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Relationship of Nitrate Reductase Activity to Leaf Yield, Protein, Sugar and Physiological Attributes in Mulberry (*Morus alba* L.)

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Ten improved mulberry varieties (V1, C1730, C2016, C2017, Anantha, RFS-175, Thallaghatapura, Vishala, S1 and S1635) were evaluated through enzyme assay and estimation of soluble protein content followed by regression analysis, grown under irrigated conditions in the alluvial soils of Gangetic plains of West Bengal in India for five successive crops in a year. The nitrate reductase (EC No. 1.6.6.1) activity (NRA, μmol NO_2^- $h^{-1}g^{-1}$ fr. wt.), total soluble protein (mg g^{-1} fr. wt.) was estimated which showed to vary significantly in the tested varieties. In addition to these, the other parameters like unit leaf fresh and dry weight (g), moisture%, unit leaf area (cm^2), specific leaf weight (g cm^{-2}), total soluble sugar (mg g^{-1} fr. wt.), leaf yield/plant (kg), shoot yield/plant (kg) and net photosynthetic rate (NPR, μmol m^{-2} s^{-1}) were also studied which showed to vary significantly in tested varieties. Among them, S1635, having higher NRA (13.25 μmol NO_2^- $h^{-1}g^{-1}$ fr. wt.), total soluble protein (39.63 mg g^{-1} fr. wt.), NPR (16.66 μmol m^{-2} s^{-1}), total soluble sugar (48.44 mg g^{-1} fr. wt.), leaf yield/plant (0.689 kg) and shoot yield/plant (1.135 kg) showed its superiority over other tested varieties. Regression and correlation coefficients were analysed, and a strong positive correlation was found between NRA & total soluble protein, NRA & NPR, NRA & total soluble sugar, NRA & unit leaf weight, NRA & specific leaf weight, NRA & leaf yield/plant, NRA & shoot yield/plant, NPR & leaf yield and NPR & specific leaf weight.

Key words: Mulberry, NRA, Photosynthesis, Leaf yield

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Introduction

Silkworm (*Bombyx mori* L.) feeds on the singular food mulberry (*Morus alba* L.) from which it ingests various nutrients to maintain/support physiological activities. Silkworm nutrition depends on the quality and quantity of food. Protein is a kind of basic substance to construct silkworm body and one of the most important composition of cell, also is important material for silk and egg production (Zuhua, 1994). Nitrate reductase, is a key enzyme in nitrogen metabolism and one of the most important regulatory enzymes that catalyzes the reduction of nitrate to nitrite (Beevers and Hageman, 1969). The enzyme NR was studied extensively in different crop plants (Schrader and Hageman, 1967; Deckard *et al.*, 1973; Nicholas *et al.*, 1976; Srivastava, 1980; Reddy *et al.*, 1985; Paliwal and Ilangovan, 1990; Karadge *et al.*, 1994; Abrol *et al.*, 1999) and its activity was positively correlated with economic yield in rice (Rao *et al.*, 1990), jute (Singh *et al.*, 1994) and in berseem (Amaresh Chandra and Roy, 1995). Eilrich and Hageman (1973) were the first who proposed to use NR activity in cereals. Also, it has been established by Johnson *et al.* (1976) that NRA is such a parameter which ultimately determines future of the actively growing plant particularly in terms of yield or leaf biomass. In recent years, NR activity has been increasingly used as an additional parameter for identifying high yielding genotypes in several crop plants (Rao *et al.*, 1990; Singh *et al.*, 1994; Amaresh and Roy, 1995). In mulberry, very few reports are available on NR activity (Paliwal and Ilangovan, 1990; Rao *et al.*, 2000)

In the present investigation, a correlation between NRA and protein content, leaf yield and leaf yield attributing parameters were studied

Material and Methods

Planting material

Well-rooted saplings of ten improved mulberry varieties

were planted under 90 × 90 cm spacing in Randomized Block Design (RBD) with 3 replications. In each replication out of 84 plants, 15 plants were utilized for observations, surrounded by one line of border excluding intervarietal border. Recommended cultural practices for cultivation under irrigated condition were applied (Ray, 1973). Data on various parameters were recorded after one year of sapling (rooted cutting) plantation.

