www.bti.org.in
ISSN 0974-1453
Short Communication

NITRATE REDUCTASE ACTIVITY IN THE LEAVES OF *SARACA ASOCA* (ROXB.), De. WILDE.

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ABSTRACT: Ashoka is the most ancient tree of India, generally known as "ashok briksh", botanist known as *Saraca asoca* (Roxb.), De.wild. It is multipurpose tree species in agriculture and ethno pharmacology. *Saraca asoca* play an important role in nitrogen assimilation study. In this study, combination of different concentration of KNO_3 substrate (0.025 M, 0.05 M, 0.10 M, 0.15 M, 0.20 M, and 0.25 M) with 0.10M and 0.20M molarities of buffers under different pH i.e. 7.0, 7.1, 7.2, 7.3, 7.4 and 7.5 solutions were tried to determine the optimum substrate and buffer solution for the maximum nitrate reductase activity in the leaves of *Saraca asoca.* On the basis of results obtained it was inferred that buffer solution of the strength 0.20 M (pH 7.2) and substrate solution 0.25M was observed suitable for the maximum nitrate reductase activity in the leaf blade of *Saraca asoca.* The aim of this study was to determine high nitrate reductase activity by using different concentration of KNO_3 as substrate in the leaves of *Saraca asoca* at suitable pH.

Key words: *Saraca asoca*, nitrate reductase activity, substrate and buffer solutions.

INTRODUCTION

Nitrogen is one of the most important elements occurring in all the living organisms. Although many plants contain only 1 or 2 percent nitrogen on a dry weight basis, the amount of this element is exceeded only by carbon, oxygen and hydrogen. Most of the nitrogen exists in proteins and amino acids, but it is also present in large number of other major compounds *viz.*, nucleic acids chlorophylls, cytochromes, alkaloids, many vitamins and phytohormones. The element, therefore, plays a central role in metabolism, growth, reproduction and heredity. It is such an essential element that life is not possible without it.

Absorption of nitrate by plant roots from the soil is a carrier-mediated, active and energy-dependent process. In most plants the reduction of nitrate occurs in the root tissues and then transported to shoot through xylem whereas in others, the process occurs in leaves and stems (Gordon and Wheeler, 1983). Primary nitrogen assimilatory enzyme responsible for the reduction of soil nitrate to nitrite during its assimilation is known as nitrate reductase (NR) activity.

In order to find out the rate of nitrate assimilation in plants, general methodology known as nitrate reductase assay is used to measure the enzymatic activity. All enzyme assays measure either the consumption of substrate or production of product over time. A large number of different methods of measuring the concentrations of substrates and products exist and many enzymes can be assayed in several different ways. The nitrate reductase assay is a form of relaxation type enzyme catalyzed reaction experiment in which an equilibrium mixture of enzyme, substrate and product is perturbed, for instance by a temperature, pressure or pH jump, and the return to equilibrium is monitored.

Ashoka is the most ancient tree of India, also known as "ashok briksh", botanist known as *Saraca asoca* (Roxb.), De.wild. *Saraca asoca* (family *Fabaceae*) is a rain-forest tree. The tree is believed to have originated in the Western Ghats and Deccan plateau. It can also be found in central and Eastern Himalayas. It is known to grow at an altitude of 750 m above the sea level. The plant grows to a height of about 9 m in length. The plant generally grows in fertile and semi-fertile areas across India. The tree belongs to *Caesal pinaceae* family. This is a perennial plant which can range from dark green to greyish green in colour. The lenticels are circular and ridged opposing. The seeds generally are reddish brown with fibres.

Ashoka tree has been mentioned in some of the oldest Indian literature apart from Ayurveda. This tree can be found all over the Indian subcontinent. Across India, Ashoka tree is believed to be sacred and apart from Ramayana, Ashoka tree is mentioned in Buddhism and Jainism as well. *Charaka Samhita* which is believed to have been composed in 1000 BC describes about Ashoka tree and its medicinal benefits. The stem bark is chiefly used in medicines and it has been reported to contain chemicals such as glycoside, flavanoids, tannins, saponins, alkanes, esters and primary alcohols. The alcoholic extracts present in the bark have showed a significant action against wide range of bacteria.

