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Beneficial effect of prey-derived N for *Drosera indica*, a rare carnivorous plant in Taiwan, growing in field and in culture

Hung-Yi HUANG[1,2,*], Wen-Yuan KAO[1,3]

***1.** Institute of Ecology and Evolutionary Biology, National Taiwan University, Taipei, Taiwan.* ***2.** Department of Habitat Conservation, Society of Wilderness, Taipei, Taiwan.* ***3.** Department of Life Science, National Taiwan University, Taipei, Taiwan.*
[*]Corresponding author's email: hunter.gd.hunter@gmail.com

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ABSTRACT: *Drosera indica*, a carnivorous plant, is currently listed as a nationally endangered species in Taiwan. Understanding the biology of *D. indica* can aid in the effective conservation and protection of this species. The percentage of leaf nitrogen derived from prey in field-grown plants and the significance of carnivory in cultivated *D. indica* were investigated in this study. Carbon ([C]) and nitrogen ([N]) contents and stable carbon ($\delta^{13}C$) and nitrogen isotope ($\delta^{15}N$) values of field-grown *D. indica*, its non-carnivorous neighboring plants and potential prey were analyzed. The one-isotope, two-source, end-member mixing model, using $\delta^{15}N$ values of potential prey and plant leaves, revealed that *D. indica* obtained approximately 57% of its leaf [N] from captured prey, indicating that prey is an important source of the supplementary N for the plant growing in its natural habitat. The prey derived N might confer *D. indica* a significantly higher N content than its non-carnivorous neighboring plants. Cultivated *D. indica* plants with access to prey had significantly enriched $\delta^{15}N$ values compared to those with prey exclusion, indicating utilization of N from prey. Significant increases in flower number, capsules and seed production, but not in photosynthetic activity and growth, were found in cultivated *D. indica* in response to prey availability. In conclusion, carnivory contributes a significant amount of nitrogen to the foliage of the plant in its natural habitat but is not indispensable for cultivated *D. indica*.

KEY WORDS: carnivorous plants, *Drosera indica*, nutrient use, stable isotope analysis, prey-derived nutrient.

INTRODUCTION

In most terrestrial ecosystems, plant growth is often limited by nitrogen (N) and/or phosphorus (P) availability. Therefore, many plants have evolved traits that are adapted and/or acclimated to low-nutrient environments. Carnivory in plants has been suggested as an adaptation to a low nutrient environment. The acquisition of additional nutrients from the captured prey could supplement root nutrient uptake (Adamec, 2002), which would benefit carnivorous plants through enhanced photosynthetic rate, increased growth, earlier flowering and increased seed production (Givnish *et al*., 1984). Therefore, prey capture provides carnivorous plants with advantages that enable them to grow and/or to outcompete sympatric non-carnivorous plants in environments with low nutrient availability. However, contrasting results regarding the benefits associated with carnivory also exist (Zamora *et al*., 1997; Jürgens *et al*., 2012). For example, prey capture has been shown to be non-essential for normal growth and development in some carnivorous plants (Small *et al*., 1977; Lüttge, 1983). These findings indicate that the reliance of carnivorous plants on nutrients from prey varies with species (Schulze *et al*., 1991; Ellison and Gotelli, 2001), nutrient status, and environmental conditions (Cook *et al*., 2018).

Drosera indica, an erect annual sundew reaching 15–50 cm in height and bearing foliar glandular trichomes that trap insects, is mainly distributed in tropical regions such as Africa, Southeast Asia, and Australia (Short, 2011). On the island of Taiwan, the only stable population of this species is found in the wetland near Lienhwa Temple, where it is maintained through the constant removal of non-carnivorous neighboring plants. *D. indica* is currently listed as a nationally endangered species (Editorial Committee of the Red List of Taiwan Plants, 2017) and therefore deserves conservation and protection. In a field manipulation experiment, Huang and Kao (2021) reported that the seedling density of *D. indica* was primarily restricted by the overgrowth of neighboring plants and might not have been limited by the efficiency of prey capture in the Lienhwa Temple wetland. However, the efficiency of prey capture and the nutrient status of the plants were not investigated in that study. The objective of the present study was therefore to examine whether carnivory contributes significantly to the nutrition of *D. indica* and whether the growth of this species is limited by nitrogen availability in the wetland.

Stable isotope techniques have been widely used in studying trophic relationships among organisms in various ecosystems. For example, the natural abundances of stable nitrogen isotopes have been used to calculate the relative contribution of two N sources－root uptake and prey derived－to the total N of *Drosera* spp. in Australia (Schulze *et al*., 1991), *D. rotundifolia* (Millett *et al*., 2003; Millett *et al*., 2012; Cook *et al*., 2018) and *Nepenthes* spp. (Moran *et al*., 2001). These studies reported that of total

Fig. 1. Photograph of a wild ***Drosera indica***, showing its insect-trapping features and morphological characteristics.

plant N derived from prey in *Drosera* spp. varies from 20 to 65%. Following the approach of the aforementioned research, we analyzed stable N isotope values of leaves of *D. indica* plants, those of non-carnivorous neighboring plants, and of potential prey, and then applied the one-isotope, two-source, end-member mixing model to evaluate the relative contributions of N originating from prey or soil to the nutrition of the carnivorous species in its natural habitat in Taiwan.

