

Studies on Nitrate Reductase Activity in *Abrus precatorius* L. (Kunch)

Ashoke Bhattacharya

Received 28 June 2017; Accepted 31 July 2017; Published online 14 August 2017

Abstract *Abrus precatorius* L. (Kunch) of the family Fabaceae is a medicinally important plant. It has the ability to accumulate considerable amount of nitrogen rich litter which decomposes quickly and improves the organic matter as well as nutrient status of the soil. In present investigation urea (as N source) was applied at the rate of 0 (Control), 10, 50 and 100 kg N/ha in pot grown plants of *Abrus precatorius* to observe the effect of N on the nitrate reductase (NR) activity in pollens, roots and nodules in different months of the year. The variation of NR activities in different months of the year was noticed. Higher NR activity was observed in pollens and roots following treatment with 100 kg N/ha in the month of April, whereas, maximum NR activity was observed in nodule following treatment with 50 kg N/ha in the month of April. NR activity in

nodule sample is greater with high logarithmic value than pollen and root samples with less logarithmic values.

Keywords Enzymes, Nitrate reductase, Pollen, Nodule, Root.

Introduction

Abrus precatorius L. (Kunch) of the family Fabaceae is a medicinal climber flowers from August to December. The root extract of this plant is used in curing nervous disorder [1]. NR or Nitrate reductase (E.C.1.7.1.2) catalyzes the first committed step of nitrate assimilation in plants [2]. Soil fertility can be maintained for optimization of productivity either by using chemical fertilizers or by planting certain leguminous plants' species which can utilize soil as well as atmospheric nitrogen more efficiently [3]. A vast majority of leguminous plants assimilate nitrogen in combined form usually as NO_3^- or NH_4^+ via their root systems [4]. Nitrogen is readily lost from the soil due to the processes of nitrification, denitrification and leaching. The predominant form of nitrogen available to the plant is NO_3^- , because in majority of soil conditions, NH_4^+ nitrogen is rapidly nitrified into NO_3^- . The utilization of soil NO_3^- nitrogen by higher plants involves several steps of

A. Bhattacharya
Department of Botany, Bidhannagar College, EB-II, Sector-I,
Salt Lake, Kolkata 700064, West Bengal, India
e-mail : abha1971@yahoo.co.in

reduction by the participating enzymes. The important key enzyme involved is nitrate reductase, which determines the rate of reduction as whole [5]. Some species reduce considerable quantities of NO_3^- in their root, whereas other translocate it to leaves and other plant parts where it is further reduced [6]. Forest soil is generally deficient in nitrogen (0.01 to 0.02%) and its non-availability is the limiting factor for the growth and development of plants [7]. It is required substantially in larger quantity than other elements and occupies a prominent position in plant nutrition. The N reduced by the plant parts is incorporated into proteins and subsequently degraded and mobilized to other developing parts. Nitrate is one of the major sources of N in higher plant, translocated to the shoot, store in vacuole and assimilate into reduced N products [8]. The process of nitrate uptake, translocation and assimilation are interdependent and closely regulated in higher plants. Because of importance of N to plant productivity, nitrate assimilation and allocation patterns have been studied in many genera of tropical annuals and perennial plants. The importance of nodules and roots in nitrate assimilation was earlier assessed by the various workers in different species [1-8]. *Abrus precatorius* is one of the most commonly grown, medicinal, drought resistant, widely distributed indigenous nitrogen fixing climber. The natural regeneration of this species is poor due to hard seed coat. The present investigation has been carried out in order to know the nitrogen assimilating behavior in roots, pollens and nodules of this species following treatment with external N source (urea) in different doses at different month intervals to increase the growth and nitrogen uptake so that this species can be recommended for afforestation programs.

