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Comparison of Nitrate Reductase Activity in the Leaves of Tropical Root and Tuber Crops

Plants meet their N requirement mainly from the soil as nitrates (NO_3^-) and ammonia (NH_4^+) (Glass et al., 2002). The absorbed nitrate is first reduced to nitrite, which is subsequently reduced to ammonia in the leaves. Ammonia is then incorporated into amino acids, glutamine glutamate using the C-skeletons produced via other metabolic pathways such as respiration and photosynthesis.

The first reaction, reduction of nitrate to nitrite, is catalyzed by the metallo-protein nitrate reductase (NR) in the cytosol. Another metallo-protein, nitrite reductase, catalyzes the second reaction in the chloroplasts and or plastids in which nitrite is reduced to ammonia. The electron donors for this sequential reduction are NAD(P)H (Ni R reaction) and reduced ferredoxin from PSII (nitrite reductase reaction). Lesser activity of NR exists in the epidermal and cortical cells of roots and leaves in plants.

Few studies have been reported on the nitrate reductase activity in tropical root crops such as sweet potato (Chowdhury and Ravi, 1987, 1988; Indira and Kabeerathumma, 1990; Naskar and Chowdhury, 1995), cassava (Pereira and Splittstoesser, 1986; Odjegba and Okunnu, 2008), tannia (Latifa and Anggarwulan, 2009) and taro (Sahoo et al., 2010). Nitrate reductase activity is usually greater in the leaves than in the roots (Eilrich and Hageman, 1973; Jiaang and Hull, 1998; Flávio José Rodrigues da Cruz et al., 2011). In the present study, to understand the efficiency of NO_3^- reduction and its relation with leaf N content and dry matter, NR activity was assayed in the leaves of tropical root and tuber crops *viz.*, cassava, sweet potato, yams, elephant foot yam, taro, tannia, Chinese potato and arrowroot.

In the present study, NR was assayed *in vivo* (substrate non-limiting) in the leaves of tropical root and tuber crops according to Jaworski (1971) method. Healthy leaf samples positioning acropetally between 5 and 10 were collected from cassava, sweet potato, yams, Chinese potato and 3rd and 4th leaf in taro, tannia, and arrowroot at their active growth period (between 3 and 4 months in cassava, yams, taro, tannia, Chinese potato and arrowroot) and during 2nd month in sweet potato. The leaf samples were pooled and from this, three samples were used for estimation of NR acitivity, dry matter and N content. Discs of fresh leaf weighing 500 mg was used for the NR activity in all the root and tuber crops, except for sweet potato where 200 mg discs of fresh leaf were used. During the assay, leaf discs were incubated for 45 minutes in a solution containing nitrate, 2-propanol and phosphate buffer. Nitrate enters the leaf discs which supply both NR and reducing power. Nitrite is released into the assay medium and is converted to a bright magenta azo dye by reaction with sulfanilamide and (SA) N-1 naphthylethylenediamine-HCL (NEED). If the nitrite content is to be measured the conversion of nitrite to ammonia must be stopped and the nitrite must be released from the cell. Under anaerobic conditions the reduction of nitrate is promoted and the reduction of nitrite is inhibited. The presence of 2-propanol in the assay medium increases the permeability of both nitrate into and nitrite out of the leaf segments and provides an essentially anaerobic environment. Leaf N content was estimated by micro Kjeldahl method using three samples of 200 mg dry leaf powder.

In the present study, NR activity was maximum in the leaves of sweet potato (33.06 – 43.07 μg NO_2^- g^{-1} fresh leaf h^{-1}) while lowest in the leaves of cassava (0.14 – 1.1 μg $NO_2^-g^{-1}$ fresh leaf h^{-1}). Next to sweet potato, elephant foot yam had greater NR activity in the leaves (9.97 – 11.67μg NO_2^- g^{-1} fresh leaf h^{-1}). Taro, tannia, arrowroot and Chinese potato leaves showed little variation in NR activity (4.12 – 6.47 μg NO_2^- g^{-1} fresh leaf h^{-1}) (Table 1). Leaf dry matter was maximum in cassava (29.82 – 36.22%) whereas it was lowest in sweet potato (15.66 and 18.17%).

