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# ECOPHYSIOLOGICAL AND GENETIC CHARACTERISTICS OF THREE WETLAND PLANT SPECIES OCCURRING IN LAKE VICTORIA REGION IN KENYA

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**ABSTRACT**

This study was carried out to investigate the ecophysiological and genetic characteristics of some selected wetland plant species in Lake Victoria basin, Kenya. Seedlings of *Cyperus esculentus* L., *Aframomum angustifolium* (Sonn.) and *Phragmites australis* (Cav.) Trin. Ex Steudel) were grown outdoors in pots. Plants were provided with four nutrient dosage levels of 0 mg [no fertilizer added], 50 mg, 100 mg, 150 mg fertilizer [N: P: K, 10:26:10] per pot and replicated five times. Data on Leaf area, number of tillers per pot, Leaf chlorophyll concentration, Gas exchange parameters and chlorophyll fluorescence parameters were determined. Separation of means was carried out to compare nutrient treatments and species. Increasing nutrient availability significantly ($P \leq 0.05$) influenced most of the morphological and physiological parameters investigated. *C. esculentus* had significantly greater leaf area compared to *A. angustifolium* and *P. australis*. Number of tillers per pot was significantly increased in *C. esculentus* than in all other species, suggesting that this species was greatly influenced by nutrient availability than the other species. *P. australis* had significantly higher chlorophyll a and b contents and carotenoids content compared to *C. esculentus* and *A. angustifolium*. *C. esculentus* showed a higher photosynthetic activity compared to *A. angustifolium* and *P. australis* at increasing nutrient availability. Stomatal conductance increased with increase in nutrient availability in all the three species, but interestingly, *P. australis* and *A. angustifolium* had higher stomatal conductance compared to *C. esculentus*. Transpiration rates also increased with increasing nutrient availability but *A. angustifolium* and *C. esculentus* had significantly higher transpiration rates compared to *P. australis*. Nutrient treatments at medium levels, 50 and 100mg, increased electron quantum yield of PS2 and ETR values in *A. angustifolium*, and *C. esculentus* while that of *P. australis* decreased. The study reveals *C. esculentus* as a physiologically superior species compared to *A. angustifolium* and *P. australis*.

**Keywords:** wetland plant species, Lake Victoria, growth, nutrient availability, gas exchange, chlorophyll fluorescence.

## INTRODUCTION

Many forces threaten to alter the productivity and biodiversity of the wetland landscapes. Within-stand cycling of nutrients is an important feature of wetland systems that provides a basis for understanding their nutrient status, productivity, and degree of eutrophication (Gathumbi *et al.,* 2005). Little is known about the combined effects of climate change with those human activities already influencing lakes and rivers. The increase of the content of nitrogen and phosphorus in wetlands plays a major part in problems of eutrophication (Rozema and Leendertse, 1991).

Conversion of the native rangelands into intensively managed pasture systems represents a wide spread land use change that has consequences for biogeochemical cycles, as well as the links between upland and wetland systems and the eutrophication of wetlands in these agricultural landscapes (Gathumbi *et al.,* 2005). With increased diversification in industrialization and extensive use of metal-based fertilizers, the concentrations of metal pollutants in wetlands continue to rise through natural run off. Presence of metal pollutants in wetland ecosystems is known to disturb the delicate balance of the ecosystems (Ipinmoroti, 1993).

If phosphorus is limiting, increased N deposition does not stimulate plant growth but rather reduces it (Gotelli and Ellison, 2002). Negative responses may reflect N toxicity (Lucassen *et al*., 2002) or increased P deficiency (Brouwer *et al*., 2001). These effects may vary among species within a community, so that some species are still stimulated by N, while others are reduced by competition, P-deficiency or N toxicity (Limpens *et al.*, 2003). Invasive species have devastated many of the world's wetland ecosystems (Kourtev, 2003), due to a combination of anthropogenic alteration and nutrient enrichment of wetlands.

Currently, very little effort has been focused on obtaining empirical data to determine the extent of nutrient enrichment on wetlands invasive plant species and how this might impact the management of these species in a changing global environment. Therefore, it is important to understand how invasive wetland plant species will be impacted by excess nutrients in their natural ecosystems. The growing interest in trees and forests under the influence of environmental pollution and changing global climate makes it desirable to select one or a few plant species to study more intensively (Posthumus, 1991).

Little is known of the ecophysiological and morphological traits, and genetic relationships among the wetland plant species in isolated wetlands in the Lake Victoria basin in Kenya. One way to avoid the loss of populations of wetland plant species would be to implement programs of reintroduction of the species in the ecosystem, although this is limited by the lack of

information regarding the ecophysiology, genetic variation and forestry management of the species. It is therefore important to gather more information on their ecophysiological strategies.

Some of the wetland species commonly encountered in Kenya, include papyrus (*Cyperus papyrus*, *Cyperus latifolius*) and *Typha domingensis*. Other wetland plant species include *Cyperus rotundus*, *Cyperus esculentus, Typha angustifolia*, *Phragmites australis*, and *Aframomum angustifolium*.

The common reed (*Phragmites australis)* (Cav.) Trin. Ex Steud (Poaceae) is an emergent grass found in wetlands around the world (Vasquez *et al.,* 2006). It is a shrub or perennial woody graminoid herb that grows annually from woody rhizomes to heights of 1.5 to 4 m (occasionally to 6 m). Common reed utilises the C3 photosynthetic pathway, although it's photosynthetic rates and the presence of mature chloroplasts in the bundle sheath bear resemblance to the C4 pathway (Farnsworth and Meyerson, 2003).

*Cyperus esculentus* L. is an erect perennial, grass-like sedge which belongs to the family Cyperaceae (Onovo and Ogaraku, 2007), and is usually 30-90 cm high, with long narrow dark-green leaves arranged in three rows around the triangular stem. *Cyperus esculentus* is a C4 plant (Bryson and Carter, 2004). The plant develops as a series of shoots, bulbs and stem tubers connected by brown wiry rhizomes which are strengthened by lignification of the inner cortex. Tubers are small, 1-2 cm in diameter, and are borne at intervals along the rhizomes.

Wild or Madagascar Cardamom (*Aframomum angustifolium* Sonn.) belongs to the family; Zingiberaceae. The plant is an herb with leafy stems to 1.90 m high, and flowers narrow in short inflorescences, pink or carmine; of moist shady places in hill savanna, across the Region from Guinea to N and S Nigeria (Royal botanic gardens, KEW,). The plants grow well in swampy environment (Author's personal observation).The fruits contain an acid pulp and many small brown seeds which are eaten fresh. The dried seeds are used like pepper to season food. The fruits are edible and are aphrodisiac (Kokwaro, 1976).

Wise decisions concerning these wetland plants would be difficult without a more complete understanding of their biology and ecophysiology. There is insufficient information to predict wetland ecosystem responses to climate and human induced stresses such as nutrient enrichment, which may profoundly impact them. Information is particularly lacking regarding growth, gas exchange, chlorophyll content, chlorophyll fluorescence and mineral relations, especially of the seasonal wetland plant species in Lake Victoria region in Kenya.