Materials and Methods

Fresh *Abrus precatorius* seeds were germinated and seedlings grown. Urea as a source of nitrogen was applied at the rate of 0 (Control), 10, 50 and 100 kg N/ha. Nitrate reductase activity of pollens, roots and nodule in individual seedlings were recorded at monthly intervals. The monthly observations were pooled. *In vivo* nitrate reductase activity in different plant parts i.e. pollens, roots and nodules were assayed periodically in the standardized incubation

medium of 0.20 M potassium nitrate and 0.20 M potassium phosphate buffer (pH, 7.5). A known amount of aliquot was taken for the estimation of nitrate reduced into nitrite during enzyme activity. NR activity was estimated using an *in vitro* assay method described by Young et al. [8]. Frozen samples were ground to a powder in liquid nitrogen and extracted in 200 m mol L^{-1} potassium phosphate buffer, pH 7.9, containing 5 m mol L^{-1} Na_2 ethylenediaminetetraacetic acid (EDTA), 0.3% (w/v) insoluble polyvinyl pyrrolidone, 2 m mol L^{-1} dl-dithiothreitol, 3% (w/v) bovine serum albumin (Fraction V) and 1% (v/v) Triton X-100. The assay mixture contained 200 m mol L^{-1} sodium phosphate buffer pH 7.9 with 200 m mol L^{-1} NADH, 20 m mol L^{-1} flavin adenine dinucleotide, 20% volume as extract and 10 m mol L^{-1} KNO_3 . The assay was incubated at 12°C and the reaction terminated by the addition of 1 M zinc acetate. NO_2^- concentration was measured spectrophotometrically in centrifuged supernatants and activity estimated by linear regression of increasing NO_2^- concentration over time.

Results and Discussion

The nitrate reductase activity in different samples of pollens, roots and nodules were recorded at monthly intervals. Maximum nitrate reductase activity was reported in pollens and roots sample during the month of April following the treatment with 100 kg N/ha (Figs. 1 and 2). The maximum NR activity was noticed in the nodule samples in the month of April following treatment with 50 kg N/ha (Fig. 3). However, minimum activity was reported in the nodules, pollens and roots during winter followed by summer and rainy seasons (Figs 1, 2 and 3). Considering the comparative account of NR activities of pollens, roots and nodules of this species, it has been observed that the nodules possess higher NR activities with greater logarithmic value $\{y = 1.722 \ln(x) + 2.043; R^2 = 0.773\}$ following treatment with 50 kg N/ha as compared to pollens and roots with less logarithmic values $\{y = 1.443 \ln(x) + 1.852; R^2 = 0.926$ and $y = 1.225 \ln(x) + 1.726; R^2 = 0.957$ respectively} followed by treatment with 100 kg N/ha during the month of April (Fig. 4). Nitrate is one of the major sources of N, taken up by roots of higher plants, translocate to the shoot, store in the vacu-

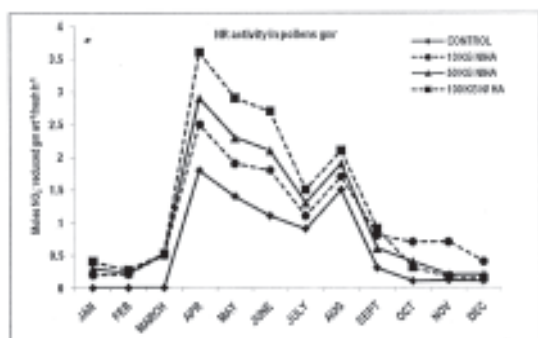


Fig. 1. Nitrate reductase activity in pollen g^{-1} sample of *Abrus precatorius* L. (Kunch).

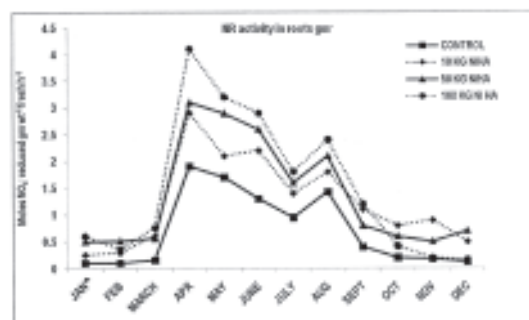


Fig. 2. Nitrate reductase activity in root g^{-1} sample of *Abrus precatorius* L. (Kunch).

