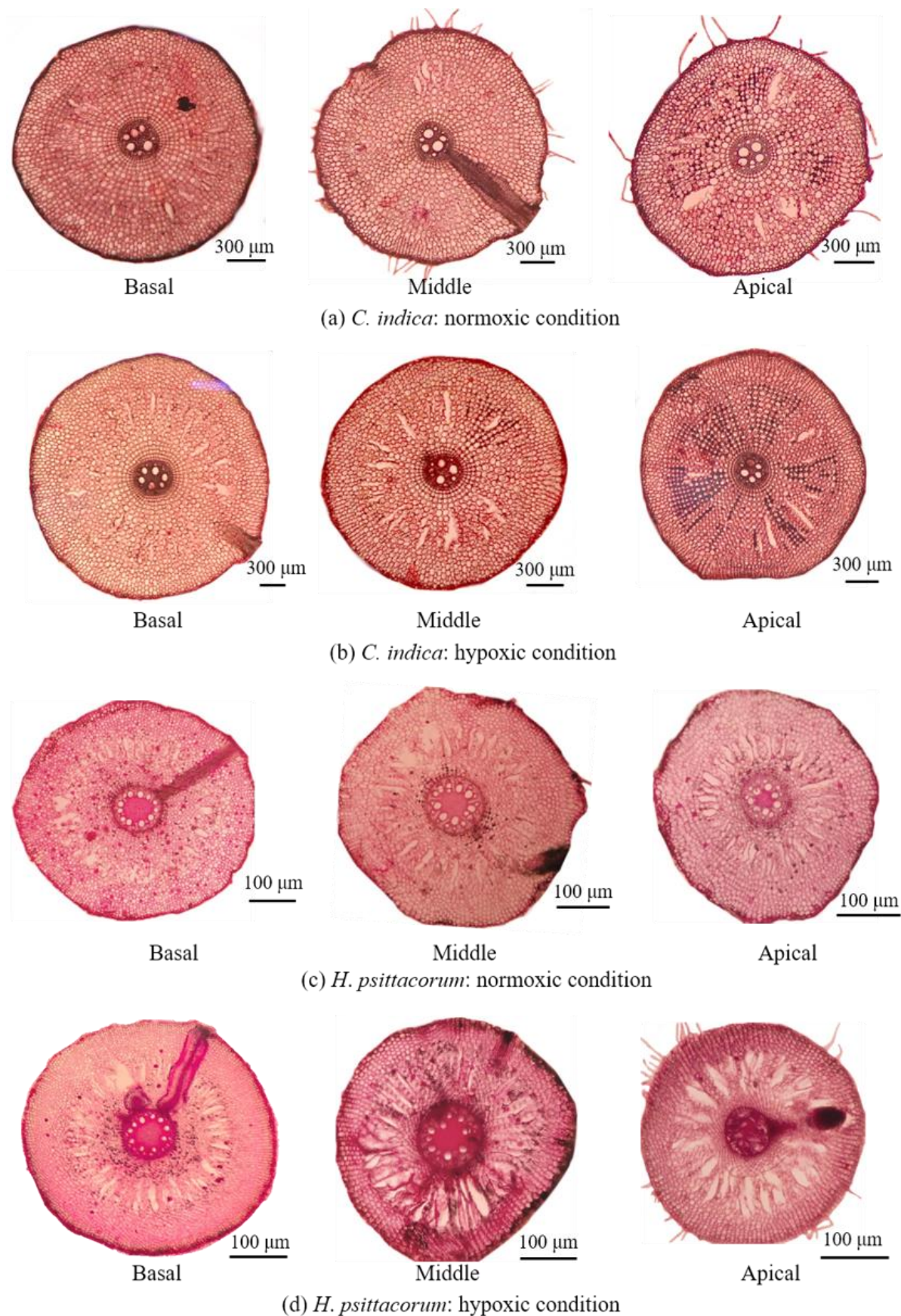


Figure 4. Root cross-sections at basal, middle and apical zone of *C. indica* and *H. psittacorum* grown under normoxic and hypoxic conditions under Fe supplied.