It was hypothesized that there are significant variations in gas exchange, growth, and chlorophyll fluorescence among the wetland plant species under changing ecological conditions induced by human activities such as excessive NPK fertilization. The main objective of this study was to investigate the ecophysiological and genetic characteristics of *Cyperus esculentus, Typha*, *Phragmites australis*, and *Aframomum angustifolium*, which are some of the dominant wetland plant species in Lake Victoria region, Kenya, by evaluating their growth, chlorophyll content, gas exchange, and chlorophyll fluorescence.

## MATERIALS AND METHODS

### Study sites and species

Field surveys were conducted at Maseno university botanic garden swamp (latitude $0^0$1'N - $0^0$12'S and longitude $34^0$25'E - 47'E, altitude is 1500m above sea level) and Nyamasaria swamp (Latitude $0^0$50'S-$0^0$45'N and longitude $33^0$20' E - $35^0$20E), to assess species diversity of the changing wetlands. Based on the initial surveys the dominant plant species from each of these two sites: *Cyperus esculentus* L. *Phragmites australis* (Cav.) Trin. Ex Steud and *Aframomum angustifolia* (Sonn.) were selected for detailed physiological and biochemical studies. The ecophysiology and genetic variation of the plants was evaluated in a pot experiment, the plants were grown under well drained conditions.

### Growth conditions and experimental design

Seeds of *Aframomum angustifolia* (Sonn.) were collected from Maseno university botanic garden and germinated within the glasshouse in small plastic containers (10 x 15 x 4 cm), containing river sand. After germination the seedlings were transplanted at the age of three weeks into polythene bags containing a 1:3 mixture of soil and river sand and were irrigated with water in a nursery for seven months.

Small rhizome sections (approximately 5 g each) of *Cyperus esculentus* L. And *Phragmites australis* were collected from Nyamasaria swamp and Maseno university botanic garden swamp, respectively. The rhizomes sections were transplanted in 3.5 litre pots, one plant per pot containing a 1:3 mixture of soil and river sand. The Pots were irrigated with tap water to initiate shoots prior to the treatments. Potted plants were acclimatized for three weeks before initiating experimental treatments. The pots were arranged in a completely randomized design under out-door conditions in the botanic garden at Maseno university. Plants were provided with four nutrient dosage levels of 0 mg [no fertiliser added], 50 mg, 100 mg, 150 mg fertilizer [N: P: K, 10:26:10] per pot modified from Pons and Westbeek (2004), and replicated five times. The plants were irrigated daily with tap water for eight weeks. Photosynthetically active radiation (PAR) during the study period ranged between 500-2020 µmol photons $m^{-2}s^{-1}$, day temperatures ranged between 22-$33^0$C and relative humidity was between 30-50%.

### Leaf area

Areas of leaves were determined according to *Jose et al.* (2000) using the following equation: $A_L = 0.73 (L_L \times W_L)$ where $A_L$ is the leaf area, $L_L$ is the length of the leaf blade and $W_L$ is the maximum width measured for each leaf. The product of both was an estimate of leaf area.

Leaf length and maximum width were measured by use of a micrometer and a transparent ruler.

**Number of tillers**

Number of tillers in each pot were counted and recorded at the end of the experiment.

**Gas exchange measurements**

Leaf gas exchange parameters [net $CO_2$ assimilation, stomatal conductance, intercellular $CO_2$ concentration and transpiration rate] were determined by use of a portable infrared gas analyzer system connected to a PLC (B) assimilation chamber (CIRAS-1, PP Systems Ltd., Herts, U.K.) on 2.5 $cm^2$ of leaf surface. The second fully expanded leaf from the top of the plant was inserted inside the leaf cuvette. Measurements were recorded after an equilibration time of 60-90 s, as soon as steady-state assimilation was reached on every measurement. Five measurements were taken for a total of five replications. Measurements were made four to seven days prior to harvesting. Data was collected between 0930 and 1300 hours at light-saturated levels between 500-2000 µmol photons $m^{-2}s^{-1}$ photosynthetically active radiation, the leaf temperatures during measurements ranged between 29-32$^0$C, relative humidity was about 30%, with an internal $CO_2$ concentration of 350 ppm and a flow of 150 µmol $s^{-1}$. Vapour pressure deficit was between 1.7-2.1 kPa.

**Chlorophyll fluorescence measurements**

The parameters of fast chlorophyll fluorescence: maximum fluorescence yield from PS2 following a saturating pulse of photons in a light-adapted plant ($F_M$'), steady state yield of PS2 fluorescence in the light (Fs), electron transport rate through PS2 (ETR), actual efficiency of PS2 [quantum yield of PS2 electron transport, (ΦPS2); Belkhodja *et al.*, 1999], were determined during the day between 1100 and 1400 hours. Quantum yield of PS2 electron transport, (ΦPS2) was calculated as [Fm'-Fs]/Fm' (Maricle *et al.*, 2007). Measurements were done on leaves which had been dark-adapted using leaf clips for 15 minutes, using a portable non-modulated fluorometer, (Hansatech, PEA, U.K.). An actinic light pulse was used to saturate the photosystems. Chlorophyll fluorescence measurements were taken four days prior to harvesting.

**Chlorophyll *a* and *b* and carotenoids content**

Chlorophyll was extracted from the third mature leaf (from the shoot apex) of one plant of each of the five replicates. 0.1 g fresh material was ground in 20 ml 0f 80% (v/v) acetone using a mortar. The suspension was filtered and Absorbance read against an 80% acetone blank at 480 nm (carotenoids), 645 nm (chlorophyll *b*) and 663 nm (chlorophyll *a*) using a spectronic 20 spectrophotometer. Chlorophylls *a* and *b* contents were calculated according to Adelusi *et al.* (2006) and Muthomi and Musyimi (2009), carotenoids were measured according to Yadegari *et al.* (2007), using the following formulas:

$C_a$ = 13.19 x A663-2.57 x A645 (mg $g^{-1}$ fresh weight)
$C_b$ = 22.1 x A645-5.26 x A663 (mg $g^{-1}$ fresh weight)
$C_{x+c}$ = 1000 x A480-2.270 x $C_a$ – 81.4 x $C_b$/227 (mg $g^{-1}$ fresh weight)

Where $C_a$ and $C_b$ are chlorophylls *a* and *b* contents, respectively, $C_{x+c}$ are carotenoids (x = xanthophylls and c = carotenes) contents, A663, A645 and A480 is the absorbance at 663nm, 645nm and 480nm, respectively.

**Data analysis**

Analysis of variance (ANOVA) was carried out on the data for the variables measured during the study period to test for differences between the treatments and plant species by use of SAS statistical computer package. Treatment means were separated using the least significant difference (LSD) test (P = 0.05).

