Diurnal Rhythm in Nitrate Reductase Activity of *Cocos nucifera* L. Leaves

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Summary

Nitrate reductase (NR) activity in the leaves of *Cocos nucifera* L. exhibited a strong diurnal rhythm with a peak at 2 p.m. This rhythm was maintained under permanent dark or light conditions indicating the endogenous control of the rhythm. The NR activity was independent of tissue nitrate level. The study suggested a possible regulatory role of sugars on leaf NR activity.

Key words: Cocos nucifera L., nitrate reductase diurnal rhythm.

Introduction

Nitrate reductase (EC 1.6.6.1 NADH: nitrate oxido reductase) is known to be induced by light (Hageman and Flesher, 1960; Sawhney and Naik, 1972), nitrate (Beevers and Hageman, 1969), and hormones (Dilworth and Kende, 1974; Kende et al., 1971). With nitrate nutrition, the mode of regulation of this enzyme is of vital importance, as it determines the amount of reduced nitrogen available for the plant. Hageman et al. (1961) were the first to report diurnal fluctuations of NR activity in corn. Subsequently similar reports on the rhythm of NR have been made in wheat (Upcroft and Done, 1972), Lolium (Bowerman and Goodman, 1971), soybean (Harper and Hageman, 1972), rice (Shibata et al., 1969), *Chenopodium* (Cohen and Cumming, 1974) and a few hydrophytes like *Lemna* (Lakshmi Devi and Maheshwari, 1979) and *Wolffia* (Inderjeet Singh Bakshi et al., 1979). To our knowledge, such a study has been made in only one perennial crop (Subbiah, 1982).

Our earlier studies indicated that the *in vivo* NR activity measured in coconut leaves showed wide fluctuations during the day though it was not very much different when measured at the same time on different days (unpublished). This finding interested us to study the diurnal variations in NR activity. In the present paper we

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Abbreviation: NR = Nitrate reductase.

report the existence of diurnal rhythm in NR and the role of nitrate and reducing sugars in the regulation of this enzyme.

Material and Methods

Two-year old coconut seedlings (*Cocos nucifera* L. Cv. West Coast Tall) grown in pots filled with red sandy loam soil were used in this study. The seedlings were supplied with a uniform dose of N, P and K (100 g each of urea, superphosphate and muriate of potash) one week prior to the beginning of the experiment and were irrigated daily. All the experimental seedlings had 4–6 fully developed leaves.

Plants were exposed to three different light/dark treatments:

1. Continuous light conditions in which the seedlings were given light between 6 p.m. and 6 a.m. from four 160 W lamps (MLL-B/53 Philips, India) kept at a height of 3 meters above the ground.
2. Continuous dark conditions in which the seedlings were maintained in a dark room at 25±2 °C.
3. Reversed schedule of light and dark conditions: Light was provided during the dark period (between 6 p.m. and 6 a.m.) and were transferred to darkness during the day (6 a.m. to 6 p.m.)

Plants growing in normal photoperiod (12 hrs light and 12 hrs dark) conditions served as experimental control.

Leaf sampling for the assay was done at 2 h intervals from the youngest fully opened leaf. The time between sampling and assay was generally less than 30 min.

Nitrate reductase was assayed *in vivo* according to Jaworski (1971) with some modifications.

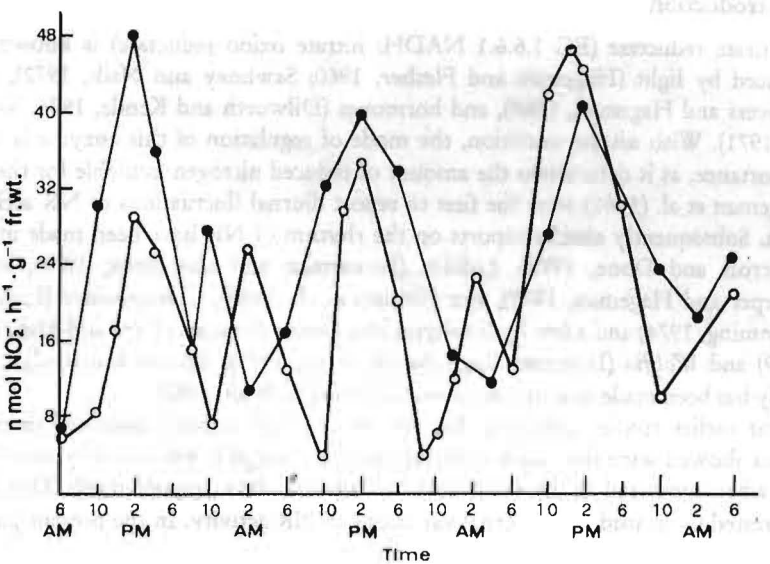

Fig. 1: Diurnal variations in NR activity under normal days of 12 h light and 12 h darkness (—●—) and in continuous light (—○—).