DISCUSSION

Generally, *C. indica* and *H. psittacorum* grew well in the aerated nutrient solution with an Fe supply. However, plant growth and total biomass drastically decreased under hypoxic conditions. Similar results were found in *Pennisetum purpureum* x *P. americanum* cv. Pakchong 1; the plants not only decreased leaf biomass but also reduced transpiration rates, photosynthetic rates, and nutrient uptake rates when grown under hypoxia (Dat et al., 2018; Muenrew and Jampeetong, 2018). In this study, no signs of iron toxicity were observed in *C. indica*. The greater ROL of this species can oxidize Fe^{2+} to an insoluble form (Fe^{3+}), so that less Fe^{2+} is taken up by the plants. In contrast, severe symptoms such as leaf chlorosis, leaf curling (epinasty), wilted leaves and defoliation were found in *H. psittacorum*, which has no ROL. Leaf chlorosis in *H. psittacorum* was probably caused by high Fe^{2+} taken up and accumulated in the tissue, which interferes with chlorophyll formation by generating reactive oxygen species (ROS), promoting chlorophyll degradation and diminishing photosynthesis (Saaltink et al., 2017). Leaf curling, corresponding to hypoxic stress, makes the plants decline in water uptake, and leaves wilted as has been found in *Physalis peruviana* L. grown under hypoxia (Aldana et al., 2014.). In general, root biomass is highly reduced under hypoxia (Jampeetong and Muenrew, 2016; Pederson et al., 2019) but in this study *H. psittacorum* and *C. indica* had no significant change of root biomass under hypoxia. However, it was found that each species had different root responses to hypoxic conditions in terms of root number and lateral root production. The primary root of *C. indica* was shorter, but it produced more lateral roots. In contrast, the primary root of *H. psittacorum* was longer but produced lower numbers of new roots and shorter lateral roots. As available oxygen declines, decrease of root number may be one strategy to reduce root O_2 requirements in order to maintain root growth and activity (Folzer et al., 2006; Jampeetong and Muenrew, 2016).

Ability to form aerenchyma is an important root trait that allows many wetland plant species to grow under hypoxic conditions. In this study, aerenchyma formation tended to increase in the basal and middle parts of the root in both species but was not significant. At the basal parts, most cells were loose parenchyma cells, providing intercellular space for air preservation (Jung et al., 2008). Normally, *C. indica* had greater root diameter than *H. psittacorum* regardless of O_2 availability. Under hypoxia, *C. indica* greatly increased root diameters in the middle parts of roots. Due to the long roots of both plant species, air transported from shoots to roots by passive O_2 diffusion is inadequate for root respiration. Increase in aerenchyma combined with increasing root diameter, leads to more space for air transportation (Brix, 1993; Khan et al., 2016; Li et al., 2013). Under hypoxia, *C. indica* well developed internal air space area ($87,254 \mu\text{m}^2$) compared with *H. psittacorum* ($22,055 \mu\text{m}^2$) likewise greater root porosity was found in *C. indica* than *H. psittacorum*. Moreover, both plants slightly increased root angle and developed internal air spaces; the roots containing air tends to be lighter than the roots containing less air, resulting in root growth upward to the water surface where O_2 levels are sufficient for aerobic processes of roots. Similar results were found in *Arabidopsis thaliana* (L.) Heynh. and *Oryza sativa* L. exposed to hypoxia, both plants species increased root air space and adjusted their primary and adventitious root angles upward to the water surface to avoid the hypoxic soil layer (Pederson et al., 2019).