## RESULTS

**Leaf area**

The nutrient availability treatments had significant effects ($P \leq 0.05$) on leaf area increments in all the three species (Figure-1). *C. esculentus* had the highest leaf area (100.504 $cm^2$) compared to *A. angustifolium* (58.660$cm^2$) and *P. australis* (40.416$cm^2$). Analysis of leaf area revealed significant interactions between nutrient treatments and species (Table-1).

**Number of tillers**

Nutrient availability significantly ($P \leq 0.05$) increased the number of tillers per pot for each species (Figure-2). *Cyperus esculentus* had the highest number of tillers followed by *A. angustifolium* and then *P. australis*. There were significant interactions between nutrient treatments and species (Table-1).

**Chlorophyll a content**

Chlorophyll a content increased in all nutrient treatments and among the species (Figure-3). There were significant differences ($P \leq 0.05$) in leaf chlorophyll a content between treatments and among species. Chlorophyll a content also exhibited a significant nutrient treatment x species interaction (Table-1). *Phragmites australis* had significantly higher chlorophyll a content compared to *A. angustifolium* and *C. esculentus*.

**Chlorophyll b content**

Chlorophyll b content increased in all nutrient treatments (Figure-4). Significant differences ($P \leq 0.05$) between treatments and among species were observed in this study. The interactions between treatments and species were also significant (Table-1). *Phragmites australis* had significantly higher chlorophyll b content compared to *A. angustifolium* and *C. esculentus*.

**Carotenoids content**

Carotenoids content of the leaves increased in all nutrient treatments (Figure-5). There were significant

differences ($P \leq 0.05$) in carotenoids contents between treatments and among the three species. The pattern of leaf carotenoids content among the three species resembled that of chlorophyll a and chlorophyll b contents. There was no significant nutrient treatment x species interactions (Table-1).

**Gas exchange parameters**

**$CO_2$ assimilation rate**

$CO_2$ assimilation rate significantly ($P \leq 0.05$) increased with increased nutrient availability in all the species, with most pronounced effects on *C. esculentus* (Figure-6). The $CO_2$ assimilation rate also depended on the day of measurement as indicated by a significant Species × nutrients × Day interaction term (Table-2). *Cyperus esculentus* had higher photosynthetic rate, a mean value of (10.52 µmol $CO_2$ $m^{-2}s^{-1}$), compared to *A. angustifolium* (9.91 µmol $CO_2$ $m^{-2}s^{-1}$) and *P. australis* (8.86 µmol $CO_2$ $m^{-2}s^{-1}$), respectively. There was a significant difference in $CO_2$ assimilation between the species, primarily reflecting somewhat higher photosynthetic rates in *C.esculentus* at high nutrient availability treatments.

**Stomatal conductance**

Analysis of stomatal conductance revealed significant species x nutrient x day interactions ($P \leq 0.05$, (Table-2). Relative to control plants, nutrient treatments significantly increased stomatal conductance in all the species (Figure-7). *Aframomum angustifolium* plants had higher average stomatal conductance (33.02 mmol $m^{-2}s^{-1}$) followed by P. *australis* (26.00 mmol $m^{-2}s^{-1}$) and lastly *C. esculentus* (20.92 mmol $m^{-2}s^{-1}$).

**Transpiration rate**

Transpiration rate was significantly ($P \leq 0.05$) increased by nutrient treatments (Figure-8). *Afromomum angustifolium* had slightly higher transpiration rate values (1.14 mmol $H_2O$ $m^{-2}s^{-1}$) compared to *C. esculentus* (0.67 mmol $H_2O$ $m^{-2}s^{-1}$) and *P. australis* (0.39 mmol $H_2O$ $m^{-2}s^{-1}$). Analysis of transpiration rates indicated significant species x nutrient x day interactions (Table-2).

**Intercellular $CO_2$ concentration**

*Cyperas esculentus and A. angustifolium* had generally higher intercellular $CO_2$ concentration compared to *P. australis* which showed a slight decrease *(*Figure-9). Species differed significantly ($P \leq 0.05$) for mean intercellular $CO_2$ concentration. Intercellular $CO_2$ concentration varied also significantly with the species x nutrient interaction (Table-2). Intercellular $CO_2$ concentration had also a significant species x nutrient x day interaction.

**Chlorophyll fluorescence parameters electron quantum yield of PS2 (actual efficiency of PS2)**

ΦPS2 increased significantly with increase in nutrient availability level ($P \leq 0.05$). Average ΦPS2 values in *C. esculentus* remained high in most of the nutrient treatments compared to *P. australis* and *A. angustifolium* (Figure-10). Pattern of variation in ΦPS2 among species differed significantly between nutrient treatments (species x nutrient interaction).

**Electron transport rate**

Electron transport rate exhibited significant differences ($P \leq 0.05$) between treatments and among the species. *Afromomum angustifolium* had higher ETR values at the medium treatments 50 and 100mg, and lower ETR values at the highest nutrient treatment (150mg) and control. ETR values reduced in *C. esculentus* and P. *australis* with increasing nutrient availability (Figure-11). There were significant interactions between nutrient treatments and species. The patterns of the changes of ETR across the nutrient treatments were not consistent.

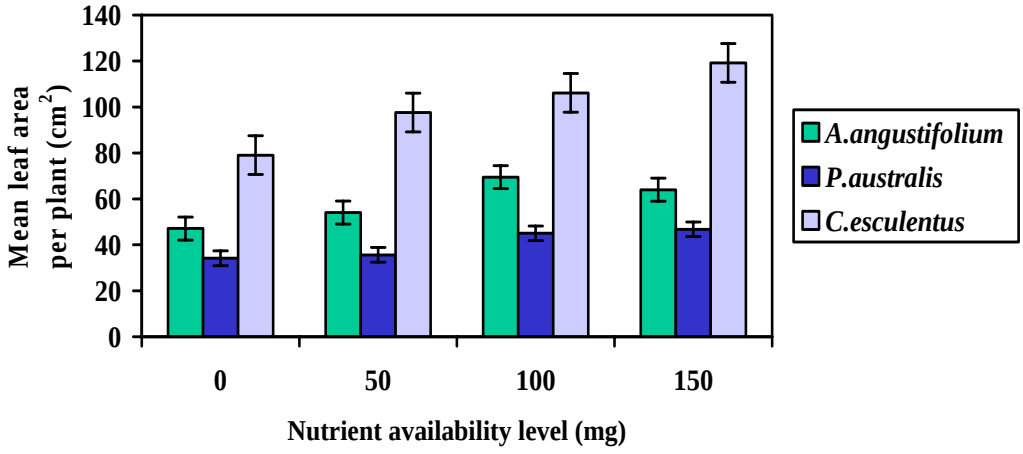


**Figure-1.** Leaf area for three wetland plant species grown at four levels of nutrient availability under outdoor conditions for 56 days (Means of five replicates ± SE). [LSD (0.05) = 6.5892].

