Ecological Engineering 73 (2014) 653–658

Contents lists available at ScienceDirect

Ecological Engineering

journal homepage: www.elsevier.com/locate/ecoleng

Short communication

Effects of inorganic nitrogen form on growth, morphology, N uptake, and nutrient allocation in hybrid Napier grass (*Pennisetum purpureum* × *Pennisetum americanum* cv. Pakchong1)

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ARTICLE INFO

Article history:
Received 24 March 2014
Received in revised form 21 August 2014
Accepted 14 September 2014
Available online xxx

Keywords:
Leaf transpiration
Napier grass
Nitrogen nutrition

ABSTRACT

Plant cultivars with high biomass production may have a high potential for being used in integrated water treatment and plant production system. The highly productive hybrid Napier grass cultivar, *Pennisetum purpureum* × *Pennisetum americanum* cv. Pakchong1, may be a candidate species for being used in such systems. We studied the effects of inorganic nitrogen form (NH_4^+, NH_4NO_3 or NO_3^-) on growth, morphology, N uptake, water content and mineral allocation in this species under hydroponic conditions at equimolar concentrations (500 μmol N L^{-1}). Generally, the N-form significantly affected growth, biomass allocation and tissue nutrient and mineral composition of the plants. The hybrid Napier grass grew better on NH_4^+ compared to NO_3^-, and the plants supplied with NH_4^+ contained three times more chlorophylls than plants supplied with NO_3^- alone or NO_3^- combined with NH_4^+. The morphology of the plants was, however, not affected by N source, except for the shoot to root ratio, which was lower in NH_4^+-fed plants. The relative water content of the leaves was lowest in the NH_4^+-fed plants, but the transpiration rate was not affected, indicating that NH_4^+ nutrition and the associated low tissue concentration of K had negative effects on the water use efficiency of the plants.

The study suggests that this hybrid Napier grass cultivar may be a new candidate species for use in integrated water treatment and plant production systems.

1. Introduction

Plant species or plant cultivars with high biomass production are widely used as forage crops for animal feed, as bio-energy crops for fuel production and for bio-remediation of contaminated soil or water (Dzantor et al., 2000; Somerville et al., 2010). In recent years, there has been an increasing interest in perennial C_4 grasses, such as Napier grass in South-East Asia, switch grass in the US and *Miscanthus* in Europe, as promising energy crops because of their high biomass yield potential and high water-use efficiency. However, these C_4 species have rarely been used in constructed wetland (CW) systems for the treatment of wastewaters. Instead high productive wetland plants species such as *Phragmites*,*Typha*, *Schoenoplectus* and *Cyperus* that are adapted to the wetland growth conditions, but with a biomass that have limited use-potential, are the most commonly used species (Leto et al., 2013; Vymazal, 2013).

Recently, there is an increased interest in integrating water treatment systems and plant production systems based on the common-sense approach of conversion of wastes into products. By integrating water treatment and plant production, it is possible to reduce wastes and associated environmental impacts, and at the same time generate an additional crop (Naegel, 1977; Quillere et al., 1995; Rakocy et al., 2006). In China, *Coix lacryma-jobi* L. has been used in CWs to remove nitrogen (N) and phosphorus (P) from polluted water and at the same time produce an edible crop (Xu and Li, 2007), and different species of leaf vegetables have been integrated with the treatment of fish aquaculture (Trang et al., 2010; Trang and Brix, 2014). The high nutrient supply from non-heavy metal contaminated wastewaters is an important source of fertilizer enhancing plant growth and increasing N content in the plant tissue, making the plants have a high quality as animal feed or green manure. Most species used for water treatments are aquatic or wetland plants. Ammonium (NH_4^+), which is the major N form in water-saturated soils, is the preferred N-form taken up

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http://dx.doi.org/10.1016/j.ecoleng.2014.09.078
0925-8574/

by wetland species like *Phragmites australis* (Cav.) Trin. ex Steudel (Tylova-Munzarova et al., 2005), whereas nitrate (NO_3^-), which is the dominant N-form in well-drained soils (Ponnamperuma, 1972; Bloom et al., 2003), is preferred by most terrestrial plant species. The form of N taken up may, however, interact with the water relations of plants (Guo et al., 2007a). Studies comparing wetland plant species to crop and terrestrial plant species have found that the N use efficiency of the plants are closely related to their water use efficiency (Caviglia and Sadras, 2001; Fan et al., 2002; Ripullone et al., 2004). However, variations in the N nutrition may affect the water relations of plants in different ways for different N sources. (NO_3^-, NH_4^+ or both) It is important to understand the interaction between plant N nutrition and water relations in order to be able to evaluate the potential applicability of an untried species in CW systems.

