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Influence of seasonal variation on oxalate accumulation in Napier Bajra hybrid under different nitrogen nutrition

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Abstract

Nitrogen fertilization is one of the most practical and effective way to improve yield and nutritional quality in crops but higher doses may lead to accumulation of anti-nutritional components like oxalates, nitrates and HCN. The present study was conducted to evaluate the effect of seasons, nitrogen doses and sources on oxalate content of Napier Bajra (NB) hybrid. The experiment was designed in randomized block design with three nitrogen sources (nitrate, amide and ammonium) at three doses (50, 75 and 100 kg N/ha) and replicated thrice. First cutting was taken after two months and subsequent cuttings were taken at an interval of 35-40 days. Total oxalate content varied with increasing doses of nitrogen fertilization and seasons, but effect was not consistent in all the cuttings. Soluble oxalate level was increased with nitrogen fertilization and nitrate form increased the content above toxic level (<20 g kg^{-1}DM). Insoluble oxalate content decreased with nitrogen fertilization.

Keywords: Cutting frequency, Napier Bajra hybrid, Nitrogen fertilization, Nitrogen sources, Oxalate

Introduction

Napier Bajra hybrid (NBH, cv. PBN-233), a hybrid of bajra (*Pennisetum glaucum*) and napier grass (*Pennisetum purpureum*) developed at Punjab Agricultural University, Ludhiana is considered as major breakthrough in green fodder production. As the area under forages is decreasing due to the ever increasing cultivation of cereal crops such as wheat and rice (Hazra, 2014), NB hybrid seems to be a promising fodder at this situation. It is fast growing multi-cut fodder crop and provides fodder from April to November. With higher yields of green fodder (2700 to 2800 q/ha) and nutritive value, it is rated as potential forage crop of coming times (Antony and Thomas, 2014). However, several cases of ruminal disorder are reported in dairy animals fed Bajra and Napier Bajra hybrid. It was suspected that this type of syndrome was due to feeding overgrown fodder or due to the presence of higher quantity of oxalates in these fodders.

Oxalate is common constitute of plants that occurs in two forms: insoluble and soluble oxalates. Many forages plants accumulate oxalate 3-10% of plant dry mass (Yu *et al.,* 2010). Excess level of oxalate in forages act as anti-nutritive factor, calcium binding component and sometimes lead to excessive mobilization of bone mineral. In general, oxalate poisoning is a complex issue. Factors such as chemical form of oxalate, age of animals, forage type, composition of diet and availability of water influence the susceptibility of animals to oxalate poisoning.

While application of nitrogen (N) strongly influence plant growth, but excessive and inappropriate use may accumulate anti-nutritive components in the edible products of the plants. Experiments carried out with tropical grasses showed that soluble oxalate content increased with increasing levels of N fertilizer (Mani and Kothandaraman, 1980). The objective of this study was to evaluate whether interactive effect of different doses of nitrogen fertilization and N sources can affect oxalate accumulation in different harvest seasons of Napier Bajra hybrid.

Materials and Methods

***Plant cultivation, experimental design and sampling procedure*:** Stem cuttings of released variety of NB hybrid (PBN 233) were planted in experimental field of Forage Research Farm, Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana, Punjab, India during *kharif* season in 2013. The crop was planted at second week of April in 30 plots each of 4.8 m x 2.4 m size. Each plot was planted with thirty-two stem cuttings at 60 cm x 60 cm row spacing. A factorial plan (3 N levels × 3 N sources) in a randomized block design with three replications was used. Nitrogen levels (50, 75, 100 kg N/ha) in the form of KNO_3, urea and $(NH_4)_2SO_4$ were applied in divided doses. First dose was added at first irrigation and second dose was applied after 10 days of planting/previous harvest. Plant tops were harvested (15 cm above the soil surface) four times, first cutting at 2 months interval and subsequent cutting at 35-40 days interval. Leaf/stem ratio was measured after separating both portions of samples. The harvested samples were chopped and dried in a oven till the constant weight was obtained. The samples were ground in a Willy mill through 1mm screen. Total and soluble oxalate was analyzed using the method of Abeza *et al.* (1968). The difference between total and soluble oxalate was considered as the level of insoluble oxalate.

