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EFFECT OF pH AND SUBSTRATE VARIABILITY ON THE NITRATE REDUCTASE ACTIVITY OF EXPERIMENTAL MOSS, LIVERWORT AND ANGIOSPERMIC PLANTS

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The enzyme nitrate reductase (NR) has been proposed as an index of nitrogen incorporation. The highest NR activity was obtained when ammonium molybdate was used as the nitrogen source. High levels of nitrate were found to be inhibitory which indicates a wide diversity of forms of NR. In the present study, the order of NR activity obtained is $(NH_4)_2Mo_7O_{24}.4H_2O > KNO_3 > NH_4Cl > NH_4NO_3$, that differs from our earlier study with *Plagiochasma,* where lower concentrations were used. Present finding suggests that NR activity is rate limiting for nitrate incorporation and also the plant species differ in their relative ability to absorb and assimilate these two nitrogenous forms (nitrate and ammonium).

Keywords: Angiosperm; Liverwort; Moss; NR activity; pH; Substrates.

Introduction

Bryophytes are important contributors to biomass and productivity in a wide variety of ecosystems, while in urban areas, *Duranta repens* and *Bougainvillea spectabilis* contributes more in live fencing of houses[1, 2]. NRA is a major contributor in productivity of plant and is an indicator enzyme of nitrogen assimilation pathway [3]. NRA (NR, E.C. 1.6. 6.1-2) have the ability to reduce nitrate to nitrite and is common virtually in higher plants, bryophytes and pteriodophytes.

Various factors like light, temperature, oxygen concentration, pH of the medium, substrate and heavy metal pollutant influences the enzyme activity to a certain extent [4, 5]. Due to proteineceous nature NRA is extremely pH sensitive. Variation in the optimal pH for NRA in different plant species signifies their variability in their nature of enzyme protein in different genera.

The NR activity is mainly determined by the concentration of nitrate in the medium[4]. The anion (NO_3^-) act both as substrate as well as inducer of the enzyme. The induction mechanism is well studied in higher plants but very little is known in bryophytes [5, 6]. The variability in NRA activity while using different nitrogen sources on liverwort *Plagiochasma appendiculatum* was found in our earlier study [6]. An important aspect of NO_3^- uptake is that it is regulated [7, 8]. Nitrate uptake decreases when plants are fed NH_4^+ or high levels of NO_3^-.

The present investigation were undertaken with the prospective to assess the impact of pH and substrate variability on some bryophytes *Racomitrium crispulum, Plagiochasma appendiculatum, Thuidium cymbifolium* and angiosperm *Duranta repens & Bougainvillea spectabilis*. It is expected that the study will provide some insight into the possible mechanism of action of nitrogen sources on NRA.

Materials and Methods

Liverwort (*Plagiochasma appendiculatum*), an acrocarpous moss (*Racomitrium crispulum*) and pleurocarpous moss (*Thuidium cymbifolium*) were collected from Mukteswar forest (Kumaon Hill) in winter season, in year 2004 and were identified in bryology Laboratory, Bareilly College, Bareilly and samples were submitted to Prof. Poes, Eager University, Hungary for their authenticity. Two higher plants *Duranta repens* and *Bougainvillea spectabilis* growing in botany department, Bareilly College, Bareilly were used.

The NRA of these bryophytes and on two flowering plants were determined by following the method of Srivastava [9] in the pH range 5 to 9. Later response of substrate variability on the activity of NR was studied by supplemental –N application. 0.2 M of each substrate i.e. ammonium molybdate, ammonium chloride, ammonium nitrate and potassium nitrate were used for treatment. As cryptogams are able to absorb substances with their whole surface, therefore, dilute solutions of each were used for incubation for 24 hours.

Application of these substrates to *Bouganvillea* and *Duranta* as foliar spray were applied during rainless period in the field *in situ*. Application of nitrogen as a spray increases the speed of application and allows for fast

uptake & translocation of the applied nitrogen [10]. Prior to and after foliar spray, the leaves were not rinsed with water, since experimental evidence with higher plants have shown a complete dissolution of NO_3^- & SO_4^- from surface deposits due to washing, followed by slower leaching of these compounds from within the leaf [11, 12]. Plant for both control and treatment were harvested after 24 hours.

All data represents means of three separate experiment ± SE. The significance (*) value with confidence limit (95%) differ from control ($P < 0.05$).

Results and Discussion

Fig. 1 shows that NRA activity varies with pH under KNO_3 as substrate. The pH optima also differ from plant to plant. Acrocarpous moss (*Racomitrium crispulum*) shows maximum activity at pH 6.9 while pleurocarpous moss (*Thuidium cymbifolium*), liverwort (*Plagiochasma appendiculatum*) and in angiospermic plant (*Duranta repens* and *Bougainvillea spectabilis*) shows at pH 7.1, 7.5, 6 & 5.2 respectively. In *Bougainvillea spectabilis, Duranta repens and* moss *Racomitrium crispulum* there were more than one peak observed under pH variation.

