Bot. Bull. Academia Sinica (1989) **30:** 285-289

Seasonal changes in the nitrogenase activity and other metabolic parameters of cycad coralloid roots

J. Marshall, N. Grobbelaar* and S. James

Department of Botany, University of Pretoria, Pretoria, 0002, Republic of South Africa

(*Received August 17, 1989; Accepted August 21, 1989*)

Abstract. The nitrogenase activity and respiration rate of Cyanobacteria-infected *Encephalartos* coralloid roots from garden plants of the Pretoria area were considerably higher in summer (November to February) than in the remainder of the year when monitored under constant conditions. The higher activity coincided with a higher sugar content of the roots although the sugar content of the roots was also high during April and May without a concomitant rise in the nitrogenase or respiratory activity. The respiratory quotient, water content and chlorophyll content of the roots did not vary considerably or in a meaningful way during the 16 months experimental period.

Key words: Coralloid roots; Cyanobacteria; Cycad; Nitrogenase; Seasonal variation; Symbiosis.

Introduction

In studying the nitrogenase activity of cyanobacteria-infected coralloid roots of some *Encephalartos* species growing in the botanic garden of the University of Pretoria, it appeared that the activity was much lower in winter than in summer. Halliday and Pate (1976) found that the nitrogenase activity of the coralloid roots of *Macrozamia riedlei* growing in a winter rainfall area was higher in winter than summer although the summer temperatures of the region corresponded better to the optimal temperature of the roots' nitrogenase activity. They concluded that for *M. riedlei,* water availability has a stronger regulating effect on nitrogenase activity than temperature.

Although Pretoria is situated in a summer rainfall area with dry winters, the plants in the botanic garden of the University are watered regularly during dry spells. The present study was therefore undertaken to determine the nitrogenase activity and certain other parameters of the metabolic activity of coralloid roots at regular intervals over a period of 16 months in an attempt to ascertain whether pronounced seasonal changes do occur.

In earlier unpublished studies it also appeared that coralloid roots lost all nitrogenase activity when homogenised. Mixing such a homogenate with whole, active coralloid roots depressed the nitrogenase activity of the roots significantly. At other times of the year the inhibitory effect of the homogenate appeared to be much smaller. Therefore it was decided to investigate this phenomenon over a period of 16 months as well.

Materials and Methods

To ensure that sufficient and comparable material would be available for the duration of the experiment, ten plants with large clusters of cyanobacteria-infected coralloid roots were selected from those growing in the National Botanic Gardens of Pretoria and the Botanic Garden of the University of Pretoria.

The plants chosen were four individuals of *Encephalartos altensteinii* Lehm., one individual that is con-

*To whom correspondence should be addressed.

sidered to be a natural hybrid between *E. villosus* Lem. and *E. altensteinii* Lehm. and one individual each of *E. arenarius* R.A. Dyer, *E. laevifolius* Stapf and Burtt Davy, *E. lehmannii* Lehm., *E. transvenosus* Stapf and Burtt Davy and *E. villosus* Lem..

About 45 g fresh coralloid roots were collected at roughly monthly intervals over a period of 16 months. Root samples were collected from the same root clusters on the 10 plants every time in a way that caused minimal disturbance to the remainder of the root clusters.

The roots were scrubbed free of soil with a soft brush under running tap water and cut transversely into pieces 0.5~1.0 cm long. The root pieces were washed in 5 changes of distilled water to minimise possible wound effects and blotted dry with paper towels. Separate samples were used for the assay of nitrogenase activity, respiratory activity, chlorophyll *a* content, water content and sugar content.

Nitrogenase activity was determined by means of the acetylene reduction technique (Dilworth, 1966; Hardy *et al.,* 1968) as outlined by Grobbelaar *et al.* (1986) except that the assays took place at 27°C. In an additional treatment the root segments were thoroughly mixed with a root homogenate immediately before determining their nitrogenase activity. The homogenate was obtained by grinding 1 g root material to a paste with a mortar and pestle in 1 ml of water.

The chlorophyll *a* content of the cyanobacteria in the coralloid roots was determined by grinding approximately 3 g roots together with 10 ml methanol and a small amount of acid washed sand with a mortar and pestle in dim light. The suspension was filtered by gravity through Whatman No. 1 filter paper and the residue washed 3 times with 5 ml methanol. The combined filtrates were made to 50 ml with methanol and the optical density of the solution measured immediately at 663 nm. The chlorophyll *a* content of the roots was calculated according to Mackinney (1941).

The rate of oxygen uptake and net gas exchange was determined with a Gilson apparatus at 27°C. Six flasks, each containing about 1 g of roots, were used at a time. KOH (0.2 ml of a 10% m/v solution) was used in the centre well of the 3 flasks which were used to determine the oxygen uptake. The results of the other flasks were used to determine net gaseous exchange and the respiratory quotient was calculated from the two sets of results.