The data was statistically analyzed using analysis of variance (ANOVA). Further mean separation of treatment effects was accomplished by using Fisher's protected least significant difference test. All data analysis was carried out using SAS software.

Results and Discussion

Oxalate content

***Total oxalate content*:** Total oxalate content varied non-significantly (P>0.05) with N fertilization doses (Table 1) as reported earlier by Rahman *et al.* (2009) in napier grass. Our results depicted higher oxalate accumulation with nitrate form of fertilization as compared to other two forms of fertilization in each harvest (P<0.001). Similar results were obtained on napier grass in nutrient solution (Rahman *et al.,* 2009). The mechanistic basis of oxalate accumulation in relation to nitrate/amide/ammonium treatment has long been a subject of interest but is little understood. Some studies also reported higher oxalate accumulation in nitrate treated plants (Tanaka *et al.,* 2002; Rahman and Kawamura, 2011). In the present study, a significant lower oxalate accumulation was observed in ammonium treatment as compared to nitrate treatment. Earlier studies also showed sole ammonium nutrition caused toxicity to many plant species (Rahman *et al.,* 2010). Other possible reason of lower oxalate accumulation may be that plants grown with ammonium fertilization, acidified the cytoplasm due to excretion of H^+ from plant roots which probably inhibit organic acid biosynthesis including oxalic acid (Schubert and Yan, 1997). Among different harvest seasons, total harvest mean (g kg^{-1} DM) was observed comparatively higher in summer (39.71) season followed by autumn (38.21), pre-winter (35.77) and monsoon (34.49) season. Though N fertilization was of same rate in each harvest, the variable oxalate level with N fertilization in each harvest might be due to seasonal variation as temperature, precipitation and relative humidity vary a lot in each harvest. Singh (2002) also observed high values of oxalate in Napier bajra hybrid during the month of June and July. A significant (P<0.001) interaction was observed between N doses and seasons. In the present study, total oxalate content in summer and monsoon seasons found to be increased with N application rates, but this effect was not consistent in the subsequent harvesting seasons. Total oxalate content decreased in autumn and pre-winter seasons with N fertilization. The results were in accordance with Rahman *et al.* (2008) where a decreasing trend of oxalate accumulation with increased fertilization rate in second harvest of Rhode grass was observed. Further variability in oxalate content with nitrogen fertilization in each harvest could also be correlated with changed leaf/stem ratio. Onset of monsoon observed a positive interaction of N fertilization rate with oxalate accumulation and leaf/stem ratio but late monsoon and onset of autumn seasons observed a negative relationship of nitrogen fertilization rate with these two factors. The declined leaf/stem ratio with increased fertilization rate in third and fourth harvest (Table 5) thus might be responsible for inverse trend of oxalate content in these harvests as stem contained less oxalate than leaves (Ji and Peng, 2005; Ugrinovic *et al.,* 2012). In first two harvests, though leaf/stem ratio was less than third harvest, but it tended to increase with higher fertilization rate. So measurement of botanical fractions was an important parameter in assessing variable trend of oxalate accumulation in NBH. Oxalate content in each harvest of Napier Bajra hybrid seems to be the result of interactive effect of seasons, N fertilization rates and N sources. The interactive effect of N sources, doses and harvest seasons for total oxalate content was non-significant (P>0.05).

