Research Findings

Experimental setup at Jamia Hamdard University. Photo by S. Umar.

Managing Nitrate Accumulation in Forage Sorghum by Potassium Fertilization

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Abstract

Excessive nitrogenous fertilization or environmental stress due to drought, cold, frost, hail, leads to nitrate (NO_3^-) accumulation in forages. Elevated nitrate levels are a major concern because they can be detrimental to animal health and have caused several mass cattle-death incidents. A pot culture experiment was conducted under greenhouse conditions to screen 16 genotypes of sorghum (*S. bicolor* L.) for leaf nitrate reductase activity (NRA) and, hence, also potential nitrate accumulation. Marked differences in NRA and nitrate concentrations were observed among the genotypes, many of which accumulated nitrate to very high concentrations. From this screening experiment a high nitrate reductase (HNR) genotype and a low nitrate reductase (LNR) genotype viz. POP-52 (V9) and EB-15 (V7), respectively, were selected to study the effect of potassium (K) application on NRA and nitrate accumulation. The two sorghum genotypes were grown in specially designed PVC drums and the plants supplied with increasing levels of K, supplied as KCl at rates of 0, 30, 60 and 120 mg K_2O kg^{-1} soil. Measurements made for leaf NRA and nitrate concentration at 30 and 60 days after

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sowing (DAS). Regardless of K treatment, NRA values increased from the 30 to the 60 day harvest and, correspondingly, nitrate concentrations decreased. At both harvests, K treatment up to 60 mg K_2O kg^{-1} soil, (K_{60}) increased leaf NRA and depressed leaf nitrate accumulation. An approximately three-fold decrease in nitrate concentration was observed in the K_{60} treatment in both genotypes from 30 to 60 DAS. Leaves of the 60 day old (K_{60}) treated plants, V9, the genotype with the higher NRA (9.916 µmol NO_2^{-1} h^{-1} g^{-1} fresh wt.), showed the lower nitrate accumulation (816.6 mg kg^{-1} fresh wt) and vice versa with the V7, the genotype with the lower NRA (5.018 µmol NO_2^{-1} h^{-1} g^{-1} fresh wt.), which showed the higher nitrate accumulation (2691.8 mg kg^{-1} fresh wt.). K application also substantially lowered the nitrate concentration in the leachate indicating that K is effective in mitigating nitrate pollution in plants and soil. The results emphasize the importance of K in increasing nitrogen use efficiency (NUE) and of balanced fertilization in combating detrimental effects of nitrate on human beings, animals and the environment.

Introduction

Production and consumption of fertilizers is the main requirement for agricultural development but addition of fertilizers alone does not ensure enhanced crop production. Mineral nutrient fertilizer constituents, particularly nitrogen (N) and phosphorus (P) can leach into ground and surface waters due to excessive fertilizer input and poor application methods (EPA, 2010), which results in environmental damage including eutrophication. In order to feed the burgeoning population and maintain sustainable development, the amount of N needs to be double that currently applied, unless the N use efficacy of crops is improved (Anjana *et al.*, 2011). In view of the close interrelationship between N and K uptake, application of N has to be balanced by adequate K supply in order to be effective in increasing crop yields (Zhang *et al.*, 2010).

Annual crops growing in well aerated soils take up and assimilate N mainly in the form of nitrate (NO_3^-). In many parts of the world, nitrate concentrations in ground water exceed the maximum limit of 50 mg l^{-1}, equivalent to 11.3 mg l^{-1} as NO_3^--N as recommended by the (WHO, 2011). The main sources of nitrate contamination in water are intensive agricultural production, domestic and industrial wastes, sewage and atmospheric nitrogen pollution. When the N input exceeds the demand by plants, it builds up in the soil, mostly as nitrates, and leaches into the groundwater (Gairola *et al.*, 2009). Nitrate leaching, due to excessive N fertilization, leads to eutrophication of freshwater bodies and marine ecosystems. Any factor that slows down the rate of plant growth can lead to increased nitrate levels in well-fertilized plants. Accumulation of nitrate in plants is thus commonly observed during drought, long periods of cloudy or cool weather, or following heavy fertilization with manures and nitrogen-containing fertilizers or herbicide applications (Tuncay *et al.*, 2011). Nitrate accumulation in plants in extreme cases has been known to induce toxicity in animals which feed on them. Intrinsically, however, nitrate is not very toxic to animals. Once within the animal body, nitrates are converted to nitrites and then to nitrosamines that are believed to be associated with gastric cancer and other complications like methemoglobinemia (condition affecting oxygen carrying capacity of red blood cells) (Fahmy *et al.*, 2010).