The water content of the roots was determined after drying at 80°C to constant mass.

The reducing sugar content of the roots was determined according to the method of Bernfeld (1955). Approximately 4~5 g roots was homogenised in a little water with a mortar and pestle. The homogenate was filtered through nylon gauze, the fibres washed once with about 5 ml water and centrifuged for 5 min at 3,500 × g. The supernatant was made up to 100 ml with water and 1 ml of the solution was used in triplicate to determine the sugar content which was expressed in terms of glucose.

Results

Acetylene Reduction

In no case could ethylene production be detected in the absence of acetylene. In the presence of acetylene, ethylene was produced during every trial (Fig. 1). The presence of the homogenate reduced the rate of acetylene reduction of the roots throughout. The highest nitrogenase activity was obtained during summer, in the presence and absence of the homogenate. Much lower rates were obtained during the winter.

Respiratory Activity

The rate of oxygen uptake and carbon dioxide release by the coralloid roots during the experimental period is illustrated in Fig. 2. The respiration rate was generally much higher during summer than winter despite the fact that the measurements were made at 27°C at all times. The respiratory quotient varied between 0.96 and 1.17 with a mean value of 1.08.

*Chlorophyll **a** Content*

The chlorophyll *a* content of the roots varied from 0.13 to 0.27 mg per g fresh mass with a mean value of 0.19 (Table 1). The variations do not appear to follow a seasonal pattern because high and low values were recorded for the same month in consecutive years.

Water Content

The water content ranged from 80.37% to 85.37% with a mean value of 83.21% (Table 1). The variations do not appear to follow a seasonal pattern.

Sugar Content

The sugar content varied from 3.29×10^{-5} to

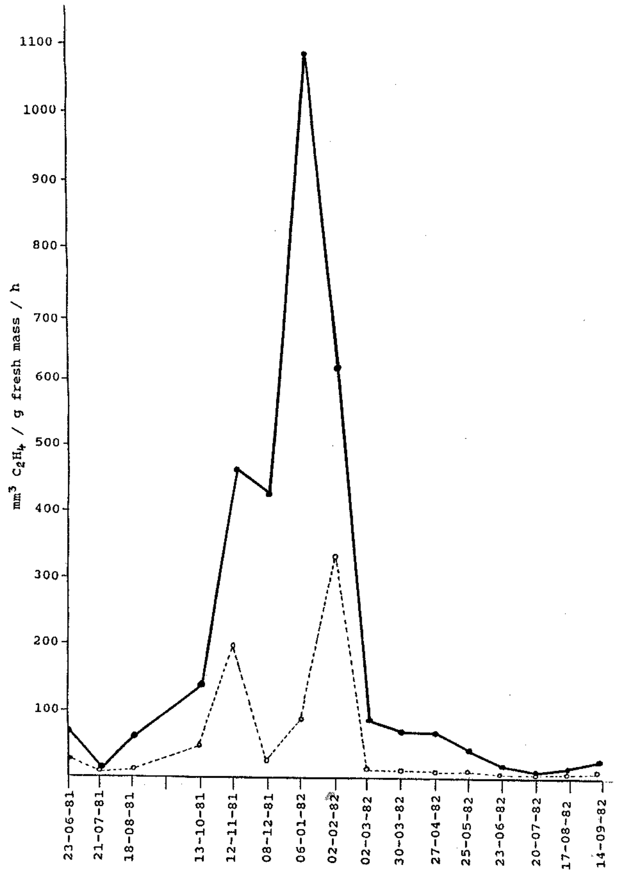

Fig. 1. Acetylene reduction rate of cycad coralloid roots from 1981-06-23 to 1982-09-14 in Pretoria. ●—● roots; ○···○ roots mixed with root homogenate.