***Soluble oxalate*:** Soluble oxalate content increased significantly (P<0.001) with N fertilization (Table 2). The oxalate level was higher at 100 kg N/ha and lower level was found with 50 kg N/ha fertilization rate in all the three N sources. Among N sources, highest soluble oxalate content (g kg^{-1} DM) was observed in KNO_3 treated plants (21.94) followed by urea (16.58) and $(NH_4)_2SO_4$ (14.58) treated plants (P<0.001). A significantly higher soluble oxalate accumulation was observed in nitrate treated plants than amide and ammonium treated plants. Soluble oxalate level was < 2% (on dry matter basis) in first three harvest seasons of KNO_3 treated plants, which could lead to acute toxicosis in ruminants (Mckenzie *et. al.,*

Table 1. Total oxalate content (g kg^{-1} DM) in whole plant of Napier Bajra hybrid

N Source	N dose (kg N/ha)	Harvest season				Pooled mean
		Summer	Monsoon	Autumn	Pre-winter	
KNO_3	50	42.33±2.08	32.00±1.00	46.33±1.53	40.66±1.53	40.33
	75	42.66±2.08	39.33±1.53	41.00±2.00	39.33±0.58	40.58
	100	45.00±1.00	40.66±0.58	39.66±2.08	36.33±1.53	40.41
	Mean	43.33	37.33	42.33	38.77	40.44
Urea	50	37.33±2.52	32.33±1.53	40.33±1.53	38.66±0.58	37.16
	75	38.66±3.06	34.00±1.00	37.00±2.00	37.33±1.53	36.75
	100	39.66±0.58	36.00±1.00	35.66±0.58	31.33±0.58	35.66
	Mean	38.55	34.11	37.66	35.77	36.52
$(NH_4)_2SO_4$	50	34.66±2.08	28.33±2.52	35.33±1.53	35.00±2.00	33.33
	75	37.00±2.00	31.66±1.53	33.33±0.58	32.33±2.52	33.58
	100	37.66±1.53	33.66±1.53	31.66±0.58	28.00±1.00	32.75
	Mean	36.44	31.22	33.44	31.78	33.22
	Harvest mean	39.71	34.49	38.21	35.77	

CD (P<0.05) A (N Sources) = 0.751, B (N doses) = NS, C (Seasons) = 0.867
AB = NS, AC = NS, BC = 1.501, ABC = NS

Overall mean of individual N dose across harvest seasons, 50= 36.94, 75= 36.97, 100= 36.27
Values are mean ± standard deviation of three replications, NS = non-significant

Table 2. Soluble oxalate content (g kg^{-1} DM) in whole plant of Napier Bajra hybrid

N Source	N dose (kg N/ha)	Harvest season				Pooled mean
		Summer	Monsoon	Autumn	Pre-winter	
KNO_3	50	25.00±2.00	20.00±1.00	18.33±1.53	17.00±1.73	20.08
	75	26.66±2.52	22.00±1.00	20.00±1.00	19.00±1.00	21.92
	100	28.66±0.58	24.66±1.53	22.33±0.58	19.66±0.58	23.83
	Mean	26.77dC	22.22cC	20.22bB	18.55aC	21.94
Urea	50	16.00±2.00	14.33±1.53	14.33±058	13.33±1.53	14.50
	75	19.33±1.53	17.00±1.00	14.66±1.53	15.33±0.58	16.58
	100	21.66±1.53	20.00±1.00	17.33±1.53	15.66±0.58	18.66
	Mean	19.00cB	17.11bB	15.44aA	14.77aB	16.58
$(NH_4)_2SO_4$	50	14.33±2.08	13.00±1.00	12.33±1.15	11.33±0.58	12.75
	75	17.00±2.00	14.66±1.15	15.33±1.15	13.33±0.58	15.08
	100	18.33±1.15	16.33±0.58	15.00±1.00	14.00±1.00	15.92
	Mean	16.55cA	14.66bA	14.22bA	12.89aA	14.58
	Harvest mean	21.16	18.30	16.85	15.63	

CD (P<0.05) A (N Sources) = 0.615, B (N doses) = 0.615, C (Seasons) = 0.710
AB = NS, AC = 1.230, BC = NS, ABC = NS

Overall mean of individual N dose across harvest seasons, 50= 15.78, 75= 17.86, 100= 19.47
Values are mean ± standard deviation of three replications, NS = non-significant
[a - d] alphabets show significant difference between the means of each nitrogen source among different harvest seasons (P<0.05).
[A - D] alphabets show significant difference between the means of each harvest season among different nitrogen sources (P<0.05).