Study of the effect of substrate with respect to control in all experimental plants undertaken, revealed that every experimental plants show maximum activity under $(NH_4)_2Mo_7O_{24}.4H_2O$, while the NR activity varies with respect to substrate undertaken i.e. ammonium chloride, ammonium nitrate and potassium nitrate from plant to plant. Present study shows that after ammonium molybdate, potassium nitrate is the best inducer for NRA (Table 1)

In vivo NR activity of cryptogams & angiosperms were assayed under different conditions to reveal its ability & biochemical characteristics to reduce nitrate to nitrite.

Maximal NRA activities at 30°C of experimental plants clearly indicates that pH is an important regulatory factor for *in vivo* NRA and varies with pH, temperature and substrate concentration. *In vitro* NR activity assayed under different conditions [18], showed maximum NR activity at pH 8, at 15°C in red algae i.e. *Gracilaria chilensis*. In supportive evidence, for getting more than one peak in *Bougainvillea spectabilis, Duranta repens and* moss *Racomitrium crispulum* , two assimilatory NR forms were reported earlier, i.e EC 1.6.6.1, specific for NADH, & EC 1.6.6.2, using either NADH or NADPH.

Experiments were also preformed to examine the response of NR activity in bryophytes and higher plants to nitrate and ammonium source. Nitrate reductase is a very sensitive plant enzyme and substrate inducible i.e. the supply of nitrate invariably increases the level of nitrate reductase. However, in present study with both angiosperm and cryptogams the enzyme activity was highest with ammonium molybdate as a substrate. The molybdenum-cofactor of NR transfers electrons to NO_3^- and seems to be required to achieve highest activity as evident by a tremendous increase in NR activity.

Further the appearance of NR activity with ammonium as the sole nitrogen source indicates that nitrate may not be necessary for NR synthesis. The appearance of NRA has also been reported earlier in green algae and diatoms when ammonium was used as a nitrogen source [13, 14] and also in *Plagiochasma* in our earlier study [6].

However, in earlier findings, in liverwort *Plagiochasma* the highest enzyme activity was noted in NH_4NO_3, followed by NH_4Cl & only slightly higher in KNO_3, over the control. Unexpectedly, an entirely different result is obtained in present study where highest activity was induced by ammonium molybdate, followed by KNO_3, NH_4Cl & NH_4NO_3. The substantial increase in NR by KNO_3 compared to NH_4NO_3 & NH_4Cl may be due to higher substrate concentration (KNO_3, 0.2 M), used in present study. Nitrate reductase (NR) triggers nitrate assimilation by reducing nitrate to nitrite is induced by NO_3^- N flux to the induction and assimilaton sites (cytoplasm) rather than by the NO_3^- N content of leaves [15, 16].

Nitrate uptake decreases when plants are fed with NH_4^+ & may therefore be responsible for lowest NR activity when supplied with NH_4NO_3 [19, 20]. Further, the degradation

Table 1. Effect of different substrate on NR (n mole NO_2 hr^{-1} g^{-1} F Wt.) activity. Each value is mean of 3 replicates ± S.E. Significance (*) differs from control ($P < 0.05$).

Plants under study	Substrates used 0.2M				
	Control	$(NH_4)_2Mo_7O_{24}.4H_2O$	NH_4Cl	NH_4NO_3	KNO_3
Racomitrium crispulum	2008±158.4	3472*±28.8	142.4*±20.8	139.2*±4.8	400*±28.8
Thuidium cymbifolium	356.8±17.6	4080*±576	396.8±38.4	150.4*±19.2	398.4±56
Bougainvillea spectabilis	2273.6±84.8	7824*±246.4	291.2*±12.8	904*±411.2	1465.6±774.4
Duranta repens	2515.2±268.8	5747.2*±172.8	875.2*±222.4	409.6*±32	1198.4*±273.6
Plagiochasma appendiculatum	273.6±129.6	5294.4*±324.8	556.8±35.2	476.8±86.4	665.6*±83.2

