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RESEARCH PAPER

Nitrate as a bio-indicator for enhancing the nitrogen use efficiency in maize

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Abstract: Maize has robust growth habit with high requirement of nutrients, particularly nitrogen. Rapid tests to assess the nutritional status on site can make easier decision of any adjustments in the nitrogen fertilization. Hence , a field experiment was conducted during *kharif* 2017 at Main Agricultural Research Station (MARS), University of Agricultural Sciences, Dharwad to assess the nitrogen requirement using nitrate as a bio-indicator in maize to enhance NUE. Plant sap nitrate content was measured on site using Cardy nitrate ion meter at critical stages under different levels of nitrogen. Difference in nitrate values between under supplied nitrogen to hundred per cent supplied nitrogen recommended were used as indices for application of deficit nitrogen. Results revealed that nitrate is a good bio-indicator of nitrogen status of maize at critical stages. Supplementing the N based on sap nitrate test has saved 28.5 per cent nitrogen and enhanced nitrogen use efficiency.

Key words: Cardy nitrateion meter, Crop growth stages, Nitrate, Nitrate nitrogen

Introduction

Nitrogen, phosphorus pentoxide and potash are common nutrients applied every year in all crops. The ideal ratio of the application is 4:2:1 and the ratio now has increased to 8:3:1, the higher increase in N rather than other two is causing grave concern to agriculture sustainability and pollution (Anon., 2018).There is enormous decrease in nitrogen use efficiency (NUE) and pushing agricultural production at high risk. Nitrogen management is one of the main concerns in maize crop production system. Nitrogen is the mineral element, plants require in greatest amounts. It serves as a constituent of many plant cell components including amino acids, nucleic acids, membranes, organelles and protoplasm and structural components of the cells since it is the most important and primary nutrient for growth and development of the crop (Leghari *et al*., 2016). Maize (*Zea mays* L.) has robust growth habit with higher consumption of nutrients, particularly nitrogen.Farmers some times apply higher doze of N without bothering to know whether inherent N in soil is high or low.Hence to economise the nitrogen use, there is a need of technology to optimise the use of nitrogen and enhancing the NUE in maize.

Plant tissue analysis currently available for all nutrients comprises of traditional digestion procedures followed by distillation and estimations with high cost involvement and labour. Further another common problem of traditional digestion laboratory analysis has time lag (few days to weeks) between sample collection and the potential use of the results by crop management specialists. Sampling and conducting the tissue analysis on the same day can overcome this problem. New methodologies involve a combination of techniques, such as measurements of stable (*i.e*., chlorophyll) and mobile (*i.e*., ammonium [NH_4^+] and nitrate [NO_3]) N forms in plant sap. Petiole / plant sap nitrate concentration has been used in several studies, and it has been demonstrated that plant nitrate concentration measurements are more sensitive to N changes. In addition, sap nitrate concentration can be used for detecting over or under fertilized plants. Timely early detection of the N deficiency and appropriate N application are important field management practices (Wu *et al*., 2007).On site measurements of nutrient concentrations can be done at least for few nutrients including nitrogen using ion meters. The Cardy NO_3 ion meters offer crop management specialists the ability to quickly evaluate tissue nutrient levels in the field. Cardy meters have been widely used in vegetable production, with NO^{-3} and K^+ thresholds established for several crops (Padilla *et al.,* 2020). Despite the acceptance and utilization of Cardy meters in vegetable production, very little research has been conducted to determine the utility of these meters in new crop production system. Several researchers (Burmester and Mullins, 1994; Smith *et al*., 1998 and Kenty *et al.,* 2003) have investigated the utility of Cardy meters as diagnostic tools in cotton. But, utility of these diagnostic tools for maize in India are meagure and not widely accepted. Ion measurements in the sap at any point of time given on the crop indicates overall efficiency of the crop to take up nutrient, soil nutrient status, type and level of nutrient applied. Technique is accurate, sensitive and holistic to determine the nutrient demand at various stages of crop development.

Literature survey has indicated that Cardy ion meters have not been previously tested for management of N in maize crop production. Hence, an attempt has been made here in the present investigation to measure maize sap nitrate content by nitrate ion meter at critical stages of crop growth as influenced by native soil nitrogen and different levels of additional N application to the soil (at sowing) and to find and validate the relation between nitrate levels and total nitrogen content. Along with, it has also been tested whether nitrogen application can be managed on site in the crop to increase the NUE and economise nitrogen use based on sap nitrate levels. Thus, to finally test whether nitrate ion meter would help during growth in managing the nitrogen requirement in maize.

