International Research Journal of Pure & Applied Chemistry

21(24): 334-340, 2020; Article no.IRJPAC.65422
ISSN: 2231-3443, NLM ID: 101647669

Effect of Crop Cover and Stage of Crop Growth on Soil L-Glutaminase Activity

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Authors' contributions

This work was carried out in collaboration among all authors. Author MBNY performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors GP and TA managed the analysis of the study. Author JAK managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IRJPAC/2020/v21i2430373
Editor(s):
(1) Dr. Wolfgang Linert, Vienna University of Technology, Austria.
Reviewers:
(1) O. P. Bansal, D. S. College, India.
(2) Ashay D. Souza, University of Agricultural Sciences, India.
Complete Peer review History: http://www.sdiarticle4.com/review-history/65422

Original Research Article

Received 25 October 2020
Accepted 28 December 2020
Published 31 December 2020

ABSTRACT

A pot culture experiment was conducted at glass house of Department of Soil Science and Agricultural Chemistry, College of Agriculture, Rajendranagar, Hyderabad. The aim of the present experiment was to study the influence of crop cover and stage of crop growth on soil L- glutaminase activity in an *Alfisol* and *Vertisol*. The experiment was under taken with six crops viz., two cereals (Rice, Maize), two legumes (Groundnut, Greengram), one oilseed (Sunflower) and one vegetable (Bhendi) crop. The experiment was conducted in Completely Randomized Block design with three replications along with the uncropped control. The results obtained with regard to the effect of these crops on soil L-glutaminase activity showed that there was an increase in enzyme activity with age of the crop upto 60 DAS and it varied with crops grown. The increased enzyme activity (µg of NH4+ released g-1 soil 4h-1) varied from 5.56 to 12.17 for groundnut, 5.58 to 11.25 for greengram, 5.43 to 10.87 for sunflower, 5.48 to 8.61 for rice, 5.39 to 8.23 maize and 5.31 to 7.92 for bhendi in *Vertisol.* In *Alfisol* the L-glutaminase activity (µg of NH4+ released g-1 soil 4h-1) under different crop cover found to vary from 6.72 to 13.59 (groundnut), 6.68 to 12.71 (greengram), 6.63 to 11.96 (sunflower), 6.61 to 10.25 (rice), 6.59 to 9.47 (maize), 6.62 to 9.26 (bhendi). A close perusal of the data indicates that the L-glutaminase activity followed the sequence groundnut > greengram > sunflower > rice > maize > bhendi, in both Alfisol and Vertisol.

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Keywords: L-glutaminase; Vertisol; Alfisol; groundnut; greengram.

1. INTRODUCTION

The enzyme L-glutaminase (L-glutamine amido hydrolase E.C. 3.5.1.2) in soils hydrolyse L-glutamine to glutamic acid and ammonium, thus it is important in making the amide form of nitrogen available to plants. Nitrogen is present in soil in the organic and inorganic forms. The organic N has two fractions, they are hydrolysable fraction (60 to 80%) and non hydrolysable fraction [1] about 24 to 37 percent of total N in surface soils could be accounted for amino nitrogen. Bremner [2] reported that after acid hydrolysis of humic substances, 7.3 to 12.6% of total nitrogen was in the form of amide nitrogen. The important enzymes involved in mineralization of amide forms of nitrogen in the soils are urease, amidase, glutaminase and asparaginase.

The presence of crop cover and the type of plant grown on the soil will have marked effect on the enzyme activities. Their effect could be directly through endoenzymes contained in the plant residues or the extracellular enzymes secreted by living roots which also may also make significant contribution to enzyme activity. This is because cropping systems that have higher carbon input or that conserve carbon inputs promotes enzyme activity. Hence crop cover and stage of crop growth plays an important role especially on characteristics which include soil micro biomass, soil enzymes and soil respiration. Hence the present experiment was conducted to study the influence of crop cover, soil L-glutaminase activity under six crops [3].