oles and assimilate into reduced N products. The process of nitrate uptake, translocation and assimilation are interdependent and closely regulated in higher plants. Maximum NR activity was observed in nodules treated with 50 kg N/ha followed by pollens and roots treated with 100 kg N/ha during the month of April. Minimum activities were recorded in the month of December and January respectively. Thus a great variation on the NR activities was observed at different months intervals as well as a seasonal variation was also noticed. Similar types of observations were reported by other researchers [6–8]. It is well known that NR represents a classical example of a substrate induced enzyme and its activity is decisively determined by NO_3^- supply [9]. The capacity of root to reduce NO_3^- appear to be particularly intense during the early stage of plant developments as the plant tends to reduce an increasing proportion of absorbed NO_3^- . The contribution of root, pollen and nodule to NR assimilation process might be dependent on the external NO_3^- concentrations to some extent. An increase in the concentration of NO_3^- in the external medium can greatly increase NR activity in nodule with a concomitant decrease in activity in the root [10]. NR activity is influenced by site factor which may affect the regulation of nitrate assimilation [11]. Differences in N induced NR activity between pollens, nodules and roots have been reported to be the major site of nitrate reduction [12]. Thus the location of nitrate assimilation varies from species to species, habitat and age of species [13], environmental factors [14] and

in most herbaceous plants the main site of nitrate reduction are the pollens [15]. The partitioning of nitrate reduction between root, pollen and nodule might not be necessarily constant for every species and may vary with plant growth conditions and plant development [16]. NR activities in different samples like roots, nodules and pollens might be known to fluctuate in response to the changes in environmental conditions, and such fluctuation usually may influences the capacity of different plant parts to assimilate nitrate. The responsiveness of any leguminous species to N fertilizers may extremely be variable and depends on species fitness, soil as well as environmental conditions of that particular area [17]. In this study the nitrate assimilation increased as the N fertilizer doses increased up to 100 kg N/ha means that this species can assimilate higher doses of fertilizer. It might be due to this species belong to tropical zone whereas, the experimental zone was in sub tropical conditions in which the growth was continued during winter season also, that's why the available N was immediately incorporated. More trials are required to be conducted to know actual demand of N in different places separately. The excessive application of N should be avoided as this leads to an economic loss of N and environmental problem. It is evident from the results derived from the present investigation that the higher N doses are not found to be effective as far as the N assimilation is concerned. Therefore, an excessive N fertilizer application in the leguminous species like *Abrus precatorius* should either be avoided or given in

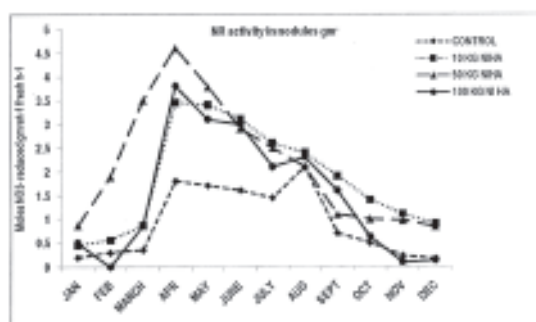


Fig. 3. Nitrate reductase activity in nodule g^{-1} sample of *Abrus precatorius* L. (Kunch).

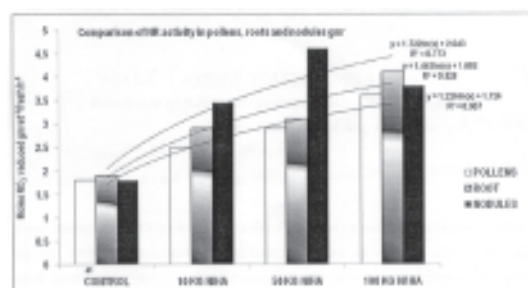


Fig. 4. Comparison of nitrate reductase activity in pollen, root and nodule g^{-1} sample of *Abrus precatorius* L. (Kunch).

more splits after assessing the requirement to maintain a consistent availability of N to the plants. Initially, low N starter dose might be helpful in boosting up the seedling growth whereas, excessive N fertilizer application may inhibit the process of N fixation on one hand and depletion through volatilization and leaching and ultimately polluting atmosphere and soil respectively on the other hand. Moreover, it is concluded that the higher N was not found to be effective in the context of nitrogen assimilation and an excessive nitrogen application should either be avoided or applied in splits after knowing the demand to maintain proper availability of N in the soil profile.

