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Effects of inorganic nitrogen availability on the sporophytes of *Acrostichum aureum* L.

R.S. PILLAI and B.-L. ONG*

Department of Biological Sciences, The National University of Singapore, Lower Kent Ridge Road, Singapore 119260, Republic of Singapore

Abstract

Plants grown at low irradiance were fertilized with 0, 60, and 600 g m^{-3} NH_4NO_3 once every fortnight. Plants treated with high N concentrations showed an increased growth, producing longer and broader fronds with larger areas, and were darker green in colour. Nitrogen also increased the content of chlorophyll (Chl) and carotenoids per leaf area unit. Different N treatments did not affect the photosynthetic efficiency of photosystem 2, as reflected by the high values of Chl fluorescence kinetics F_v/F_m, ranging between 0.81 to 0.84, and F_v/F_0 of 4.30 to 5.10. An increase in photochemical quenching (q_P), accompanied by a decrease in non-photochemical quenching (q_N), was observed in sporophytes fertilized with increased concentrations of NH_4NO_3. Nitrogen availability allowed sporophytes of *Acrostichum aureum* to become more established under natural conditions.

Additional key words: chlorophyll fluorescence; fern; fertilization; growth; photosynthesis.

Introduction

The effects of N fertilization are, in general, promotive on both plant growth and development. Nitrogen deficiency could result in a decrease of vegetative and reproductive growth (*e.g.*, Hocking 1995, Ranjith and Meinzer 1997), reduced photosynthetic capacity and lowered contents of photosynthetic pigments (Evans and

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*Author for correspondence; fax: (+65)-874-2852, e-mail: dbsongbl@nus.edu.sg

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Abbreviations: $\Delta F/F_m'$, photochemical efficiency of photosystem 2 in light-adapted state; $\Delta F/F_m' \times$PPFD, relative electron transport rate at steady state; F_0, minimal chlorophyll fluorescence yield; F_m, maximal chlorophyll fluorescence yield; F_v, variable yield of chlorophyll fluorescence; F_v/F_m, potential photochemical efficiency of photosystem 2 in dark-adapted state; F_v/F_0, variable fluorescence ratio; PPFD, photosynthetic photon flux density; PS, photosystem; Q_A primary quinone electron acceptor of PS2; q_P, photochemical quenching; q_N, nonphotochemical quenching; $1-q_P$, indicator of the pool size of reduced Q_A.

Terashima 1988, Bowman 1991). Nitrogen may also affect responses of plants to different abiotic stress factors. Ramalho *et al.* (1997) showed that the capacity to cope with high irradiance increased in coffee plants fertilized with nitrogen. Nitrate effectively alleviates chloride damage in citrus orchards irrigated with high-chloride containing water (Bar *et al.* 1996).

Acrostichum aureum L. is a mangrove fern found near the landward-side of Southeast Asian mangroves. In general, mangrove sediments are rich in nitrogen, phosphorus, and potassium. The amounts of these elements vary from place to place (Kathiresan *et al.* 1994, Li 1997). The concentration of nutrients in the sediment declines with the distance from the sea (Li 1997). The aim of the present study was to determine the effects of nitrogen on growth and photosynthesis of the sporophytes of *A. aureum*. Such studies would definitely enhance our understanding of the physiology of mangrove plants and, more importantly, allow us to understand how N availability affects the establishment of *A. aureum* in nature.

Materials and methods

Sporophytes of *A. aureum* were raised from spores and allowed to grow to the stage when two to three juvenile fronds were produced; the plants were about 6-7 cm tall. They were then transplanted into vermiculite-containing pots (diameter 13 cm, height 10.4 cm, volume 850 cm^3), and were left in the shade in open air [with photosynthetic photon flux density (PPFD) of 100 $\mu mol\ m^{-2}\ s^{-1}$ at mid-day]. The inorganic N source was supplied in the form of NH_4NO_3. 0 (0N), 60 (LN), and 600 (HN) $g(NH_4NO_3)\ m^{-3}$ were dissolved in modified N-free 10 % Hoagland solution (Hoagland and Arnon 1950) containing 200 μM $MgSO_4$, 100 μM KH_2PO_4, 500 μM KCl, 500 μM $CaCl_2$, 500 μg Fe EDTA; 0.1 cm^3 of micronutrient solution was added to 1000 cm^3 of this solution. The micronutrient stock solution contained 2.86 g H_3BO_3, 1.81 g $MnCl_2 \times 4\ H_2O$, 0.22 g $ZnSO_4 \times 7\ H_2O$, 0.08 g $CuSO_4 \times 5\ H_2O$, 0.025 g $Na_2MoO_4 \times 2\ H_2O$ per 1000 cm^3.