1988). Soluble oxalate can bind with calcium in the intestine to form insoluble calcium oxalate crystals thus lowering calcium serum levels. In a nutrient solution study on napier grass, Rahman *et al.* (2009) also observed that nitrate treated napier grass accumulated significantly higher soluble oxalate content than ammonium treated plants. Soluble oxalate content of NB hybrid was < 20 g kg^{-1} DM in all levels of N fertilization and harvest seasons in amide and ammonium treated plants and thus could be used safely in relation to oxalate toxicity level.

Irrespective of N sources, soluble oxalate content was higher in summer (21.16 g kg^{-1} DM) i.e. the month of June and decreased afterwards in the subsequent harvest seasons (P<0.001). Similar trend of soluble oxalate in different cutting of napier grass was also depicted by Rahman *et al.* (2014). A significant interaction (P<0.001) was observed between harvest seasons and N forms. Overall interaction of N sources, N doses and harvest seasons was found to be non-significant (P>0.05).

Table 3. Insoluble oxalate content (g kg^{-1} DM) in whole plant of Napier Bajra hybrid

N Source	N dose (kg N/ha)	Harvest season				Pooled mean
		Summer	Monsoon	Autumn	Pre-winter	
KNO_3	50	17.33±0.06	12.00±0.17	28.00±0.26	23.66±0.12	20.25
	75	16.00±0.35	17.33±0.25	21.00±0.26	20.03±0.12	18.67
	100	16.33±0.15	16.00±0.17	17.33±0.25	16.66±0.15	16.58
	Mean	16.55aA	15.11aA	22.11cB	20.22bB	18.50
Urea	50	21.33±0.45	18.00±0.26	26.00±0.17	25.33±0.15	22.67
	75	19.33±0.21	17.00±0.00	22.33±0.21	22.00±0.17	20.17
	100	18.00±0.10	16.00±0.10	18.33±0.21	15.66±0.12	17.00
	Mean	19.55bB	17.00aB	22.22cB	21.00bC	19.94
$(NH_4)_2SO_4$	50	20.33±0.29	15.33±0.15	23.00±0.26	23.66±0.21	20.58
	75	20.00±0.35	17.00±0.17	18.00±0.17	19.00±0.30	18.50
	100	19.33±0.21	17.33±0.21	16.66±0.12	14.00±0.10	16.83
	Mean	19.89bB	16.55aB	19.22bA	18.89bA	18.64
	Harvest mean	18.55	16.19	21.36	20.14	
CD (P<0.05)	A (N Sources) = 0.997, B (N Doses) = 0.997, C (Seasons) = 1.151 AB = NS, AC = 1.993, BC = 1.993, ABC = NS					

Overall mean of individual N dose across harvest seasons, 50= 21.17, 75= 19.08, 100= 16.80
Values are mean ± standard deviation of three replications, NS = non-significant
[a - d]alphabets show significant difference between the means of each nitrogen source among different harvest seasons
[A - D]alphabets show significant difference between the means of each harvest season among different nitrogen sources

Table 4. Nitrogen (N) content (g kg^{-1}DM) in leaves of Napier Bajra hybrid

N Source	N dose (kg N/ha)	Harvest season				Pooled mean
		Summer	Monsoon	Autumn	Pre-winter	
KNO_3	50	13.44±0.97	9.80±0.00	11.48±1.84	12.95±0.99	11.92
	75	16.61±0.70	10.92±1.01	12.51±1.13	13.26±0.84	13.33
	100	17.45±2.12	12.20±1.17	12.69±1.44	14.75±0.81	14.27
	Mean	15.83	10.97	12.23	13.65	13.17
Urea	50	14.84±1.01	9.99±0.70	12.13±0.98	12.04±0.84	12.25
	75	15.84±0.67	10.17±0.81	13.07±0.81	13.56±0.62	13.16
	100	18.08±0.61	10.36±0.74	13.81±0.58	14.01±0.76	14.07
	Mean	16.25	10.17	13.00	13.20	13.16
$(NH_4)_2SO_4$	50	14.37±0.86	9.99±0.58	12.32±1.48	12.63±0.69	12.33
	75	16.14±2.33	10.80±1.32	13.00±0.75	12.97±0.90	13.23
	100	19.32±1.96	11.39±0.70	15.48±0.85	15.40±1.01	15.40
	Mean	16.61	10.73	13.60	13.67	13.65
	Harvest mean	16.20	10.62	12.88	13.49	
CD (P<0.05)	A (N Sources) = NS, B (N Doses) = 0.506, C (Seasons) = 0.584 AB = NS, AC = NS, BC = 1.01, ABC = NS					