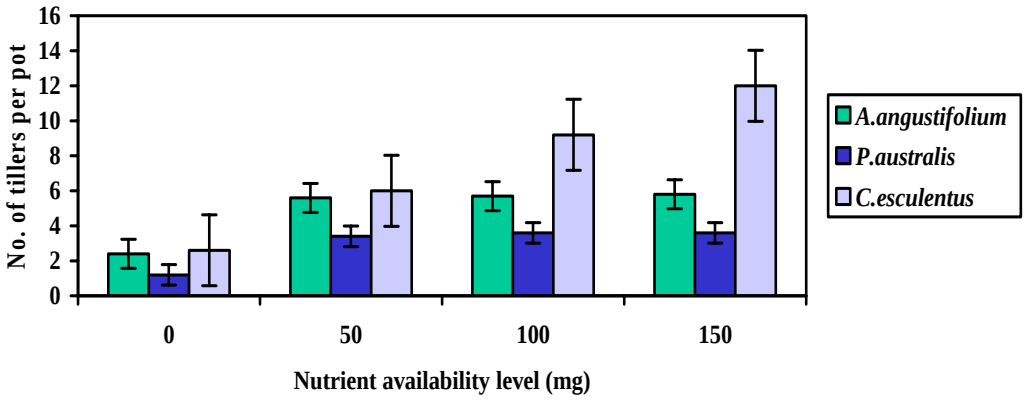


**Figure-2.** Number of tillers per pot for three wetland plant species grown at four levels of nutrient availability under outdoor conditions for 56 days (Means of five replicates ± SE). [LSD (0.05) = 0.9813].

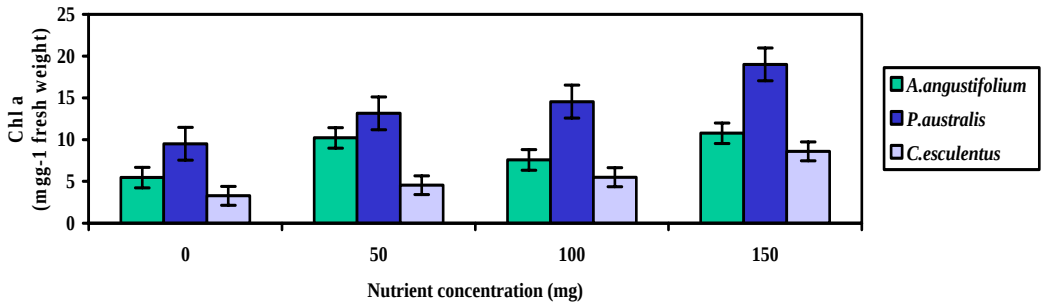


**Figure-3.** Chlorophyll a content for three wetland plant species grown at four levels of nutrient availability under outdoor conditions (Means of five replicates ± SE). [LSD (0.05) = 0.9214].

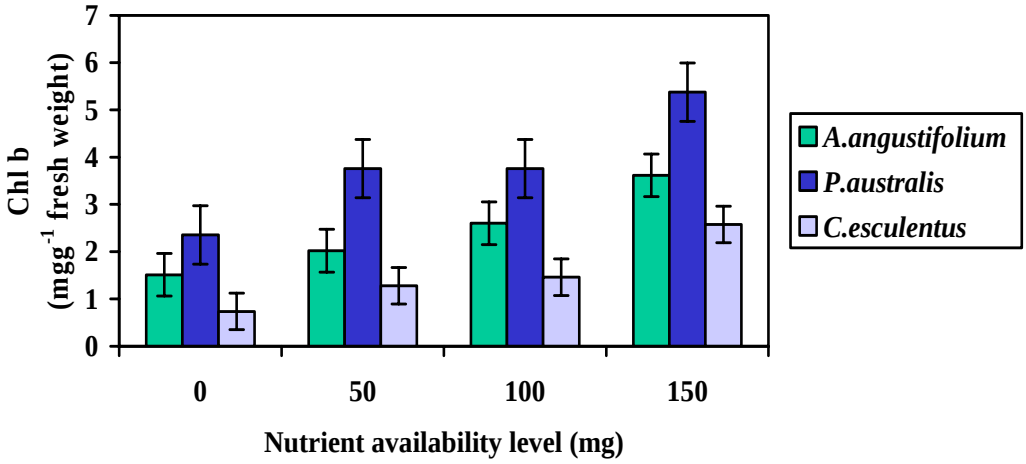


**Figure-4.** Chlorophyll b content for three wetland plant species grown at four levels of nutrient availability under outdoor conditions for 56 days (Means of five replicates ± SE). [LSD (0.05) = 0.3291]

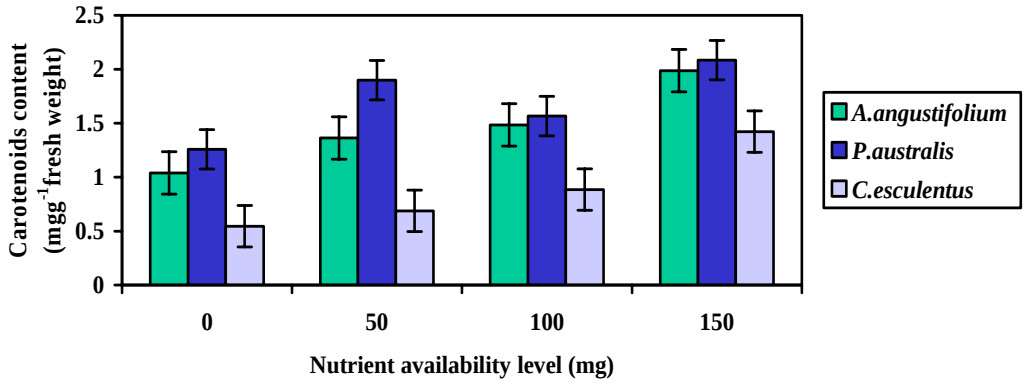


**Figure-5.** Carotenoids content for three wetland plant species grown at four levels of nutrient availability under outdoor conditions for 56 days (Means of five replicates +SE). [LSD (0.05) = 0.2109].

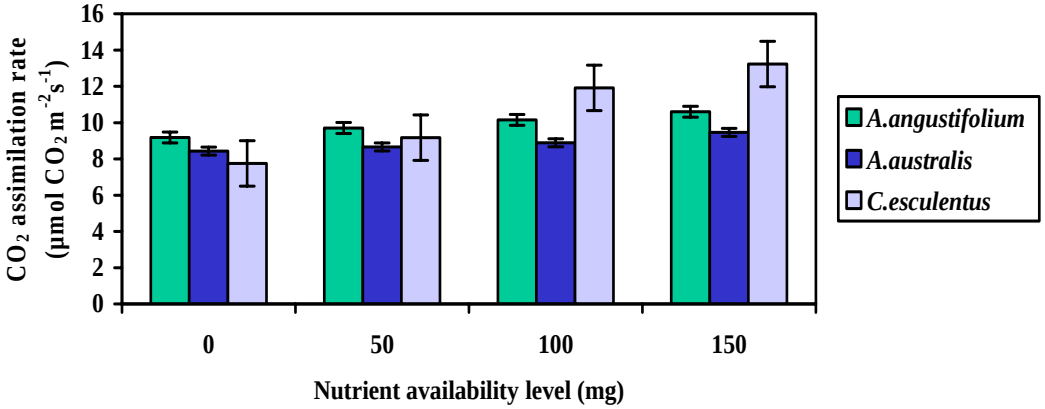


**Figure-6.** $CO_2$ assimilation rate for three wetland plant species grown at four levels of nutrient availability under outdoor conditions for 56 days (Means of five replicates ± SE). [LSD (0.05) = 0.108].

