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Effect of Salinity Stress on Dry Matter Yield and Oxalate Content in Napiergrass (*Pennisetum purpureum* Schumach)

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ABSTRACT : Sodium is involved in elevation of oxalate content in some plant species and this element is abundant in saline soils. Oxalate causes precipitation of insoluble calcium oxalate in the rumen and kidneys. The intention of this study was to evaluate the effect of soil salinity stress on dry matter yield and oxalate content in pot-grown napiergrass (*Pennisetum purpureum* Schumach). Plants were cut three times at 56, 118 and 179 d after transplanting to the pots. Five salinity treatments were used containing various concentrations of NaCl solution as follows: 0, 100, 300, 600 and 900 mM. At 28, 42, 84, 98, 146 and 160 d after transplanting, plants were irrigated with one liter of the particular treatment for each application. Dry matter yield of napiergrass was not affected ($p>0.05$) by salinity treatments. Plants treated with 100 mM NaCl exhibited a higher soluble oxalate content compared to other treatments, but the differences were not statistically significant ($p>0.05$). Although salinity treatments had significant ($p<0.05$) effects on insoluble and total oxalate contents in plant tissue between the 100 and 900 mM NaCl treatments, the differences were too small to be considered biologically important. The present study indicates that where the soil is high in NaCl, napiergrass will tend to grow well and be low in oxalate. (**Key Words :** Napiergrass, Salinity, Sodium Chloride, Soluble Oxalate, Total Oxalate)

INTRODUCTION

There are a range of plants that grow in saline soils and these have been used as animal feed. It is not unusual for plants growing in saline areas to accumulate secondary compounds (e.g., oxalates, tannins etc.), and these can adversely affect palatability, feed intake and animal health (Masters et al., 2001; Masters et al., 2005).

Napiergrass (*Pennisetum purpureum* Schumach) can grow in a wide range of soils with a pH of 4.5-8.2, performing best in fertile and well drained soils, but cannot tolerate flooding or water logging. It establishes well in clay or sandy loam, and fertile loam soils produce best growth and yields (Skerman and Riveros, 1990). Recently, this plant has been cultivated in the high salinity soil of northeastern Thailand (Pongtongkam et al., 2006) and southern Bangladesh (BLRI, 2002) for animal feeding. Salt tolerance dwarf napiergrass has recently been developed with several positive characters, i.e. high fresh yield, narrow leaf, high crude protein and can be grown for years (Pongtongkam et al., 2006). Although napiergrass can be used safely as animal feed under most conditions (Gwayumba et al., 2002), sometimes this plant accumulates relatively high levels (≥3.0%) of soluble oxalate (Lal et al., 1966; Dhillon et al., 1971; Rahman et al., 2006). Soluble oxalate interferes with the absorption of calcium in animals, and a substantial portion of insoluble oxalate is also dissolved during digestion in ruminants (Libert and Franceschi, 1987). Marais (2001) reported that acute toxicity occurred in adapted ruminants fed with kikuyugrass (*Pennisetum clandestinum*) containing relatively low oxalate (0.39-2.44%). In another study, McKenzie et al. (1988) observed that 2% or more soluble oxalate can lead to acute toxicosis in ruminants. Cymbaluk et al. (1986) also reported that certain prairie forages with moderate amounts of oxalate (1.3-1.8%) could cause subclinical bone diseases.

Soil salinity can alter the level of oxalate in some plants. For example, the soluble oxalate content in halogeton (*Halogeton glomeratus*) was greatly increased by adding sodium chloride (NaCl) to the nutrient solution, and it was shown that Na is more effectively used than potassium (K) in the production of soluble oxalate salts (Williams, 1960). In our previous study, fertilizing Rhodesgrass, Guineagrass