Successful in situ conservation of *D. indica* requires an understanding of the factors limiting its population size, which has been investigated in a previous study (Huang and Kao, 2021). However, due to the loss of habitats, *ex situ* conservation and field transfer might also be necessary for conserving this species. An exploration of the biology of *D. indica* can help in growing this rare and endangered species in controlled conditions. The effect of light availability and temperature on growth and seed germination of *D. indica* was previously investigated (Huang and Kao, 2021). However, nutrient metabolism of *D. indica* has not been studied. The second objective of this study was to investigate whether carnivory is dispensable for the growth and development of *D. indica* under cultivated conditions. To achieve the objective, we compared photosynthetic activity (potential and effective quantum yield of photosystem II), growth (accumulated biomass), and fecundity (seed production) of cultivated *D. indica* with and without access to prey in an outdoor experimental farm.

MATERIALS AND METHODS

Field experiment

Study site and sampling: Lienhwa Temple wetland (24°52′ 40″ N, 120°57′42″ E) is located in a valley in northern Taiwan. Detailed information about the site has been described in a previous report (Huang and Kao, 2021). *Drosera indica* (Fig. 1), accompanied by non-carnivorous species of Cyperaceae and Gramineae, is the dominant carnivorous plant in the wetland. *Drosera spatulata,* another carnivorous plant species, was also present in the wetland but its population size was very small. Destructive sampling would have had a devastating impact on the population. Therefore, isotope analysis was not conducted on this species. This study focused solely on studying *D. indica*.

Insects representing potential prey were collected once every week from August to Sept. of 2014. "During each sampling week, flying insects were collected using an insect net at five randomly selected locations in the wetland. Insects < 7 mm in length were picked, stored and frozen in plastic bags, transferred to the laboratory, observed and identified under a dissecting microscope. The collected insects belonging to the same order during each survey were pooled into a sample for carbon and nitrogen and stable isotopes analyses.

Healthy and mature leaves of *D. indica* and non-carnivorous neighboring plants (as reference) were sampled in Sept., 2014. Ten plants of each species were collected. Since *D. indica* was not evenly distributed within the wetland, mature plants with a height of 20 – 30 cm or taller were randomly selected. For each plant, 3 - 4 mature leaves, the 3th – 6th leaf from the top of the shoot, were sampled. The collected leaf samples were stripped of any prey and rinsed twice with distilled water.

Analyses of N and C contents and $\delta^{13}C$ and $\delta^{15}N$ values

Leaf and insect samples were put into an oven (temperature maintained at 60 °C) within 24 hours after collection, dried for 72 hours, and then ground into a fine powder using a mortar and a pestle. The analyses of nitrogen and carbon content and the stable carbon ($\delta^{13}C$) and nitrogen isotopes ($\delta^{15}N$) of the grounded samples were performed using an isotopic ratio mass spectrometer (Thermo Scientific™ Delta V™ Advantage) coupled with an elemental analyzer (ThermoFisher Scientific Flash EA 1112) at TechComm Laboratory, College of Life Science, National Taiwan University. Stable isotope data were reported as the relative difference between ratios of a sample (R_{sample}) and standards ($R_{standard}$) in standard notation as:

$$\delta X\ (‰) = [(R_{sample}/R_{standard}) - 1] \times 10^3,$$

where $R={}^{13}C/{}^{12}C$ or ${}^{15}N/{}^{14}N$. $\delta^{13}C$ or $\delta^{15}N$ is the per-mil (‰) deviation of that sample from the recognized isotope standard, i.e., PeeDee Belemnite (PDB) limestone for $\delta^{13}C$ and atmospheric N_2 for $\delta^{15}N$ (Gonfiantini *et al*., 1995).

Calculation of relative contribution of N derived from prey to total leaf N of *D. indica*

Following the one-isotope, two-source, end-member mixing model, the relative contribution of N derived from prey to total leaf N of *D. indica* (N_{dfp} %) was calculated as

$$N_{dfp}\% = [(\delta^{15}N_D - \delta^{15}N_r)/(\delta^{15}N_p - \delta^{15}N_r)] \times 100$$

where, $\delta^{15}N_D$, $\delta^{15}N_r$ and $\delta^{15}N_p$ represent the mean $\delta^{15}N$ values of *D. indica*, reference plants and prey, respectively. Theoretically, the value of $\delta^{15}N_r$ should be that of *D. indica* with100% soil-derived N. Since no *D. indica* with 100% soil-derived N was available in the field, we used mean $\delta^{15}N$ of its neighboring plants (excluding N_2 fixing plants) as a surrogate. Similar approach has been used in Millett *et al*. (2003, 2012, 2015) and Moran (2001) to estimate the contribution of N derived from prey to total leaf N of carnivorous plants.

Experimental design for culture experiment

Seeds collected from field-grown *D. indica* were sown in peat-filled plug trays. Seedlings of ca. 14 days old were transplanted into plastic pots (diameter of 11 cm and height of 9 cm) filled with peat (Klasmann Deilmann mbH, Geeste, Germany) and cultivated in a growth chamber by tray method (http://www.growsundews.com/tray_method_carnivorous_plants.html) with the potting peat kept constantly moist. During the seedling stage, the seedlings ceased growth after germination, showing no increase in height and appearing pale, likely due to nutrient deficiency. Fertilization was therefore applied to the seedlings, after which their color gradually turned green and they resumed growth.