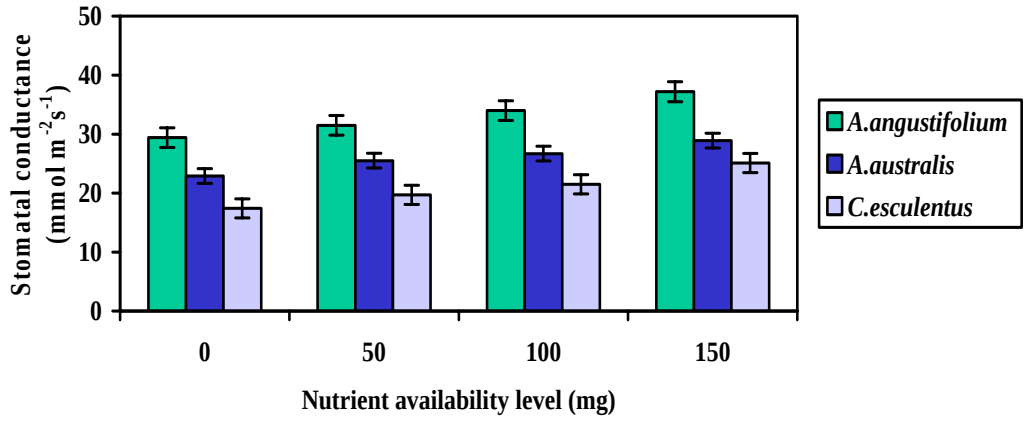


**Figure-7.** Stomatal conductance for three wetland plant species grown at four levels of nutrients availability under outdoor conditions for 56 days (Means of five replicates ± SE). [LSD (0.05) = 0.3629].

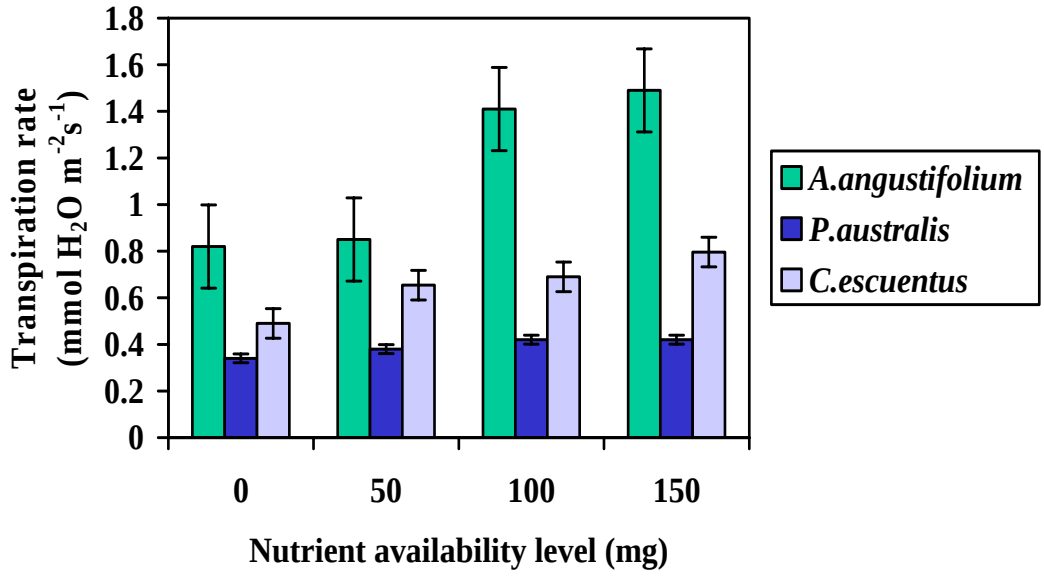


**Figure- 8.** Transpiration rate for three wetland plant species grown at four levels of nutrients availability under outdoor conditions for 56 days (Means of five replicates ± SE). [LSD (0.05) = 0.0121].

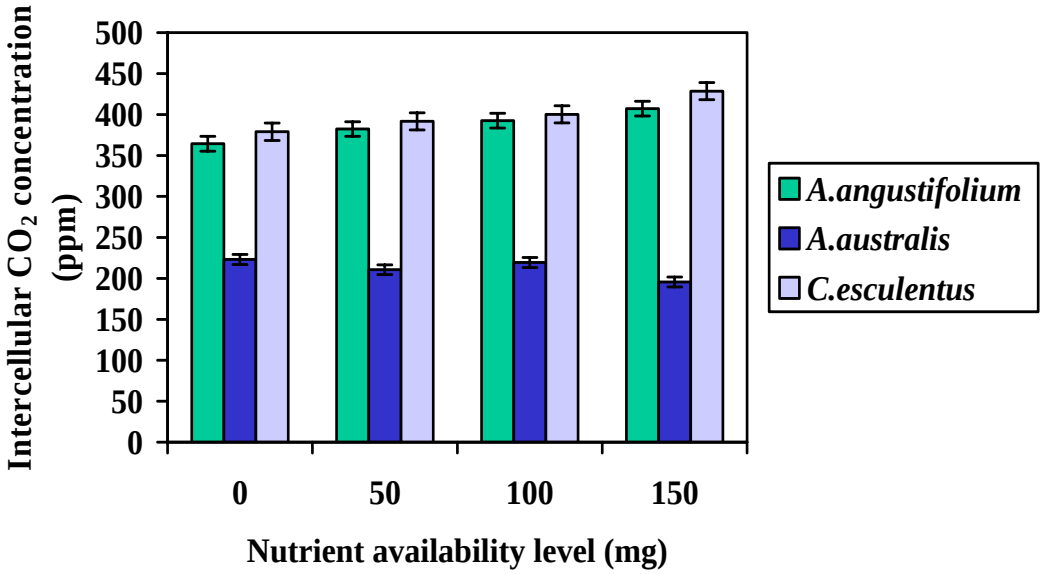


**Figure-9.** Intercellular $CO_2$ concentration for three wetland plant species grown at four nutrients availability under outdoor conditions for 56 days (Means of five replicates ± SE). [LSD (0.05) = 9.3939].