Material and methods

A field experiment was conducted at the Main Agricultural Research Station, Dharwad during *Kharif*, 2017. The experiment was laid out in simple randomized block design consisting seven treatments with four replications. Prior to the imposition of treatments, soil in all the plots was collected and analyzed for available nitrogen. Soil nitrogen ranged from medium to high in the experimental plots (Table 2).Though the variation in soil available nitrogen existed among the plots, it has not been accounted since, the plant sap was tested for nitrate content, which was inclusive of nitrogen of the soil also. Seven treatments comprised of four N levels in the form urea *viz*., at 100 (T_1), 50 (T_2), 25 (T_3) and 0 (T_4) kg ha^{-1} to study variation in NO_3 levels in plant sap at critical stages of growth including three treatments such as 50 % recommended N + rest soil application (T_5), 0 N + rest soil application (T_6) and 0 N + rest foliar application (T_7) (2% urea), based on tissue nitrate tests (*i,e* on crop demand). P and K were applied commonly in all the treatments to the soil @75 and 25 kg through SSP and MOP. The seeds of the hybrid maize CP 818 were dibbled at spacing 30 x 60 cm on 23rd June, 2017 after imposing the treatments.

Plants were randomly selected in each treatment for plant sap collection for ion meter readings. Plant sap was collected using garlic hydraulic press from 10 cm long stalk cut from 25cm above the soil surface (Wilhelm *et al*., 2000). The nitrate content was measured within one or two minutes of extraction of fresh sap collected from stalk between 9-11 A. M. and reading was recorded in ppm after stabilization of display reading in Nitrate ion meter (B-731 "LAQUA twin" Cardy meter, Horiba Scientific, Irvine, CA). Nitrate readings were thus made at three stages *viz*., V_6 stage (30 DAS), prior to tasseling (55 DAS) and cob formation stage (70 DAS). Further the readings were converted to nitrate-nitrogen values by dividing the nitrate value by 4.43 factor.

To manage the N requirement in the treatments T_5, T_6 and T_7, the nitrate values in these treatments were deducted from the nitrate values of T_1 (100 % N application) at respective stages. Further, the deficit balance N was applied at 35 and 60 DAS through soil and foliar application as per the treatments. Since, the values were on par between treatments T_1,T_5 and T_6 at 70 DAS, further application of N was withheld.

The NUE was calculated by using the formula (Fageria and Baligar, 2005) as given below

$$NUE = \frac{Y_f - Y_u}{N_a}$$

Where, Y_f - Yield of fertilized plot (Grain / stover)

Y_u - Yield of unfertilized plot (Grain / stover)

N_a - Quantity of N applied

Results and discussion

Nitrate is a major fraction of nitrogen metabolism in plant sap, accumulates and transient as it further metabolically converts into nitrite and ammonia and finally as amino acids as building blocks of cellular organelles. Since, nitrate is major form of nitrogen available in the soil and most of the plants take up nitrate from the soil through roots. It is translocated through xylem. Thus, xylem of petiole, midrib and stem have major amount of nitrate. Maize sap contains approximately sixty percent nitrate form of nitrogen in its sap (Taize and Zeiger, 1988). Hence, nitrate is a good indicator of nitrogen status in maize. Though, the nitrate content in plant sap varies due to temperature, light, moisture and metabolism, but at a given time and space the differences are mainly influenced by the native soil nitrogen, management, genetic potential and phenological stage of the crop growth.

The nitrate content measured by Cardy nitrate ion meter was initially high at 30 DAS (719) and subsequently decreased at 55 DAS (483) and 70 DAS (379) (Table 1). Maize plants have taken up highest nitrogen up to 30 DAS, indicating the early 30 days period is the most active period of uptake and hence is a crucial point of measuring nitrogen requirement of maize. Reduction in nitrate values at 55 and 70 DAS with advancement of growth (High dry matter accumulation) suggests utilization of nitrate in metabolism and plant growth and development. Similar results were reported by Lopez *et al*. (2010) in cotton petioles and Prasad and Spiers, 1985; Hochmuth, 1994;

Table 1. Plant sap nitrate content, nitrate nitrogen, grain yield and stover yield as influenced by different nitrogen levels and tissue nitrate test based application of nitrogen of maize at different growth stages