Pennisetum species are annual or perennial grasses belonging to the family Poaceae. They are native to Africa, but have been introduced to most tropical and subtropical countries where they have become naturalized and used as forage crops (Farrell et al., 2002). The perennial *Pennisetum purpureum* Schumach (Napier or elephant grass) readily cross with the annual *Pennisetum americanum* (L.) Leeke (pearl millet) and the resultant interspecific hybrids are more vigorous than the parent species. Hence, several hybrid Napier grass cultivars (*P. purpureum* × *P. americanum*) have been developed (Premaratne and Premalal, 2006). Because of the high biomass production and very high forage quality for livestock, growth of these hybrids is widely promoted in subtropical and tropical countries including Thailand (Tudsri et al., 2002; Premaratne and Premalal, 2006). Recently, a very productive cultivar (Pakchong1) of this hybrid grass has been developed and marketed by Thailand's Department of Livestock Development. The Pakchong1 cultivar is claimed to have several advantages over other Napier grass cultivar, as it grows taller, has a higher content of crude protein, has an annual yield exceeding 500 t $year^{-1}$, and can be harvested 5–6 times per year.

Both of the parent species of this hybrid Napier, *P. purpureum* and *P. americanum*, have been used in CWs for wastewater treatments (Chikafumbwa, 1996; Kivaisi, 2001; Yang et al., 2001, 2007; Prasad, 2010), But information about growth and physiological responses under different conditions is still lacking, and in particular the N nutrition and its interaction with water relations are not well understood. The hybrid Napier grass is presently being tested for use in CWs in Thailand (unpublished data), but the basic ecophysiology of this cultivar has barely been studied. Therefore, this study aimed to assess growth, morphological characteristics and water relations of hybrid Napier grass (*P. purpureum* × *P. americanum* cv. Pakchong1), as affected by N source (either NO_3^- or NH_4^+ and combined NH_4NO_3) in order to gain further knowledge on its nutritional ecology. This is, to our knowledge, the first study to investigate the N nutrition of this Napier grass cultivar.

2. Materials and methods

2.1. Plant material and experimental design

Plant stocks of hybrid Napier grass (*P. purpureum* Schumach × *P. americanum* (L.) Leeke cv. Pakchong1) were obtained from the Energy Research and Development Institute–Nakornping, Chiang Mai University, Thailand. In order to produce similar-sized plants for the experiment, the stems were cut into 200–300 mm sections and laid in shallow water until new shoots and adventitious roots were initiated from the stem nodes. Then the stems were cut at both sides of the nodes, and 18 similar-sized plants (approximately 70–90 mm tall; 14–18 g fresh mass) were placed in 5-L plastic buckets (diameter 0.25 m; height 0.22 m) containing a full strength standard N and P free growth medium prepared according to Smart and Barko (1985) to which 100 μM $KHPO_4^{2-}$ and a commercial plant micronutrient solution (Tropica, Egaa, Denmark) were added. pH was adjusted to 6.5 ± 0.2 using HCl and NaOH. Nitrogen was added as $(NH_4^+)_2SO_4$ and KNO_3 at equimolar (500 μmol N L^{-1}) concentrations to create the following three treatments: (i) 500 μM NH_4^+ (ii) 250 μM NH_4NO_3 and (iii) 500 μM NO_3^- ($n = 6$). The plants were placed in the greenhouse at the Department of Biology, Faculty of Science, Chiang Mai University, Thailand. During the experimental period, the temperature and light regimes in the greenhouse were 26 ± 10 °C:14 ± 5 °C day:night temperature and approximately 10 h light/14 h dark. The growth medium was renewed every 3 days in order to minimize the depletion of nutrients from the growth medium.