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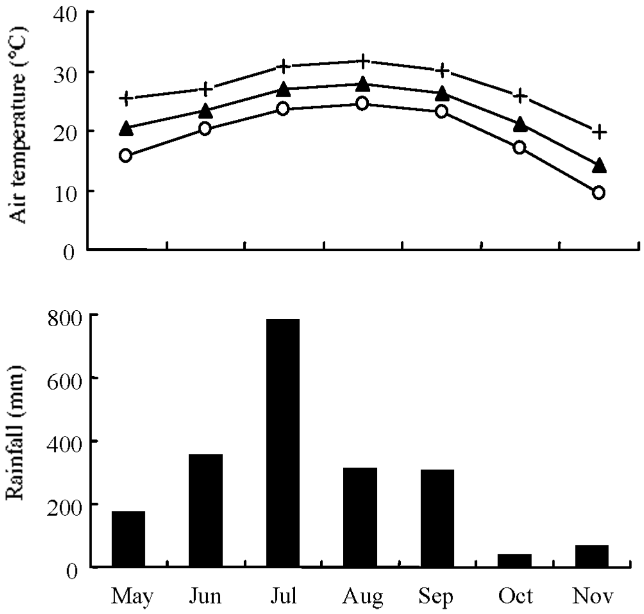

Figure 1. Means of maximum (+), mean (▲) and minimum (O) daily temperatures (°C), and monthly total rainfall (mm) during the study.

and Sudangrass with urea did not result in excessive oxalate accumulation in the forage (Rahman et al., 2008). However, there is very little information about the impact of salinity stress on oxalate content in tropical forage in the literature. Therefore, this study aimed to observe the effect of salinity stress on oxalate content in napiergrass and to assess dry matter yield.

MATERIALS AND METHODS

Napiergrass (cv. Dwarf-late) was grown by transplanting a rooted tiller into Wagner's pot (size 1/2,000 a; diameter 25 cm; depth 30 cm) containing 10 kg commercial heated soil at a density of one plant/pot/m^2 in an experimental field of the Faculty of Agriculture, University of Miyazaki, Japan (31°50′N, 131°24′E) on May 18, 2007. Climatic conditions during the plant growth period were recorded at the Miyazaki Meteorological Station about 10 km north from the experimental site (Figure 1). Plants were cut three times (12 July, 12 September and 12 November) at approximately 2-month intervals. Each pot was fertilized with N (10 g), P_2O_5 (30 g) and K_2O (30 g) before transplanting, and then 10 g of N was applied to each pot after each cutting. The five salt treatments which were used contained various concentrations of NaCl as follows: 0, 100, 300, 600 and 900 mM. In all treatment groups, plants were treated at 28, 42, 84, 98, 146 and 160 d after transplanting i.e. plants received salinity 2 times before each cutting. At each time, one liter of the respective salinity treatment was applied to each pot. Watering was done when necessary with a plastic vase set at the outlet of a Wagner's pot to protect against runoff of nutrients in water. Four plants were used per treatment (4 pots×1 plant) and arranged in a completely randomized design with five treatments and four replications. The fertilizers were used as ammonium nitrate for N, superphosphate for P_2O_5, and potassium chloride for K_2O. The cutting height was 15 cm above the ground surface. Data were collected on dry matter (DM) of biomass after oven-drying (70°C) samples for 48 h.

Dried samples were milled to pass through a 1-mm screen using a Wiley mill (Toyo Inc. Ltd., Tokyo, Japan). Samples were analyzed for total oxalate and soluble oxalate using high-performance liquid chromatography (Rahman et al., 2007). Insoluble oxalate was estimated as the difference between total oxalate and soluble oxalate.

Effects of salinity on DM yield and oxalate content in plants were subjected to a completely randomized design and analyzed by analysis of variance using the general linear model procedures of SPSS (version 12.0, SPSS Inc, Chicago, USA). The significance of the differences among treatment groups with variable means was determined by least significant difference and significance was considered at $p<0.05$.