Treatments	Plant sap NO_3 (ppm)			Plant sap NO_3 – N (ppm)		
	30 DAS	55 DAS	70 DAS	30 DAS	55 DAS	70 DAS
T_1- 100 % recommended N(100: 75: 25 kg N, P_2O_5 and K_2O ha^{-1})	1037^{a}	660^{a}	502^{a}	234^{a}	149^{a}	113^{a}
T_2- 50 % recommended N(50:75:25 kg N, P_2O_5 and K_2O ha^{-1})	757^{bc}	546^{ab}	457^{ab}	143^{c}	123^{ab}	103^{ab}
T_3- 25 % recommended N(25:75:25 kg N, P_2O_5 and K_2O ha^{-1})	620^{cd}	426^{b}	231^{c}	140^{cd}	96^{b}	52^{c}
T_4- 0 % N (0:75:25 kg N, P_2O_5 and K_2O ha^{-1})	435^{d}	258^{c}	185^{d}	98^{d}	58^{c}	42^{d}
T_5- 50 % recommended N + rest soil application based on tissue nitrate test.	903^{ab}	552^{ab}	465^{ab}	204^{ab}	124^{ab}	105^{ab}
T_6- 0 % N + rest soil application based on tissue nitrate test.	647^{c}	472^{b}	445^{ab}	146^{c}	107^{b}	101^{ab}
T_7- 0 % N+ rest foliar N application based on nitrate tissue test	632^{cd}	465^{b}	368^{b}	171^{bc}	105^{b}	83^{b}
Mean	719	483	379	162	109	86
S. Em.±	63	42	32	14	9	7
LSD @ 5 %	188	124	96	42	28	21

Note: DAS- Days after sowing

Table 2. Available soil N in experimental plots before sowing and quantity of N applied based on the nitrate values at different stages of maize crop growth

Treatments	Available N in soil (kg ha^{-1}) before sowing	Nitrogen applied (kg ha^{-1}) on respective dates for each treatment based on sap nitrate values				Total quantity (kg ha^{-1})	Nitrogen saved over control (kg ha^{-1})
		23-June (Sowing)	17-July (25 DAS)	27- July (35DAS)	22-August (60 DAS)		
T_1- 100 % recommended N (100: 75: 25 kg N, P_2O_5 and K_2O ha^{-1})	382.8	50	50	NA*	NA	100	0
T_2- 50 % recommended N (50:75:25 kg N, P_2O_5 and K_2O ha^{-1})	439.4	25	25	NA	NA	50	50
T_3- 25 % recommended N (25:75:25 kg N, P_2O_5 and K_2O ha^{-1})	686.3	12.50	12.50	NA	NA	25	75
T_4- 0 % N(0:75:25 kg N, P_2O_5 and K_2O ha^{-1})	313.0	0	0	0	0	0	100
T_5- 50 % recommended N + rest soil application based on tissue nitrate test.	578.1	25	25	NA	21.43	71.40	28.60
T_6- 0 % N + rest soil application based on tissue nitrate test.	543.9	NA	NA	39.50	32.23	71.80	28.30
T_7- 0 % N+ rest foliar N application based on tissue nitrate tests at critical stages	431.5	NA	NA	12.00	20.00	32.00	68.00

NA*: Not applied

Goffart *et al*., 2008 in vegetable crops, in that the concentration of petiole nitrate increased at all sampling dates as the rate of applied N increase and concentration declined as the season progressed. As it is known nitrate content in plant sap depends on supply of nitrogen and as well on native nitrogen in the soil. Application of 100 % N at the time of sowing to the soil resulted in significantly higher sap nitrate content (1037 ppm) and was least in 0 % application of nitrogen (435 ppm). The sap nitrate content at 30 DAS not only varied due to variation in the levels of application of nitrogen at the time of sowing but also varied due to variation in the native soil N content. Soil analysis of experimental plot before sowing (Table 2) has revealed that the T_3 imposed plot had highest nitrogen content (686 kg ha^{-1}) followed by T_5 (578.1) and T_6 (543.9). The higher values of sap nitrate in T_5 was due to 50 % of N applied at sowing and higher native soil N content. The same has not been represented in T_3 due to application of only 25 % of N at the time of sowing. Therefore, it is noticed that sap nitrate content is an indicator of both soil and applied nitrogen to the plant.

As in T_3 and T_6 sap nitrate values remained higher, which was basically attributed to higher available N in soil (Table1). Hence, the sap nitrate values indicate soil available N status too. Thus, values of sap nitrate in maize at 30 DAS of all the treatments are indicator of native soil available, applied N to the soil and phenological potential of the genotype. The nitrate test values have been further used for compensation of nitrogen in the treatments, where partial supply of N was provided in the treatment T_5, T_6 and T_7 at the time of sowing (Table 2). The difference in the nitrate values of 100 per cent N applied (T_1) and other treatments *viz*., T_5, T_6 and T_7 were worked out and T_5 had nitrate on par with T_1, hence nitrogen was not supplied on 35 DAS (27th July, 2017). Since, there was no significant difference in nitrate concentration in the treatments with 50 per cent application of recommended N and 100 per cent recommended N at sowing, it could be concluded that 50 per cent application of recommended N at sowing was sufficient for uptake and maintained required N for plant growth. Whereas in T_6 and T_7 w here no application of N at sowing was done, nitrate values were significantly lower suggesting plants were in need of N. Accordingly, soil application of 39.5 kg ha^{-1} and foliar application of urea (12 and 32 kg ha^{-1}) were made, respectively in T_6 and T_7, (Table 2). Thus, nitrate values measured could be used for application of N based on need or demand of the maize crop.