To fertilize the seedlings, the tray was filled with nutrient solution, a 1:500 dilution of Hyponex 2 (N: P: K = 20: 20: 20), and replaced at a weekly interval. The conditions inside the growth chamber were controlled at a relative humidity of 70%, a 12 h/12 h light/dark cycle and photosynthetic photon flux density of 100 – 200 µmol m^{-2} s^{-1} at light period, and temperature regimes of 25/20 °C during light/dark cycle. Twenty days after the transplanting, plants were transferred from the growth chamber to an outdoor experimental farm at National Taiwan University for the prey availability treatments. Thereafter no fertilizer was given to the plants till the end of the experiment. Totally 20 potted plants were assigned to two trays filled with tap water without fertilizer. Prey availability was controlled by completely or partially covering the cultivated trays with one layer of pearl yarn (mesh opening size, 0.85 mm, 70 cm high) supported by a frame locating on the top of the tray. One tray containing 10 pots of plants was completely covered by the yarn (prey excluded treatment, PE). The other tray containing the remaining 10 pots was partially covered (prey available treatment, PA) by partially covering the supporting frame with the yarn, with ca. 20-cm height left uncovered at the bottom of the frame for the entrance of prey. Field observations indicated that at certain sun angles when the uncovered portion exceeded 20 cm height, light availability would be different between the two treatments. Numerous insects were observed stuck on the leaves of plants in the PA treatment. Sampling and measurements were conducted after 2 months of treatments. During the experimental period the light availability at the NTU experimental farm was about 680–810 µmol m^{-2} $month^{-1}$, similar to the light environment (650–800 µmol m^{-2} $month^{-1}$) during the growing season of *D. indica* in the field without shading (Huang and Kao, 2021).

Leaf chlorophyll content and photosynthetic activity measurements

Chlorophyll content ([Chl]) and photosynthetic activity was measured on mature leaves of the cultivated plants, 70 days after the initiation of the prey availability treatments. For the [Chl] analysis, 3 – 5 leaves were ground into powder using liquid nitrogen and mixed with 1 mL of 95% ethanol and saturated $MgCO_3$ and incubated for at least 24 hours in a 4 °C refrigerator. Then the liquid mixture was centrifuged at 7000 rpm (Eppendorf Centrifuge 5415C, Labequip, Canada) for 15 minutes. The absorbance of the supernatant was measured at wavelengths of 665 nm (A_{665}) and 649 nm (A_{649}) with a spectrophotometer (Helios, Thermo, USA), and total chlorophyll content (Tchlorophyll) was calculated following Wintermans and De Mots (1965):

$$\text{Tchlorophyll } (\mu g\ mL^{-1}) = 6.10 \times A_{665} + 20.04 \times A_{649}$$

Leaves of *D. indica*, approximately 1 – 3 mm in width, are extremely slender and delicate. They were easily broken or detached from the shoot after being clamped into the chamber of a gas exchange system. Thus, it is difficult measure the photosynthetic rate of the plant directly using a gas exchange system. Therefore, photosynthetic activity was evaluated using a chlorophyll fluorometer, which does not damage the leaves during measurement. Effective quantum yield of photosystem II (Φ_{PSII} = (Fm'-Ft) / Fm', where Fm' is maximum fluorescence and Ft steady-state fluorescence of the illuminated leaf) was measured, with a chlorophyll fluorometer (Mini-Pam, Walz, Germany), every two hours from 7:00 am to 6:00 pm (sunset). Maximal quantum yield of PSII (Fv/Fm), Fm: maximal fluorescence yield of a dark-adapted sample, Fv: variable fluorescence = Fm - Fo (minimal fluorescence yield of a dark-adapted sample) was measured 2 hours after sunset.

N and C contents and the stable carbon and nitrogen isotopes analyses

Mature and senescent leaves (n = 6) were sampled for N and C content analyses and the stable C and N isotopes analyses following the methods described above.

The reutilization efficiency of N per leaf (N_{reu}) was calculated following Adamec (2002, 2014). Briefly,

$$N_{reu}(\%) = [(N_{mature} - N_{senescent} \times 0.71) / N_{mature}] \times 100$$

where N_{mature} and $N_{senescent}$ represent leaf N content of mature and senescent leaves, respectively.

To account for the decrease in dry weight of senescent leaves, a correction factor of 0.71 was applied, representing the DW of senescent leaves relative to that in mature leaves (Adamec, 2014)