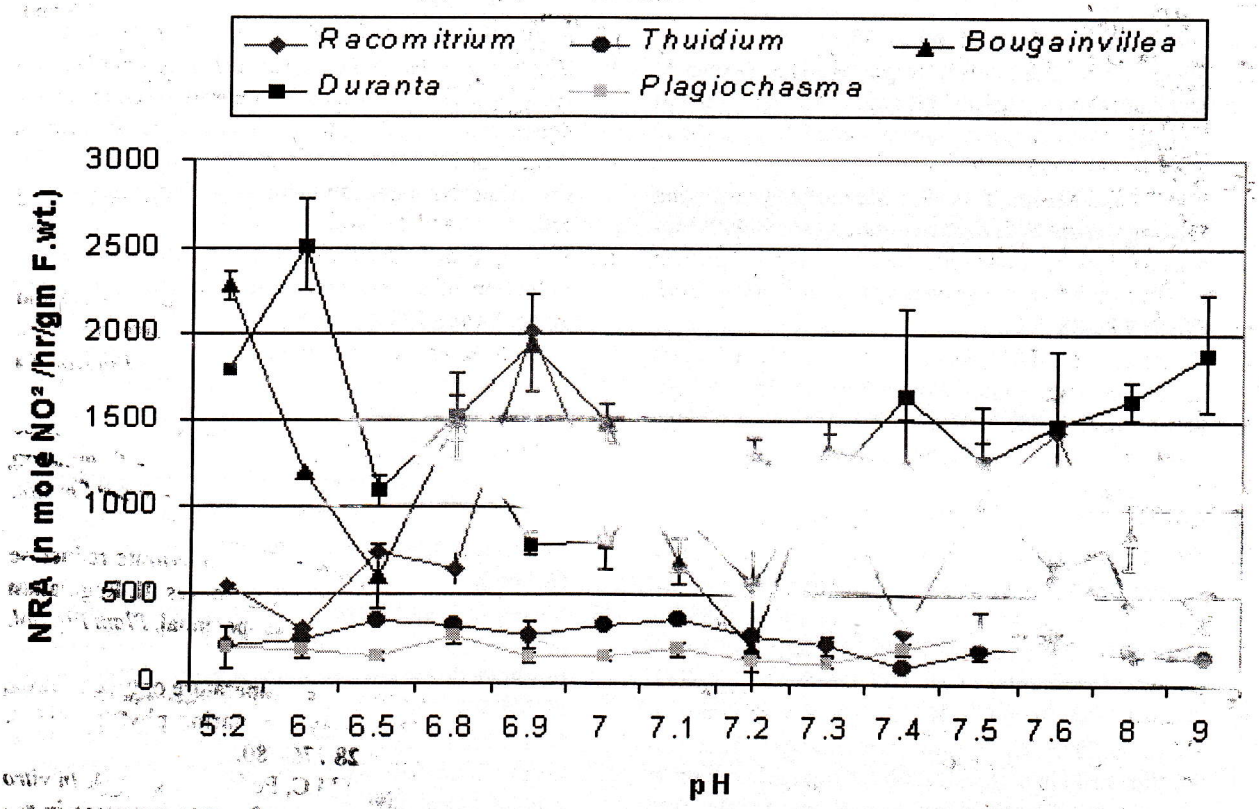

Fig.1. Effect of pH variation on NRA (n mole NO_2 hr^{-1} g^{-1} F Wt.) of undertaken experimental plant species. Each value is mean of 3 replicates ± S.E.

of NR also occurs in the presence of reduced nitrogen forms (ammonium being the highest reduced form). The inhibitory effect of ammonium on NR activity is established in many algal groups. Ammonium may also act on the enzyme directly at the level of synthesis or post-translational modification. Similar trend was also observed in moss *Thuidium cymbifolium*.

In the acrocarpous moss *Racomitrium crispulum*, higher NR activity was observed for ammonium molybdate over the control after 24 hours but decreases significantly for other substrates. Lowest activity was observed for NH_4Cl & NH_4NO_3. The NR was not induced by KNO_3 in *Racomitrium crispulum*. The decrease could be due to decrease in nitrate uptake, when higher level of NO_3^- is [illegible] shoot nitrogen [illegible] also observed in both *Duranta repens* and *Bougainvillea spectabilis* over control. There is a threshold of nitrate concentration in metabolic pool to induce NRA.

The assumption that NR activity is rate limiting for nitrate incorporation appears to be justified well in present study. The diversity in terms of NR is evident by the use of different substrates, present study also suggests that plant species differ in their relative abilities to absorb and assimilate these two nitrogenous forms (Nitrate and ammonium). The NR activity for *Plagiochasma appendiculatum* and *Thuidium cymbifolium* were lowest compared to other plants used in present study. It is therefore suggested that for both, the assay temperature should be investigated, since 30°C is remarkably higher than that at which these lower cryptogams live. Thus, the assay performed at higher temperature may not be related to those conducted at *in situ* temperature [17].

This study has demonstrated the critical importance of examining NR activity assays for different species in the laboratory before attempting its application to diverse plant assemblages in field situations.

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