Sorghum is the fifth most important cereal crop grown in the world and is also valued for its fodder and stover. It is a known accumulator of toxic levels of nitrate even at moderate N fertility levels. In India, forage sorghum is grown on 2.6 million ha predominantly in the states of western Uttar Pradesh (UP), Haryana, Punjab, Rajasthan and Delhi, which fulfills over two thirds of the fodder demand during the Kharif (summer) season.

Sorghum plant grown in the experimntal drum (column). Photo by S. Umar.

K is one of the essential mineral elements for plant growth and development and plays a key role both in the uptake of nitrate and at various steps during N assimilation and metabolism, as well as in numerous other biochemical and physiological processes (Marschner, 2012). It thus has a major impact on agricultural ecosystems. According to Shrotriya (1998), balanced application of N, P and K could increase sorghum yield in India by up to 122%. Thus, imbalanced fertilization with an increase in N at the cost of a decrease in K has a major detrimental impact on the utilization of N itself.

Improving crop performance through balanced fertilization by application of K is a prerequisite for minimizing environmental risks due to N losses as well as nitrate poisoning in ruminants. In this work we report on a greenhouse study on sorghum to investigate the influence of increasing supply of K on two selected genotypes differing greatly in leaf nitrate reductase activity (NRA) and nitrate accumulation. We also report on the effects of increasing K supply on nitrate leaching.

Materials and methods

The experiments

A greenhouse pot experiment was conducted in the Herbal Garden of Jamia Hamdard, New Delhi, during the Kharif (summer) season of 2010-2011 in order to screen 16 genotypes of sorghum (*S. bicolor* L.) for both nitrate reductase activity (NRA) and nitrate concentration in the leaves. Seeds for the 16 genotypes, namely: CSV 15, CSV 21F, CSV23, E-68-1, E73, E77, EB-15 (V7), HC-308, POP-52 (V9), SPSSV 5, SPSSV 6, SPSSV 7, SPSSV 20, SPSS 422, SPV 462, and SPV 913 were obtained from the Sorghum Research Institute, Hyderabad (India). The plants were grown in earthen pots of about 25 cm diameter (with 4 plants per pot) with three replicates per genotype (a total of 48 pots). Prior to sowing, the pots were lined with polythene bags and filled with 8 kg of soil taken from the Herbal Garden, which had been thoroughly mixed with a uniform basal dressing of fertilizers for all the pots. This comprised N (120 mg kg^{-1}) as urea, P (30 mg kg^{-1}) as single super phosphate, K (80 mg kg^{-1}) as muriate of potash (KCl) and Zn (25 mg kg^{-1}) as $ZnSO_4 \cdot 7H_2O$. The Herbal Garden soil (Lukhi soil series of Gurgaon) is a sandy loam (83.6% sand, 6.8% silt and 9.6% clay) with a neutral pH 7.1 and is low in available K (40 mg kg^{-1} soil) N (30 mg kg^{-1}) and P (4 mg kg^{-1}). Thirty days after sowing (DAS), fully expanded leaves from the same position were analyzed simultaneously for NRA and nitrate concentration. The genotypes POP-52 and EB-15 were identified as highest and lowest in NRA, respectively and these two genotypes were used in the second experiment.

The purpose of the second experiment was to test the effects of increasing supply of K on the activities of NRA and nitrate accumulation in the leaves of these two very different sorghum genotypes in NRA activity. The experiment also allowed a simultaneous investigation of possible differences in nitrate leaching as influenced by the two genotypes in relation to K supply. In this experiment the plants were grown in much larger containers: PVC drums 25 cm × 120 cm (diameter × height) with a total capacity of 60 liters using the same amended Herbal Garden soil as that of the pot experiment except for the K supply. Increasing levels of potassium were tested: 0, 30, 60 and 120 mg K_2O kg^{-1} soil (K_0, K_{30}, K_{60} and K_{120} respectively, applied as KCl). Three drums were used per treatment for both the genotypes at 30 and 60 DAS so that there was a total of 24 drums in the experiment. Ten seeds each of high and low NRA genotypes were sown, which were thinned down to four plants per drum. The experimental drums were fitted with drainage systems at three different sites on the drums (30, 60 and 100 cm height of the soil column). Glass wool filled the lower opening of the drum above a 10 cm height filling of washed fine gravel. Plastic funnels (10 cm diameter) with PVC tubes (5 mm diameter) were fitted to each drain to collect the leachate. Before planting, the soil was irrigated with sufficient water for seed germination. The pots were weeded and scarified weekly. Fully expanded leaves from the same position were sampled in triplicates per drum at two different stages, viz. 30 and 60 DAS. The schematic diagram of the experimental set up is given (Fig. 1).