The assay medium contained in a final volume of 5.0 ml, 1.0 mM phosphate, 0.8 mM KNO_3 and 0.5 mg of diethyl dithiocarbamate (Subbiah and Shivashankar, unpublished). The nitrite produced at the end of 1 hour was estimated colorimetrically, (Snell and Snell, 1949).

Tissue nitrate was extracted in phosphate buffer and assayed by the method of Onken and Sunderman (1977). Reducing sugars were estimated according to Somogyi (1952).

Results and Discussion

The NR activity increased rapidly from 6 a.m. to 2 p.m. and thereafter declined continuously (Fig. 1). The peak activity at 2 p.m. was 4–6 times higher than that obtained in the dark period. The appearance of the enzyme peak at 2 p.m. was

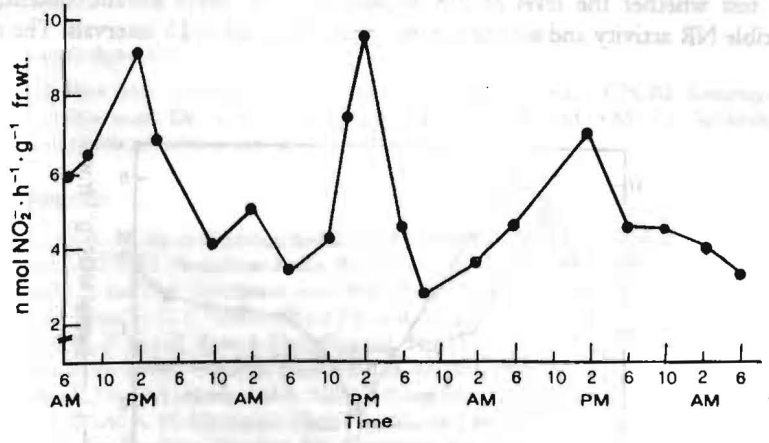

Fig. 2: Rhythm in NR activity under continuous dark conditions.

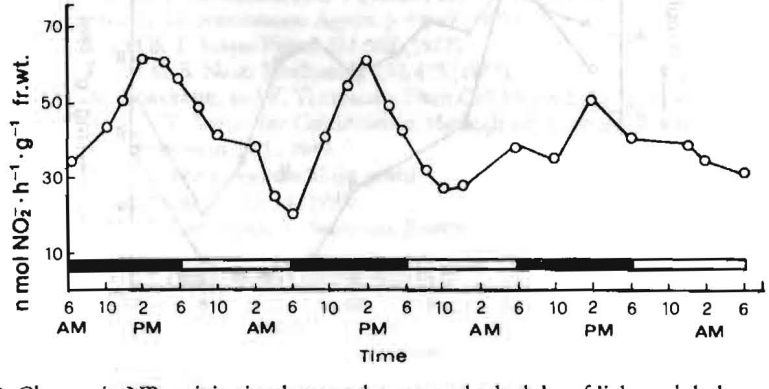

Fig. 3: Changes in NR activity in plants under reversed schedules of light and darkness. Plants were exposed to light from 6 p.m. to 6 a.m. and to darkness from 6 a.m. to 6 p.m.

consistent in a 72 h experimental period. These observations show that light has a strong stimulatory effect on NR activity.

The pattern of NR activity was then tested in plants growing under continuous light or dark conditions. The peak continued to appear at 2 p.m. irrespective of the treatment (Figs. 1 and 2) though with decreased amplitude in continuous darkness. The activity profile under reversed schedule of light and dark conditions is presented in Fig. 3 where too, the peak activity was at 2 p.m.

The above observations indicate that there is an endogenous rhythm in NR activity of coconut leaves. It may be noted here that specific leaf weight and starch content in coconut leaf were also reported to be at their maximum at 3 p.m. by Kasturi Bai et al. (1981).