Leaf yield parameter

Leaf yield/plant was recorded 5 times per year (February, April, July, September and November) in accordance with the silkworm rearing schedules in West Bengal. The yield attributing characters like unit leaf fresh weight, dry weight, leaf area, leaf yield and shoot yield per plant were also recorded as per schedule.

Biochemical analyses

Total soluble protein was determined in fresh leaves as described by Lowry *et al.* (1951) using bovine serum albumin as standard. The *in vivo* nitrate reductase activity was assayed as per Hagemen and Hucklesby (1971). Total soluble sugar was measured spectrophotometrically using anthrone reagent (Morris, 1948). All the biochemical constituents were determined in triplicate and repeated twice in fresh leaves on 60th day after pruning.

Gas-exchange parameters

Net photosynthetic rate (NPR) was measured from fifth expanding leaves using a portable photosynthetic system (LI-COR model 6200; Licor Instrument Inc, USA). The whole experiment was carried out between 11 – 12 hrs under natural condition with ambient temperature range of 28 – 30°C, relative humidity of 70 – 80% and photoperiod of 12 hrs.

Statistical analyses

The data were analyzed statistically and the simple linear correlation coefficients between NR activity/NPR and different characters were computed (Gomez and Gomez, 1983).

Results and Discussion

Among the genotypes S1635, C1730 and Anantha exhibited high NR activity and soluble protein content in leaves. NR activity was found maximum in S1635 (13.25 µmol $NO_2^- h^{-1}g^{-1}$ fr. wt.) and minimum in C2016 (7.85 µmol $NO_2^- h^{-1}g^{-1}$ fr. wt.). Likewise S1, V1, RFS-175, Thallaghatapura and Vishala with moderate NR activity had moderate protein content and the remaining two genotypes, C2016 and C2017 showed low NR activity and low protein content, which clearly indicates that the level of NR activity is closely related with that of total protein content (Table 1). It was reported that any factor (nutrient uptake) that affects the leaf NR activity will directly affect its protein content (Ghosh *et al.*, 1994). As the enzyme (NR) is believed to be rate limiting in overall assimilation of nitrate (Beevers and Hageman, 1969), the genotypes having more NR activity will be having more in-built nitrogen utilization efficiency (Amaresh and Roy, 1995), which may probably enhance the protein content (Paliwal and Ilangovan, 1990). In the present study a significant positive correlation ($R^2 = 0.9579$) was observed between NR activity and protein content (Fig. 1).

The NPR *i.e.*, net photosynthetic rate and total soluble

Table 1. Nitrate reductase activity, total soluble protein, net photosynthetic rate and total soluble sugar in ten improved mulberry varieties

Mulberry variety	Nitrate reductase activity [µmol $NO_2^- h^{-1} g^{-1}$ fr. wt.)	Total soluble protein (mg g^{-1} fr.wt.)	Net photosynthetic rate (µmol $m^{-2} s^{-1}$)	Total soluble sugar (mg g^{-1} fr.wt.)
V 1	9.84	33.31	10.72	44.09
C 1730	10.09	34.40	12.12	45.62
C 2016	7.85	30.04	9.53	40.00
C 2017	7.92	30.25	9.92	41.03
Anantha	10.66	35.58	12.46	46.76
RFS 175	9.82	32.90	10.57	44.51
Thallaghatapura	9.34	32.15	10.20	43.29
Vishala	9.33	32.00	9.71	43.22
S 1	9.97	35.00	11.17	44.27
S 1635	13.25	39.63	14.66	48.44
CD at 5%	0.214	0.121	1.05	0.982