2.2. Growth and morphological study

After 48 days, all plants were harvested and cleaned. The total biomass, plant height, number of new shoots, number of leaves and average area of four mature leaves, number of roots and the maximum root length were recorded. Then all plants were fragmented into shoots and roots and freeze-dried. The relative growth rate (RGR; g g^{-1} $days^{-1}$) in each treatment was calculated by the formula: $RGR = (\ln W_2 - \ln W_1)/(t_2 - t_1)$, where W_1 and W_2 are the initial and final dry mass (DM), and t_1 and t_2 are initial and final time (days). The shoot elongation rate (SER; mm $days^{-1}$) was calculated as the total increase in shoot length throughout the experiment divided by the number of days.

2.3. Nitrogen uptake

Three days before harvest, the N uptake of plants from each treatment was determined. Selected plants ($n = 4$) were pre-incubated in a N-free growth medium for 18 h in the same growth conditions as the growth study. After pre-incubation, the plants were placed in 500-mL beakers containing the basic growth medium with either 500 μM NH_4^+ or NO_3^-. The solutions in the beakers were mixed by continuous air bubbling. The NO_3^- and NH_4^+ uptake rates were estimated based on the N depletion in the solution during 6 h (Konnerup and Brix, 2010). The NH_4^+ concentration in the samples was analyzed using a modified salicylate method (Quikchem Method no. 10-107-06-3-B; Lachat Instruments, Milwaukee, WI, USA). The NO_3^- concentration was analyzed from the absorbance at 202 nm and 250 nm (Cedergreen and Madsen, 2002). After the uptake experiment, all plants were separated into shoots and roots, freeze-dried and weighed. The N uptake rates were calculated from the depletion curves with linear regression analyses and related to root DM.

2.4. Leaf desiccation

The transpiration rate and relative water content (RWC) of leaves were determined according to Nejad and van Meeteren (2005). The 3rd or 4th leaf of each plant counted from the apical shoot was sampled ($n = 4$). All leaves were soaked with distilled water under dark conditions for 2 h to bring them to maximum fresh mass. The leaves were then gently dried using blotting paper, weighed and placed on a sieve in light conditions at room temperature. The fresh mass of the leaves were then recorded every 5 min for 3 h. The transpiration rates were calculated from the weight loss between two consecutive measurements and expressed per leaf area. The RWC was calculated according to González and González-Vilar (2001): RWC (%) = [(fresh mass − dry mass)/(saturated mass − dry mass)] × 100. The RWC express the water content in percent as related to the water content at full turgor.

2.5. Leaf epidermal studies

Mature leaves (3rd or 4th counted from the apical shoot) were taken for epidermal study. The leaves were cut into small pieces (25 × 25 mm) and placed into vials containing bleaching solution (6% sodium hypochlorite: distilled water; 1:1) for 24 h. The samples were then rinsed with tap water and preserved in 70% ethanol. The density of epidermal cells and stomata as well as the length of guard cells on both the upper and lower sides of the leaves were determined using 40× light transmission-microscopy according to Hansen et al. (2007). The stomatal index was calculated as the number of stomata per unit leaf area divided by the number of epidermal cells plus guard cells per unit leaf area, then multiplied by 100 (Willmer and Fricker, 1996).

2.6. Pigments

5 mg samples of freeze-dried leaves were weighed, cut into small pieces and extracted in 96% ethanol in the dark at room temperature. After 24 h, the extracts were thoroughly mixed by vortexing and their absorbance measured at 648.6 nm and 664.2 nm using a UV–vis spectrophotometer (Lambda 25 version 2.85.04, USA). Chlorophyll a (Chl *a*), chlorophyll b (Chl *b*) and total chlorophyll (total Chl *a* + *b*) were calculated according to Lichtenthaler (1987).

2.7. Tissue mineral and nutrient concentrations

The concentrations of Ca, Fe, K, Mg, Mn, Na and P in leaves and roots were analyzed in subsamples (approximately 110–120 mg) of finely ground freeze-dried plant materials. The subsamples were digested with 4 mL concentrated HNO_3 and 2 mL H_2O_2 in a microwave sample preparation system (Multiwave 3000, Anton Paar GmbH, Austria), and the element concentrations analyzed using inductively coupled plasmaspectrometry (Optima 2000 DV, PerkinElmer Instruments Inc., CT, USA). The total carbon (C) and nitrogen (N) contents of the leaves and roots were analyzed in the ground plant samples (1.8–3.0 mg) using a CHN analyzer (Na2000, Carlo Erba, Italy).