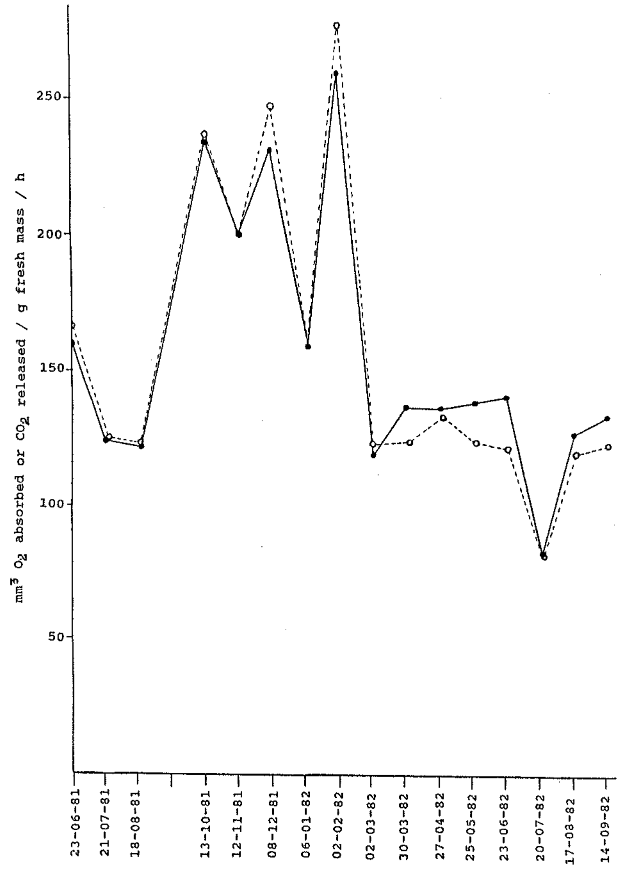

Fig. 2. Oxygen uptake (●—●) and carbon dioxide evolution (○ ··· ○) by cycad coralloid roots from 1981-06-23 to 1982-09-14 in Pretoria.

8.51×10^{-5} mol glucose per g fresh coralloid roots with a mean value of 5.38×10^{-5} (Fig. 3). The sugar content during the late winter and early spring of both years studied were relatively low. This pattern could therefore be a regular feature of the roots' metabolism.

During the summer (November to February) the sugar content was high. Although the sugar content also reached a low value during March, the values for April and May were high.

Discussion

Halliday and Pate (1976) studied the symbiotic nitrogen fixation of the coralloid roots of *Macrozamia riedlei* in Perth, Australia. They determined the rate of acetylene reduction monthly for just over a year and found that the nitrogenase activity was considerably higher during the cooler, moist winter months than during the warmer, drier summer months. They also examined the influence of temperatures between 0°C and 50°C on the nitrogenase activity of the coralloid roots. A broad temperature optimum ranging from 15°C to 30°C was found. Because the ambient temperature during winter falls outside this range, they concluded that the higher nitrogenase activity during winter is mainly due to the greater availability of water during that season and that temperature plays a lesser role than water availability in regulating the rate of nitrogen fixation by the coralloid roots.

The material used in the present study was however all obtained from well watered gardens. Conse-

Table 1. *RQ-values, chlorophyll* ***a*** *content and water content of cycad coralloid roots over a period of 16 months in Pretoria*

Date	RQ values	mg Chl *a* per g fresh mass	Water content (%)
81/06/23	1.14	0.26	80.6
81/07/21	1.10	0.19	82.0
81/08/18	1.11	0.17	80.3
81/10/13	1.14	0.22	82.0
81/11/12	1.10	0.22	80.2
81/12/08	1.17	0.18	85.2
82/01/06	1.10	0.13	84.6
82/02/02	1.17	0.15	85.3
82/03/02	1.13	0.17	83.8
82/03/30	1.00	0.15	83.3
82/04/27	1.08	0.18	84.1
82/05/25	0.98	0.15	84.5
82/06/23	0.96	0.16	83.5
82/07/20	1.10	0.27	83.5
82/08/17	1.03	0.26	85.4
82/09/14	1.01	0.21	83.1
Mean	1.08	0.19	83.2

