IN VIVO NITRATE REDUCTASE ACTIVITY IN THE LEAVES OF APPARENTLY HEALTHY AND ROOT (WILT) AFFECTED COCONUT PALMS

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ABSTRACT

The *in vivo* nitrate reductase activity (NRA) was studied in apparently healthy and root (wilt) diseased coconut palms. The enzyme activity was more in the leaves of the middle whorl, followed by those of the outer whorl, than either in the spindle leaf or the first fully open leaf. In all the leaves studied, the diseased palms had higher activity than in the apparently healthy palms.

The enzyme activity was influenced by the light intensity. Irrespective of the palm condition the NRA was higher at high light intensity (1250 to 1400μE $m^{-2}s^{-1}$) than that at low light intensity (250 to 500μE $m^{-2}s^{-1}$). Again, the activity of enzyme in apparently healthy palms was less than that in the diseased palms.

With increase in the level of nitrogen application there was increase in NRA both in apparently healthy and diseased palms, the latter recording relatively high activity. The response of palms to simulated stress indicated that the enzyme was adversely affected in both the palms. The study thus revealed disturbed nitrogen metabolism caused by the root (wilt) disease.

INTRODUCTION

Several physiological and biochemical changes have been observed in the root (wilt) affected coconut palms. The leaves of

diseased palms had a higher respiration rate than those of apparently healthy palms (Michael, 1978). The content of sugars was relatively high in the diseased palms (Chacko Mathew, 1977). As compared to healthy palms, the diseased palms showed variations in different fractions of proteins (Padmaja et at., 1981. However, detailed investigation on the nitrogen assimilation is lacking. The first rate limiting enzyme in nitrogen assimilation is nitrate reductase (NR), which catalyses the reaction between nitrate and nitrite. Nitrate reductase activity is inducible both by light and the substrate (Beevers et al., 1965; Travis et al., 1970; Sawhney and Naik, 1972; Vijayaraghavan et al., 1979). Though some aspects of NRA had been studied in coconut (Shivasankar and Rajagopal, 1983; Shivasankar and Ramadasan, 1983), the activity of the enzyme in relation to the root (wilt) disease has not been investigated earlier. The present paper reports certain aspects of the enzyme activity in the apparently healthy and diseased palms.

MATERIALS AND METHODS

Coconut palms (*Cocos nucifern* L. var. West Coast Tall) were grown in the Institute Farm with the usual cultural and agronomic practices. The palms were supplied with the recommended doses of N : P : K (500 : 320 : 1200 g/palm/year). In one of the experiments, palms grown with a higher level of nitrogen (750g) were also studied. Palms of uniform age (15 to 20 years) were selected in the two categories, namely apparently healthy (disease index less than 10 per cent and middle diseased palms (disease index 25 to 35 per cent).

Three experiments were designed, so that the first one was concerned with the determination of NR activity in the leaves of different maturity. In the second experiment the influence of light intensity on NRA was studied. Determination of enzyme activity in palms with two levels of nitrogen supply formed the third experiment. In addition, the effect of simulated stress (with PEG, MW 6000, 20 per cent) on the activity of NR both in apparently healthy and diseased palms was investigated.

The *in vivo* nitrate reductase was assayed in accordance with the method described by Klepper et al. (1971) and nitrite estimation by Evans and Nason (1953). In the preliminary experi-

ments, the quantity of leaf material, the substrate concentration required for the optimum activity, the time of incubation and the aliquot used for colour development were standardised (data not presented now). Based on this, all the assays were carried out. Details pertaining to each experiment are described in Tables 1, 2 and 3.

RESULTS AND DISCUSSION

The activity of NR was higher in the middle leaves than in other leaves irrespective of the palm condition (Table 1). Shivasankar and Ramadasan (1983) also observed increase in NR activity with maturity up to 14 leaves followed by a decline. Since the leaf water content differs between the apparently healthy and diseased palms, the enzyme activity is also expressed on unit dry weight basis. The trend in NRA remained the same on both unit fresh and dry weight bases. There was relatively high activity both in young and mature leaves of diseased palms as compared to healthy palms. For instance, the spindle leaf of diseased palms had 88.7 per cent higher NRA than that in the apparently healthy palm (dry weight basis).

Table 1. NR activity in the leaves of different whorls of apparently healthy and root (wilt) affected coconut palms. Values are mean of six palms

Leaf position	NRA—n. moles g^{-1} fwt h^{-1}		NRA—n. moles g^{-1} dwt h^{-1}	
	App. Healthy	Diseased	App. Healthy	Diseased
Spindle	52.5	60.2	214.6	405.1
First whorl	58.3	81.2	168.9	260.0
Middle whorl	123.7	126.8	323.3	332.6
Outer whorl	73.1	101.7	185.3	235.7

The enzyme activity was determined during 'cloudy' (250 to 500μE $m^{-2}s^{-1}$) and 'bright' (1250 to 1400μE $m^{-2}s^{-1}$) days (Table 2). Irrespective of the palm condition and leaf position, the NRA was less under low than under high light intensity. Under both light intensities, diseased palms had higher enzyme activity (59.1

per cent and 46.9 per cent, and 86 per cent and 6 per cent in the first and middle leaves respectively) than the apparently healthy palms. Variations in NR activity in coconut between the 'summer' and 'wet' seasons were also observed (unpublished). The influence of light on NR activity is well established in many crops (Beevers et al., 1965; Sawhney and Naik, 1972). NRA had also exhibited diurnal and seasonal fluctuations in different species (Rajagopal et al., 1977; Steer, 1974; Shivashankar and Ramadasan, 1983). These indicate the relationship between the enzyme activity and photosynthesis. Maximum activity of NR in coconut leaves treated with nitrate exposed to light was reported recently (Shivashankar and Ramadasan, 1983).