Table 1
Growth, morphological characteristics and chlorophyll contents (mean ± SD) of hybrid Napier grass (*Pennisetum purpureum* × *P. americanum* cv. Pakchong1) grown with either NO_3^-, NH_4^+ or NH_4NO_3 at equimolar N concentration (500 μM N) and results of one-way ANOVA (*F*-ratios).

	N source			*F*-ratio
	NO_3^-	NH_4 NO_3	NH_4^+	
Relative growth rate (g g^{-1} days^{-1})	0.023 ± 0.003^a	0.030 ± 0.001^b	0.030 ± 0.001^b	15.4**
Shoot elongate rate (mm days^{-1})	2.8 ± 0.1	3.3 ± 0.7	3.1 ± 0.2	1.6
Shoot:root ratio	5.3 ± 1.3^c	4.4 ± 0.7^{ab}	3.8 ± 0.4^a	4.4*
Average leaf area (cm^2)	37 ± 1	42 ± 14	35 ± 7	0.5
Leaf number	7.2 ± 1.4	7.2 ± 0.7	7.8 ± 0.7	0.8
Root number	23 ± 4	25 ± 5	22 ± 4	1.2
Root length (cm)	17 ± 4	18 ± 3	28 ± 15	2.7
New shoot	0.16 ± 0.04	0.5 ± 0.8	0.0 ± 0.0	1.4
Chl *a* (mg g^{-1} DM)	2.1 ± 1.3^a	2.5 ± 1.2^a	10.7 ± 1.4^b	77.8***
Chl *b* (mg g^{-1} DM)	0.7 ± 0.4^a	0.9 ± 0.4^a	3.6 ± 0.5^b	86.8***
Total Chl *a* + *b* (mg g^{-1} DM)	2.8 ± 1.8^a	3.4 ± 1.5^a	14.3 ± 1.9^b	80.4***

Different letters superscripts between columns indicate significant differences between treatments.
* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$

2.8. Data analysis

Statistical analysis was performed using Statgraphics Plus version 4.1 (Manugistics, Inc., MD, USA). One-way analysis of variance (ANOVA) was used to determine the effects of treatments. Differences between treatments were identified using the Tukey's HSD post hoc procedure at the 5% significance level.

3. Results

3.1. Growth and tissue composition

The N form supplied to the plants affected the growth of hybrid Napier grass significantly (Table 1). The plants supplied with NH_4^+ or NH_4NO_3 had higher RGRs than the NO_3^- fed plants. The number of roots, the root length and the average leaf area were not affected by N form. But the shoot:root ratio was affected, as plants supplied with NH_4^+ had relatively more biomass allocated to roots than plants supplied with NO_3^-. The concentrations of chlorophylls in the leaves, however, were approximately four times higher in NH_4^+-fed plants than in NO_3^- and NH_4NO_3-fed plants.

Table 2
Total C, total N, C/N ratio and concentrations of Ca, K, Mg, Fe, Mn, P and Na (mean ± SD) in leaves and roots of hybrid Napier grass (*Pennisetum purpureum* × *P. americanum* cv. Pakchong1) grown with either NO_3^-, NH_4^+ or NH_4NO_3 at equimolar N concentration (500 μM N) and results of one-way ANOVA (*F*-ratios).