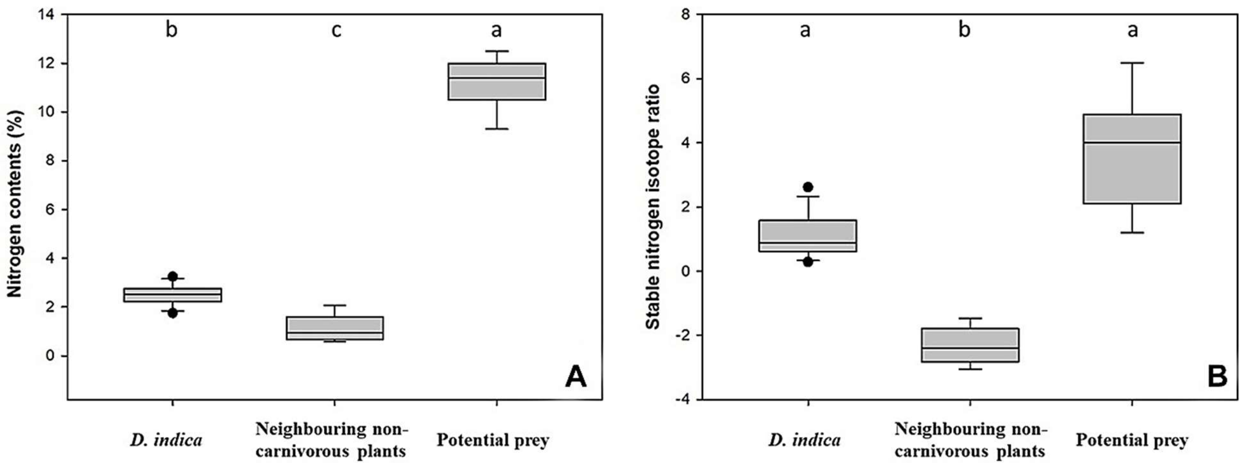

Fig. 2. Nitrogen (N) content (Fig. 2A) and stable nitrogen isotope ratio ($\delta^{15}N$) of ***Drosera indica*** (mean ± S.D., n = 15), neighboring non-carnivorous plants (n = 9), and potential prey (n = 7) collected from Lienhwa Temple wetland (Fig. 2B), northern Taiwan. (Kruskal-Wallis One Way Analysis, Dunnett's T3 post hoc test, $p < 0.05$). Values with different letters indicate significant differences.

Biomass measurement

Plants were harvested 70 days after the treatments. Prior to harvesting, the number of inflorescences per plant, flowers per inflorescence, capsules per plant, and seeds per capsule were recorded. Seed counts were performed under a dissecting microscope. For the biomass measurement, plants were carefully dug out from the pots and excised roots were thoroughly washed with tap water until all attached soil particles were removed. Root length was measured using a plastic ruler. Roots, stems, leaves and inflorescences were separated, dried (at 60°C) and weighed to the nearest 0.01 mg.

Seed germination

Seeds produced by *D. indica* cultivated with access to prey and those from the prey exclusion treatment were collected, respectively, and germinated in the aforementioned plastic pots, with 100 seeds per pot, four pots for each treatment, filled with peat (Klasmann Deilmann mbH, Geeste, Germany). To maintain constant soil moisture, pots containing seeds were placed into a tray (length x width x height = 43 cm × 33cm × 11 cm) filled with tap water to 6 cm depth. The tray, containing 8 pots of germinating seeds, was then transferred to a growth chamber controlled at a relative humidity of 70%, a 12 h/12 h light/dark cycle and photosynthetic photon flux density of 100 - 200 μmol m^{-2} s^{-1} at light period, and temperature regimes of 25/20 °C during light/dark cycle, respectively. Seed germination was recorded every 2–3 days, and cumulative germination percentage was calculated as the number of seeds germinated divided by 100. Seeds were considered germinated when roots were visible to the naked eye.

The maximum germination percentage (seed germinated/100 seeds sowed) and time required for 50% of maximum germination (T_{50}) were calculated using the following equation. $G = G_{max}/\{1+\exp [- (T - T_{50}) /G_{rate}]\}$ where G is the cumulating germination (%) at time T, G_{max} is the maximum germination (%), T_{50} is the time taken to reach 50% of the final germination, and G_{rate} indicates the slope of the germination curve.

RESULT

N contents and $\delta^{13}C$ and $\delta^{15}N$ for field collected samples

The mean $\delta^{13}C$ of *D. indica* was -27.2 ± 0.6 ‰ (mean ± S.D.). $\delta^{13}C$ of the non-carnivorous neighboring plants, dominated by graminoids, could be divided into two groups, one -12 ~ -11 ‰ and the other -32 ~ -30 ‰ (Supplementary Table S1).

$\delta^{15}N$ of the non-carnivorous neighboring plants varied from -1.5 to -3.1 ‰, whereas those of potential prey ranged from 1.2 to 6.5 ‰ (Supplementary Tables S1, S2). Significant differences in $\delta^{15}N$ values and nitrogen content were detected between neighboring plants and *D. indica* (Fig 2). Leaves of *D. indica* were $\delta^{15}N$ enriched and contained higher N than those of non-carnivorous neighboring plants.

For field-grown *D. indica*, the mean proportion of total leaf N obtained from prey was 57% calculated using the mean $\delta^{15}N$ values of non-carnivorous neighboring plants (-2.33 ‰), *D. indica* (1.14 ‰) and prey (3.73 ‰) (Fig. 2).

Effect of the prey availability on cultivated *D. indica*

In comparisons between cultivated *D. indica* plants with and without prey access, plants with prey access (PA plants) had significantly higher leaf chlorophyll and N contents and more positive $\delta^{15}N$ values than those with prey exclusion (PE plants) (Table 1). In contrast, mature leaves of PA and PE plants had similar Fv/Fm (Table 1) (measured on dark adapted leaves). No significant differences were detected between leaves of PA and PE plants in Φ_{PSII}, measured at the same time during the light period, except the values at 10 am (Fig 3).