2. MATERIALS AND METHODS

A pot culture experiment was conducted at, College of Agriculture, Rajendranagar, Hyderabad during the year 2019 with two soils (Alfisol and Vertisol), six crops viz., two cereals (Rice, Maize), two legumes (Ground nut, Greengram), one oilseed crop (Sunflower) and one vegetable (Bhendi). The experiment was conducted in Completely Randomized Block design with three replications along with the uncropped control. The soil samples were collected at 10 days interval from 0 DAS to 90 DAS and at harvest and were assayed for L-glutaminase activity. The activity of L-glutaminase was assayed by Frankenberger and Tabatabai [4] and the rate of NH_4^+ released was quantified by the modified Indophenol method as given by Dorich and Nelson [5].

2.1 REAGENTS

2.1.1 THAM buffer (0.1 M)

It was prepared by dissolving 12.28 g of THAM (Tris hydroxy methyl amino methane) in about 800 mL of distilled water and adjusting the pH by adding 0.1N HCl or 0.1N NaOH to the desired pH and then makes up the volume to 1 litre with water.

2.1.2 L-Glutamine (0.125M)

The solution was prepared by dissolving 18.25g of L- glutamine in 1 litre of THAM buffer of desired pH.

2.1.3 Potassium chloride (2.5M)-silver sulphate (100ppm) KCl-Ag_2SO_4 solution

It was prepared by dissolving 100 mg of Ag_2SO_4 in 700 mL of distilled water and dissolving 186.4 g of KCl and diluting the solution to one litre with distilled water.

2.1.4 Ethylene diamine tetra acetic acid (EDTA) 6%

This was prepared by dissolving 6 g of EDTA in distilled water and made up to 100 mL. The pH of the solution was adjusted to 7 with diluted 0.1 N NaOH.

2.1.5 Phenol-nitroprusside

7 g of Phenol and 34 mg of sodium-nitroprusside was dissolved in distilled water and diluted to 100 mL with distilled water.

2.1.6 Buffered hypochlorite

This was prepared by dissolving 14.8 g of NaOH and 49.8 g of Na_2HPO_4 in 400 mL of distilled water, adding 400 mL of NaOCl (4-5%), adjusting the pH to 11.8 and the volume was made up to 1 litre.

2.1.7 Standard Ammonium Solution

Primary stock solution of 100 µg mL^{-1} of ammonium was prepared by dissolving 0.4717 g

of ammonium sulphate in distilled water and was made up to 100 ml with distilled water.

2.2 Procedure

Soil sample (10 g) was taken in a 150 mL conical flask and adds 0.4 mL of Toluene, to which 12 mL of 0.1 M THAM buffer of pH 8 was added. The flasks were gently swirl to mix the contents followed by addition of 8 ml of 0.125 M L-glutamine were added, so that concentration substrate was 50 mM. The flasks were gently shaken for few seconds and covered with polythene paper. Then the contents were incubated at 37±0.5^{o}C for 4 hours in BOD incubator. After incubation, reaction was terminated by addition of 30 mL of 2.5 M KCl - (100 ppm) Ag_2SO_4 solution. The contents were agitated on mechanical shaker for 30 min to release all NH_4^+ formed and the suspension was allowed to settle and filtered. In the controls the same procedure described above was followed but the L-glutamine solution was added after deactivating with KCl-Ag_2SO_4 reagent. The incubation time of 4 hours was chosen because, preliminary investigations carried out various time intervals indicated an incubation time of 4 hours to be optimum time for the assay of enzyme during the investigation. Similarly the amount of 10 grams of soil was based on a preliminary investigation taking 2,4,6,8,10,12 and 15 grams soil sample for assaying L-glutaminase activity out of which 10 grams was found to be optimum for assay.