	N source			*F*-ratio
	NO_3^-	NH_4 NO_3	NH_4^+	
Total C (%)				
Leaves	34.0 ± 1.2^a	36.5 ± 0.8^b	38.9 ± 0.6^c	42.5***
Roots	40.6 ± 0.2^a	40.7 ± 1.5^a	41.6 ± 0.6^b	6.7*
Total N (%)				
Leaves	3.1 ± 0.3^a	4.0 ± 0.6^b	4.0 ± 0.4^b	7.8**
Roots	2.4 ± 0.0^a	2.7 ± 0.2^b	3.8 ± 0.2^b	8.9**
C/N ratio				
Leaves	11.1 ± 1.2^b	9.2 ± 1.2^a	10.0 ± 0.9^{ab}	4.6*
Roots	16.9 ± 0.3^b	15.1 ± 0.8^a	14.9 ± 0.5^a	9.7*
Ca (mg g^{-1} DM				
Leaves	8.5 ± 3.2^b	8.3 ± 3.0^b	6.6 ± 2.0^a	0.9
Roots	3.2 ± 0.3^b	3.0 ± 0.5^b	1.9 ± 0.2^a	10.7**
K (mg g^{-1} DM				
Leaves	86.4 ± 7.2^c	63.4 ± 10.8^b	10.0 ± 0.9^{ab}	4.6*
Roots	16.9 ± 0.3^b	15.1 ± 0.8^a	14.9 ± 0.5^a	9.7*
Mg (mg g^{-1} DM)				
Leaves	3.6 ± 0.8^b	3.4 ± 1.0^{ab}	2.6 ± 0.6^a	2.8*
Roots	1.6 ± 0.1^a	1.6 ± 0.1^a	2.0 ± 0.1^b	14.8**
Fe (mg g^{-1} DM)				
Leaves	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.0	0.2
Roots	2.1 ± 0.7	4.1 ± 1.5	3.0 ± 1.0	2.2
Mn (mg g^{-1} DM)				
Leaves	0.09 ± 0.03	0.11 ± 0.05	0.08 ± 0.02	0.8
Roots	4.5 ± 1.2^b	6.5 ± 2.5^b	0.7 ± 0.2^a	10.3*
P (mg g^{-1} DM)				
Leaves	23.9 ± 4.7	26.2 ± 4.3	24.7 ± 1.7	0.6
Roots	15.9 ± 1.1^{ab}	17.8 ± 1.8^b	15.0 ± 0.5^a	3.9
Na (mg g^{-1} DM)				
Leaves	1.1 ± 0.3^{ab}	1.4 ± 0.8^b	0.7 ± 0.4^a	2.4*
Roots	1.0 ± 0.1^a	1.1 ± 0.1^a	2.1 ± 0.4^b	19.9**

Different letters superscripts between columns indicate significant differences between treatments.
* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$.

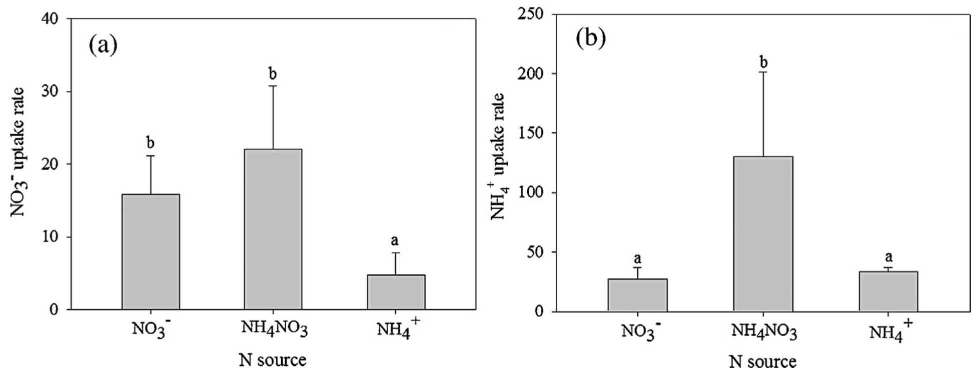

Fig. 1. (a) Average (± SD) NO_3^- uptake rate (μmol NO_3^- g^{-1} root DM h^{-1}) and (b) NH_4^+ uptake rate (μmol NH_4^+ g^{-1} root DM h^{-1}) of hybrid Napier grass (*Pennisetum purpureum* × *P. americanum* cv. Pakchong1) grown with either NO_3^-, NH_4^+ or NH_4NO_3 at equimolar N concentration (500 μM N). Different letters above columns indicate significant differences between treatments.

The tissue concentrations C and N were significantly affected by N source as NH_4^+-fed plants had higher concentrations of both C and N than NO_3^- fed plants (Table 2). The concentrations of Ca, K, Mn and Mg generally were lower in the plants supplied with NH_4^+ compared with plants supplied with NO_3^-. In particular, the K concentration was affected, as the K concentration in leaves of NH_4^+-fed plants was eight times lower than the concentrations in NO_3^- fed plants. Na in roots of NH_4^+-fed plants was higher than in the two other treatments, and tissue P and Fe concentrations were unaffected by the N supply.

3.2. Nitrogen uptake

The uptake rates of NH_4^+ were generally much higher than the uptake rates of NO_3^-, particularly in plants supplied with NH_4^+ in combination with NO_3^- (Fig. 1). The highest NH_4^+ uptake rate (130 ± 71 μmol NH_4^+ g^{-1} root DM h^{-1}) found in NH_4NO_3-fed plants, was approximately four times higher than the uptake rates in NH_4^+ and NO_3^- fed plants. The plants supplied with NO_3^- had higher NO_3^- uptake rates than plants supplied with NH_4^+ alone.