Sporophytes were irrigated with tap water everyday and were supplied with 100 cm^3 of 0N, LN, and HN solutions [or 0.0, 0.1 and 1.0 $g(NH_4NO_3)$ per kg of rooting medium] once a fortnight. After three months of fertilization, experiments were conducted with newly matured fronds. Length, breadth, and area of mature fronds, and plant height above soil level were determined. Chlorophylls and carotenoids were extracted with 100 % (v/v) acetone and their concentrations were determined spectrophotometrically at 460, 645, and 663 nm according to Arnon (1949) and Embry and Nothnagel (1988). Fronds of the sporophytes were also collected and oven dried at 80 °C for a week. Areal leaf mass (ALM) was expressed as $g(d.m.)\ m^{-2}$(frond area).

Undetached, newly matured fronds were used for Chl fluorescence studies with the Fluorescence Monitoring System II (*FMSII, Hansatech*, Norfolk, U.K). Preliminary experiments were done to optimize the duration of dark adaptation of these fronds. Fluorescence parameters did not differ for the dark adaptation varying from 15 min to 2 h (values not shown). However, we darkened the fronds for 30 min to ensure complete oxidation of photosystem 2 (PS2).

Chl fluorescence parameters were determined as follows: initial Chl fluorescence yield, F_0, was measured at a PPFD lower than 0.1 μmol m^{-2} s^{-1} after 30 min of dark adaptation. Maximum Chl fluorescence yield, F_m, was determined by giving a 700 ms saturation pulse of "white light" at 10 000 μmol m^{-2} s^{-1}. After this, actinic radiation at 32 μmol m^{-2} s^{-1} was switched on. Maximum Chl fluorescence yield in the light-adapted state, F_m', was determined by applying a 700 ms saturation pulse (10 000 μmol m^{-2} s^{-1}) at every 60 s interval till the steady state was reached (usually about 30 min). Minimal Chl fluorescence yield in the light-adapted state, F_0', was determined by turning on the far-red radiation at the end of 30 min. Photochemical (q_P) and non-photochemical (q_N and NPQ) quenchings were calculated according to Schreiber *et al.* (1994).

All experiments were replicated five times and results are presented as means ± S.E. (standard error). A least significant difference test (Fisher's LSD at $p \leq 0.05$) was used to test the effect of nitrogen on the different physiological parameters determined.

Results and discussion

Sporophytes fertilized with various concentrations of NH_4NO_3 showed obvious differences in plant growth and photochemistry of PS2. Fronds of sporophytes treated with 0N and LN were pale green, while those of plants supplied with HN had very green fronds. Fronds of plants supplied with 0N and LN also showed earlier senescence of matured fronds. This phenomenon was found also in *Beta vulgaris* (Nevins and Loomis 1970) and *Spinacia oleracea* (Evans and Terashima 1988). Fronds of plants fertilized with HN remained green and were attached to the sporophytes for longer periods. Petiole length of these fronds was double that of other treatments (values not shown).

Table 1. Effects of NH_4NO_3 (0N, LN, HN = 0, 60, 600 g m^{-3}) on growth and contents of photosynthetic pigments of sporophytes of *Acrostichum aureum* L. ALM = areal leaf mass of fronds. Means in the same row followed by the same letter do not differ significantly at the 0.05 level of probability. Numbers in brackets indicate average percent change.