References

- Verma AK, Kumar M, Bussmann R. W (2007) Medicinal plants in an urban environment : The medicinal flora of Banaras Hindu University, Varanasi. Uttar Pradesh. J Ethnobiol and Ethnomed 3 : 35—48.
- Chaukiyal SP, Pokhriyal TC (2005) Nitrogen fertilization vis-à-vis nitrate assimilation in different plant parts of *Pongamia pinnata* Pierre seedlings. Annals of for 13 : 31—43.
- Chaukiyal SP, Khatri N, Kannoja P, Bhatia P (2014) *In vivo* nitrate reductase activity in the *Myrica esculenta* buch. Ham. D. Don seedlings under nursery conditions. Octa J Environm Res 3 : 185—195.
- Kandpal J, Chaukiyal SP, (2016) Effect of nitrogen fertilizers on growth and biomass production of *Albizia procera* seedlings under nursery conditions. Octa J Environm Res 4 : 41—50.
- Jabeen N, Ahmad R (2011) Foliar application of potassium nitrate affects the growth and nitrate reductase activity in sunflower and safflower under salinity. Notulae botanicae Horti Agrobotanici Cluj-Napoca 39 : 172—178.
- Chaukiyal SP, Khatri NP, Bhatia, Pokhriyal TC (2014) Standardization of *in vivo* nitrate reductase activity and its pattern in the individual leaf blades of *Myrica esculenta* buch. Ham. Ex.D. Don. Ind J Pl Physiol 19 : 287—291.
- Chaukiyal SP, Shams PM (2014) Some soil enzymatic studies in five semi-arid plant species of Rajasthan. Ind For 140 : 162—170.
- Young EB, Lavery PS, van Elven B, Dring MJ, Berges JA (2005) Dissolved inorganic nitrogen profiles and nitrate reductase activity in macroalgal epiphytes within seagrass meadows. Marine Ecol Prog Series 288 : 103—114.
- Jabeen N, Ahmad R (2012) Foliar application of potassium nitrate affects the growth and nitrate reductase activity in sunflower and safflower under salinity. Notulae Botanicae Horti Agrobotanici Cluj-Napoca 40 : 165—173.
- Mir RA (2012) To study the seasonal effects on nodulation and nitrogen assimilation behavior of seedling vis-à-vis cutting raised plants in Shisham (*Dalbergia sissoo* Roxb.). Pl Physiol Biochem 34 : 609—620.
- Chaukiyal SP, Pokhriyal TC (2005) Effects of nitrogen treatment and seasonal variation on growth and biomass production in *pongamia pinnata* Pierre seedlings. In : Multi purpose trees in the tropics : Management and improvement strategies. Arid Forest Research Institute Jodhpur, India. Sci Publ Jodhpur, pp 244—251.
- Kumar P (2005) Nitrogen fixation and assimilation behavior in relation to seasonal variation in *Acacia catechu* Willd (Khair). J Hort Sci 67 : 273—281.
- Chaukiyal SP, Khatri N, Bhatia P (2014). The partitioning of nitrate assimilation between root and shoot of higher plants. Pl Cell Environ 9 : 511—519.
- Chaukiyal SP, Shams PM (2015) Soil enzymatic studies in some semi-arid plant species of Rajasthan. Ind For 141 : 92—99.
- Ratrey A, Chaukiyal SP, (2013) Assessment of nitrate reductase activity in the leaves of *Erythrina blakei* R. Parker. Ind J For 36 : 191—196.
- Vandana, Chaukiyal SP, Uniyal P (2016) Assessment of nitrate reductase activity in leaf of *Albizia chinensis* (Os.) Merr. Octa J Environm res 4 : 92—99.
- Kandpal J, Chaukiyal SP (2016) Effect of nitrogen fertilizers on growth and biomass production of *Albizia procera* seedling under nursery conditions. Octa J Environm Res 4 : 63—70.