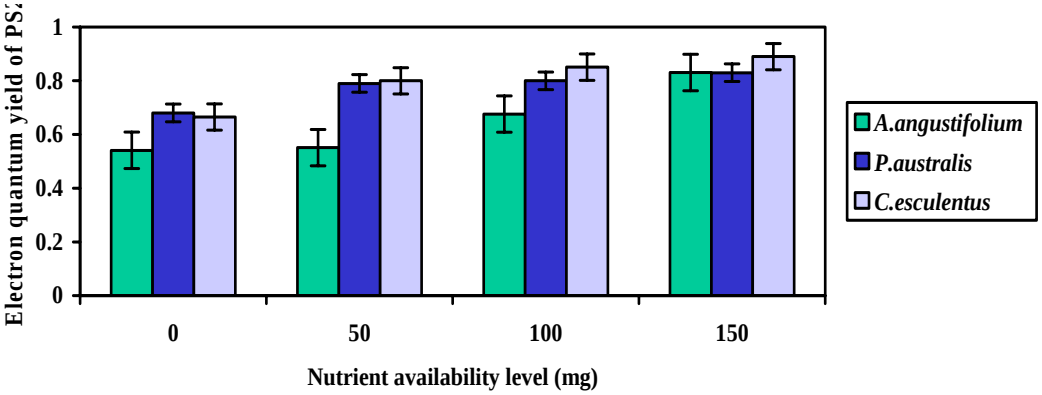


**Figure-10.** Electron quantum yield of photosystem 2 of three wetland plant species grown at four levels of nutrient availability under outdoor conditions for 56 days (Means of five replicates ± SE). [LSD (0.05) = 0.0479].

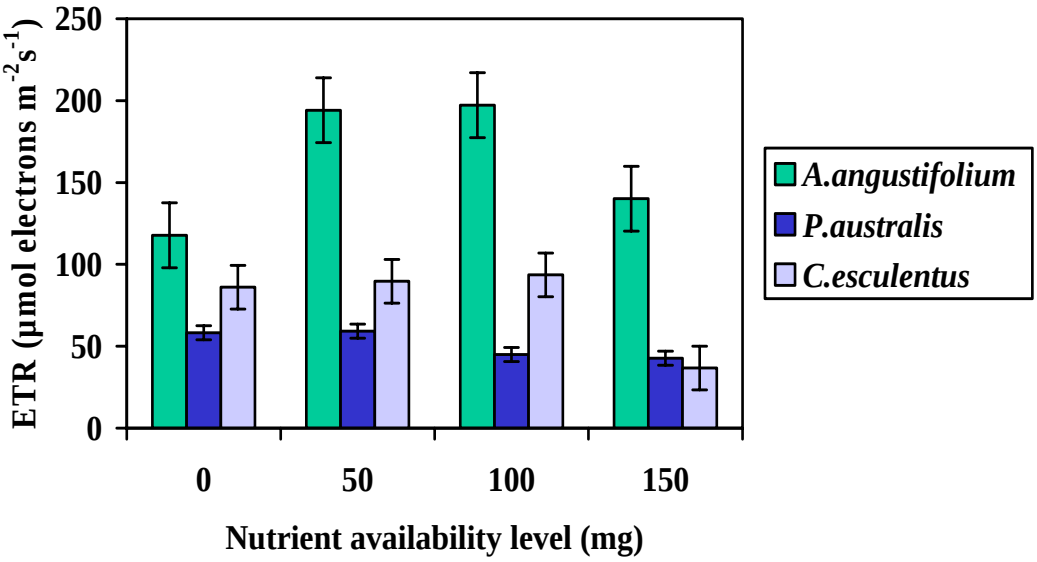


**Figure-11.** Electron transport rate of three wetland plant species grown at four levels of nutrients availability under outdoor conditions for 56 days (Means of five replicates ± SE). [LSD (0.05) = 42.981].

**Table-1.** Analysis of variance in leaf area, number of tillers, chlorophyll a, chlorophyll b and carotenoids contents of *C. esculentus, A. angustifolium* and *P. australis* grown under various levels of nutrient availability for 56 days. P values are shown in bold face type.

| Parameter | Source | DF | MS | F | P > F |
|---|---|---|---|---|---|
| Leaf area (cm) | Species (S) | 2 | 18981.445 | 236.76 | < 0.0001 |
| | treatment (T) | 3 | 1700.356 | 21.21 | < 0.0001 |
| | S X T | 6 | 211.73036 | 2.64 | 0.0283 |
| | error | 44 | 80.17085 | | |
| Number of tillers | Species (S) | 2 | 101.31667 | 56.98 | < 0.0001 |
| | treatment (T) | 3 | 72.6000 | 40.83 | < 0.0001 |
| | S X T | 6 | 19.916667 | 11.20 | < 0.0001 |
| | error | 44 | 1.7780303 | | |
| Chlorophyll a (mg/g) | Species (S) | 2 | 378.3660707 | 241.36 | < 0.0001 |
| | treatment (T) | 3 | 112.7952289 | 71.95 | < 0.0001 |
| | S X T | 6 | 10.2181388 | 6.52 | < 0.0001 |
| | error | 44 | 1.567653 | | |
| Chlorophyll b (mg/g) | Species (S) | 2 | 26.1160767 | 130.62 | < 0.0001 |
| | treatment (T) | 3 | 13.3278424 | 66.66 | < 0.0001 |
| | S X T | 6 | 0.45950228 | 2.30 | 0.0513 |
| | error | 44 | 0.1999363 | | |
| Carotenoids (Mg/g) | Species (S) | 2 | 3.54695030 | 43.19 | < 0.0001 |
| | treatment (T) | 3 | 1.98837312 | 24.21 | < 0.0001 |
| | S X T | 6 | 0.09928121 | 1.21 | 0.3198 |
| | error | 44 | 0.08212121 | | |

**Table-2.** Analysis of variance in $CO_2$ assimilation rate, stomatal conductance, intercellular $CO_2$ concentration, transpiration rate ,electron quantum yield of photosystem 2 and electron transport rate of *C. esculentus, A. angustifolium* and *P. australis* grown under various levels of nutrient availability for 56 days. P values are shown in bold face type.