Overall mean of individual N dose across harvest seasons, 50= 12.17, 75= 13.24, 100= 14.58
Values are mean ± standard deviation of three replications, NS = non-significant

Insoluble oxalate: In almost all the cuttings, the insoluble oxalate content decreased with N fertilization (P<0.001) (Table 3). In contrast to higher level of total and soluble oxalate in nitrate treated plants, no consistent trend was observed in case of insoluble oxalate level. Insoluble oxalate level (g kg^{-1} DM) was higher in amide (19.94) treated plants than ammonium (18.64) and nitrate (18.50) treated plants. Among the seasons, insoluble content (g kg^{-1} DM) was highest in autumn (21.36) and lowest in monsoon (16.19) season (P<0.001). A significant interaction of harvest season with N doses (P<0.001) and N forms (P<0.05) was observed. But the interactive effect of N sources, N doses and harvest seasons in terms of insoluble oxalate content was non-significant (P>0.05).

Nitrogen content

Nitrogen content increased significantly (P < 0.001) with

Table 5. Leaf to stem ratio of Napier Bajra hybrid

N Source	N dose (kg N/ha)	Harvest season				Pooled mean
		Summer	Monsoon	Autumn	Pre-winter	
KNO_3	50	0.73±0.06	0.59±0.08	2.97±0.15	1.09±0.10	1.35bB
	75	0.83±0.03	0.61±0.01	1.64±0.15	0.92±0.08	1.00aA
	100	0.90±0.09	0.76±0.11	1.53±0.06	0.86±0.06	1.01aA
	Mean	0.82bA	0.65aA	2.05dB	0.96cA	1.12
Urea	50	0.74±0.10	0.54±0.11	1.90±0.00	1.16±0.12	1.08aA
	75	0.81±0.12	0.57±0.06	1.60±0.10	1.02±0.14	1.00aA
	100	0.85±0.05	0.66±0.23	1.42±0.13	0.92±0.07	0.96aA
	Mean	0.80bA	0.59aA	1.64dA	1.03cB	1.01
$(NH_4)_2SO_4$	50	0.84±0.17	0.59±0.08	2.03±0.06	1.13±0.06	1.15bA
	75	0.93±0.15	0.63±0.04	1.47±0.06	0.99±0.09	1.00aA
	100	1.09±0.20	0.74±0.04	1.36±0.12	0.90±0.17	1.02aA
	Mean	0.96bB	0.65aA	1.62dA	1.01cB	1.06
	Harvest mean	0.86	0.63	1.77	1.00	
CD (P<0.05)	A (N Sources) = 0.051, B (N Doses) = 0.051, C (Seasons) = 0.059 AB = 0.088, AC = 0.101, BC = 0.101, ABC = 0.176					

Overall mean of individual N dose across harvest seasons, 50 = 1.19, 75 = 0.99, 100 = 0.99

Values are mean ± standard deviation of three replications

[a - d] alphabets show significant difference between the means of each nitrogen source among different harvest seasons (P<0.05)

[A - D] alphabets show significant difference between the means of each harvest season among different nitrogen sources (P<0.05)

[a - c] alphabets show significant difference between means of N doses in each N source (P<0.05)

[A - C] alphabets show significant difference between means of same N dose within different N sources (P<0.05)

Table 6. Correlation coefficients between oxalate content of whole plant, nitrogen content and leaf/stem ratio in Napier Bajra hybrid