One mL of supernatant from the soil suspension after incubation with L-glutamine and deactivation with KCl - Ag_2SO_4 was transferred to 25 mL volumetric flask. To this, 1 mL of 6% EDTA was added followed by addition of 2 mL of Phenol-nitroprusside and 8 mL of buffered hypochlorite reagent. The volume was then made up to the mark, mixed thoroughly by inverting several times and placed in water bath for 30 min at 40^{o}C for colour development. The flasks were removed and brought to room temperature and the absorbance of blue colo ur was measured at 636 nm using UV-1800 spectrophotometer. The L-glutaminase activity was measured with respect to the amount of NH_4^+ liberated and expressed as µg of NH_4^+ released g^{-1} soil $4h^{-1}$.

3. RESULTS AND DISCUSSION

The effect of crop cover and crop growth on L-glutaminase activity were presented in Table 1and 2. There was an increase in enzyme activity with age of the crop and it varied with plant species grown. The activity of L-glutaminase increased from 0 to 60 DAS which was coincides with maximum flowering stage of crops and then decreased upto harvest. The activity varied for crops grown in *Vertisol* and *Alfisol*. The L-glutaminase activity was consistently high with groundnut followed by greengram cover crop. The enzyme activity (µg of NH_4^+ released g^{-1} soil $4h^{-1}$) varied from 5.56 to 12.17 for groundnut, 5.58 to 11.25 for greengram, 5.43 to 10.87 for sunflower, 5.48 to 8.61 for rice, 5.39 to 8.23 maize and 5.31 to 7.92 for bhendi in *Vertisol* (Table1 and Fig. 1). In *Alfisol* the L-glutaminase activity (µg of NH_4^+ released g^{-1} soil $4h^{-1}$) under different crop cover (Table 2 and Fig. 2) found to vary from 6.72 to 13.59 (groundnut), 6.68 to 12.71 (greengram), 6.63 to 11.96 (sunflower), 6.61 to 10.25 (rice), 6.59 to 9.47 (maize), 6.62 to 9.26 (bhendi).

The trends indicated that the L-glutaminase activity was higher in *Alfisol* as compared to *Vertisol*. A close perusal of the data indicates that the L-glutaminase activity followed the sequence groundnut > greengram > sunflower > rice > maize > bhendi. The significantly higher L-glutaminase activity under groundnut could be due to continuous growth and extensive root system and high release of extracellular enzymes secreted by groundnut roots thus effecting substrate concentration in the rhizosphere. In addition, crop cover increased the biochemical variables like substrates related to microbial activity and increased the activity of L-glutaminase due to increase in carbon turnover and nutrient availability. The increased L-glutaminase activity under groundnut and greengram during flowering might be due to well developed root nodules where nitrogen fixation occurs. Hence it is possible that the ammonical nitrogen fixed during atmospheric nitrogen fixation is reduced to nitrogen rich organic compounds like glutamine and this might have a stimulatory effect on L-glutaminase activity in soil [6].

Table 1. Effect of crop cover on soil l-glutaminase activity in *vertisol*

Crops	Days after sowing / Days after transplanting											
	L-glutaminase activity (µg of NH_4^+ released g^{-1} soil $4h^{-1}$)											
	0	10	20	30	40	50	60	70	80	90	Harvest	Mean
Rice	5.48	5.81	6.28	6.81	7.59	8.12	8.61	8.03	7.65	6.58	5.59	6.95
Maize	5.39	5.54	5.91	6.27	7.13	7.78	8.23	7.91	6.83	6.49	5.57	6.64
Groundnut	5.56	6.31	7.97	9.15	10.49	11.47	12.17	11.26	9.13	7.34	5.79	8.76
Greengram	5.58	6.17	7.54	8.47	9.59	10.31	11.25	10.17	8.93	6.95	5.73	8.25
Sunflower	5.43	6.04	7.24	8.11	9.26	9.98	10.87	9.84	8.64	6.62	5.61	7.96
Bhendi	5.31	5.56	5.78	6.01	6.68	7.43	7.92	7.31	6.78	5.91	5.54	6.37
Control	5.42	5.39	5.36	5.38	5.32	5.42	5.43	5.35	5.29	5.27	5.31	5.36
Mean	5.45	5.83	6.58	7.17	8.00	8.62	9.2	8.55	7.60	6.45	5.6	