RESULTS AND DISCUSSION

Dry matter yield

The effects of salinity stress on DM yield are shown in Table 1. Dry matter yield of dwarf napiergrass was not affected ($p>0.05$) by salinity treatments. In the first cut, the lowest (137 g/m^2) and highest (174 g/m^2) DM yield were observed at 0 and 600 mM NaCl application, respectively. However, no such trend was found in the second or third cut forages. Masters et al. (2001) reported that many saline areas are capable of producing large quantities of dry matter, because of salt tolerant plants. Table 1 shows that DM yield

Table 1. Effect of salinity stress on DM yield (g/m^2) in napiergrass

Cutting number	Concentrations of NaCl (mM)					SEM	Level of significance
	0	100	300	600	900		
First cut	137	163	144	174	151	7.42	NS
Second cut	346	315	291	342	366	18.82	NS
Third cut	195	218	185	161	194	8.50	NS
Total	678	696	619	676	711	23.67	NS

NS = Not significant; SEM = Standard error of the mean.

Table 2. Effect of salinity stress on oxalate content (% DM) in napiergrass

Cutting number	Type of oxalate	Concentrations of NaCl (mM)					SEM	Level of significance
		0	100	300	600	900		
First cut	Soluble	1.49	1.84	1.69	1.64	1.57	0.098	NS
	Insoluble	0.25	0.19	0.20	0.31	0.32	0.043	NS
	Total	1.74	2.03	1.89	1.95	1.89	0.125	NS
Second cut	Soluble	0.95	1.01	0.94	0.88	0.94	0.029	NS
	Insoluble	0.25^{ac}	1.15^{d}	0.62^{b}	0.52^{bc}	0.04^{a}	0.044	***
	Total	1.20^{ab}	2.15^{d}	1.56^{c}	1.40^{bc}	0.98^{a}	0.043	***
Third cut	Soluble	0.95	1.03	0.97	0.89	0.85	0.051	NS
	Insoluble	0.44^{b}	0.06^{a}	0.17^{ab}	0.23^{ab}	0.09^{a}	0.042	*
	Total	1.39^{b}	1.09^{ab}	1.14^{ab}	1.12^{ab}	0.94^{a}	0.054	*
Average	Soluble	1.15	1.35	1.22	1.16	1.15	0.061	NS
	Insoluble	0.31^{ab}	0.44^{b}	0.35^{ab}	0.37^{ab}	0.15^{a}	0.041	*
	Total	1.46^{ab}	1.79^{b}	1.57^{ab}	1.53^{ab}	1.30^{a}	0.069	*

* $p<0.05$; *** $p<0.001$; NS = Not significant; SEM = Standard error of the mean.

$^{a, b, c, d}$ Means in the same row with different superscripts differ ($p<0.05$).

of dwarf napiergrass was 2 times higher in the second than in the first or third cut, which was also observed in napiergrass by Mukhtar et al. (2003). The present study indicated that dwarf napiergrass was able to survive and accumulate high amounts of biomass even under high soil salinity.

Oxalate content

Effects of different salinity treatments on the oxalate content in napiergrass are shown in Table 2. In all cuts, statistical analysis showed that there were no significant ($p>0.05$) differences in the soluble oxalate content of plants among all treatments. However, the content of soluble oxalate in plant tissue tended to increase with increasing salinity from 0 to 100 mM NaCl, while this value tended to decrease with further (from 300 to 900 mM NaCl) increases in salinity in all cut forages.

In the first cut, there were no significant ($p>0.05$) differences in the insoluble and total oxalate contents of plants among all treatments. It was observed that the insoluble oxalate content was lower (0.19%) at 100 mM NaCl application than 0 mM NaCl (0.25%), but this value tended to increase with further (from 300 to 900 mM NaCl) increases in salinity. In the second cut, salinity treatment had a significant ($p<0.001$) effect on insoluble and total oxalate contents in plant tissue (Table 2). The insoluble and total oxalate contents of napiergrass were significantly ($p<0.001$) increased with increasing salinity from 0 to 100 mM NaCl, while these values decreased significantly ($p<0.05$) with further (from 300 to 900 mM NaCl) increases in salinity. The 100 mM NaCl treatment produced higher insoluble (1.15%) and total oxalate (2.15%) contents than all other treatments. In the third cut, there were no significant ($p>0.05$) differences in the insoluble and total oxalate contents of plants in all other treatments, except for the 900 mM NaCl treatment which produced significantly ($p<0.05$) lower insoluble (0.09% vs. 0.44%) and total oxalate (0.94% vs. 1.39%) contents than 0 mM NaCl treatment.