The tree is used as folk medicine for the treatment of various diseases. *Saraca asoca* is highly regarded as a universal panacea in the ayurvedic medicine. It is one of the universal plant having medicinal activities. This versatile plant is the source of various types of compounds. In the present scenario many plant are used to treat many diseases. But Ashoka is ancient and reliable source of medicine so Ashoka is used in many pharmacological activities like

anti cancer, anti hemorrhagic, anti oxytoxic, anti – microbial activity and have extend uses in ayurveda, unani and homeopathy. It has many uses like to treat skin infections, CNS function, genitor-urinary functions .as the global scenario is now changing towards the use of nontoxic plant product having traditional medicine use, and development of modern drug from *Saraca asoca* should be emphasized for the control of various diseases (Pradhan, 2009).

Due to these properties its nitrogen assimilation behavior is to be studied. The actual buffer and substrate solution for this study is to be required. Therefore, an attempt was made to standardize the buffer and substrate solution to study its nitrogen assimilation study in near future.

MATERIALS AND METHODS

Fresh leaves of *Saraca asoca* were collected from the F.R.I. campus, Dehradun. The leaves were cut and chopped into small pieces. From chopped tissue 0.50gm was taken in incubation vials of 30 ml capacity, containing 3 ml phosphate buffer (KH_2PO_4) and 3 ml substrate (KNO_3) embedded in ice trays. The initial solutions of buffer and substrate was chosen from the earlier work done by Pokhriyal and Abrol (1980), Pokhriyal and Raturi (1984), Chaukiyal and Pokhriyal (1996), Chaukiyal (2008a, b), Chaukiyal and Mir (2010), Semwal *et al*. (2012), Rautela *et al.* (2013). These tubes were evacuated with the help of vacuum pump for about 2-3 minutes. The process was repeated until the plant tissue was fully submerged into the incubation medium. These tubes were then transferred into a shaking water bath at 30^0C in dark for incubation. The tubes were removed after one hour and immersed into a boiling water bath for 4 minutes to stop the reaction and effective removal of the nitrite accumulated in the plant tissue. The same method was adopted for nitrate reductase (E.C. 1.6.6.1)

activity as earlier described by Klepper *et al.* (1971) with some modification by Nair and Abrol (1977).

Estimation of *in-vitro* NR activity

End product, the amount of nitrate reduced into nitrite during enzyme activity was determined by the method earlier described by Evans and Nason (1953). A required amount of an aliquot was pipetted in clean test tube; 1 ml sulphanilamide (1 per cent, sulphanilamide in normal hydrochloric acid) was added and shaken well, followed by 1 ml of NEDD (0.01% N, 1- Nepthyl ethylene diamine dihydrochloride) and mixed thoroughly. Colour was allowed to develop for 25 minutes and final volume was made 6 ml with the help of distilled water. A change in color intensity was estimated at 540 nm in UV spectrophotometer model Lamda 2S.

RESULTS AND DISCUSSION

Different pH (7.0 - 7.5) solutions of KH_2PO_4 (0.10M) along with different substrate (KNO_3) concentrations (0.025M – 0.25M) were tested to find out a suitable incubation medium for the estimation of maximum nitrate reductase activity in the leaves of *Saraca asoca* (table 1stage I). It was observed that as the substrate concentration is increased, the NR activity decreased up to the substrate concentration 0.20M and there after it increased simultaneously. But these conditions chained in every moment (Table 1, stage 1). Whereas, increasing the buffer pH from 7.0 to 7.5 NR activity increased up to pH 7.2 with the substrate combination 0.025M.

Table 1 (Stage 1): *In-vitro* assay of nitrate reductase (n moles NO_3^- reduced g^{-1} fresh weight h^{-1}) activity in different incubation medium (containing different buffers and substrates) concentrations in *Saraca asoca.*

KH_2PO_4 (0.1 M)	**Substrate concentrations (KNO_3)**					
pH	**0.025M**	**0.05M**	**0.10M**	**0.15M**	**0.20M**	**0.25M**
7.0	182.98 ± 13.05	178.72 ± 9.19	159.57 ± 12.07	108.51 ± 24.83	155.32 ± 12.07	155.32 ± 15.60
7.1	206.38 ± 12.52	163.83 ± 27.65	117.02 ± 15.72	146.81 ± 27.33	170.21 ± 17.33	221.28 ± 32.74
7.2	212.77 ± 10.77	206.38 ± 12.07	131.91 ± 19.40	114.89 ± 14.74	182.98 ± 21.01	178.72 ± 23.07
7.3	105.32 ± 26.50	163.83 ± 11.89	119.15 ± 16.14	161.7 ± 21.01	231.91 ± 17.88	168.09 ± 20.42
7.4	90.43 ± 36.90	161.7 ± 14.52	178.72 ± 19.13	144.68 ± 12.62	214.89 ± 21.23	172.34 ± 2.85
7.5	197.87 ± 15.01	144.68 ± 10.25	110.64 ± 19.95	146.81 ± 23.93	189.36 ± 19.05	180.85 ± 21.28