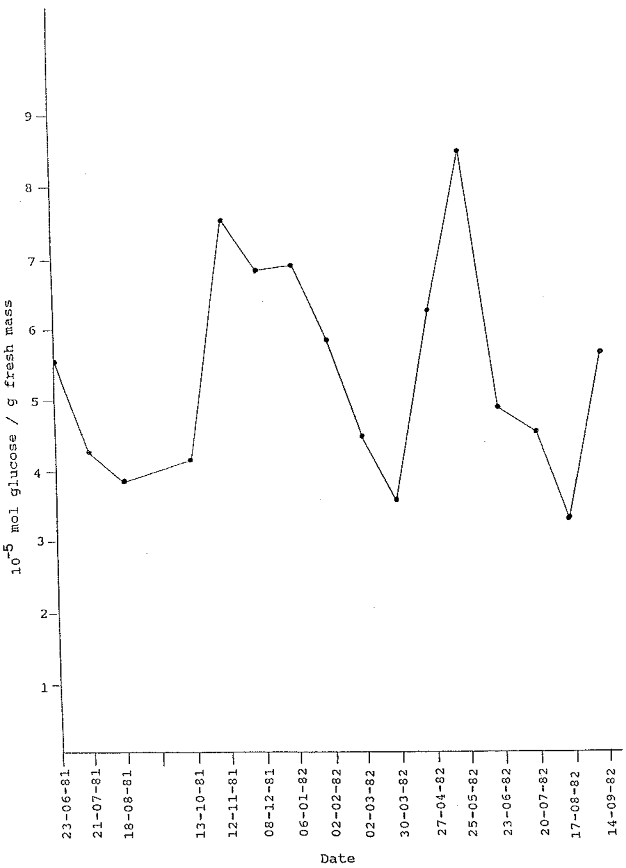

Fig. 3. Reducing sugar content of cycad coralloid roots from 1981-06-23 to 1982-09-14 in Pretoria expressed in mole glucose per g fresh corraloid roots.

quently it is not surprising that the water content of the roots did not vary significantly during the experimental period. It would therefore seem that water availability could not have had an important regulating function in the present study. Although the assays were always done at 27°C, the ambient air temperature differs considerably between winter and summer. The mean temperature at the University of Pretoria's Experimental Farm which is midway between the two gardens from which the experimental material was gathered, is 21.5°C during November to February and 12.8°C for May to August (Anonymous, 1988). Under natural conditions the difference between the summer and winter rates of nitrogen fixation as well as respiration therefore probably is considerably greater than was obtained in the present study.

The higher nitrogenase activity and respiration rate observed in the summer probably resulted from a greater availability of photosynthate in the coralloid roots during November to February. A similarly high sugar content in the roots during April and May however did not coincide with a peak in the nitrogenase activity or respiration rate. An increase in the sugar content of the coralloid roots therefore apparently do not automatically result in an increase in the roots' respiration rate and/or nitrogenase activity.

Because acetylene reduction is an energy consuming process, an increased nitrogenase activity is probably only possible when there is a concomitant increase in the roots' respiration rate. The higher respiration rate could have been made possible by the increased sugar content of the roots which in turn probably resulted from a higher photosynthetic rate of the cycad in summer when the days are longer and the light intensity and temperature are higher than in winter.

The homogenate reduced the rate of acetylene reduction throughout the experimental period. It is possible that the homogenate contained an inhibitor of nitrogenase activity. On the other hand, it is possible that by forming a slimy layer around the root segments, the homogenate might have inhibited nitrogenase activity by impeding the root's gas exchange.

A seasonal pattern do not appear to exist in the variation of the chlorophyll *a* content of the coralloid roots. In as far as the chlorophyll *a* content can be used as a criterion of the cyanobiont content of the roots, it would therefore appear that the cyanobiont content of the roots did not vary significantly with the seasons.

Acknowledgements. The financial assistance by the Foundation of Research Development and the University of Pretoria to one of us (N.G.) is gratefully acknowledged.

Literature Cited

Anonymous. 1988. Climate of South Africa. Government Printer, Pretoria, p. 229.

Bernfeld, P. 1955. Amylases, α and β. *In* S.P. Colowick and N.O. Kaplan (eds.), Metods of enzymology, vol. 1. Academic Press, New York.

Dilworth, M. J. 1966. Acetylene reduction by nitrogen-fixing preparations from *Clostridium pasteurianum*. Biochim. Biophys. Acta **127:** 285-294.

Grobbelaar, N., W. Hattingh, and J. Marshall. 1986. The occurrence of coralloid roots on the South African species of the Cycadales and their ability to fix nitrogen symbiotically. S. Afr. J. Bot. **52:** 467-471.

Halliday, J. and J. S. Pate. 1976. Symbiotic nitrogen fixation by coralloid roots of the cycad *Macrozamia riedlei*: Physiological characteristics and ecological significance. Aust. J. Plant Physiol. **3:** 349-358.

Hardy, R. W. F., R. D. Holsten, E. K. Jackson, and R. C. Burns. 1968. The acetylene-ethylene assay for N fixation: Laboratory and field evaluation. Plant Physiol. **43:** 1185-1207.

Mackinney, G. 1941. Absorption of light by chlorophyll solutions. J. Biol. Chem. **140:** 315-322.

鐵樹根瘤固氮活性與其他代謝作用之季節變化

J. Marshall, N. Grobbelaar and S. James

Department of Botany, University of Pretoria, Pretoria, 0002
Republic of South Africa

南非 Pretoria 地區庭園所栽植之鐵樹 *Encephalartos*，其與藍綠藻共生之根瘤的固氮活性與呼吸速率在夏季(11 月～12 月)遠高於其他季節。固氮活性強弱與根部含糖量高低大體相一致，只是 4～5 月根部含糖量仍高，但固氮活性却沒有相伴地上升。根部之呼吸商，含水量與葉綠素含量在連續 16 月的實驗中並無明顯變化。