		Total oxalate	Soluble oxalate	Insoluble oxalate	Nitrogen content
Soluble oxalate	(S)	.988**			
	(M)	.895**			
	(A)	.500			
	(P)	.401			
Insoluble oxalate	(S)	-.906**	-.961**		
	(M)	.222	-.237		
	(A)	.732*	-.225		
	(P)	.772*	-.273		
Nitrogen content	(S)	.146	.182	-.235	
	(M)	.702*	.554	.319	
	(A)	-.786*	-.162	-.757*	
	(P)	-.608	.373	-.897**	
Leaf/stem ratio	(S)	-.155.	-.108	.017	.806**
	(M)	528	.500	.058	.884**
	(A)	.766*	-.003	.864**	-.711*
	(P)	.453	-.613	.901**	-.848*

** = Significant (P<0.01), * = Significant (P<0.01), S = Summer, M = Monsoon, A = Autumn, P = Pre-winter

nitrogen fertilization rates (Table 4). Overall effect of N application for N content was in the range of 11.92 to 15.40 g kg^{-1} DM. Increase in N content with higher level of nitrogen application in quinoa (*Chenopodium quinoa* wild) and barley was observed by Kakabouki *et al.* (2014) and Meena and Mann (2010), respectively. Previous studies reported total nitrogen content of sorghum varied due to nitrogen and sulphur application (Mondal and Nad, 2011). Among N sources, there was marginal increase of N content in $(NH_4)_2SO_4$ treated plants (P>0.05). Interaction between nitrogen sources and fertilization rates were found to be non-significant (P>0.05). In different harvest seasons, overall harvest mean showed maximum N level (g kg^{-1} DM) in summer (16.23) which was decreased in monsoon (10.62) but further level was increased in autumn (12.94) and pre-winter (13.51) with nitrogen fertilization (P<0.001). N sources, doses and harvest seasons did not interact significantly (P>0.05).

Leaf/stem ratio

Leaf/stem ratio is an important component of forage quality. Young plants have high leaf/stem ratio compared to mature plants. It varied significantly (P<0.001) with nitrogen (N) fertilization in each harvest season, but changes were not consistent over all harvest seasons (Table 5). Leaf to stem ratio was increased with N application upto 80 kg N/ha and then decreased at 120 kg N/ha in oat fodder (Luikham *et al.,* 2012). It varied significantly (P<0.001) among different N sources and observed maximum with nitrate (1.12) form of fertilization followed by ammonium (1.06) and amide (1.01) form of fertilization. Harvest mean across N sources showed highest leaf/stem ratio in autumn (1.77) and lowest ratio recorded in monsoon (0.63). A significant (P<0.001) interaction was observed between N doses and seasons. Leaf/stem ratio was tended to increase with nitrogen fertilization in summer and monsoon season, but a significant decreasing trend was observed in autumn and pre-winter. Similar decrease in leaf/stem ratio with N fertilization in last two harvest period of pearl millet was observed by Shahin *et al.* (2013). Nitrogen sources, doses and harvest seasons (P<0.001) interacted positively for this trait.

Correlation studies

Total oxalate content positively correlated to nitrogen content in monsoon season whereas negatively correlated in autumn season (Table 6). Total oxalate content was also found to be positively correlated to leaf/stem ratio in autumn season at 5% level of significance. On the other hand, insoluble oxalate content revealed negative correlation with nitrogen content in autumn and pre-winter season whereas positive correlation with leaf/stem ratio in autumn and pre-winter season at 1% level of significance.

Conclusion

High N application rates did not significantly increase the oxalate accumulation in Napier Bajra hybrid but difference was significant in different harvests of N treatment groups. Soluble oxalate content was <20 g kg^{-1} DM in urea and ammonium treated plants in all the levels of N fertilization and harvest seasons and thus could be used safely for feeding in ruminant animals, avoiding oxalate toxicity. No consistent trend was observed in case of insoluble oxalate content. Leaf/stem ratio played a significant role in total oxalate accumulation with N fertilization.

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