Nitrate reductase activity has been reported to vary

between 6.5 and 10.77 μmol NO_2^- $h^{-1}g^{-1}$ fresh leaf in mulberry (Misra et al., 2009), 2 and 6 μmol NO_2^- h^{-1} g^{-1} fresh leaf in maize (Reed et al., 1980), 5.5 μmol NO_2^- $h^{-1}g^{-1}$ fresh leaf in Kentucky Blue grass cultivars (Jiang and Hull, 1998), 4.0 and 21.5 μmol NO_2^- $h^{-1}g^{-1}$ fresh leaf in wheat (Croy and Hageman, 1970). In cassava leaves, NR activity of 0-2.5 μmol of NO_2^- $g^{-1}h^{-1}$ was reported (Pereira and Splittstoesser, 1986) with no activity in the younger leaves (leaf 1 on top). A very low NR activity ranging between 0.0033 and 0.025 μ mol NO_2^- $h^{-1}g^{-1}$ fresh leaf was reported in cassava (Cruz et al., 2004). The NR activity increased with increase in NO_3^- concentration and incubation time in leaves of cassava (Cruz et al., 2004) and tannia (Latifa and Anggarwulan, 2009). In tannia, a very high *in vivo* NR activity (153.97 – 176.9 μmol NO_2^- $h^{-1}g^{-1}$ fresh leaf) was reported when the leaves were incubated for 2 hours (Latifa and Anggarwulan, 2009). In sweet potato, NR activity in the leaves, among 24 genotypes, was maximum during the first month of crop growth period (8.41 – 23.15 μmol of NO_2^- $g^{-1}h^{-1}$) and it declined at 4th month of crop growth period to values varying between 0.53 and 7.15 μmol of NO_2^- $g^{-1}h^{-1}$) (Naskar and Chowdhury, 1995). The level of NR activity observed in the leaves of tropical root and tuber crops in the present study is in agreement with these findings, except in the case of tannia. In the case of cassava, we observed nearly 10 fold greater NR activity than that reported by Cruz et al. (2004). In the present study, because NR activity was assayed with the externally supplied NO_3^-, the variation in the NR activity in the leaves of tropical root and tuber crops is attributed to the genetic variation of the enzyme, flux rate of nitrate into the leaf blade cells, availability of reductant and stability of NR *in situ*. Variations in NR activity could also result from limitations in carbon supply (Oaks, 1994).

Despite the wide difference in NR activity in the leaves of tropical root and tuber crops, the leaf N content showed little variation (Table 1). Nitrate reductase activity was found to increase in response to added N and paralleled with an increase in reduced N in vegetative parts in wheat (Eilrich and Hageman, 1973) and cassava (Cruz et al., 2004). The level of NR activity in the leaves was also found to be positively related to N content in the leaves of potato (Kapoor and Li, 1982). In the leaves of tannia, no consistent relation was found between NR activity and total N content (Latifa and Anggarwulan, 2009). Thus, in the present study, there was no definite relation between the level of NR activity and total N content in the leaves of tropical root and tuber crops. The lack of relation between NR activity and the accumulation of total N in the leaves indicates that the level of the enzyme activity, as measured, is not a valid index of cumulative accumulation of total N. This is in agreement with Reed et al. (1980). There was no mathematical correlation between NR activity and NO_3^- content of the leaves in maize (Reed et al., 1980) and potato (Davies et al., 1987). The current data show that other factors may be involved. The total N content in the leaves of cassava varieties reported here (3.99 - 4.75%) are in agreement with Cruz et al. (2004).

Linear relationship between NR activity in the leaf and total soluble protein and shoot dry matter has been reported in sorghum (Flávio José Rodrigues da Cruz et al., 2011). In the present study, no definite relation was found between the level of NR activity and leaf dry matter of tropical root and tuber crops. However, relation between the level of NR activity and total plant biomass warrants investigation. Nitrate reductase activity *in vivo* was found to be influenced by light, temperature, NO_3^- uptake rate by the root, NO_3^- supply to the leaves and light duration (Jiang and Hull, 1998). Light penetration into the plant canopy and the rate of nitrate flux into the cells of the leaf blade have been shown to affect induction and activity of NR activity more than total leaf nitrate content in maize leaves (Shaner and Boyer, 1976). It is not known how these factors influence NR activity *in vivo* in the leaves of tropical root and tuber crops.

Crop yields were often correlated with high NR activity assayed in the leaves of barley, cotton, maize, pearl millet, sorghum, wheat (Jiang and Hull, 1998 and references cited there in) and sweet potato (Naskar and Chowdhury, 1995). But, a clear relationship between plant productivity and NR activity was not found in Kentucky Blue grass cultivars (Jiang and Hull, 1998). Inter and intraspecific variations in the NR activity have been reported in the leaves of maize (Reed et al., 1980) and Kentucky Blue grass cultivars (Jiang and Hull, 1998).

