Visual evidence of nitrate reductase exudation from plant roots

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To prove the nitrate reductase exudation, roots of 22 plant species were kept in contact with filter sheet impregnated with *N*-(1-naphthyl)ethyl-diamine dihydrochloride, sulphanilamide and potassium nitrate. Nitrate reductase exudation was indicated by the appearance of root impressions in red colour. Nitrate reductase exudation was observed from the roots of 13 plant species even though roots of all species showed its activity.

NITRATE reductase (NR) catalyses the conversion of NO_3 to NO_2 (first step in denitrification process)[1,2] and its high activity may lead to high N losses from soil[3,4]. Like other soil enzymes, i.e. urease and phosphatase, microorganisms are known to be the source of its activity in soil[2]. Plants may also effect the enzyme status in soil either by affecting the microbial population in rhizosphere or by directly contributing towards its pool as root exudates as in the case of urease and phosphatase[5,6]. However, it is not known whether plant contributes directly towards NR activity in soils. Such an information may be useful in devising strategies for reducing NR activity in soil and controlling denitrification losses. Here, we describe a technique to find out whether grasses, crops and trees (Table 1) contribute directly towards NR activity in soil as root exudates.

A sheet of Whatman No. 1 filter paper was spread on a glass plate (60 × 45 cm) in a slanting position and clamped. Distilled water (100 ml) was gradually poured from the upper end of the sheet three to four times for washing. The sheet was then allowed to dry at room temperature. Subsequently, a mixture of *N*-(1 naphthyl) ethyl diamine dihydrochloride (NED, 500 ppm aqueous solution) and sulphanilamide (SA, 1% w/v aqueous solution), mixed in equal proportion was sprayed on the sheet through a chromatographic sprayer and was then dried in dark. Once again, the sheet was sprayed with potassium nitrate solution (100 ppm NO_3–N) and dried in dark. Next, the sheet was cut into 25 × 10 cm pieces and fixed on glass plate (20 × 7.5 cm) by folding and sticking the excess portion of the sheet on the reverse side of the plate with a cellophane tape.

Selected plants were irrigated and this facilitated uprooting of these plants with their roots intact. Subsequently, roots were washed, first under flowing tap water and then with distilled water to remove adhering soil particles. Roots were mildly jerked to remove water droplets. Thereafter, roots were spread on to the glass plate and covered with a similar type of plate. Both plates were held together by rubber bands and kept in dark for 4 h.

It was hypothesized that if exuded, NR will reduce NO_3 present on filter sheet to NO_2. NO_2 would then diazotize with NED and SA and give the characteristic red colour[7]. Roots of *Cymposis tetragonoloba* (var. Maru guar), *Vigna radiata* (var. S-8), *Vigna aconitifolia* (var. Maru moth), *Vigna unguiculata* (var. Pusa aseem), *Cicer arietinum* (var. BCG-9), *Zea mays* (var. Megha), *Triticum aestivum* (var. PBW 175), *Azadirachta indica*, *Albizia lebbek*, *Prosopis juliflora*, *Ficus religiosa*, *Simmondsia chinensis* and *Acacia ampliceps* left perfect impression in red colour on the filter paper (Figure 1). These impressions indicated the exudation of NR from roots.

However, theoretically the impressions would have also formed had NO_2 been exuded from roots. To explore this possibility roots were kept in contact with freshly prepared filter sheet sprayed only with NED and SA and not with KNO_3. Absence of red impressions would confirm exudation of only NR by ruling out NO_2 exudation. However, we observed red-coloured root impressions in this case also. Though, exudation of NO_2 appears highly unlikely as it is toxic, highly unstable and is rapidly converted to NH_4 in plant[8], but without discounting the possibility of its exudation, NR exudation cannot be proved. To test this, inhibition of root NR by sodium tungstate was tried[9]. The hypothesis was that

Table 1. Activities of nitrate reductase in roots of different plants

Species	Nitrate reductase (μg NO_2^-–N/g root tissue/h)	Root impression
Crops		
Vigna unguiculata	1.00	Yes
Cymopsis tetragonoloba	7.71	Yes
Vigna radiata	2.48	Yes
Vigna aconitifolia	1.31	Yes
Triticum aestivum	5.80	Yes
Zea mays	9.90	Yes
Cicer arietinum	9.90	Yes
Pennisetum glaucum	3.13	No
Sesamum indicum	3.40	No
Ricinus communis	9.89	No
Trees		
Albizia lebbek	1.31	Yes
Acacia ampliceps	1.16	Yes
Ficus religiosa	0.87	Yes
Azadirachta indica	0.74	Yes
Simmondsia chinensis	0.44	Yes
Prosopis juliflora	0.54	Yes
Acacia nilotica	0.64	No
Dichrostachys nutans	0.55	No
Colophospermum mopane	0.65	No
Acacia aneura	0.58	No
Grasses		
Lasiurus sindicus	9.45	No
Cenchrus ciliaris	10.39	No

Figure 1. Root impressions showing of nitrate reductase. '+' and '–' indicate exudation and no exudation of NR respectively.

appearance of red-coloured impressions even after NR inhibition would demonstrate NO_2 exudation while lack of it would show NR exudation. For this, plants were irrigated with 100 µM solution of sodium tungstate (Na_2WO_4) twelve hours before uprooting. Inhibition of root NR was confirmed by *in vivo* assay[10]. Roots irrigated with Na_2WO_4 did not produce red impressions on filter paper impregnated with (i) NED + SA only or (ii) NED + SA + NO_3. Absence of coloured root impression in (ii) demonstrated complete inhibition of root NR by sodium tungstate because otherwise we would have observed at least a faint impression. These observations led us to conclude that NO_2 is not exuded. Therefore, our earlier observation (roots with uninhibited NR in contact with NED + SA only) of red root impressions on filter sheet suggests the formation of NO_2 on root surface. This is possible only if both NO_3 and NR are exuded. Exudation of NO_3 has also been reported in *Lolium perenne*[11]. Further, Figure 1 suggests that NR is exuded from the entire root system rather than any specific site.

Root of *Pennisetum glaucum* (var. MH 179), *Sesamum indicum* (var. C 50), *Ricinus communis* (var. Aruna), *Colophospermum mopane*, *Acacia aneura*, *Dichrostachys nutans* and *Acacia nilotica* however, did not exude NR.

Estimation of NR in roots[10] indicated its activity in all plants (Table 1). However it was surprising that 7 crops out of 10, 6 trees out of 10 and none of the tested grasses showed its exudation. Plants studied belonged to different families but all plants of even one family did not exude NR. Therefore, it appears that exudation or lack of it was clearly a character of individual plant species.

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