To find out the maximum NR activity, all the substrate combination of table 1 with same pH of 0.20 M buffer along with substrate (KNO_3) concentration (0.025M - 0.25M), were tested. It was observed that as the substrate concentration increased, the NR activity also increased up to the concentration 0.25M in all the combination

(Table 2 stage 2), and after that the NR activity was observed to decrease at 0.30M in all combination. Further it was noticed that within 0.20M, 0.25M and 0.30M substrate concentration, higher activity was noticed in pH 7.2 with substrate concentration 0.25M (Table 2 stage 2).

Table 2 (stage 2): *In-vitro* assay of nitrate reductase (n moles NO_3^- reduced g^{-1} fresh weight h^{-1}) activity in different incubation medium (containing different buffers and substrates) concentrations in *Saraca asoca.*

KH_2PO_4 (0.2 M)	**Substrate concentrations (KNO_3)**						
pH	**0.025M**	**0.05M**	**0.10M**	**0.15M**	**0.20M**	**0.25M**	**0.30M**
7.0	93.62 ± 25.24	170.22 ± 20.59	161.70 ± 18.54	187.23 ± 7.84	234.04 ± 25.40	476.59 ± 17.64	439.26±21 .45
7.1	131.91 ± 16.70	204.25 ± 12.65	244.68 ± 20.16	234.04 ± 7.12	238.30 ± 27.51	521.28 ± 20.17	491.64±16 .32
7.2	231.91 ± 23.40	197.87 ± 20.76	227.65 ± 12.94	246.80 ± 29.04	321.27 ± 36.35	524.89 ± 29.97	498.23±25 .31
7.3	190.42 ± 29.40	144.68 ± 18.25	257.44 ± 12.07	268.08 ± 31.27	376.59 ± 38.21	429.78 ± 49.95	409.32±17 .21
7.4	150.00 ± 22.77	212.76 ± 13.19	270.21 ± 20.42	242.55 ± 35.03	376.59 ± 49.52	459.57 ± 18.28	393.26±36 .34
7.5	221.27 ± 35.30	237.02 ± 18.54	263.82 ± 13.85	297.87 ± 51.57	409.57 ± 23.87	419.14 ± 6.59	249.33±43 .12

Hence in third step only three substrate with pH 7.1 to 7.4 was considered and observed the activity. In this step higher activity was noticed in the pH 7.2 and substrate concentration 0.25M. (Table 3 stage III) among all the pH and substrate concentration.

Table 3 (stage III): *In-vitro* assay of nitrate reductase (n moles $NO_3^{-\ reduced}$ g^{-1} fresh weight h^{-1}) activity in different incubation medium (containing different buffers and substrates) concentrations in *Saraca asoca.*

KH_2PO_4 (0.2 M)	**Substrate concentrations (KNO_3)**		
pH	**0.20M**	**0.25M**	**0.30M**
7.1	335.43 ± 16.85	350.38 ± 51.85	280.85 ± 53.05
7.2	355.31 ± 28.03	478.72 ± 47.16	200.65 ± 24.76
7.3	217.02 ± 40. 30	256.57 ± 48.32	221.27 ± 31.27
7.4	311.45 ± 23.17	321.89 ± 32.09	255.31 ± 27.23

On comparing all three Tables it was observed that higher nitrate reductase activity was observed in substrate concentration 0.25M with buffer solution pH 7.2. Therefore, on the basis of the result given in Table 1, 2 and 3, it was concluded that a buffer solution of 0.25 M (pH 7.2) and substrate (KNO_3) solution of 0.20 M was found suitable incubation medium for nitrate reductase activity in Saraca *asoca* leaves.

