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AGE-DEPENDENT CHANGES IN *IN VIVO* NITRATE REDUCTASE ACTIVITY IN BLACK PEPPER (*PIPER NIGRUM* L.)*

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ABSTRACT

Nitrate reductase activity (NRA) was studied in the leaves and berries of black pepper during growth and development. The mature leaves from the runner shoots showed higher NRA per leaf than the young leaves, though the latter showed higher activity on unit weight basis. The enzyme activity in the flag leaf opposite to the spikes bearing the berries and in the berry, during its development was investigated. NRA in the berry increased with the time of development while that in the flag leaf declined. The activity in the leaves of runner shoots was found to be high during the day time and negligible during the night.

INTRODUCTION

Nitrate reductase (EC 1.6.6.1), an important enzyme in the assimilation of nitrate is regulated both by light (Beevers et al. 1965, Sawhney and Naik, 1972, Vijayaraghavan, Sopory and Guha Mukerjee, 1979) and its substrate nitrate (Beevers et al. 1965, Travis, Jordan and Huffaker, 1970). The effect of light on this enzyme appears to be phytochrome-mediated (Rao et al. 1980). Diurnal rhythm in NRA had been reported in some crops (Steer, 1975; Rajagopal, Balasubramanian and Sinha, 1977).

There are several reports in the literature on various aspects of NRA in the annual crops, both cereals and pulses, but to a less extent in plantation crops (Shivashankar and Rajagopal, 1983; Subbaiah and Balasimha, 1983). However the activity of NRA in black pepper has not been investigated. Varietal differences in black pepper exist as far as the dependence of light on yield is concerned. The performance of the hybrid Panniyur 1 is very poor under shaded conditions (Nambiar, 1977). The level of NRA in many crops has been positively correlated with their productivity (Deckard, Lambardt and Hageman, 1973; Oh, Warner and Kleinhofs, 1980). The present paper aims at investigating the activity of the NRA in the leaves and berries of pepper at different stages of maturity.

MATERIALS AND METHODS

Eight-year-old vines of the hybrid black pepper Panniyur 1, which was grown as an intercrop in coconut plot in the Institute Farm, was used for the sampling. The vines were supplied with 100g N, 40g P_2O_5 and 140g K_2O.

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Nitrate reductase activity was assayed by the *in vivo* method of Klepper, Flesher and Hageman (1971). 0.25g of leaf/fruit tissue (berries) $2cm^2$ or $2mm^2$ (in the case of berries) pieces, infiltrated under vacuum with 5.0ml of 0.2M KNO_3 in 1.0mM potassium phosphate buffer at pH 7.5 and incubated at 30°C for 1h in the dark. The blank contained buffer and the plant material. After the incubation period, 1.0 ml aliquotes were taken for nitrite estimation by the method of Evans and Nason (1953).

RESULTS AND DISCUSSION

The fully open young leaf (3–4 weeks old) had a mean area of $32cm^2$ and the slightly mature (10–11 weeks old) leaf had 56.4 cm^2, while the mature (16–17 weeks old) had three times higher area than the young ones (Table I). Fresh and dry weights also exhibited a similar trend among the leaves of different ages.

Nitrate reductase activity in the young leaves of black pepper was higher per unit weight than in the mature leaves (Table I). However, the nitrate reducing potential of the whole leaf is proportional to its total weight. When the enzyme activity per leaf was considered the mature and slightly mature leaves had 2.5 and 2 times the activity of young leaves, respectively. Generally the enzyme activity is expressed on unit weight basis, but when the leaves of different physiological ages are used it is necessary to know the relative contribution of leaves of different maturity to nitrate reducing potential. In the present case, the mature leaves contributed more to the nitrate reducing potential of the plant. In wheat, Grover, Nair and Abrol (1978) reported that of the upper three leaf blades, the flag leaf had significantly higher NRA than the other two leaf blades.

Berries from the spikes of different maturity were collected along with their opposite leaves (flag) from the floral shoots and NRA was estimated. The flag leaf, irrespective of its own stage of maturity, had higher NRA during the early stages of berry development and it decreased with the maturation of the berries (Table II). The activity per unit fresh weight of berries increased from 125 to 213 nmoles with the develop-

Table I. *Nitrate reductase activity in the leaves of different maturity. Values are means of five determinations with three leaves each.* ± *denote S. E. of the mean*

Stage		Leaf area, cm^2	Fresh wt.(g)	Dry wt.(g)	NR activity, nmoles NO^-_2 formed g^{-1}fwt h^{-1}	g^{-1}dwt h^{-1}	$leaf^{-1}$ h^{-1}
Young		32.8	0.75	0.13	1131	6525	848
	±	2.8	0.09	0.01	92	569	74
Slightly mature		56.4	1.44	0.30	1071	5142	1542
	±	4.1	0.11	0.02	104	493	149
Mature		92.5	2.40	0.53	891	4035	2138
	±	8.7	0.36	0.05	85	373	205

Table II. *Nitrate reductase activity in the berries and the flag leaves at different maturity of the former. Values are means of four determinations, each with three sets of leaves and berries. ± denote S. E. of the mean*