To test whether the level of NR is controlled by tissue nitrate content, both inducible NR activity and nitrate content were estimated at 2 h intervals. The nitrate

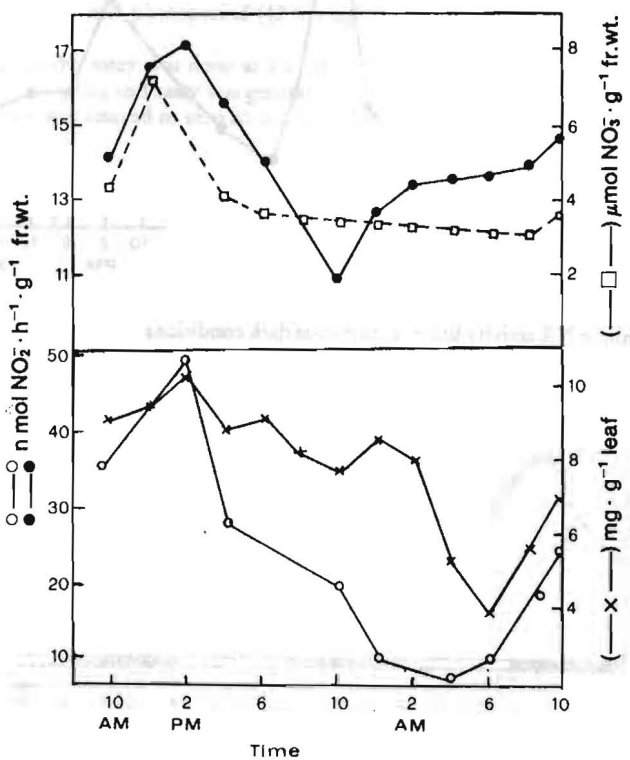

Fig. 4: Relationship of NR activity with levels of nitrate (□ --- □) and reducing sugars (× --- ×) in a 24 h cycle. --- ○ --- Inducible NR activity, --- ● --- Endogenous NR activity.

level increased from 6 a.m. to noon followed by a gradual decline thereafter till 8 a.m. the next morning. Both induced and endogenous activities (Fig. 4) did not show any relationship with tissue nitrate level. Similar results were reported by Pearson and Steer (1977) and Bakshi et al. (1979).

In a study conducted earlier, we observed that the addition of glucose to the medium enhanced the activity of NR of dark-treated leaves (unpublished). Udayakumar et al. (1981) reported enhancement of NR activity in dark-treated *Helianthus annuus* plants by the addition of glucose. These findings prompted us to study the level of sugars in the leaf tissue in relation to NR activity. As shown in Fig. 4 the content of total sugar followed a trend similar to that of NR activity during the experimental period with a peak at 2 p.m. which suggests a possible role of sugars in the regulation of NR activity.

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References

Bakshi, I. S., A. H. Abad Farooqi, and S. C. Maheshwari: Plant Cell Physiol. *20*, 957 (1979).
Beevers, L. and R. H. Hageman: Annu. Rev. Plant Physiol. *20*, 495 (1969).
Bowerman, A. and P. J. Goodman: Ann. Bot. *35*, *353 (1971)*.
Cohen, A. S. and B. G. Cumming: Plant Physiol. *52*, 2351 (1974).
Dilworth, M. F. and H. Kende: Plant Physiol. *54*, 821 (1974).
Hageman, R. H. and D. Flesher: Plant Physiol. *35*, 700 (1960).
Hageman, R. H., D. Flesher, and A. Gitter: Crop Sci. *I*, 20 (1961).
Harper, J. E. and R. H. Hageman: Plant Physiol. *49*, 146 (1972).
Jaworski, E. G.: Biochem. Biophys. Res. Commun. *43*, 1274 (1971).
Kende, H., Heinz Hahn, and S. K. Kays: Plant Physiol. *48*, 702 (1971).
Kasturi Bai, K. V., A. Ramadasan, and K. V. Satheesan: J. Indian bot. Soc. *60*, 352 (1981).
Lakshmi Devi, S. and S. C. Maheshwari: Physiol. Plant *45*, 467 (1979).
Onken, A. B. and H. D. Sunderman: Agron. J. *69*, 49 (1977).
Pearson, C. T. and B. T. Steer: Planta *137*, 107 (1977).
Sawhney, S. K. and M. S. Naik: Biochem. J. *130*, 475 (1972).
Shibata, M., M. Kobayashi, and E. Takahashi: Plant Cell Physiol. *10*, 337 (1969).
Snell, F. D. and C. T. Snell: In: Colorimetric Methods of Analysis. Vol. II pp 804. D. Van Nostrand Co., Princeton, N.J., 1949.
Subbiah, C. C.: Proc. Treephysindia 82 (in press).
Somogyi, M.: J. Biol. Chem. *195*, 19 (1952).
Udayakumar, M., R. Devendra, V. Srinivasa Reddy, and K. S. Krishna Sastry: New Phytol. *88*, 289 (1981).
Upcroft, J. A. and J. Done: FEBS Lett. *21*, 142 (1972).