Parameter	0N	LN	HN
Frond length [cm]	14.89 ± 0.76^a (100 %)	17.23 ± 0.71^{ab} (115 %)	19.67 ± 1.31^b (132 %)
Frond breadth [cm]	1.86 ± 0.08^a (100 %)	2.34 ± 0.10^b (125 %)	2.40 ± 0.11^b (129%)
Frond area [cm^2]	52.56 ± 3.27^a (100)	66.37 ± 3.08^{ab} (126%)	74.31 ± 7.60^b (141%)
ALM [g m^{-2}]	39.69 ± 1.07^a (100%)	42.65 ± 1.75^a (107%)	49.57 ± 0.29^b (125 %)
Plant height [cm]	18.35 ± 1.33^a (100 %)	21.93 ± 1.27^{ab} (119 %)	26.38 ± 0.07^b (143 %)
Chlorophyll (*a*+*b*) [mg m^{-2}]	453.98 ± 29.17^a (100 %)	473.57 ± 39.88^a (104 %)	485.97 ± 37.52^a (107 %)
Carotenoids [mg m^{-2}]	74.14 ± 4.88^a (100 %)	73.07 ± 3.73^a (98 %)	106.72 ± 18.26^b (144 %)
Chl/Carotenoids	6.13 ± 0.02^b	5.94 ± 0.33^b	4.55 ± 0.04^a

Frond length, breadth and area, and plant height increased with higher amounts of inorganic N supplied (Table 1, Fig. 1). When the sporophytes were fertilized with HN, frond length, frond area, and plant height increased by 32, 41, and 43 %, respectively (Table 1). Nitrogen fertilization also increased leaf length in *Helianthus annuus* (Radin and Boyer 1982) and leaf area in *Trifolium subterraneum* (Bouman 1970), *Diplacus aurantiacus* (Gulmon and Chu 1981), flag leaves of *Triticum aestivum* (Evans 1983) and *Zea mays* (Costa 1991). Unlike Robinson (1997), who did not observe any change in ALM upon N fertilization of *Spinacia oleracea*, the availability of N increased the ALM of sporophytes from 39.69 to 49.57 g m^{-2}. The ALM increase was likely due to an increase in the photosynthetic CO_2 assimilation capacity of the fronds (Jurik 1986). This higher CO_2 assimilation could be reflected by higher values of relative (non-cyclic) electron transport rate, indicated by $\Delta F/F_m' \times PPFD$ (Schreiber *et al.* 1994) where PPFD refers to the growth irradiance. Since all plants were grown under the same irradiance, the rate of electron transport depended on the value of $\Delta F/F_m'$ (Table 2), the effective quantum yield of PS2. The increase in leaf area and ALM of the fronds correlated well with the changes in $\Delta F/F_m'$ (Fig. 2).

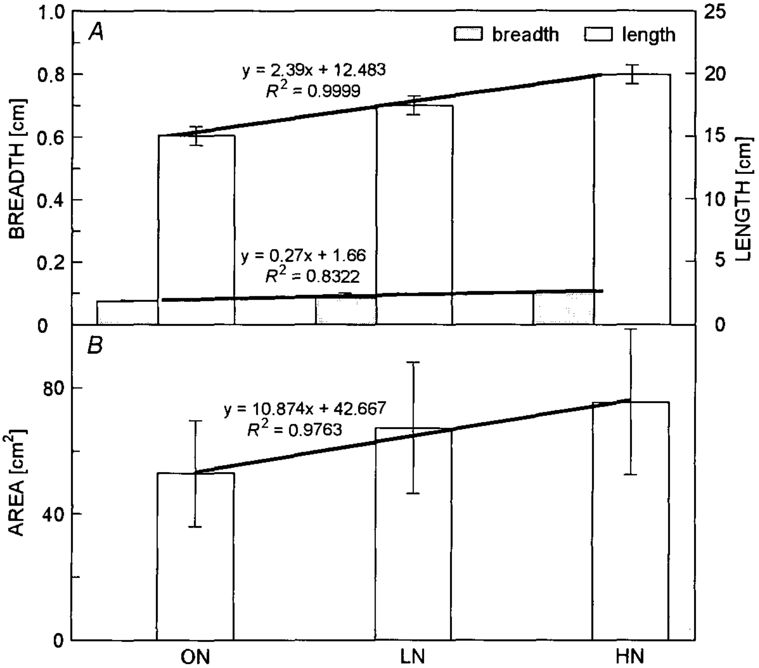

Fig. 1. Effects of nitrogen fertilization on frond length and breadth (*A*) and frond area (*B*). 0N, LN, HN = 0, 60, 600 $g(NH_4NO_3)\ m^{-3}$.