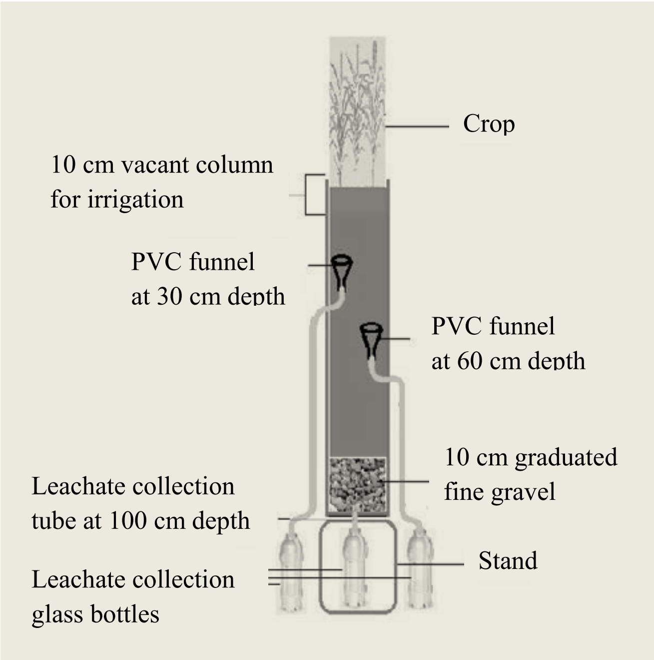

Fig. 1. Schematic sketch of the column used showing the collection of leachate at various depths of the soil.

Chemical estimations

Nitrate was extracted from ground dried leaf material using the method of Grover *et al.* (1978) followed by the reduction hydrazine method of Downes (1978). This is based on the reduction of nitrate to nitrite followed by diazotization from the addition of sulphanilamide and naphthyl ethylene diamine dihydrochloride to produce a pink colored solution - the intensity of which depends on the nitrite concentration and is measured using the spectrophotometer at 540 nm. Nitrate concentration is expressed as mg g^{-1} fresh weight of leaves. Nitrate reductase activity (NRA) in the leaves was determined by the intact tissue assay method of Jaworski (1971) using the method of estimating nitrite as described above. The estimation of NRA is dependent on the rate of formation of nitrite and is expressed as µmol NO_2^- h^{-1} g^{-1} fresh wt. Biomass nitrogen utilization efficiency was calculated using the formula: NutE = Biomass (dry matter) (g $plant^{-1}$)/Total N content (g $plant^{-1}$). Leachate was collected after each irrigation for nitrate analysis, which was determined using the same basic method as described above. Available N, P and K values in the Herbal Garden soil were determined by soil extraction methods described by Kalra and Maynard (1994), Oslen *et al.* (1954) and Hanway & Heidal (1952), respectively.

Results and discussion

Marked variation was evident in the leaf NRA in the 30 day old plants of the 16 genotypes as detailed in Table 1. POP-52 (V9) genotype had the highest level of NR activity (7.024 µmol NO_2^{-1} h^{-1} g^{-1} fresh wt.) with minimum nitrate concentration, while the lowest level of NR activity was observed in EB-12 (V7) genotype (1.813 µmol NO_2^{-1} h^{-1} g^{-1} fresh wt.) with the maximum nitrate concentration. Leaf nitrate concentrations also varied significantly among the 16 sorghum genotypes in the 30 day old plants. This observation is in keeping with the inter- and intra-species variations in leaf nitrate content reported in many crop plants (Anjana *et al.*, 2007). A significant negative relationship (r = -0.913) between the NRA level and leaf nitrate concentration was found among the 16 genotypes (Fig. 2).

Leaf nitrate concentration under K application

There is evidence in the literature that K stimulates N assimilation so that increased K fertilization can depress nitrate accumulation as observed, for example, by Nurzynska-Wierdak *et al.*, (2012) in rocket leaves. Our study findings also confirm that K application decreased nitrate accumulation significantly ($p<0.05$) and that the decrease was substantial in the leaves of both the sorghum genotypes studied (Table 2). A reduction in nitrate concentration at $K_{60,}$ 35.24% (V9) and 26.0% (V7), occurred over the control (K_0) at 30 DAS. An approximately three-fold difference was observed between leaf nitrate concentrations at 30 and 60 DAS. Genotype V9 (816.6 mg of nitrate kg^{-1} fresh wt.) showed the lowest nitrate concentration at K_{60} (Table 2).