Under normoxic conditions, *C. indica* had higher ROL than *H. psittacorum* even though both plant species had the same ROL pattern. Under hypoxic conditions, *C. indica* greatly increased O_2 loss from its roots. The O_2 release was observed from the main roots and lateral roots especially at the basal and middle parts of roots where there were great increases of aerenchyma formation and root porosity. In *C. indica*, higher ROL was clearly influenced by increasing root porosity. Likewise, the ROL was positively correlated to root porosity under hypoxia in some wetland plants such as *Juncus effusus* L., *Rumex crispus* L., *Rumex palustris* Sm. and *Salix nigra* Marshall (Visser et al., 2000; Li et al., 2006; Manzur et al., 2014). Unlike *C. indica*, O_2 released from the roots of *H. psittacorum* under hypoxia was lower than normoxia even though aerenchyma formation increased. It might be because *H. psittacorum* produced fewer

new roots and lateral roots when compared with normoxia. In many wetland plants, the greatest ROL has been found in subapical parts of active young roots and lateral roots compared to old roots. Moreover, leaf wilting and stomata closure leading to reduced O_2 entering the aerial parts of the plants along with reducing lateral root density, result in less area for gas movement and limit permeability of ROL (Li et al., 2013; Pincam et al., 2020).

The results of growth, biomass production, aerenchyma formation, root porosity and ROL suggest that with sufficient oxygen, both *C. indica* and *H. psittacorum* could be used in CWs. Under low O_2 condition, *C. indica* may be preferred over *H. psittacorum* as it overcomes hypoxia and Fe rich wastewater by developing extensive aerenchyma and increasing ROL greatly. According to Cheng et al. (2014), plant species with higher ROL correlate with higher growth and photosynthesis rate, leading to higher nutrient removal efficiency in CWs. Moreover, the oxygenation of the rhizosphere promotes the growth of microorganisms and facilitates nutrient uptake (Khan et al., 2016; Sandoval et al., 2019). In addition, *C. indica* has abundant lateral roots which provide surface area for microorganism attachment and might lead to improving nutrient removal by microbial nutrient cycle processes, e.g., nitrification. The higher ROL also leads to tolerance to pollutants as root released O_2 neutralizes toxic substances such as Fe^{2+} , Mn^{2+} and H_2S through oxidation or aerobic microorganism in the rhizosphere. As plants permit O_2 released from the roots, Fe^{2+} tends to be oxidized into Fe^{3+} causing iron precipitation onto root surfaces, called iron plaque. Tai et al. (2018) pointed out that amounts of iron plaque on *C. indica* increase with the concentration of Fe^{2+} in the medium, varying from 0-200 $mg L^{-1}$. It has been documented that iron plaque can be either a barrier or a reservoir. Functioning as a barrier, iron plaque can prevent excess uptake, leading to metal tolerance in plants (Jia et al. 2018; Khan et al., 2016). In this study, *C. indica* showed little rust-color on new roots when growing in hypoxic conditions and did not show leaf chlorosis from excessive iron accumulation in leaves. On the other hand, the iron plaque on the root surface provided more surface for nutrient uptake. As mentioned by Tai et al. (2018) root iron plaque formation can enhance phosphorous uptake. However, this role might result from Fe plaque thickness and composition (Zhang et al., 2020). In contrast, *H. psittacorum* should not be used for long-term hypoxia and Fe contaminated wastewater treatment since it showed toxic symptoms, leading to a releasing of organic matter from root to the solution. From this situation, DO was dramatically declined which could lead to a death of plant and worse water quality.

CONCLUSION

In summary, under low O_2 availability, both species decreased growth rates, biomass, and leaf areas. *C. indica* responded effectively to hypoxia and Fe stress by increasing of internal air space and radial oxygen loss. Whereas *H. psittacorum* seems to be not well adapted to low O_2 with high Fe availability. Under such conditions, this species showed stress symptoms e.g. leaf chlorosis, leaf curling, and root rotting combined with reduction of ROL. Thus, this study suggests that *C. indica* is more satisfactory for Fe contaminated wastewater treatment under low O_2 conditions.

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AUTHOR CONTRIBUTIONS

Tanapong Suriyakaew conducted the experiments, performed the statistical analysis, data visualization and wrote original draft of the manuscript. Arunothai Jampeetong designed and supervised T. Suriyakaew, conducted the experiments, commented and edited the manuscript. All authors have read and approved of the final manuscript.

CONFLICT OF INTEREST

The authors declare that they hold no conflict of interests.

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