3.3. Leaf desiccation

The leaf water loss rates decreased over time (Fig. 2) and were consistently higher in NH_4NO_3-fed plant than in NH_4^+ and NO_3^- fed plants (Fig. 2). At the termination of the desiccation experiment after 3 h, the RWC of the NH_4^+-fed plants (59.0%) was significantly lower than the RWC of the NO_3^- fed plants (66.6%) and NH_4NO_3-fed plants (65.5%).

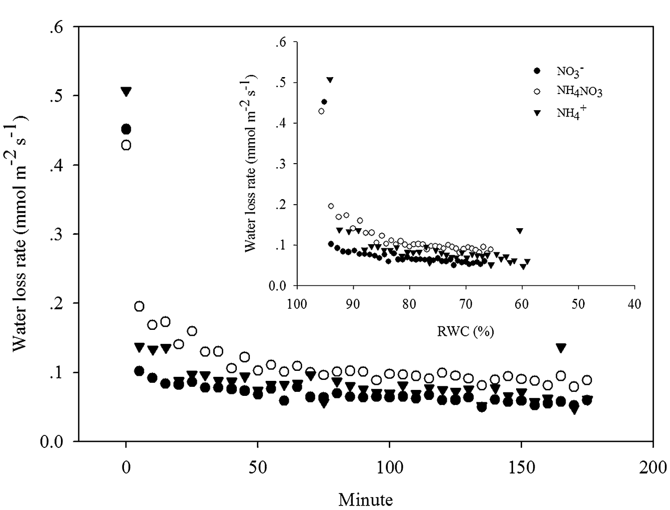

Fig. 2. Changes of water loss rate in hybrid Napier grass (*Pennisetum purpureum* × *P. americanum* cv. Pakchong1) grown with either NO_3^-, NH_4^+ or NH_4NO_3 at equimolar N concentration (500 μM N). The relationship between water loss rate and relative water content (RWC) is shown as an insert.

Table 3
Epidermal density, stomatal density, stomatal index and stomatal length of the upper and lower leaf surface (mean ± SD) of hybrid Napier grass (*Pennisetum purpureum* × *P. americanum* cv. Pakchong1) grown with either NO_3^-, NH_4^+ or NH_4NO_3 at equimolar N concentration (500 μM N) and results of one-way ANOVA (*F*-ratios).

		N source			*F*-ratio
		NO_3^-	NH_4NO_3	NH_4^+	
1.	Epidermal density (mm^{-2})				
	Upper leaf surface	338 ± 26^b	265 ± 74^a	309 ± 23^{ab}	3.00^*
	Lower leaf surface	333 ± 67	281 ± 30	316 ± 52	1.34
2.	Stomatal density (mm^{-2})				
	Upper leaf surface	74 ± 22	67 ± 15	65 ± 13	0.39
	Lower leaf surface	73 ± 12	73 ± 11	70 ± 13	0.07
3.	Stomatal index (%)				
	Upper leaf surface	18 ± 4	21 ± 6	18 ± 4	0.71
	Lower leaf surface	18 ± 4	21 ± 3	19 ± 5	0.52
4.	Guard cell length (μm)				
	Upper leaf surface	30 ± 3^a	44 ± 3^c	37 ± 5^b	83.6^{***}
	Lower leaf surface	29 ± 4^a	42 ± 2^c	39 ± 2^b	114.9^{***}

Different letters superscripts between columns indicate significant differences between treatments.
* $P < 0.05$.
*** $P < 0.001$.

3.4. Leaf epidermal studies

The density of epidermal cells on the upper side of the leaves was affected by N form, but there was no difference in epidermal density on the lower side of the leaves (Table 3). Stomatal density and stomatal index of both sides of the leaves were also not affected by the treatments. However, the length of stomata (guard cell length) differed significantly between the treatments both on the upper and lower side of the leaves. The longest guard cells were found in NH_4NO_3-fed plants followed by NH_4^+-fed plants and NO_3^- fed plants, respectively.