Leaf NRA under K application

Strategies for improving nitrogen assimilation require an understanding of the N-assimilation pathway. NRA, which reduces nitrate to nitrite, is assumed to be the rate-limiting step for nitrate assimilation in plants. A positive and linear relationship was shown to occur between NRA and K-fertilization up to K_{60} in both genotypes at 30 and 60 DAS. At the highest K application (K_{120}), activity appeared to be depressed in both genotypes. The

Table 1. Variation in nitrate reductase activity (NRA) (µmol nitrite h^{-1} gm^{-1} fresh wt. of leaves) and nitrate concentration (mg kg^{-1} fresh wt. of leaves) in leaves of 30 day old plants of 16 *Sorghum bicolor* L. genotypes.

Sl. no.	Code no.	NRA	Nitrate
1	V1	3.536±0.097	3726.67±29.72
2	V2	5.063±0.079	2406±27.18
3	V3	4.448±0.096	2870.33±36.11
4	V4	2.905±0.092	4508.67±36.42
5	V5	2.582±0.12	5397±46.01
6	V6	2.152±0.095	6492±40.19
7	V7	1.813±0.031	7121.33±22.63
8	V8	3.573±0.049	3466.67±32.8
9	V9	5.989±0.096	2308.67±24.25
10	V10	2.380±0.067	6107.67±44.4
11	V11	4.360±0.039	2643.33±25.3
12	V12	2.449±0.046	5731.67±36.4
13	V13	4.288±0.087	2476±12.5
14	V14	4.039±0.046	3218.67±33
15	V15	2.635±0.05	5147.67±26.74
16	V16	2.778±0.057	4880±27.60

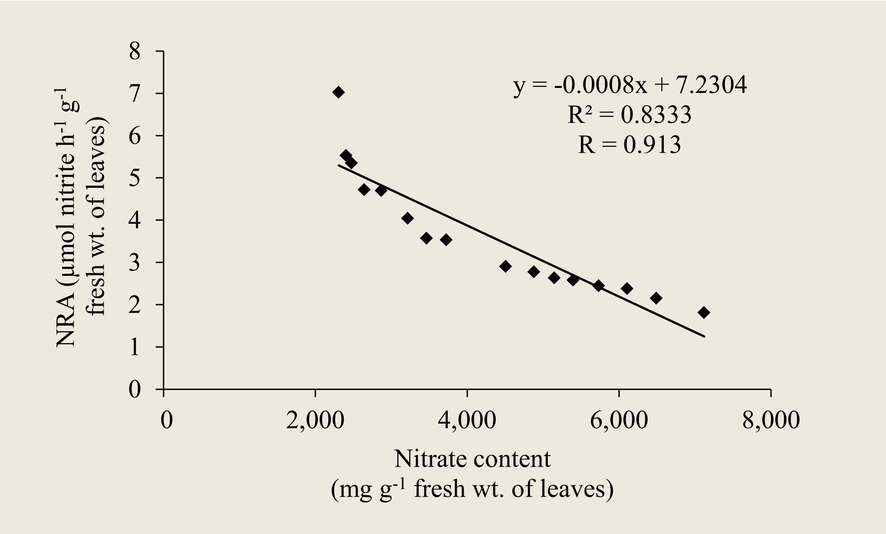

Fig. 2. Relationship between nitrate concentration and nitrate reductase activity in leaves of sixteen sorghum genotypes in 30 day old plants.

Table 2. Effect of applied potassium on nitrate reductase activity (NRA) (*in vivo*) (µmol nitrite h^{-1} gm^{-1} fresh wt.), nitrate concentration (mg kg^{-1} fresh wt.) at 30 and 60 DAS, nitrate concentration (mg l^{-1}) of leachate at 30 cm, 60 cm and 100 cm depths and biomass nitrogen utilization efficiency (NUE) (g $plant^{-1}$).

Treatments	NR activity at 30 days		NR activity at 60 days	
	HNR	LNR	HNR	LNR
K_0	3.21±0.035d	1.646±0.022d	7.203±0.203c	3.154±0.065d
K_{30}	3.416±0.032c	1.814±0.028c	7.462±0.104c	3.738±0.059c
K_{60}	5.978±0.042a	2.174±0.039a	9.916±0.212a	5.018±0.053a
K_{120}	4.918±0.026b	1.984±0.037b	8.142±0.078b	4.178±0.059b