	C.D. (5 %)	SE(m) ±
L-glutaminase	0.194	0.069
Crop Cover	0.154	0.055
L-glutaminase X Crop Cover	0.512	0.183

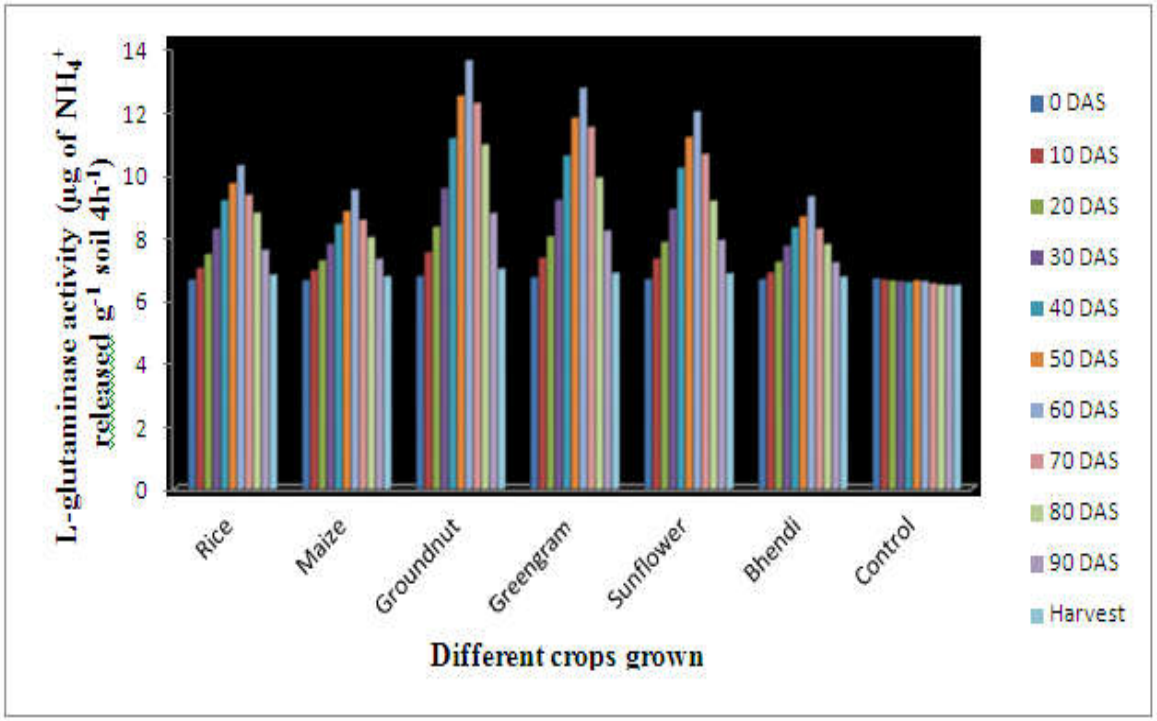

Fig. 1. Effect of crop cover on soil l-glutaminase activity in *vertisol*

As compared to uncropped control higher activity of L-glutaminase was observed with crops. Various factors like rhizosphere effect, age of crop, nature of crops influenced L-glutaminase activity in soil. L-glutaminase activity increases from 0 to 60 days after sowing which coincides with the active growth stage of the crop, enhanced root activity and the release of cellular enzyme in to soil solution during the active growth phase which resulted in higher rate of mineralization of nutrients in the soil. Vandana et al.[7]; Pavani [8] and Kumari et al. [9] observed the difference in enzyme activity mainly due to type of vegetation. The enzymes secreted by plant roots and microorganisms associated in rhizosphere release large amount of enzymes into soil. In addition plant type has been reported to be dominant factor affecting soil microbial and L-glutaminase activity [10-11]. Besides, the higher enzyme activity under crop cover could be due to the

incorporation of organic residues coupled with greater microbial activity in cover areas [12]. Thus a large and complex number of factors during crop growth contribute to an increased L- glutaminase activity.