| Parameter | Source | DF | MS | F | P > F |
|---|---|---|---|---|---|
| $CO_2$ assimilation rate ($\mu$mol $CO_2$ $m^{-2}s^{-1}$) | Species (S) | 2 | 42.2873889 | 629.28 | < 0.0001 |
| | treatment (T) | 3 | 62.1177037 | 924.38 | < 0.0001 |
| | S X T | 6 | 20.0699815 | 298.66 | < 0.0001 |
| | day (D) | 2 | 150.0890556 | 2233.49 | < 0.0001 |
| | S X D | 4 | 30.1112222 | 448.09 | < 0.0001 |
| | T X D | 6 | 5.9689815 | 88.83 | < 0.0001 |
| | S X T X D | 12 | 5.0782593 | 75.57 | < 0.0001 |
| | error | 140 | 0.0671992 | | |
| Stomatal conductance (mmol $m^{-2}s^{-1}$) | Species (S) | 2 | 2214.838889 | 2922.20 | < 0.0001 |
| | treatment (T) | 3 | 412.044444 | 543.64 | < 0.0001 |
| | SXT | 6 | 4.527778 | 5.97 | < 0.0001 |
| | error | 2 | 940.772222 | 1241.23 | < 0.0001 |
| | | 4 | 844.955556 | 1114.81 | < 0.0001 |
| | | 6 | 2.35000 | 3.10 | 0.0070 |
| | | 12 | 7.333333 | 9.68 | < 0.0001 |
| | | 140 | 0.75794 | | |
| Intercellular $CO_2$ concentration (ppm) | Species (S) | 2 | 659067.289 | 1297.46 | < 0.0001 |
| | treatment (T) | 3 | 4145.769 | 8.16 | < 0.0001 |
| | S X T | 6 | 4788.563 | 9.43 | < 0.0001 |
| | day (D) | 2 | 780455.406 | 1536.42 | < 0.0001 |
| | S X D | 4 | 305210.289 | 600.84 | < 0.0001 |
| | T X D | 6 | 3110.257 | 6.12 | < 0.0001 |
| | S X T X D | 12 | 9135.685 | 17.98 | < 0.0001 |
| | error | 140 | 507.968 | | |
| Transpiration rate (mmol $H_2O$ $m^{-2}s^{-1}$) | Species (S) | 2 | 8.69744889 | 10333.0 | < 0.0001 |
| | treatment (T) | 3 | 1.28392889 | 1525.37 | < 0.0001 |
| | S X T | 6 | 0.45182222 | 536.79 | < 0.0001 |
| | day (D) | 2 | 0.00003556 | 0.04 | 0.9587 |
| | S X D | 4 | 1.25434389 | 1490.23 | < 0.0001 |
| | T X D | 6 | 0.07226222 | 85.85 | < 0.0001 |
| | S X T X D | 12 | 0.08096389 | 96.19 | < 0.0001 |
| | error | 140 | 0.00084171 | | |
| Electron quantum yield of photosystem 2 | Species (S) | 2 | 0.12674220 | 29.92 | < 0.0001 |
| | treatment (T) | 3 | 0.13165966 | 31.08 | < 0.0001 |
| | S X T | 6 | 0.01489678 | 3.52 | 0.0063 |
| | error | 44 | 0.00423649 | | |
| Electron transport rate ($\mu$mol electrons $m^{-2}s^{-1}$) | Species (S) | 2 | 61095.1970 | 17.91 | < 0.0001 |
| | treatment (T) | 3 | 11159.2942 | 3.27 | 0.0299 |
| | S X T | 6 | 14354.7775 | 4.21 | 0.0020 |
| | error | 44 | 3411.1502 | | |

## DISCUSSIONS

There was an increase in leaf area with increase in nutrient availability (Figure-1). The results agree well with the studies by Oberbauer *et al.* (2006), who found that the addition of fertilizer caused large increases in leaf area production for all species tested. Increase in leaf area with increased nutrient availability may be a consequence of increased meristematic activities, due to increased supply of nitrogen to drive shoots growth and photosynthesis, and also of increased cell expansion. The observed differences among plant species in leaf area growth may result from differences in total carbon assimilation or nutrient uptake. Leaf area affects dry matter production and plant growth through its role in light interception (Jones, 1992). Vos and Van der Putten (1998) reported a decrease in the area of individual leaves with decreasing N fertility. The relative impact of N on cell division and cell expansion depends on the developmental stage of the leaf (Del Amor, 2006). Physiological characteristics such as leaf area may also impact the invasiveness of plants (Smith *et al.,* 2008). Increased nutrient availability therefore may likely stimulate growth of these species and improve their competitiveness for invasion.

Number of tillers per pot increased with increase in nutrient availability (Figure-2), this increase may be associated with increased mineral uptake and hence increased biomass accumulation and growth.

Both chlorophyll a and b contents were increased to a similar extent in all species. *Phragmites australis* had significantly higher chlorophyll a and b contents in comparison to *A. angustifolium* and *C. esculentus* (Figures 3 and 4). The decrease of chlorophyll a and b in some of the treatments might be due to the decreases in carotenoid concentration because carotenoids may protect chlorophylls from photooxidative destruction (Yao *et al.*, 2008). The higher chlorophyll contents found in plants leaves at high nutrient availability levels compared to control treatment, suggests that higher photosynthetic rates occurred, resulting in greater growth of the plants than in control treatments. Since nitrogen is closely related to photosynthetic pigment content and activity of Rubisco, nitrogen nutrition strongly affects the processes of photosynthesis and related gas exchange (Yao *et al.,* 2008).

Nitrogen limitation in plants mainly affects chlorophyll concentration and thus absorptance and light harvesting, while phosphorus limitation mainly affects the functioning of PSII (De Groot *et al.,* 2003). The amount of solar radiation absorbed by a leaf is largely a function of the foliar concentrations of photosynthetic pigments, and therefore low concentrations of chlorophyll can directly limit photosynthetic potential and hence primary production (Richardson *et al.,* 2002). At low growth irradiance plants invest more in chlorophyll protein complexes rather than into Calvin-cycle enzymes [including Rubisco] (De Groot *et al.,* 2003).

There was an increase in carotenoids contents of the plant leaves with increasing nutrient availability (Figure-5). Other factors such as increase in irradiance levels might have also contributed to the variation in carotenoids concentration among the three species, since previous studies indicate that increases in irradiance increase both carotenoids and chlorophyll content in barley plants and in wheat plants (Kopsell *et al.,* 2005). In chloroplasts, the carotenoids function as accessory pigments in light harvesting, but a more important role is their ability to detoxify various forms of activated oxygen and triplet chlorophyll that are produced as a result of excitation of the photosynthetic complexes by light (Yadegari *et al.,* 2007). Carotenoids provide photoprotective functions in photosynthesis by facilitating the conversion of excess absorbed light energy to heat. The decrease in Carotenoids in some of the treatments may be directly associated with the damage caused by excess irradiation.

Leaf $CO_2$ assimilation rate increased with increasing nutrient availability (Figure-6), in agreement with the results reported by Oberbauer *et al.* (2006), however *P. australis* had lower $CO_2$ assimilation compared to the other two species, suggesting other factors operating in the plants rather than leaf N and P contents contributed to the decrease in photosynthetic rate of *P. australis*. Previous studies have shown that nitrogen is a component of the photosynthetic machinery and its limitations affects $CO_2$ fixation directly through effects on photosynthetic structures rich in nitrogen like chlorophyll, the light-harvesting complex and Rubisco (Evans and Poorter, 2001). N limitation also leads to decreased growth. There is a strong relationship between photosynthesis and nutrient availability since the photosynthetic machinery (i.e Rubisco) accounts for more than half of the N in leaves (Amaya-Carpio *et al.,* 2005). Previous studies have indicated a correlation between increased plant nutrient levels, particularly P, and increased photosynthesis and plant dry matter (Amaya-Carpio *et al.,* 2005). A high rate of net carbon assimilation can lead to higher biomass accumulation, favouring future growth and reproduction as well as competitive ability of *C. esculentus*.