Treatments	Nitrate at 30 days		Nitrate at 60 days	
	HNR	LNR	HNR	LNR
K_0	3,272.04±215.169a	9,600.97±313.494a	1,726.8±46.067a	5,179±49.79a
K_{30}	2,533.96±116.899b	8,073.31±405.298b	1,507.4±41.966b	3,627.8±43.22b
K_{60}	2,118.89±114.216c	7,104.06±235.615c	816.6±45.181d	1,969.4±38.47d
K_{120}	2,112.80±147.157c	7,096.18±346.563c	1,050.8±40.59c	2,199.4±39.20

Treatments	Leachate nitrate concentration at 30 cm		Leachate nitrate concentration at 60 cm		Leachate nitrate concentration at 100 cm	
	HNR	LNR	HNR	LNR	HNR	LNR
K_0	20.49±0.38a	23.11±0.19a	26.65±0.23a	28.75±0.25a	20.45±0.31a	21.67±0.15a
K_{30}	18.11±0.22b	21.56±0.16b	23.38±0.27b	25.36±0.39b	16.54±0.33b	20.04±0.34b
K_{60}	14.17±0.18c	17.69±0.23c	20.46±0.26c	23.12±0.46c	14.1±0.25c	17.80±0.20c
K_{120}	13.90±0.25d	14.76±0.14d	20.12±0.22d	22.00±0.27d	12.52±0.21d	14.86±0.15d

Treatments	Biomass NUE	
	HNR	LNR
K_0	37.1±0.55d	31.05±0.24d
K_{30}	41.43±0.43c	33.86±0.24c
K_{60}	45.93±0.36a	39.09±0.37a
K_{120}	44.68±0.25b	38.18±0.28b

Note: Values represent mean ± SE. Rows showing different letters (a-d) indicate significant differences according to Duncan's test at $p<0.05$.

increase in NRA was significant at ($p<0.05$) with increasing levels of K application. The V9 genotype showed the highest NRA at K_{60} at 30 and 60 DAS, anthe lowest at K_0 (Table 2).

Biomass NUE in leaves at 60 DAS

The most effective way to improve the efficiency of N fertilizers is adequate and balanced use of fertilizer nutrients. Biomass nitrogen utilization efficiency (NUE) significantly ($p<0.05$) increased from K_0 to K_{60} in both genotypes. In V9, biomass NUE of (45.93) was greater than V7 (39.09) at K_{60}. The minimum biomass NUE was recorded at K_0 both in V9 (37.1) and V7 (31.05) (Table 2). Brar *et al.* (2012) also reported that application of K effectively increased the NUE of maize. A higher NUE implies a more efficient utilization of N and little wastage. Improving NUE with K means that a lower amount of N can be applied without affecting yield, thereby preventing land and water contamination.

Nitrate content in the leachate

Imbalanced nitrogen fertilization leads to nitrate leaching as it shows a negligible interaction with the negatively charged matrix of most topsoil, especially in sandy soils with minimum nutrient retention capacity. As evident from Table 2, the leachate nitrate decreased significantly ($p<0.05$) with increasing K levels (from K_0 to K_{60}) at all soil depths (30, 60 and 100 cm). The lowest nitrate concentrations were recorded with K_{60} in both the genotypes at all soil depths; however, the value was comparatively lower in V9. This decrease in nitrate concentration in the leachate can be attributed to the greater utilization of the available nitrate as a consequence of K application.

Conclusions

Nitrate concentration in the leaves was the highest at the younger stage (30 DAS). However, with K application, especially at K_{60}, leaf nitrate concentration decreased considerably, while the NRA appeared at the highest level in both the genotypes (V9 and V7). Genotype V9 showed better growth seemingly through higher NRA and lower nitrate concentration in the leaves and leachate compared to V7 at both the growth stages. K application showed the greatest effect after 60 DAS. Thus, balanced nutrient management with K application at 60 mg K_2O kg^{-1} soil (K_{60}) appears to lower nitrate accumulation and sustain growth and productivity of sorghum. This lowering of nitrate is of benefit in preventing toxicity in animals and decreasing the load on ground water. Furthermore, it is advantageous to the farmer to attain a cost effective strategy for fertilizer management without wastage of fertilizers. Further studies are required to validate these findings on a larger scale in the field using different crops at various locations under different climatic conditions.

Acknowledgements

We gratefully acknowledge Prof. M. Iqbal, Dept. of Botany, Jamia Hamdard, and Dr. Patricia Imas for their assistance in shaping the manuscript. Generous help was rendered by the Sorghum Research Institute, Hyderabad, in providing sorghum seeds, and is gratefully acknowledged. The authors also thank the International Potash Institute (IPI), Switzerland, for funding this study.

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The paper "Managing Nitrate Accumulation in Forage Sorghum by Potassium Fertilization" also appears on the IPI website at:

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