In a study conducted by Balezentiene and Klimas [13] the legume grass mixtures formed an even cover over the soil during the year of sowing and improved the micro-biological properties of the soil. The grasses form better conditions for organic matter decomposition and hence, urease and saccharase activities were found to be highest in the grass grown soils compared to other crops.

Table 2. Effect of Crop Cover on soil L-glutaminase activity in *Alfisol*

Crops	Days after sowing / Days after transplanting											
	L-glutaminase activity (µg of $NH4^+$ released g^{-1} soil $4h^{-1}$)											
	0	**10**	**20**	**30**	**40**	**50**	**60**	**70**	**80**	**90**	**Harvest**	**Mean**
Rice	6.61	6.98	7.42	8.23	9.14	9.68	10.25	9.31	8.74	7.56	6.77	8.27
Maize	6.59	6.91	7.22	7.75	8.38	8.79	9.47	8.51	7.96	7.27	6.72	7.78
Ground nut	6.72	7.48	8.29	9.52	11.11	12.46	13.59	12.23	10.91	8.73	6.96	9.82
Greengram	6.68	7.31	7.99	9.15	10.56	11.76	12.71	11.47	9.86	8.17	6.83	9.29
Sun flower	6.63	7.28	7.81	8.86	10.17	11.15	11.96	10.61	9.12	7.87	6.81	8.93
Bhendi	6.62	6.84	7.18	7.69	8.27	8.62	9.26	8.23	7.74	7.16	6.71	7.67
Control	6.65	6.61	6.58	6.56	6.53	6.59	6.56	6.49	6.45	6.44	6.44	6.54
Mean	6.64	7.11	7.5	8.25	9.17	9.86	10.54	9.54	8.63	7.6	6.75	

	C.D. (5 %)	**SE(m) ±**
L-glutaminase	0.115	0.041
Crop Cover	0.092	0.033
L-glutaminase X Crop Cover	0.306	0.109