Stage	Age of berries, weeks after anthesis	Berry size dia. mm	NR activity, nmoles NO^-_2 formed g^{-1}fwt h^{-1}	
			Flag leaf	Berry
1	Immediately after anthesis	—	495 ±29	—
2	4	2.83 ± 0.13	580 ± 68	125 ± 16
3	8	4.49 ± 0.26	381 ± 24	184 ± 23
4	10	5.03 ± 0.75	300 ± 16	213 ± 21

ment of the berries. In mung bean NRA in the leaves increased during early stages, but during pod-filling the activity declined (Sinha et al., 1978), similar to the situation obtained with pepper. Chatterjee, Pokhriyal and Abrol (1980) have studied the role of flag leaf in grain nitrogen accumulation in barley. A similar role can be attributed to the leaf opposite to the berry spike. Results in Table II suggest that this leaf might play an important role in the translocation of nitrogen to the berries during the early developmental stages of the latter. Also, an increase in the enzyme activity of the berries with a concomittant decline of the same in the leaves is attributable to the greater 'sink' activity of berries and depletion of the reduced nitrogen at the 'source'. Since CO_2 fixation is closely related to nitrogen assimilation (Solomonson and Spehar, 1977), the translocation of photosynthates and reductants from this leaf to berries is very important for the development of the latter.

Leaves from runner shoots were collected at 3h intervals over a period of 24h and NRA estimated. Peak light intensity was between 13 and 14h. With the increase in light intensity during the day, there was a corresponding increase in the enzyme activity, with the maximum of 425 nmloes NO^-_2 g^{-1} fresh weight h^{-1} and the minimum of 48 nmoles NO^-_2 g^{-1} fresh weight h^{-1} at 12 and 18h, respectively (Fig. 1). The activity during the night was low. Diurnal rhythm in NRA was reported in different crops (Steer, 1974; Roth-Bejerano and Lips, 1970; Rajagopal, et al. 1977), including coconut (Shivashankar and Rajagopal, 1983).

Thus the study had indicated the variation in the nitrate-reducing potential of leaves of different maturity and also the relationship between the 'source' and 'sink'.

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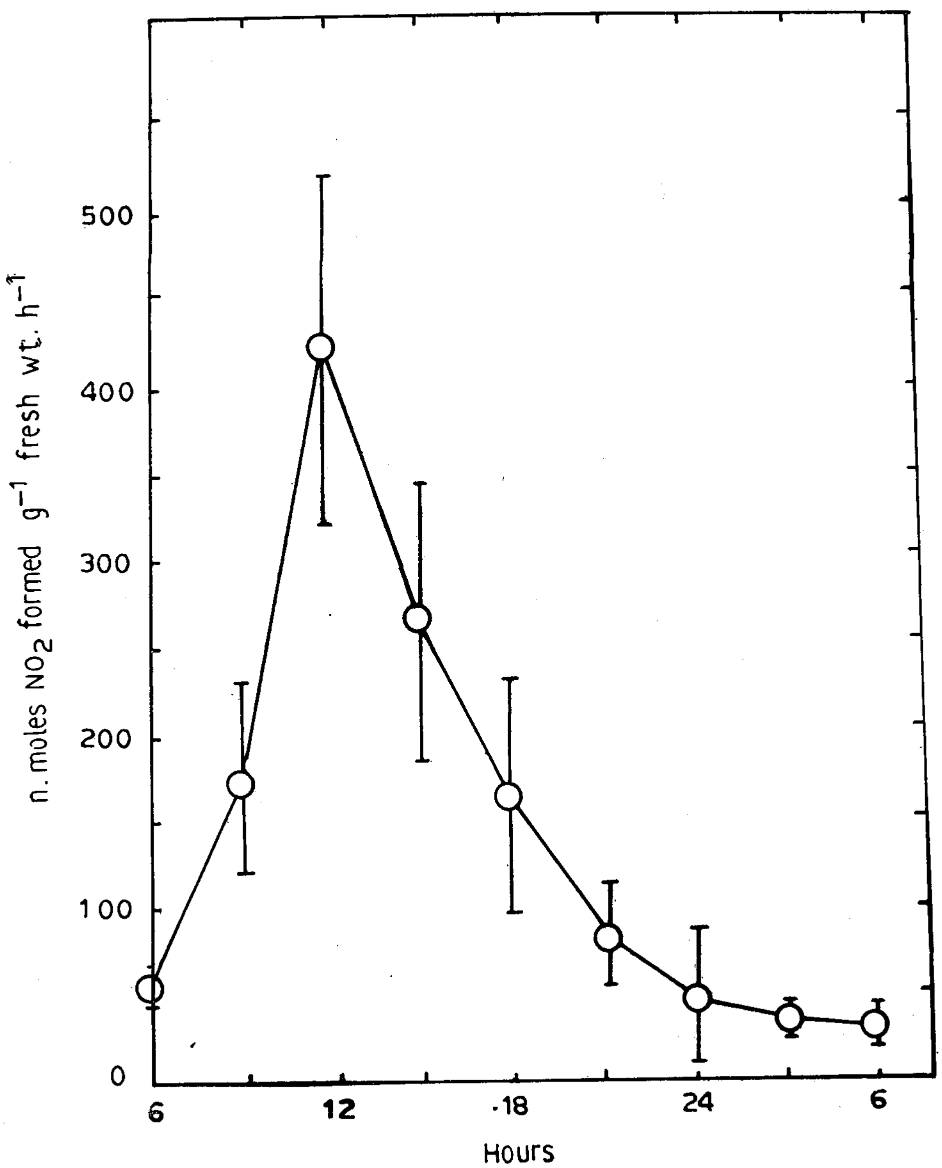
1. Diurnal fluctuations in NR activity during the 24 h cycle. Values are means of two determinations of four leaves each. Vertical bars indicate S. E. of the mean



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