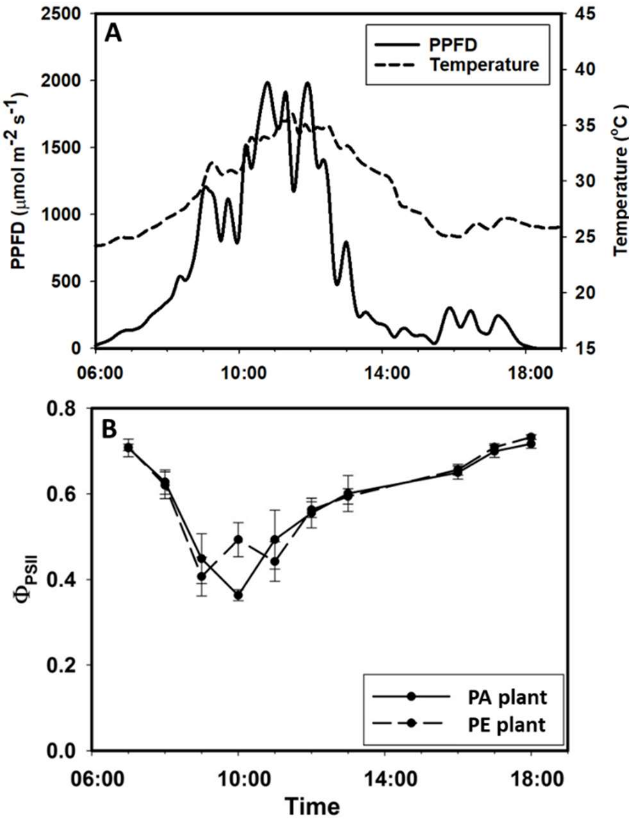

Fig. 3. Diurnal changes in photosynthetic photon flux density (PPFD) and air temperature (Fig. 3A) during the measurement of the effective quantum yield of PSII (Φ_{PSII}) (Fig. 3B) of *Drosera indica* cultivated under treatments of prey available (PA) and prey excluded (PE).

Comparisons between mature and senescent leaves revealed significant reductions in leaf nitrogen content in senescent leaves (Table 1). The reduction in leaf N was significantly higher in leaves of PA plants than in those of PE plants. Consequently, leaves of PA plants had significantly higher N reutilization efficiency than leaves of PE plants.

At harvest, PE plants were significantly taller than PA plants. However, no significant difference in root length, shoot, root and total biomass (Table 2), and the number of inflorescences per plant were detected between PE and PA plants (Fig 4). In partitioning of total biomass to different organs, *D. indica* had allocated the most biomass to leaf and the least to roots (Table 2). In comparison between PE and PA plants, PA plants allocated significantly a higher percentage of biomass to inflorescence but a lower percentage to leaf than PE plants.

No significant difference was observed between PE and PA plants in the average number of inflorescence produced by each plant (Fig 4). However, PA plants produced significantly more flowers per inflorescence, more capsules per plant, more seeds within each capsule and total number of seeds for each plant than PE plants (Fig 4).

Table 1. The potential quantum yield of PSII (Fv/Fm), total chlorophyll content ([Chl], mg g^{-1} dry weight), carbon ([C]) and nitrogen ([N]) contents, stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope ratio of mature and senescent leaves collected from ***Drosera indica*** cultivated under treatments of prey available (PA) and prey excluded (PE), and the calculated leaf level N reutilization efficiency (mean ± S.D., n = 5). Values with different letters indicate significant difference between treatments.

Mature leaf	PA plant	PE plant
Fv/Fm	0.75±0.02	0.75±0.01
[Chl]	2.23±0.19[a]	1.51±0.13[b]
[C] (%)	42.59±0.50	41.92±1.88
δ13C (‰)	-28.84±0.57	-29.37±0.27
[N] (%)	2.16±0.20[a]	1.52±0.09[b]
δ15N (‰)	1.99±0.56[a]	-0.14±0.98[b]
Senescent leaf		
[C] (%)	44.24±1.91	43.93±2.22
[N] (%)	1.34±0.28	1.20±0.07
N reutilization (%)	54.2±7.7[a]	42.7±4.2[b]

Table 2. Shoot height (length) and root length, dry mass of leaf, stem, root and inflorescence, total biomass (TB), and biomass partitioning (ratio) into leaf, stem, root and inflorescence of ***Drosera indica*** plants cultivated under treatments of prey available (PA) and prey excluded (PE). (mean ± S.D., n = 6, pair t-test, $p < 0.05$). Values with different letters indicate significant differences between treatments.

Length (cm):	PA plant	PE plant
Shoot	15.28±1.99[b]	22.27±5.56[a]
Root	5.83±1.70	6.37±0.95
Dry mass (mg):		
Shoot	304.80±65.00	316.90±86.29
Leaf	135.53±36.89	175.57±56.09
Stem	68.90±11.26	73.63±10.01
Inflorescence	100.37±26.56	67.7±36.27
Root	17.30±1.76	21.09±7.40
Total biomass (TB):	322.10±66.12	337.99±91.98
Biomass partitioning (%)		
Leaf / TB	41.70±5.20[b]	51.17±7.99[a]
Stem / TB	21.66±1.96	23.67±10.11
Root / TB	5.54±1.07	6.14±1.66
Inflorescence / TB	31.10±3.98[a]	19.01±5.75[b]

Seeds started germinating ca. 20 days after sowing and about 90% of the seeds were germinated 60 days after sowing (Fig 5). No significant difference was found in the final cumulative germination percentage between seeds produced by PE and PA plants (Fig 6). However, seeds of PA plants had a faster germination rate, the time (day) for 50% of the germinating seed population to germinate (T_{50}), than those of PE plants (Fig 6).

DISCUSSION

This study demonstrated a significant effect of prey capture on the nutrient status of *D. indica* growing in the field and in cultivated plants with access to prey in the experimental farm. The implications of these results are discussed below.