Table 2. Effect of light on the NR activity in the leaves of apparently healthy and diseased palms. The samples were collected on a 'cloudy' and 'bright' day and the light measurement was determined with a steady state porometer attached with a light sensor between 930 and 1130 AM. Values are means of six palms

Light, $Em^{-2}s^{-1}$	Leaf position	NRA – n. moles g^{-1} dwt h^{-1}	
		App. Healthy	Diseased
250–500	First whorl	63.5	101.0
	Middle whorl	59.2	110.1
1250–1400	First whorl	439.0	645.0
	Middle whorl	297.0	383.0

The response of the enzyme to nitrogen level was determined both in the apparently healthy and diseased palms (Table 3). An increase in nitrogen supply from the normal level of 500 g to 750 g per palm resulted in enhanced enzyme activity in young and mature leaves of both apparently healthy and diseased palms. A two-fold increase in the enzyme activity was observed in both the leaves of diseased and in the matured leaves of apparently healthy palms, while the increase was over three-fold in the first leaves of apparently healthy palms. Enhanced activity with a high level of nitrogen supply could be attributed to substrate induction, as NR is a substrate inducible enzyme (Beevers et al., 1965).

As observed in the previous cases, diseased palms had always higher enzyme activity than the healthy palms. Influence of different levels of nitrogen supply on the NRA of sorghum varieties was reported (Rajagopal et al., 1976), which showed that there are genotypic variations to nitrogen response. While some genotypes exhibited increase in NR activity with increase in nitrogen supply from 50 to 200 kg ha^{-1}, other genotypes had the opposite trend.

Table 3. Effect of nitrogen levels on the NR activity in the leaves of apparently healthy and diseased palms. Values are means of six palms

N. levels, g/palm	Leaf position	NRA—n. moles g^{-1} dwt h^{-1}	
		App. Healthy	Diseased
N1, 500	First whorl	190.8	463.7
	Middle whorl	342.1	375.6
N2, 750	First whorl	675.9	851.9
	Middle whorl	602.7	775.3

The consistent observation of relatively high NRA in the leaves of diseased palms as compared to healthy palms shows that at least the first step in nitrogen assimilation is not adversely affected as a result of root (wilt) disease. This might indicate that the nitrate supply from the roots to leaves is maintained at a high level in the diseased palms. This perhaps is possible through a high transpiration stream, which is characteristic of root (wilt) affected palms (Rajagopal et al., 1984). Though the nitrate reduction step in diseased palms is above the normal level of apparently healthy palms, there is variation in different protein fractions (Padmaja et al., 1981). Increase in non-protein nitrogen content with a concomittant fall in water-soluble nitrogen and protein nitrogen in the leaves of diseased palms was reported (Varkey et al., 1969). Pillai and Shanta (1965) reported accumulation of acidic amino acids and their amides in the leaves of diseased palms.

Significantly high levels of sugars in the diseased levels (Chacko Mathew, 1977) could also have caused higher NR activity.

Recently, Shivashankar and Rajagopal (1983) showed the activity of NR during different times of the day following the same trend as that of the total sugar level. This suggests a possible role of sugars in the regulation of NRA. Thus, an above-normal supply of nitrate and high level of sugars might have favoured relatively high activity in the leaves of diseased palms.

Moisture stress adversely affected the enzyme activity in the leaves of both apparently healthy and diseased palms (Table 4). It is interesting to note that though the diseased palms had maintained higher NRA under normal conditions, imposition of stress resulted in greater loss of activity of NR in the diseased palms. (64 per cent reduction on 'control') than in the apparently healthy palms (30 per cent reduction on 'control'). This indicates the poor stability of the enzyme in the diseased compared to apparently healthy palms. NR activity has been shown to be highly sensitive to moisture and salt stress in different crops (Balasubramanian et al., 1974; Sinha and Rajagopal, 1975, 1977).

Table 4. Effect of simulated stress on the NR activity in the middle leaves of apparently healthy and diseased palms. The leaflets from the middle whorl were excised and kept in half the strength of Hoagland's nutrient solution or 20 per cent polyethyleneglycol, 6,000 prepared in nutrient solution for 24 hours in the laboratory near the window under diffused light. At the end of the treatment, NR activity was determined both in the control and stressed leaflets. Representative samples were kept for dry weight. Values are mean of five palms

Treatment	NRA – n. moles g^{-1} dwt h^{-1}	
	App. Healthy	Diseased
Control	143.4	183.0
Stressed	100.2	61.1
Percentage of control	69.9	35.7

It is evident from the foregoing discussion that the root (wilt) disease greatly affects the nitrogen metabolism of coconut palms. Though the reduction of nitrate is maintained at a high level,

further steps in the nitrogen assimilation appears to have been greatly hindered in the diseased palms, as exemplified by accumulation of certain amino acids (Pillai and Shanta, 1965) and by large variations in protein fractions (Padmaja et al., 1981). This would mean that other enzyme systems involved in the protein synthesis are probably affected by the disease. Thus, the overall effect of root (wilt) disease is reflected on the disturbed nitrogen metabolism.

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