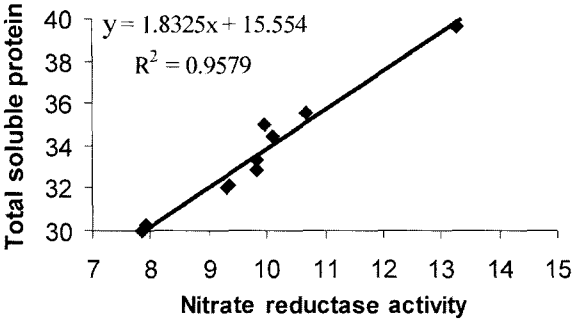

Fig. 1. Regression and correlation coefficients between nitrate reductase activity (μmol $NO_2^- h^{-1}g^{-1}$ fr. wt.) total soluble protein (mg g^{-1} fr. wt.).

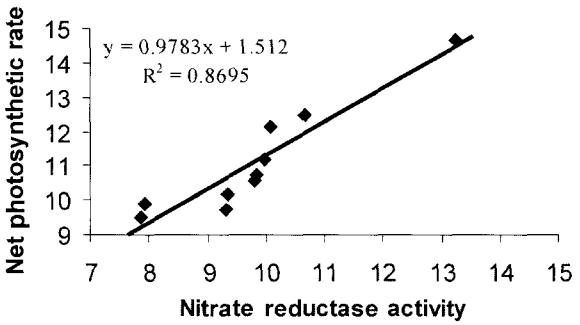

Fig. 2. Regression and correlation coefficients between nitrate reductase activity (μmol $NO_2^- h^{-1}g^{-1}$ fr. wt.) net photosynthetic rate (μmol $m^{-2} s^{-1}$).

sugars were recorded higher in those genotypes where NRA is high. The improved levels of other parameters like NPR, total sugars might result from a general improvement in the metabolic activity of the cells (Ghosh and Srivastava, 1995). A significant positive correlation (R^2 = 0.8695) was observed between NRA & NPR (Fig. 2) and

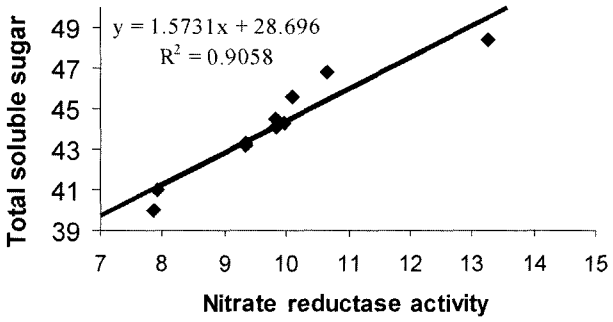

Fig. 3. Regression and correlation coefficients between nitrate reductase activity (μmol $NO_2^- h^{-1}g^{-1}$ fr. wt.) total soluble sugar (mg g^{-1} fr. wt.).

between NRA & total soluble sugar (R^2 = 0.9058) as in Fig. 3.

Data recorded on different morphological characters of ten improved mulberry genotypes are summarized in Table 2. Genotypes *viz.*, S1635 followed by Anantha and C1730 showed maximum leaf yield/plant which may be mainly due to their increased unit leaf fresh weight, leaf area, specific leaf weight and shoot yield/plant. In the present study, the genotypes with high NR activity showed high leaf protein content and leaf yield, which may be due to their greater ability of nutrient uptake and more in-built nitrogen utilization efficiency. In order to confirm the positive associations obtained between NR activity and other characters, a simple linear correlation coefficient was computed and the analysed data are presented graphically. NR activity showed highly significant positive correlation (Fig. 4) with unit leaf fresh weight (R^2 = 0.6897), specific leaf weight (R^2 = 0.6680, Fig. 5)), leaf yield / plant (R^2 = 0.8007, Fig. 6), shoot yield/plant (R^2 = 0.6895, Fig. 7). In addition to these NPR also showed

Table 2. Unit leaf fresh weight (g), unit dry weight (g, moisture (%), unit leaf area (cm^2), specific leaf weight (g m^{-2}), leaf yield / plant (kg) and shoot yield (leaf + stem)/plant (kg) in ten improved mulberry varieties