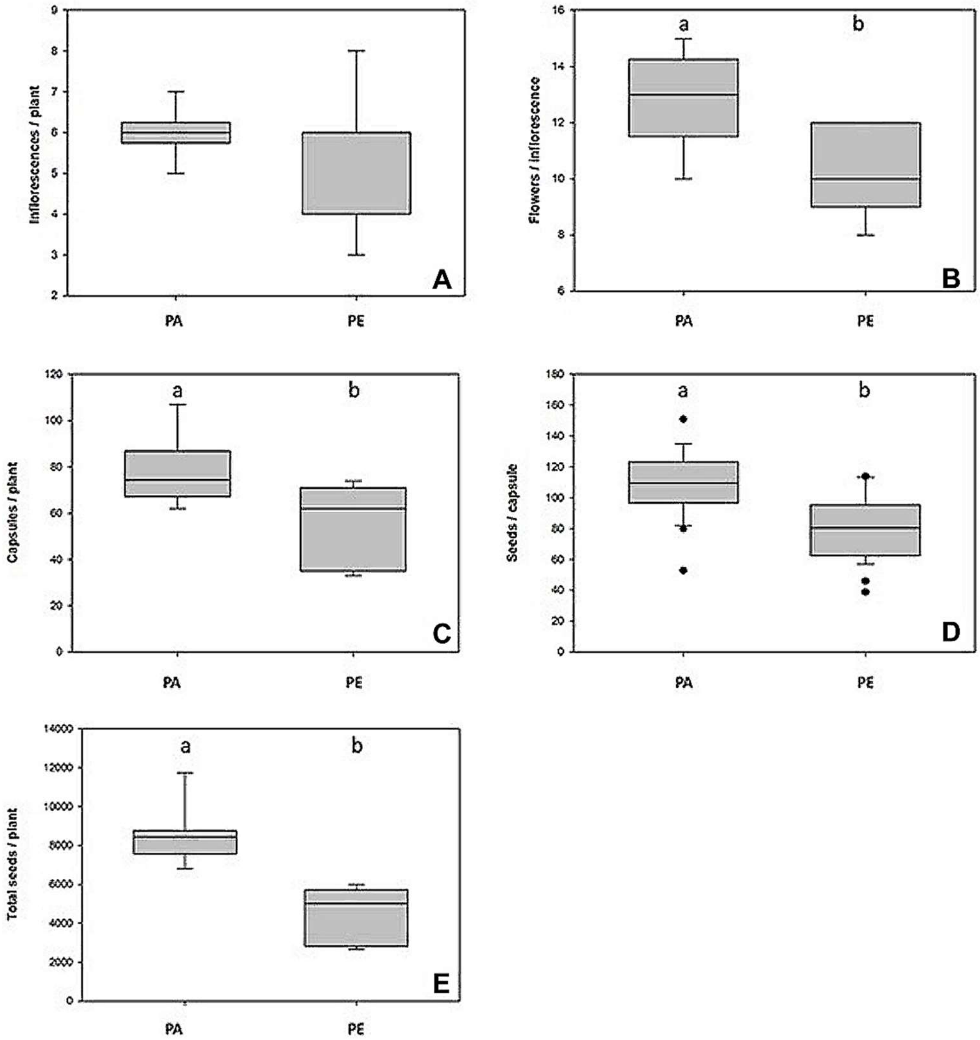

Fig.4. A summary of reproduction output, including the mean number of inflorescence produced by each plant (Fig. 4A), the number of flower on each of the inflorescence (Fig. 4B), total number of capsules produced by each plant (Fig. 4C), the number of seeds in each capsule (for these parameters n = 26 in PA, n = 32 in PE) (Fig. 4D), and the total number of seeds produced by each plant (Fig. 4E) (n = 6 in PA, n = 7 in PE) of ***Drosera indica*** cultivated under treatments of prey available (PA) and prey excluded (PE). Values with different letters indicate significant differences between treatments. (One Way ANOVA, $p < 0.05$)

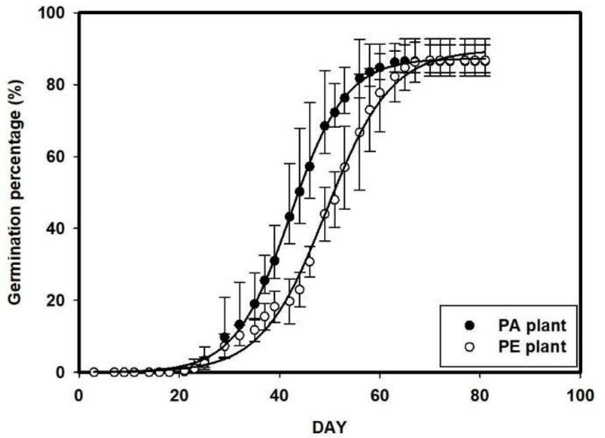

Fig. 5. Cumulative germination percentage of seeds collected from ***Drosera indica*** plants cultivated under treatments of prey available (PA plant) and prey excluded (PE plant).