In the field of agriculture comprehensive work has been done on nitrogen assimilation study. But in forestry very little work is done. In a few economically important forestry species like *Eucalyptus*, *Populus deltoids*, *Albizia*

lebbeck, *Acacia nilotica* and *Dalbergia sissoo*, *Pongamia pinnata*, *Terminalia chebula*, *Grewia optiva* standardization of buffer and substrate has been done. On the basis of standardization carried out in different species it is inferred that different species required different strength of buffer and substrate solution. Therefore, in *Saraca asoca* also 0.20 M buffer (pH 7.2) and 0.25 M substrate solution was observed suitable for maximum nitrate reductase activity in the leaves. This type of study to be extended in other economically important trees and shrub species so that their nitrogen assimilation potential, like *Saraca asoca*, can be screened and best species to be exploited for different purposes.

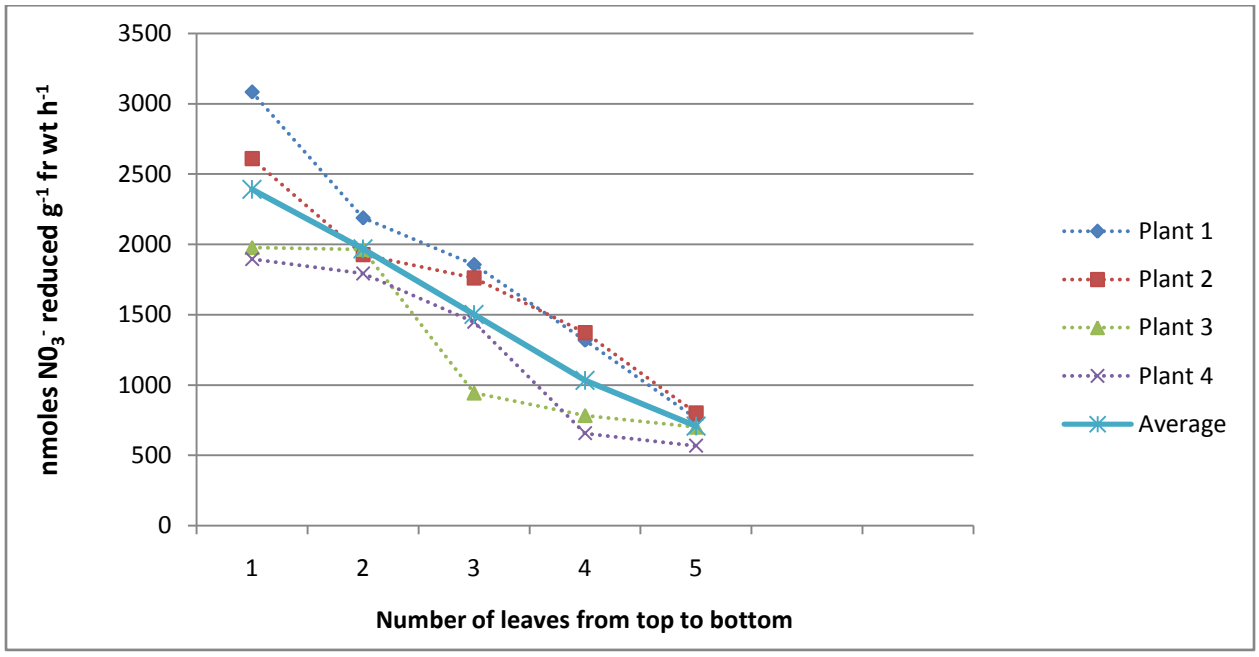

Figure 1. Nitrate reductase activity in the Single leaf blade of *Saraca asoca*

NR activity in individual leaf blade of *Saraca asoca*

Nitrate reductase activity was also studied in the individual leaf blade from top to downwards till the seventh leaf to know that which number of leaf is more active physiologically. It was observed that topmost fully expanded mature leaf was highly active than second, third and so on and least activity was recorded in the lowest leaf (fig 1). Generally the nitrate reductase activity increased from top to 6th, 7th to 8th leaf blade in some cases (Chaukiyal and Pokhriyal, 1996) then decreased but in this case higher activity is recorded in first leaf and then decreases simultaneously. But somewhat similar results were observed by Pokhriyal and Raturi (1984), Semwal *et al.* (2012) which support our finding in this species.

ACKNOWLEDGEMENT

We would like to thank the Forest Research Institute, Dehradun and Graphic Era University, Dehradun for supporting this research.

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