Stomatal conductance was higher in *A. angustifolium* and *P. australis* compared to *C. esculentus* (Figure-7). It was interesting to note that *C. esculentus* had the lowest stomatal conductance yet with the highest $CO_2$ assimilation rate (Figure-6). These findings may be partly linked to the differences in leaf thickness among the three plant species since earlier studies by Brock and Galen (2005), have revealed that thicker leaves should efficiently assimilate internal $CO_2$ while limiting stomatal conductance, and this has been attributed to higher concentration of photosynthetic proteins per area. In this study *C. esculentus* was found to have thicker leaves, followed by *A. angustifolium* and then *P. australis*.

The reduction of stomatal conductance was expected to decrease the intercellular $CO_2$ concentration in *C. esculentus*, and then the $CO_2$ assimilation rate. However, the increase in $CO_2$ assimilation rate was accompanied by an increase in intercellular $CO_2$ concentration, indicating that higher assimilation capacity was mainly ascribed to nonstomatal limitation, such as the increase of mesophyll cell's photosynthetic activity. This finding is supported by the fact that the electron quantum yield of PS2 in *C. esculentus* remarkably increased with increase in nutrient availability compared to *A. angustifolium* and *P. australis* (Figure-10). The better photosynthetic performance of *C. esculentus* resulted in a greater ability to accumulate biomass.

Transpiration rate increased significantly with increased nutrient availability among the species (Figure-8). The results are not consistent with the results from earlier studies involving sweet pepper plants, where leaf transpiration was more greatly affected by N fertilisation, and was progressively reduced by 16.7% and 26% in the integrated and organic treatment respectively (Del Amor, 2006). A low nutrient availability decreases a plant's nutrient uptake per unit leaf dry mass and usually reduces its transpiration per unit leaf dry mass (Poorter and Nagel, 2000).

Intercellular $CO_2$ concentration was significantly increased with nutrient availability in *C. esculentus* and *A. angustifolium* (Figure-9), this increase in intercellular $CO_2$ concentration may explain the higher photosynthetic rates

in these two species. A high intercellular $CO_2$ results in a high photosynthetic rate at the same size of the photosynthetic apparatus (Pons and Westbeek, 2004). A slight decrease in intercellular $CO_2$ concentration with increase in nutrient availability occurred in *P. australis*. Similar finding has been reported by Pons and Westbeek (2004), in other plant species. Low intercellular $CO_2$ concentration and decrease in the quantum efficiency of PS2 photochemistry in *P. australis* may explain the low photosynthetic capacity of this species than in other species.

In this study, the actual efficiency of PS2 (ΦPS2), also known as electron quantum yield of PS2 showed a remarkable increase in *A. angustifolium* and *C. esculentus* with increasing nutrient availability (Figure-10), while *P. australis* did not reveal any pattern or effect on ΦPS2. The results are not in agreement with the findings reported by Maricle *et al.* (2007), where the studies indicated a decrease of ΦPS2 with increasing nutrients and attributed their findings to increases in bundle sheath leakage of $CO_2$. It is possible that some variation in ΦPS2 among the three species may have occurred due to changes in irradiance levels, since previous studies have indicated a decrease in ΦPS2 with increase in irradiance (Maricle *et al.,* 2007). The ΦPS2 values in this study compare well with that reported by Maricle *et al.* (2007) in C4 estuarine grasses.

Generally, *A. angustifolium* and *C. esculentus* had higher electron quantum yield of PS2 (ΦPS2) compared to *P. australis* (Figure-10), this is also reflected in the higher $CO_2$ assimilation rates in *A. angustifolium* and *C. esculentus* than *in P. australis*. Increase in light intensity may result to decrease in ΦPS2; this decrease may be accelerated at low temperatures (Fracheboud *et al.,* 1999). The parameter ΦPS2 is equivalent to the fraction of radiant energy absorbed by PS2 that is utilised in PS2 photochemistry (Belkhodja *et al.,* 1999).

If the quantum efficiency of $CO_2$ fixation decreases relatively more than the PS2 quantum efficiency, this implies that the quantum efficiency of $CO_2$ fixation is being affected by factors other than or in addition to PS2 quantum efficiency (De Groot *et al.,* 2003). Electron quantum yield of PS2 (ΦPS2) is directly related to the rate of electron transport (Fracheboud *et al.,* 1999).

Decrease in nutrients, especially N reduces the transport and / or utilisation of assimilates, and photosynthesis thus becomes limited by the lack of a direct sink for photoassimilates produced (Rogers *et al.,* 1998). Rubisco activation state may regulate downwards to match any electron transport limitation (Dwyer *et al.,* 2007).

Plants are known to respond to changes in irradiance by regulating their absorption cross-section of photosystem 2 (PSII) and by means of state transition (Kroon, 1994). Increase in PS2 efficiency under nutrient treatments may be associated with increased efficiency of energy transfer from the antenna to the reaction centres and /or activation of PS2 reaction centres.

The ETR values in *C.esculentus* were also relatively higher at 50 and 100mg compared to *P. australis* (Figure-11). ETR increased with increasing nutrient availability upto 100mg for *A. angustifolium* and decreased for *P. australis* and *C. esculentus*. This variation may be attributed to increases in temperature which has been shown to induce higher ETR values in some species, due to increased enzymatic activity (Vieira and Necchi, 2006). Estimates of ETR describe the ability of photosystems to use incident light thereby giving an estimate of the overall photosynthetic capacity of the plant (Uku and Björk, 2005).

Certainly PS2 is the main target of photoinhibitory damage (Zanella *et al.,* 2004). Sun plants have been shown to have higher electron transport rates than shade plants (Zhang *et al.,* 1995). It is however interesting to note that *P. australis* which is a sun plant exhibited lower ETR values in comparison to *A. angustifolium* and *C. esculentus*, probably this may have been influenced by irradiance levels, since light intensity during the measurements changed with cloud cover thus providing a range of natural light intensities.

## CONCLUSIONS

The results of this study confirm that nutrients availability in wetlands affect the growth and physiology of wetland plant species. The difference in growth and photosynthetic capacity among the three species studied at high nutrients could for the largest part be explained from their difference in electron transport rate and the actual efficiency of PS2 (ΦPS2). The data also demonstrate that many ecophysiological characters differ significantly among the different species.

*Cyperus esculentus* appeared to be more adapted to increase in nutrient availability than *A. angustifolium* and *P. australis*. The results suggest that wetland nutrient enrichment may potentially lead to alteration of the species composition and functions of the wetland ecosystems. It would be worthwhile to extend our studies on other wetland plant species in other wetlands in L. Victoria basin in order to predict the future of these species in the phase of increasing nutrient enrichments in wetland areas. The three species could be introduced in wetland areas to reinforce the conservation and management of the degraded wetlands.

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