Mulberry variety	Unit leaf fresh wt. (g)	Unit leaf dry wt. (g)	Moisture (%)	Unit leaf area (cm^2)	Specific leaf wt. (g m^{-2})	Leaf yield/ plant (kg)	Shoot yield/ plant (kg)
V 1	3.43	0.74	78.42	223.09	33	0.528	0.855
C 1730	3.89	0.74	80.97	239.23	31	0.593	1.022
C 2016	2.84	0.64	77.46	215.15	30	0.453	0.732
C 2017	3.00	0.62	79.33	202.66	31	0.463	0.710
Anantha	3.99	0.87	78.19	270.67	32	0.641	1.029
RFS 175	3.66	0.96	73.77	297.69	32	0.502	0.782
Thallaghatapura	2.47	0.50	79.75	164.52	30	0.484	0.750
Vishala	2.75	0.79	71.27	265.53	30	0.459	0.760
S 1	2.90	0.50	82.75	143.43	35	0.565	0.990
S 1635	4.92	1.15	76.62	305.70	38	0.689	1.136
CD at 5%	0.553	0.091	1.034	30.07	0.984	0.107	0.191

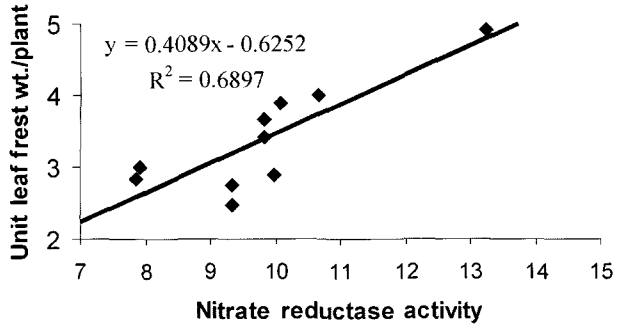

Fig. 4. Regression and correlation coefficients between nitrate reductase activity (μmol NO_2^- $h^{-1}g^{-1}$ fr. wt.) and unit leaf fresh weight (g).

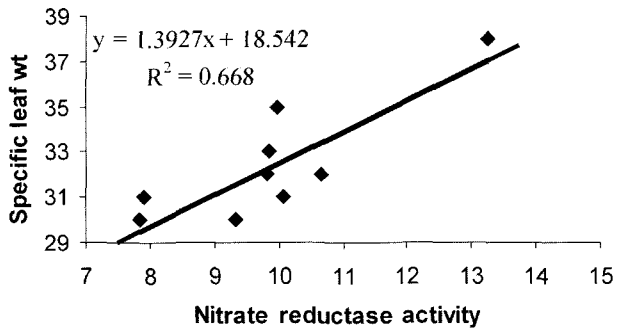

Fig. 5. Regression and correlation coefficients between nitrate reductase activity (μmol NO_2^- $h^{-1}g^{-1}$ fr. wt.) and specific leaf fresh weight (g m^{-2}).

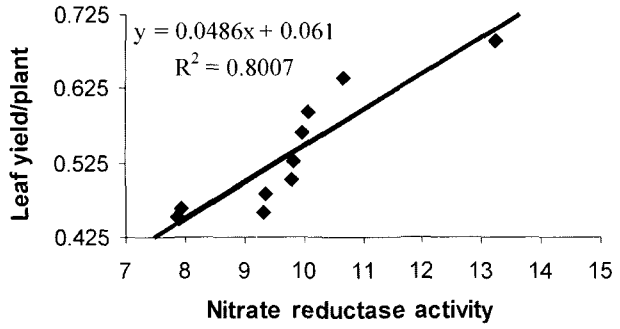

Fig. 6. Regression and correlation coefficients between nitrate reductase activity (μmol NO_2^- $h^{-1}g^{-1}$ fr. wt.) and leaf yield/ plant (kg).

highly significant positive correlation with leaf yield (R^2 = 0.9479, Fig. 8) and specific leaf weight (R^2 = 0.6680, Fig. 9).