The darker green colour of fronds of sporophytes fertilized with HN was due to an insignificant increased amount of Chls per leaf area unit (Table 1). An increase in total Chl content with increasing nitrogen, under low irradiance, was also observed in leaves of *Flindersia brayleyana* (Thompson *et al.* 1988) and *Hordeum vulgare* (Hák *et al.* 1993). A linear correlation was found between Chl content and the amount of nitrogen supplied to *Lactuca sativa* (Fontes *et al.* 1997). This was not observed in our study. An increase in Chl content leads to increases in plant growth and dry matter accumulation (Evans 1983, Costa 1991); this link was also observed in our study.

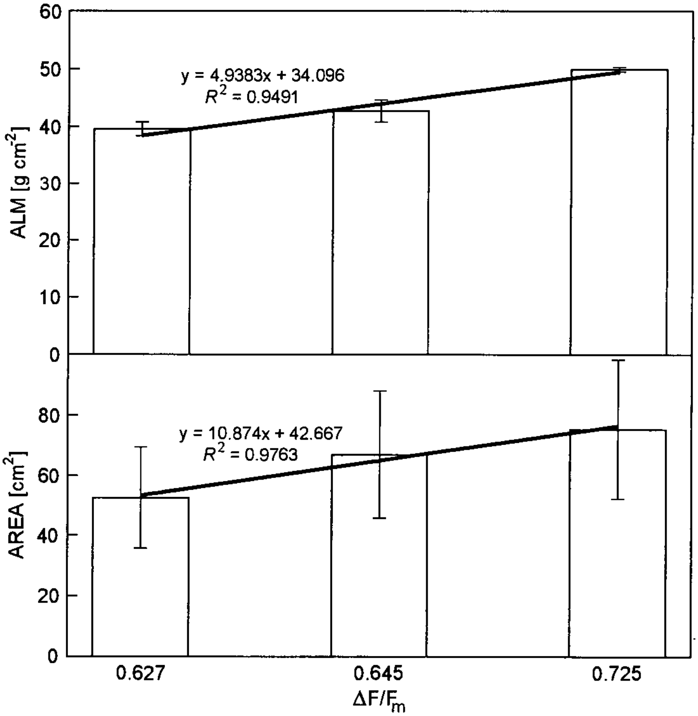

Fig. 2. Correlation of frond ALM (*A*) and area (*B*) with $\Delta F/F_m'$ of nitrogen supplied *A. aureum*. For details see Fig. 1.

Table 2. Chlorophyll fluorescence parameters of sporophytic fronds of *Acrostichum aureum* grown under different concentrations of nitrogen (0N, LN, HN = 0, 60, 600 g m^{-3} NH_4NO_3). Means in the same row followed by the same letter do not differ significantly at the 0.05 level of probability.

Fluorescence parameter	0N	LN	HN
F_0	250±6^a	262±7^a	326±55^a
F_m	1427±55^{ab}	1392±27^a	1979±313^b
F_v	1176±55^a	1130±20^a	1653±258^b
F_v/F_m	0.83±0.01^{ab}	0.81±0.00^a	0.84±0.00^b
F_v/F_0	4.72±0.25^{ab}	4.32±0.08^a	5.11±0.08^b
q_P	0.94±0.01^a	0.94±0.01^a	0.96±0.00^b
q_N	0.44±0.02^b	0.39±0.03^{ab}	0.31±0.02^a
NPQ	0.53±0.04^b	0.45±0.05^{ab}	0.35±0.04^a
$1 - q_P$	0.06±0.01^b	0.06±0.01^b	0.04±0.00^a
$\Delta F/F_m'$	0.63±0.02^a	0.65±0.02^a	0.73±0.00^b

Under natural conditions, sun plants contain higher amounts of carotenoids for the protection of PS2 against photooxidation by high irradiance (Björkman *et al.* 1988, Demmig-Adams 1998). Content of total carotenoids increased in sporophytes under HN, leading to a decrease in Chl/carotenoid ratio from 6.13 to 4.55 (Table 1); such an increase in carotenoid content might have aided the dissipation of excess radiant energy not used by photochemistry (Demmig-Adams 1998), thus decreasing the value of q_N (Tables 1 and 2). These values of Chl/carotenoids were within the range normal for green plant tissues (Schindler *et al.* 1994).

