PHOTOSYNTHETICA 34 (1): 147-149, 1997

BRIEF COMMUNICATION

Relationship between fern development and CAM in *Pyrrosia piloselloides* (L.) Price

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Abstract

Pyrrosia piloselloides (L.) Price is a constitutive CAM plant in the sporophytic phase of its life-cycle. Newly developed sporophytes, still attached to the gametophytes, showed signs of CAM expression in terms of diurnal changes in titratable acidity of the tissues. The gametophytes did not exhibit CAM.

Key words: Crassulacean acid metabolism; gametophytes; sporophytes.

The life-cycle of ferns consists of two stages - the gametophytic and the sporophytic one. Although both stages are autotrophic, the very young sporophyte is still dependent on the gametophyte. The fronds of the sporophytic stage of the epiphytic fern, *Pyrrosia piloselloides* (L.) Price, exhibit the CAM-mode of photosynthesis (Ong *et al.* 1986); the capacity to fix CO_2 nocturnally increases with the age of the fronds (Ong 1993). The sporophyte is a constitutive CAM plant (Ong *et al.* 1986). However, it is not known whether the gametophytes and the newly formed sporophytes of *P. piloselloides* exhibit CAM or not.

Spores were collected from naturally growing populations of *P. piloselloides* and surface-sterilized with 5 % *Clorox*™ for 3 min. The spores were then transferred to 10 cm^3 autoclaved 1/10 strength Hoagland's solution (Tuite 1969) in a Petri-dish (9 cm diameter). The gametophytes were then allowed to grow in 1/10 strength Hoagland's solution. When approximately 80 % of the surface area of each Petri-dish were covered by gametophytes, subculturing was conducted to avoid overcrowding. All cultures were kept under an irradiance of 40 $\mu mol\ m^{-2}\ s^{-1}$ (12 h light/12 h dark) at 25±2 °C.

Received 10 January 1996, *accepted* 4 March 1996.
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Acknowledgement: This study was supported by a research grant awarded by The National University of Singapore (grant RP 870338).

To test whether CAM was exhibited by *P. piloselloides* at various stages of its life-cycle, titratable acidity levels of fern samples were determined (Ong *et al.* 1986). Samples for determination of acidity levels were collected in the morning (08.00 h) and the evening (17.00 h) at the following stages of development: 60 d-old gametophytes, 120 d-old gametophytes each with a single sporophytic frond attached, and mature sterile fronds of the fern growing under natural conditions at an irradiance of 70-210 μmol m^{-2} s^{-1}. The samples were boiled in distilled water for 20 min, cooled and titrated against 0.01 M sodium hydroxide solution with phenolphthalein as the indicator. All values are means of ten replicates ± standard error; the replicates were obtained from different plates of culture.

Spores germinated 5 d after sowing in Hoagland's solution; mature cordate-shape gametophytes were obtained 60 d after sowing. In 90 % of these gametophytes, both archegonia and antheridia were observed 75 d after sowing. Sporophytic initials were observed in these gametophytes 100 d after sowing; further development of these initials produced the first sporophytic fronds 10 d later. Stomata which were not observed in gametophytes were found on these newly formed fronds. Newly formed single frond-sporophytes, still attached to the gametophytes, were 0.3-0.5 cm long. No sexual organs and sporophytes were formed in the remaining 10 % of the gametophytes, even after 120 d in culture.

The evening levels of titratable acidity of the 60 d-old gametophytes were slightly higher than that in the morning (Table 1); this was also observed for the 120 d-old gametophytes without sporophytes (results not shown). Evening titratable acidity level of the 120 d-old gametophytes, each with one sporophytic frond attached, was lower than the morning level (Table 1). For comparison, the newly formed sporophytic fronds were detached from their gametophytes. Such gametophytes showed acidities similar to those without sporophytes formed (Table 1). The removal of fronds from gametophytes showed that changes in titratable acidity could be entirely attributed to the presence of the newly formed sporophytes (Table 1). The morning and evening levels of titratable acidity of mature sterile fronds were much higher than those of the newly formed sporophytes; the morning level was much higher than the evening level indicating that the nocturnal CO_2 fixation occurred (Table 1).

Table 1. Titratable acidity [mol(H^+) kg^{-1}(d.m.)] of gametophytes (G) and sporophytes (S) of *Pyrrosia piloselloides*. All data expressed are means ± S.E.; n = 10.

Titratable acidity	G (60 d-old)	G with S (120 d-old)	G with S detached	Detached S	Mature sterile fronds
Morning	4.19±0.17	5.63±0.04	4.29±0.07	5.38±0.09	23.37±1.58
Evening	4.31±0.18	3.98±0.03	4.44±0.04	3.21±0.10	13.09±0.52
Morning evening	0.12±0.05	1.65±0.01	-0.15±0.01	2.17±0.03	10.28±0.53

The changes in the morning and evening levels of titratable acidity in the newly formed sporophytic fronds and mature sterile fronds of naturally growing sporophytes suggested that *P. piloselloides* could fix CO_2 nocturnally only in its sporophytic phase of the life-cycle. Unlike *P. longifolia*, in which immature sporophytes lacked CAM (Martin *et al.* 1995), immature sporophytes (20-d-old) of *P. piloselloides* exhibited CAM. The capacity to fix CO_2 nocturnally increases with the age of the sporophytic fronds (Ong 1993). Gametophytes of *P. piloselloides* did not exhibit CAM; exposure of these gametophytes to drought also did not result in CAM activity (not shown) as found in other plant species (*Mesembryanthemum crystallinum* - Piepenbrock *et al.* 1994). Hence, the results suggested that both the gametophytes and sporophytes of *P. piloselloides* exhibited different mechanisms of adaptation to stress under natural growing conditions. Also, research to explain the induction mechanism of CAM in this fern is needed.

References

Martin, C.E., Allen, M.T., Haufler, C.H.: C_3 photosynthesis in the gametophyte of the epiphytic CAM fern *Pyrrosia longifolia* (*Polypodiaceae*). - Amer. J. Bot. **82**: 441-444, 1995.

Ong, B.L.: Growth and photosynthetic characteristics of *Pyrrosia piloselloides* (L.) Price. - In: Physiological and Molecular Aspects of Plant Growth and Differentiation. Proceedings of a JSPS-NUS Seminar. Pp. 91-97. 1993.

Ong, B.L., Kluge, M., Friemert, V.: Crassulacean acid metabolism in the epiphytic ferns *Drymoglossum piloselloides* and *Pyrrosia longifolia*: studies on responses to environmental signals. - Plant Cell Environ. **9**: 547-557, 1986.

Piepenbrock, M., Albert, C. von, Schmitt, J.M.: Decreasing leaf water content induces Crassulacean acid metabolism in well-irrigated *Mesembryanthemum crystallinum*. - Photosynthetica **30**: 623-628, 1994.

Tuite, J.: Plant Pathological Methods. Bacteria and Fungi. - Burges Publishing Co., Minneapolis 1969.