Nitrate reductase activity in the leaves of arrowroot was

Table 1. Nitrate reductase activity, total N and dry matter content in the leaves of tropical root and tuber crops

Crop and variety	NR activity in leaf* (μg NO_2^- g^{-1} fresh leaf h^{-1})	Leaf dry matter* (%)	N content in dry leaf* (%)	N content in fresh leaf (%)
Sweet potato				
Sree Arun	34.51 ± 4.14	18.17 ± 0.31	5.54 ± 0.36	1.01
Kanjanghad	33.06 ± 5.31	16.46 ± 0.37	5.06 ± 0.55	0.84
Sree Kanaka	38.59 ± 5.9	15.66 ± 0.25	5.02 ± 0.54	0.78
Sree Vardhini	43.07 ± 6.35	16.9 ± 0.29	4.81 ± 0.52	0.81
Elephant foot yam				
Gajendra	9.97 ± 3.28	20.62 ± 2.21	4.45 ± 0.48	0.95
Sree Padma	11.67 ± 1.75	21.83 ± 1.94	4.04 ± 0.67	0.86
Sree Athira	10.24 ± 1.13	21.4 ± 0.4	4.82 ± 0.46	1.03
Peerumade Local	10.84 ± 1.19	20.9 ± 0.01	4.75 ± 0.37	0.99
Taro				
Topi	4.37 ± 0.66	18.19 ± 1.57	4.2 ± 0.12	0.78
Telia	6.47 ± 0.85	17.57 ± 0.98	4.62 ± 0.08	0.81
Muktakeshi	4.19 ± 0.96	20.15 ± 1.48	5.0 ± 0.33	1.03
Jhankdi	4.61 ± 0.93	21.07 ± 1.02	4.89 ± 0.3	1.04
Sree Kiran	4.94 ± 1.36	19.29 ± 1.09	4.91 ± 0.39	0.96
Sree Rashmi	5.39 ± 1.86	18.89 ± 0.99	4.96 ± 0.39	0.93
Tannia	4.12 ± 0.39	20.36 ± 1.11	3.31 ± 0.19	0.66
Yams				
Greater yam (Sree Keerthi)	6.86 ± 1.94	17.85 ± 0.16	4.31 ± 0.36	0.77
Lesser yam (Sree Latha)	12.34 ± 2.054	25.7 ± 0.77	3.3 ± 0.25	0.86
White yam (Sree Priya)	0.96 ± 0.36	27.35 ± 1.69	3.78 ± 0.32	1.05
Arrowroot	5.61 ± 0.47	23.56 ± 0.25	4.02 ± 1.12	0.95
Chinese potato	4.76 ± 1.08	8.66 ± 0.78	3.57 ± 0.58	0.31
Cassava				
Sree Vijaya	0.3 ± 0.1	34.24 ± 1.39	4.37 ± 0.28	1.48
H-165	0.33 ± 0.15	31.57 ± 2.04	4.35 ± 0.31	1.39
M4	0.17 ± 0.08	36.22 ± 3.03	4.75 ± 0.22	1.68
Sree Prakash	0.28 ± 0.06	32.95 ± 2.86	4.32 ± 0.37	1.4
H-1687	0.43 ± 0.13	35.51 ± 2.19	4.55 ± 0.29	1.61
H-226	0.54 ± 0.1	31.16 ± 0.96	4.21 ± 0.35	1.32
Sree Padmanabha	0.28 ± 0.67	34.6 ± 1.03	4.55 ± 0.22	1.56
Triploid 4-2	1.1 ± 0.82	29.82 ± 1.05	4.63 ± 0.16	1.4
CE-534	0.14 ± 0.03	32.64 ± 0.37	3.99 ± 0.44	1.29
CI-60	0.29 ± 0.1	31.83 ± 1.83	4.39 ± 0.39	1.41

*Mean of three samples of pooled leaves

greater under 75% shade (of trees) conditions as compared to plants under open conditions but the NR activity in elephant foot yam was similar in both plants under open and shade of trees (75% shade) (Table 2). Shade levels (50 and 75%) did not have a consistent influence on the NR activity of leaves in tannia (Latifa and Anggarwulan, 2009). Interestingly NR activity greatly enhanced in arrowroot under shade.

Table 2. Nitrate reductase activity in the leaves of elephant foot yam and arrowroot under open and shade

Crop	NR activity (μg NO_2^- g^{-1} fresh leaf h^{-1})	
	Open	Tree shade (75%)
Elephant foot yam var. Gajendra	9.97	9.42
Arrowroot	5.61	11.17

In cultivars which are responsive to applied N, regulation of NR activity will pay a major role in biomass accumulation and yield. Hence NR activity may assume greater significance. Therefore, further studies are needed to understand the factors regulating the NR activity in tropical root and tuber crops such as level of soil plant NO_3^-, rate of nitrate uptake by roots, the seasonal and genotypic variation. Studies are also needed to understand the relation between NR activity with the various N pools in the leaves and vegetative parts, N use efficiency and yield. Moreover, the possibility of enhancing the activity of the enzyme by management or suitable varietal selection needs to be explored.

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