From the average values of all cut forages, it was observed that salinity treatments did not affect ($p>0.05$) the soluble oxalate content of napiergrass. There was a trend to higher value at 100 mM NaCl (1.35%) application than 0 mM NaCl (1.15%), but this value tended to decrease with further (from 300 to 900 mM NaCl) increases in NaCl application. There were no significant ($p>0.05$) differences in the insoluble and total oxalate contents of plants in all other treatments, except for the 100 mM NaCl treatment which produced significantly ($p<0.05$) higher insoluble (0.44% vs. 0.15%) and total (1.79% vs. 1.30%) oxalate contents than 900 mM NaCl treatment. This result may be explained by a salinity effect on physiological responses of plants. For example, Gul et al. (2000) reported that net photosynthesis increased at low salinity (200 mM NaCl), but photosynthesis at other salinities (400, 600, 800 and 1,000 mM NaCl) was not significantly different from the control (without NaCl). Parida et al. (2004) also reported that photosynthetic rate in *Bruguiera parviflora* increased at low salinity and decreased at high salinity. In the present study, the trend for higher oxalate content at 100 mM NaCl application than for other salinity treatments may be due to photosynthetic responses. Fujii et al. (1993) proposed that inhibition of photorespiration may lower the rate of biosynthesis of oxalate in spinach.

Williams (1960) observed that control (without NaCl) treatment produced significantly lower (1.09%) soluble oxalate content in halogeton plants than treatments of 0.001 N (3.13%), 0.01 N (3.66%) and 0.1 N NaCl (3.90%). It is not unusual for plants to accumulate the secondary compound (such as oxalates, tannins, nitrate, betaine, coumarins and possibly other triterpenoids, steroids, glycosides, saponins and alkaloids) under salinity stress condition (Masters et al., 2001), but this study failed to produce higher oxalate accumulation at higher levels of salinity stress. However, this finding was in agreement with other studies reported by Singh (1974), Williams et al. (1991), Fowler et al. (1992) and Ala et al. (1995). Singh (1974) reported that if chloride or other anions are absorbed by plants, these anions compete for cations and depress oxalate synthesis. Fowler et al. (1992) observed that oxalate levels of Russian thistle (*Salsola iberica*) were reduced by salinity stress. Williams et al. (1991) reported that the content of soluble oxalate was slightly lower (2.8%) or similar (3.1%) to that of controls (3.1%) in kikuyugrass treated with 0.001 M or 0.01 M NaCl, respectively. It seems that accumulation of oxalate in plants in the presence of salinity might be affected by the chlorine ion as well as by photosynthesis rate.

In the current study, the 100 mM NaCl treatment was most effective in promoting oxalate formation, but the differences among treatments were too small to be considered toxic for ruminants. Moreover, the 100 mM NaCl application (cumulative) of this study roughly equaled 0.6% of the salt content of saline soil, which can be considered practical for low levels of salinity in coastal areas. For example, Tóth et al. (1995) reported that total salt content was over 0.8% at 0-10 cm and remained constant at around 0.5% at depths below 40 cm in saline soil of the Hai River Basin in China.

In conclusion, compared to all other treatments, dwarf napiergrass treated with 100 mM NaCl exhibited a higher soluble oxalate content but the differences were not significant. Across all cut forages, although salinity stress had significant effects on insoluble and total oxalate contents between the 100 and 900 mM NaCl treatments, the differences were too small to be considered biologically important. The present study indicates that where the soil is high in NaCl, dwarf napiergrass will tend to grow well and be low in oxalate.

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