An overall view of the results indicated that NR activity has positive correlation with leaf yield and many of the qualitative and yield attributing characters. Thus, it can be concluded that the level of NR activity may be employed as an additional parameter for identifying superior mulberry genotypes.

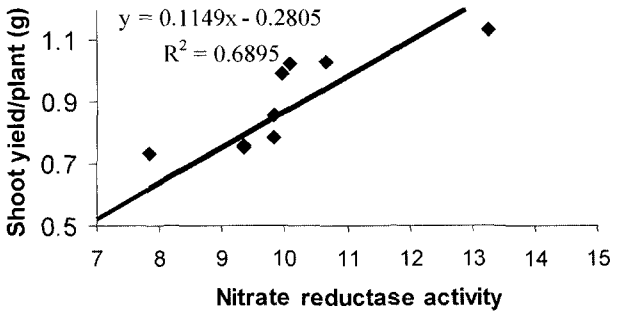

Fig. 7. Regression and correlation coefficients between nitrate reductase activity (μmol NO_2^- $h^{-1}g^{-1}$ fr. wt.) and shoot yield/ plant (kg).

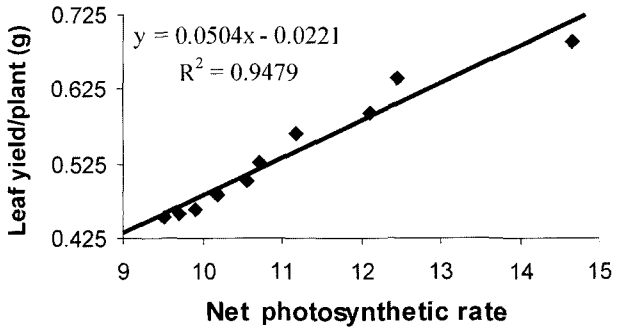

Fig. 8. Regression and correlation coefficients between net photosynthetic rate (μmol m^{-2} s^{-1}) and leaf yield/plant (kg).

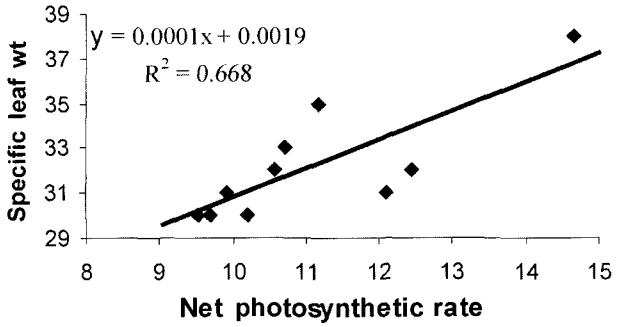

Fig. 9. Regression and correlation coefficients between net photosynthetic rate (μmol m^{-2} s^{-1}) and specific leaf weight (g m^{-2}).

References

Abrol, Y. P., S. R. Chatterjee, K. P. Ananda and J. Vanitha (1999) Improvement in nitrogen use efficiency: Physiological and molecular approaches. *Curr. Sci.***76**, 1357-1364.

Amaresh, C. and A. K. Roy (1995) Nitrate reductase activity in diploid genotypes and induced tetraploids of berseem. *Indian J. Plant Physiol.* **38**, 184-185.

Beevers, L. and R. H. Hageman (1969) Nitrate reduction in higher plants. *Annu. Rev. Plant Physiol.* **20**, 495-522.

Deckard, L., R. J. Lambert and R. H. Hageman (1973) Nitrate reductase activity in corn leaves as related to yields of grain and grain protein. *Crop Sci.* **13**, 343-350.

Eilrich, G. L. and R. G. Hageman (1973) Nitrate reductase activity and its relationship to accumulation of vegetative and grain nitrogen in wheat. *Crop Sci.* **13**, 59-66.