Nutritional benefit from prey capture in the field

Measurements of stable N isotopes in field samples revealed that *D. indica* obtained a substantial portion of its leaf N from captured prey, suggesting that prey was an important source of the supplementary N for the plant growing in the wetland. Ellison (2006) compiled data from references and reported that leaf N contents in carnivorous plant are generally lower than those in non-carnivorous plants. In contrast to these compiled data (Ellison, 2006), leaf N content of field-growing *D. indica* (25 mg g^{-1}) was significantly much higher than that of its neighboring plants (Fig. 2) and that of the median value of literature data for carnivorous plants (= 12.6 mg g^{-1}, n = 28). Nitrogen is often regarded as a limiting factor to plant growth in various ecosystems. Thus, the ability to acquire nutrient from captured prey might confer *D. indica* a higher leaf N

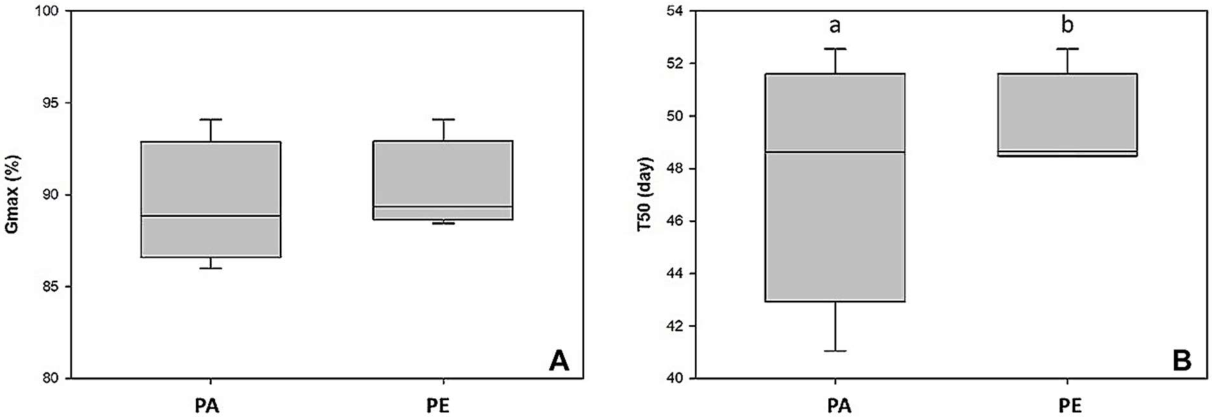

Fig. 6. Germination percentage (G_{max}) (Fig. 6A) and the time (day) for 50% of the germinating seed population to germinate (T_{50}) (Fig. 6B) of ***D. indica*** seeds sowed under treatments of prey available (PA) and prey excluded (PE). Values (means ± standard deviations, n = 4) with different letters indicate significant differences between treatments. (One Way ANOVA, $p < 0.05$).

content and consequently improved growth in the wetland. The cultivation experiment also revealed that access to prey could increase reproductive output of the *D. indica*. Our calculation of prey N contribution to the leaf total N of *D. indica* growing in natural habitat is consistent with previous in situ estimates of approximately 50% for erect *Drosera* spp. in Australia (Schulze *et al*., 1991) and the mean proportion of N derived from insects in three British populations of *D. rotundifolia* (Millett *et al*., 2003). The results indicate that prey was clearly a highly significant source of N nutrition for *D. indica* plants in its natural habitat even though they were highly shaded by neighboring plants (Huang and Kao, 2021). Most of carnivorous plants possess a weakly developed root system (Lüttge, 1983; Juniper *et al*., 1989). The strong dependence on prey-derived nitrogen also suggests that root-derived nitrogen is limited for *D. indica* growing in the wetland, potentially due to the low availability of soil nitrogen and/or the limited nitrogen uptake capacity of its small root system (Table 2). Regardless of the limiting factor, foliar nutrient acquisition from prey not only compensates for the plant's restricted capacity to absorb nitrogen from soil but also confers a significantly higher leaf nitrogen content than that of its neighboring plants.

The stable carbon isotope value ($\delta^{13}C$) of both field-growing (Supplementary Table S1) and cultivated *D. indica* plants indicate that *D. indica* is a C3 plant. One potential benefit of carnivory might be the partial replacement of autotrophy by heterotrophy as a source of chemical energy (Givnish *et al*., 1984). Is there an indication of *D. indica* might direct uptake of organic substance from the capture prey as a partial substitute of photosynthesis? Compared with the non-carnivorous neighboring C3 plants, *D. indica* exhibited enriched $\delta^{13}C$ value. In addition, farm cultivated *D. indica* with access to prey also had a higher $\delta^{13}C$ than that with prey exclusion (Table S1). A more positive $\delta^{13}C$ in C3 plants may result from increased CO_2 diffusion limitation during photosynthesis (Farquhar *et al*., 1982) and/or from uptake of organic substance from captured prey (as insect generally has more enriched ^{13}C). Without measuring gas exchange, we cannot exclude the possibility that *D. indica* directly uptake of organic substances from the capture prey as a partial substitute for photosynthetic carbon uptake.

Carnivory is not indispensable but enhances sexual reproduction for cultivated *D. indica*

The observation that viable seeds were produced by cultivated *D. indica* without access to prey demonstrates that *D. indica* is not an obligate insectivore. Nevertheless, carnivory confer certain benefits to cultivated *D. indica* (Fig 4).