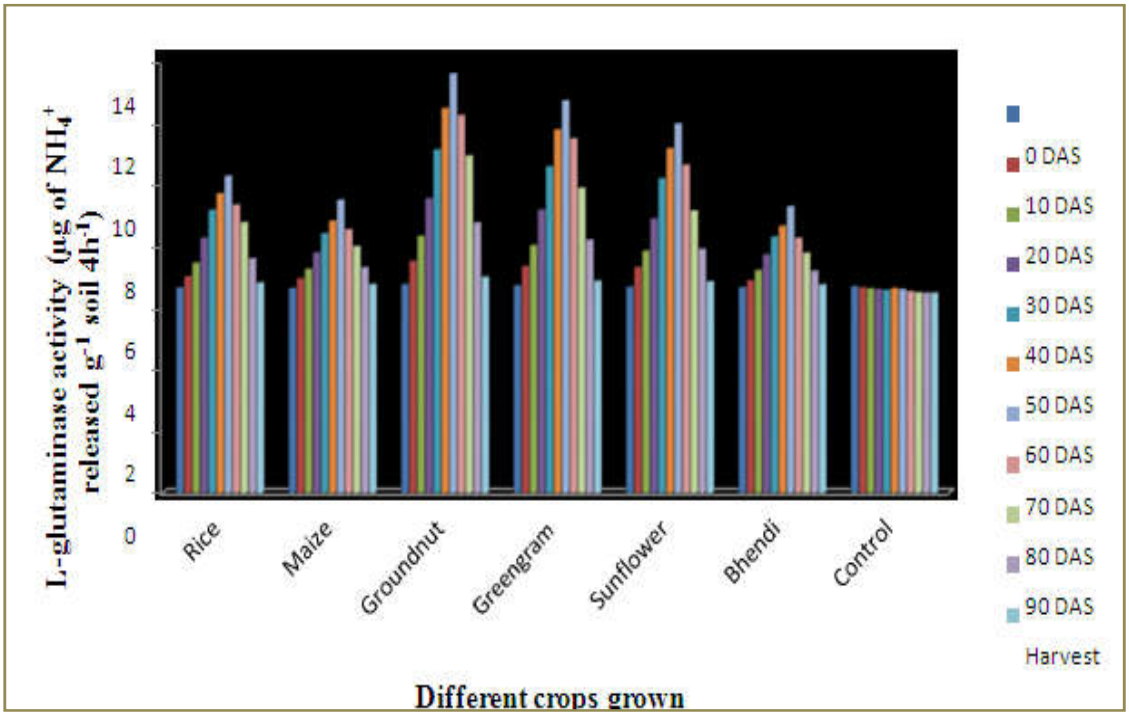

Fig. 2. Effect of crop cover on soil l-glutaminase activity in *alfisol*

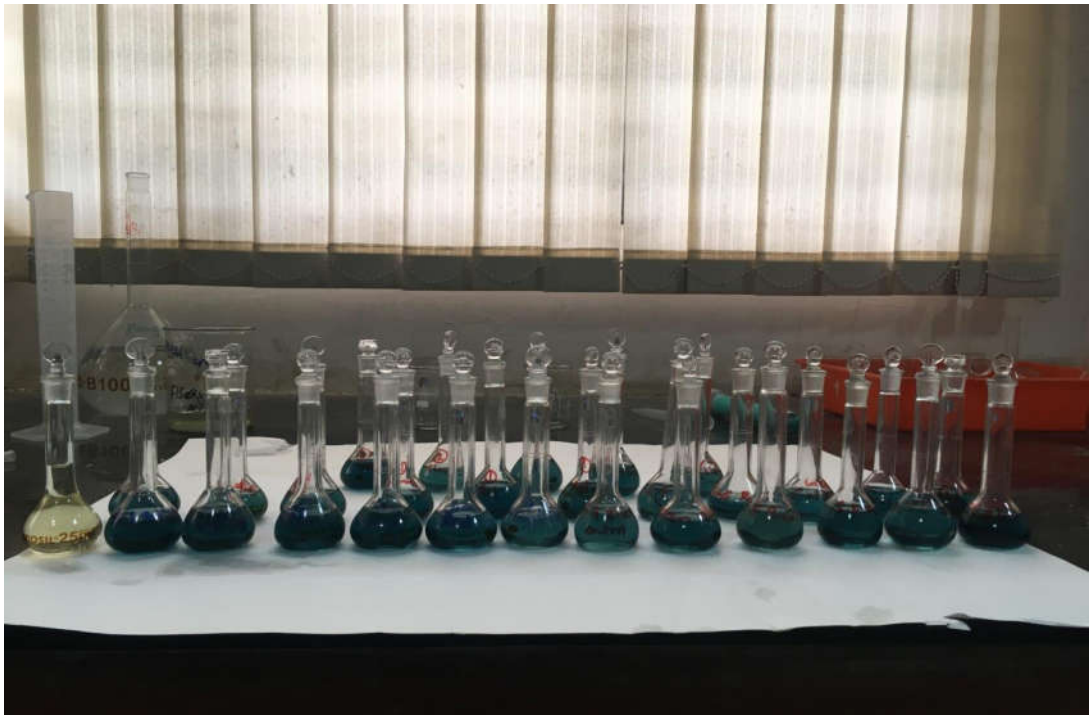

Plate 1. Assessment of l-glutaminase activity by modified indophenol method

Plate 2. Pot culture experiment

4. CONCLUSION

L-glutaminase activity increases from 0 to 60 days after sowing which coincides with the active growth stage of the crop, enhanced root activity and the release of cellular enzyme in to soil solution during the active growth phase which resulted in higher rate of mineralization of nutrients in the soil. The increase in L-glutaminase activity with the age of crop varied with the type of crop grown. The enzyme activity was higher under legume crop cover

conditions. There was a higher activity of L-glutaminase in *Alfisol* as compared to all the crops grown in *Vertisol*.

ACKNOWLEDGEMENTS

The authors are extremely thankful to Professor Jayashankar Telangana state Agricultural University, Rajendranagar, Telangana (India) for financial help and technical guidance to carry out this research work.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/65422