Ghosh, M. K., M. K. R. Noamani, C. M. Babu, P. K. Das and R. C. Srivastava (1994) Role of Mg, Zn and Mo salts on *in vivo* nitrate reductase activity in leaves of *Quercus serrta* THUN. *Indian J. Seric.* **33**, 118-121.

Ghosh, M. K. and R. C. Srivastava (1995) Effect of KCl on chlorophyll, total sugars, soluble protein, and *in vivo* nitrate reductase activity in leaves of *Quercus serrata* and correlation studies. *Israel J. Plant Sci.* **43**, 63-68.

Gomez, K. A. and A. A. Gomez (1983) Statistical procedure for agricultural research. 2nd Edn., John Wiley and Sons, New York.

Hageman, R. H. and D. P. Hucklesby (1971) Nitrate reductase from higher plants; in *Methods in Enzymology.* San Pietro, A. (ed.), pp. 491-503, Academic Press, London

Johnson, C. B., W. J. Whittington and G. C. Blackwood (1976) Nitrate reductase as a possible predictive test for crop yield. *Nature* **262**, 133-134.

Karadge, B. A., A. B. Upadhya and R. R. Thrombre (1994) Nitrate reduction and CO_2 incorporation in the developing leaves of plants differing in photosynthetic pathways. *Indian J. Plant Physiol.* **37**, 65-68.

Lowry, O. H., N. J. Rosenbrough, A. L. Farr and R. J. Randall (1951) Protein measurement with Folin-phenol reagent. *J. Biol. Chem.* **193**, 265-275.

Morris, D. I. (1948) Quantitative determination of carbohydrate with drey woods anthrone reagent. *Science* **107**, 254-255.

Nicholas, J. C., J. E. Harper and R. H. Hageman (1976) Nitrate reductase activity in soyabeans (*Glycine max* L. Merr.). *Plant Physiol.* **58**, 731-735.

Paliwal, K. and M. Ilangovan (1990) Factors influencing *in vivo* determinations of nitrate reductase (EC 1.6.6.1) activity in mulberry (*Morua alba* L.). *Sericologia* **30**, 369-379.

Ray, D., L. N. Mondal, A. K. Pain and S. K. Mondal (1973) Effect of NPK and farm yard manure on the yield and nutritive value of mulberry leaf. *Indian J. Seric.* **12**, 7-12.

Rao, D. M. R., A. Sarvesh and T. P. Reddy (1990) Relationship of NR activity to grain protein, grain yield and yield component in some induced SD mutants of rice (*Oryza sativa* L.). *J. Cytol. Genet.* **25**, 43-48.

Rao, D. M. R., M. P. Reddy, B. K. Reddy and N. Suryanarayana (2000) Nitrate reductase (NR) activity and its relationship with protein content, leaf yield and its components in mulberry (*Morus* spp.). *Indian J. Seric.* **39**, 86-88.

Reddy, L. A., R. K. Janardhan and K. Vaidyanath (1985) Nitrate reductase activity in certain induced mutants of *Setaria italica* Beav. *Indian J. Bot.* **8**, 169-173.

Schrader, L. E., and R. H. Hageman (1967) Regulation of nitrate reductase activity in corn seedling by endogenous metabolites. *Plant Physiol.* **42**, 1750-1756.

Singh, V. K., P. Palit and P. P. Yadav (1994) NR activity and its relationship with yield characters in the jute. *Indian J. Plant Physiol.* **37**, 195-197.

Srivastava, H. S. (1980) Regulation of nitrate reductase activity in higher plants. *Phytochemistry* **19**, 725-753.

Zuhua S. (1994) Silkworm nutrition and Physiology; in *Silkworm Physiology.* Junliang, X., L. Shunin, M. Yungen, Z. Chuanxi, W. Xiaofeng, S. Zuhua and Z. Fangyue (eds.), pp. 73-80, Dept. of Sericulture, Zhejiang Agricultural University, China.