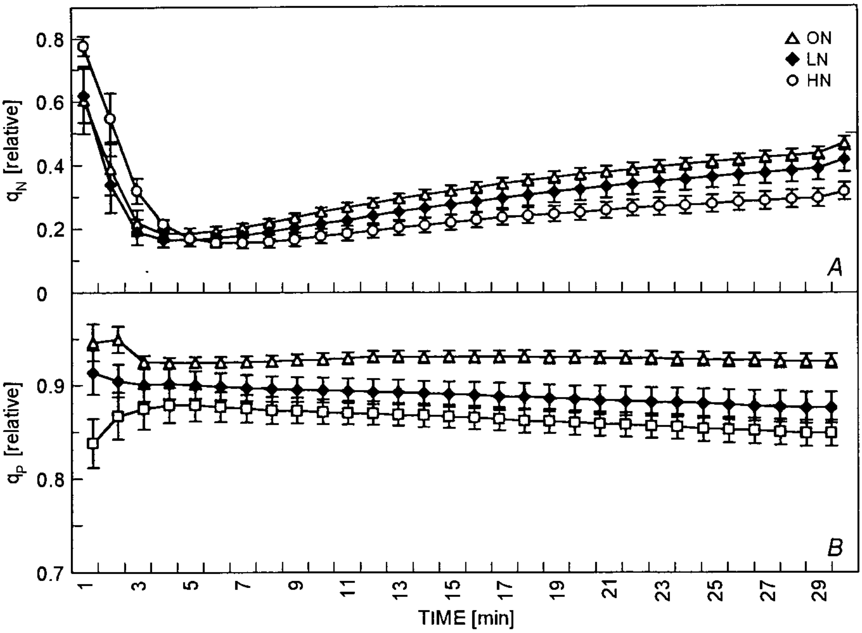

Fig. 3. Relative nonphotochemical (q_N) and photochemical (q_P) quenching of different concentrations of nitrogen-treated sporophytes of *A. aureum*. For details see Fig. 1.

The potential efficiency of PS2 (F_v/F_m) of the sporophytic fronds did not vary with the inorganic N available to the plants (Table 2); F_v/F_m remained high, between 0.81 and 0.84. Values of F_0 and F_m increased with inorganic N fed to the sporophytes especially at HN (Table 2). The effective efficiency of PS2 ($\Delta F/F_m'$) under light-adapted conditions increased with increased N availability and was highest in plants supplied with HN (Table 2). According to Babani and Lichtenthaler (1996), the variable Chl fluorescence ratio F_v/F_0 is a much better indicator of changes in the photosynthetic quantum conversion than the F_v/F_m ratio. In our study, F_v/F_0 of the sporophytes treated with different concentrations of inorganic N differed significantly (Table 2). The high values of F_v/F_0 indicated that the photosynthetic apparatus was functional in all three N treatments. The q_P was almost similar for all treatments, but nonphotochemical quenching (q_N and NPQ) decreased with increased amounts of N applied to the plants (Table 2, Fig. 3). Most of the energy generated by photosynthesis is utilized not only for the assimilation of carbon, but also for the assimilation of nitrogen. Some plants could even utilize up to 55 % of the carbon fixed in nitrogen assimilation and metabolism (Huppe and Turpin 1994). Also, increased CO_2 reduction in the presence of increased N has been correlated to a higher content of ribulose-1,5-bisphosphate carboxylase (Karvou and Maranville 1993). The increased metabolic activities and the increased assimilation of N into proteins could have increased the demand of ATP by the sporophytes of *A. aureum*. This could lead to a decrease in the transmembrane pH gradient in the thylakoids and, thus, less energy was dissipated as heat (decreasing nonphotochemical quenching) in N-fertilized sporophytes. Decreased nonphotochemical quenching led to lower $1\text{-}q_P$ values, especially in HN-treated sporophytes (Table 2); $1\text{-}q_P$ indicated the pool size of reduced Q_A and the level of excitation pressure (Schreiber *et al.* 1994). Such a decrease in $1\text{-}q_P$ with increased N availability was also reported in eight other plant species by Demmig and Winter (1988).

The amounts of N supplied to the sporophytes were fairly low in our study which showed that *A. aureum* could grow in soils poor in N supply and that an increase in N availability resulted in increased vegetative growth and photosynthetic capacity in the sporophytes. The availability of low amounts of N would definitely allow sporophytes of *A. aureum* to become more established in their natural habitats.

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