Peak growth of maize coincides at 55 DAS, which is more important at which the values of nitrate content were found reduced with higher growth compared to initial stage, 30 DAS. The nitrate has been utilized for the growth and development of the plant. However, the nitrate values differed significantly among the treatments based on initial level of nitrogen supplied. Both at 55 and 70 DAS, sap nitrate values reduced when N level has decreased from 100 % N to 0 % N but remained on par with 100 % N and 50 % N. From this, it is suggested that even 50 % of N at the time sowing has full filled the requirement of sap nitrate or nitrogen level on par with 100 % N. Sap nitrate contents at 70 days in all the treatments except T_3 and T_4 and T_7 were on par with each other ranging from 445 - 502 ppm, the growth of the maize also remained on par among these treatments. In treatment T_7 without external soil nitrogen application, nitrogen requirement has been managed by means of 2 % foliar application of urea. Foliar application of urea in combination of soil application can also be thought. However, here in the present investigation this combination has not been tried. From the above discussion, it is suggested that the most optimum concentration of nitrate in maize sap can be 900-1000, 450-600 and 450-500 ppm at 30, 55 and 70 DAS in maize, respectively. Lower values of nitrate than these in the sap at these critical stages naturally would reduce growth and yield of maize provided other conditions are favourable.

Table 3. Grain yield, stover dry weight and NUE (grain and total dry matter) of maize as influenced by different nitrogen levels and tissue nitrate test based N application

Treatments		Grain yield (q ha^{-1})	Stover yield (q ha^{-1})	Grain NUE	Total dry matter NUE
T_1	100 % recommended N(100: 75: 25 kg N, P_2O_5 and K_2O ha^{-1})	106.9^{a}	100.1^{a}	35.79^{a}	30.83^{a}
T_2	50 % recommended N(50:75:25 kg N, P_2O_5 and K_2O ha^{-1})	95.1^{ab}	89.1^{ab}	24.09^{bc}	19.84^{c}
T_3	25 % recommended N(25:75:25 kg N, P_2O_5 and K_2O ha^{-1})	78.1^{bc}	80.5^{bc}	10.19^{cd}	10.23^{d}
T_4	0 % N(0:75:25 kg N, P_2O_5 and K_2O ha^{-1})	71.1^{c}	67.5^{c}	0.01^{e}	0.01^{e}
T_5	50 % recommended N + rest soil application based on tissue nitrate test	106.7^{a}	92.7^{ab}	35.54^{ab}	24.04^{b}
T_6	0 % N + rest soil application based on tissue nitrate test.	95.6^{ab}	80.8^{bc}	24.41^{b}	11.72^{cd}
T_7	0 % N+ rest foliar N application based on nitrate tissue test	92^{ab}	69.5^{c}	21.87^{c}	3.06d^{e}
Mean		92.2	83.2	21.70	12.24
S. Em. ±		5.5	3.1	1.34	1.01
LSD @ 5 %		9.2	5.9	3.98	3.00

NUE: nitrogen use efficiency

The maize yield differed significantly among the treatments depending on the supply and availability of nitrogen and has increased with increase in N content (Kappes *et al*. 2013). Significantly higher grain yield of 106.9 q ha^{-1}was recorded with the application of 100 per cent recommended N, however, it was found on par with 50 per cent recommended N + rest soil application based on tissue sap nitrate test (106.7 q ha^{-1}). Nitrate test based application of nitrogenous fertilizer has helped to manage grain yield on par with 100 percent N and also saved 28.6 per cent nitrogen. Same trend has been noticed as regards to stover yield (Table 3).

Nitrogen use efficiency has significantly differed and was found on par between 100 per cent recommended N and 50 per cent recommended N + rest soil application based on tissue nitrate test (Table 3). Thus, it is noticed that, NUE has decreased with decrease doze of N application. These results corroborate with the findings of Singh *et al*., 2002. But, NUE has been managed based on nitrate test values saving 28.6 per cent nitrogen in T_5.

Conclusion

From the results, it is concluded that nitrogen application can be managed using nitrate as Bio- indicator of N requirement. Plant sap nitrate could be measured with Nitrate ion meter. NO_3 sufficiency ranges in maize are 900-1100, 500- 670 and 400- 500 ppm at V_6, silking and tasseling stages, respectively. Use of Nitrate ion meter has increased NUE without compromising grain yield besides, saving 28.6 per cent nitrogen.

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