4. Discussion

The form of N supplied to the hybrid Napier grass cultivar (*P. purpureum* × *P. americanum* cv. Pakchong1) significantly affected growth, biomass allocation and tissue nutrient and mineral composition of the plants. The parent species, *P. americanum*, is very drought tolerant and susceptible to water logging (Premaratne and Premalal, 2006; Ayub et al., 2009), whereas the other parent species, *P. purpureum* can grow in soils that are water saturated for most of the year (Cheng et al., 2009). The hybrid Napier grass have inherited traits from both parent species and thus may be able to acclimate to the different soil conditions, including the prevailing form of N present in different soils. The hybrid Napier grass grew better on NH_4^+ compared to NO_3^- as the RGR of plants supplied with NH_4^+ alone or NH_4^+ combined with NO_3^- was higher than the RGR of plants supplied with NO_3^- alone. Also the plants supplied with NH_4^+ contained much more chlorophylls than plants supplied with NO_3^- alone or NO_3^- combined with NH_4^+. The morphology of the plants was, however, not affected by N source, except for the shoot to root ratio, as NH_4^+-fed plants had relatively more biomass allocated to roots than plants supplied with NO_3^-.

Other studies have reported contrasting results concerning N nutrition preference of plants. For instance, the free-floating water fern *Salvinia natans* (L.) All. (Jampeetong and Brix, 2009) and the smooth flatsedge *Cyperus laevigatus* L. (Piwpuan et al., 2013) are both reported to prefer NH_4^+ over NO_3^- as the main N source. However, the growth rate of the common reed, *P. australis* (Cav.) Trin. Ex Steudel, were unaffected by the N-form available, but the plant had a higher uptake capacity for NH_4^+ compared to NO_3^-, even in fully induced NO_3^- fed plants (Tylova-Munzarova et al., 2005). Similar results are reported for *Canna indica* L. (Konnerup and Brix, 2010). The present study showed that the hybrid Napier grass cultivar (*P. purpureum* × *P. americanum* cv. Pakchong1) grew well with NH_4^+ or NH_4NO_3, but the uptake rate of NH_4^+ was higher than that of NO_3^- and resulted in higher concentrations of N in the tissues of NH_4^+-fed plants compared to plants supplied with NO_3^- alone. Hence, this hybrid Napier grass cultivar has a preference for NH_4^+ over NO_3^-, which is consistent with the fact, that most C_4 grasses are reported to prefer NH_4^+ (Guo et al., 2007a; Taylor et al., 2009).

The relative water content (RWC) of NH_4^+-fed plants was lower than that of the NO_3^- and NH_4NO_3-fed plants. The NH_4NO_3-fed plants had the largest stomata size, and the plants maintained a higher RWC compared to NH_4^+-fed plants with smaller stomata. Thus, the stomatal size seems not related to the RWC. Raab and Terry (1994) reported that the RWC in leaves of the beet *Beta vulgaris* L. supplied with NH_4^+ was lower than in plants supplied with NO_3^-. Several studies have reported negative effects of NH_4^+ nutrition on plant water uptake rate (Guo et al., 2007a,b,c,c,c). This may be associated with the reduced cation uptake, especially K, of NH_4^+-fed as the osmotic regulation of the leaves as well as stomata movement is partly controlled by K. The concentration of K in leaves of NH_4^+-fed hybrid Napier grass was 6–8 times lower than the concentration in the leaves of plants supplied with NO_3^-, and the concentrations of other cations (Mg, Na) were also lower. Hence, the N nutrition likely interacted with water uptake as Guo et al. (2007b) showed that NH_4^+ nutrition significantly affected aquaporin expression in the common bean *Phaseolus vulgaris* L.

As the hybrid Napier grass (*P. purpureum* × *P. americanu* cv. Pakchong1) grew very well on NH_4^+, this hybrid Napier grass cultivar may have great potential for bioremediation in CW systems used to treat various types of wastewaters. Its extensive root system and high growth rate may result in a significant uptake of nutrients from the polluted water, and at the same time the large amount of biomass produced can be harvested and used for varies purposes. The species seems to have potential for being used in an integrated water treatment and plant production system. However, further studies with this hybrid Napier cultivar under realistic CW conditions must be carried out to assess the real potential of using this plant species in CWs.

Acknowledgments

This research was funded by the Institute for the Promotion of Teaching Science and Technology (IPST), Thailand. Finally, thanks to the Danish Council for Independent Research - Natural Sciences, via a grant to Hans Brix.

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