Leaves of cultivated *D. indica* plants with access to prey (PA plants) had significantly higher $\delta^{15}N$ values than plants with prey exclusion (PE plants) (Table 1) suggesting that PA plants gained significant proportion of their leaf total N from captured prey, based on the theory of trophic isotopic enrichment (DeNiro and Epstein, 1976; Boecklen *et al*., 2011). Accordingly, the additional leaf N content (6.4 mg g^{-1}) gained in PA plants (Table 1) may have been acquired directly through foliar uptake of nitrogen and/or via stimulation of root nutrient uptake by foliar mineral nutrient supply (Hanslin and Karlsson, 1996; Adamec 1997, 2002). In addition to the increases in leaf N content, chlorophyll content also increased in response to prey availability. In contrast, the photosynthetic activity, the potential and the effective quantum yield of PSII, of cultivated *D. indica* was not affected by prey availability. The failure of the enhancement of prey capture on photosynthetic activity indicates that photosynthetic capacity in leaves of PE plants was not limited by N availability, likely due to the effect of fertilization before the prey availability treatment. For example, laboratory studies and in-situ experimental studies of carnivorous plant nutrition have shown that prey N is less valuable for nutrient replete plants (Ellison, 2006). However, contrasting results of the effect of carnivory on photosynthetic activity have been reported among *Drosera* spp. (Mendez and Karlsson, 1999; Pavlovič *et al*., 2014). Because foliar uptake not only N but also P nutrients from prey (Millet *et al*., 2015), Pavlovic *et. al*. (2014) attributed the improved photosynthetic performance in *D. capensis* to the alleviation of P-limitation by feeding on fruit flies

An increase in the photosynthetic rate in leaves is considered a prerequisite for faster plant growth in carnivorous plants in response to prey availability (Givnish, 1984; Adamec *et al*., 2021). In this study, because PE and PA plants exhibited similar photosynthetic activity and leaf biomass, consequently, no significant difference was found in the total biomass accumulated between *D. indica* plants cultivated with and without access to prey. In contrast to the no effect on total biomass, prey availability had a significant influence on pattern of biomass allocation in cultivated *D. indica*, in which biomass allocation to leaves was significantly reduced but that to inflorescence increased in response to prey availability (Table 2). Through the increases in the number of flowers on each inflorescence and the number of seeds within each capsule, the total number of seeds produced by each of cultivated PA plants was about two fold of that of PE plants. Thus, direct contribution of the absorbed nutrients from prey to sexual reproduction (apart from photosynthetic efficiency) seems to operate in *D. indica*. The stimulation of reproduction by prey availability was also reported in *D. filiformis* and *D. rotundifolia* (Kraft and Handel, 1991). Dixon *et al*. (1980) reported that *D. erythrorhiza* did not allocate insect N equally throughout the plants. Millet *et al*. (2003) found that flowers contain higher proportion of prey derived N than leaves and roots of *D. rotundifolia*. Accordingly, *D. indica* might allocate a higher proportion of the nutrients derived from prey to reproduction organ than to vegetative organs, thereby enhancing flower and seed production.

Interestingly, seeds produced by *D. indica* cultivated with access to prey exhibited a faster germination rate than those from plants under prey exclusion. Seed size is one of the key factors influencing the germination rate. However, we did not determine the mass of individual seeds produced by the cultivated *D. indica* owing to the light weight of its seeds.

Cultivated *D. indica* reutilized ca. 50% of N from senescing leaves (Table 1), which is the median value for N resorption in plants of all growth forms (Aerts and Chapin, 1999) but is much lower than what were reported in *D. capensis* (82%), *D. peltata* (77.8%) and *D. scorpioides* (69.8%) (Adamec, 2002). Root systems and plant nutrient status have been suggested as important factors determining the extent of N resorption. For example, Adamec (1997) proposed that carnivorous plants, due to their relatively small root system, should re-utilize mineral nutrients more efficiently than accompanying non-carnivorous plants with a greater root system, as an adaptation to low soil mineral content. In comparison to other carnivorous plants, *D. indica* has a rather low biomass allocation to root. Its root/total biomass ratio was ca. 6%, which is within the range found in various carnivorous plants (3.4% to 23%) (Karlsson and Carlsson, 1984; Karlsson and Pate, 1992; Adamec *et al*., 2002). Thus, root system cannot explain the lower efficiency of N resorption found in *D. indica*. Several studies reported that the proportion of nutrients resorbed from senescing leaves decreases as plant nutrient status improves (Aerts *et al*., 1999; Adamec, 2002). The cultivated *D. indica* indeed had higher leaf N content than most of other carnivorous plants. However, the result that PA plants exhibited a higher N re-utilization efficiency despite exhibiting higher leaf N content than PE plants implies that nutrient status might not be the factor affecting the N reutilization efficiency of the species. In summary, some undefined factors appear to be more important than leaf N concentration and the proportion of root biomass in explaining the low N re-utilization and the treatment differences in N reutilization efficiency in the cultivated *D. indica*.

The result that cultivated *D. indica* with access to prey had significantly higher leaf-level N reutilization efficiency than that with prey exclusion suggests that carnivory might stimulate the N reutilization in *D. indica*. This possibility warrants future investigation.

CONCLUSION

The beneficial effects of prey capture on nutrient status and sexual reproduction of *D. indica* were demonstrated in this study. Field-grown *D. indica* obtained ca. 57% of its leaf N from captured prey, indicating that prey was a highly significant source of N nutrition for the carnivorous plant in its natural habitat. Cultivated experiments showed that the growth and reproduction of *D. indica* is not absolutely dependent on the acquisition of N from prey, rather the additional prey-derived N significantly enhances sexual reproduction through increases in flower number and seed production.

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