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Polyamine levels during the onset of "CAM" in *Opuntia F. indica* (Miller)*

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Summary. Polyamines have been related to the "Crassulacean Acid Metabolism" (CAM) in higher plants. Such relationship was however observed in plants where CAM activity is inducible by external factors. Results presented here indicate that, in *Opuntia F. indica*, cladodes where onset of CAM is dependent on internal conditions, i.e. leaf age, the concentration of putrescine increases in parallel to the acidity of the cytoplasm. The parallel increase of putrescine concentration and acidity (malic acid concentration) can be best evaluated during the onset of CAM (young cladodes), while such correlation is not observed in mature cladodes where CAM is already in it's full function. Spermidine and spermine show no correlation with CAM activity neither during the onset of CAM nor during it's full function. However, spermidine levels correlate negatively to CAM activity when cladodes attain >30 days of age. The results suggest that putrescine in free form could possibly counteract the increase of cellular acidity during onset of CAM in *Opuntia F. indica*; the possible roles of spermidine are discussed.

Keywords: Amino acids – Polyamines – CAM – *Opuntia F. indica*

Abbreviations: PA: polyamines; put: putrescine; spd: spermidine; spm: spermine; HPLC: high performance liquid chromatography.

Introduction

Polyamines (PA) play important roles in higher-plant physiological processes such as floral and fruit development, senescence and tissue growth. These topics have been recently reviewed by Evans and Malmberg (1989). Polyamines have

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also been shown to be involved in stress responses in plants, such as osmotic stress in cereal leaves (Flores, 1985) and heat and drought stress in alfalfa and cotton cultivars (Philips et al., 1989; Kuehn et al, 1990). Morel et al. (1980) found a direct correlation between putrescine (put) and malic acid levels in plants grown under artificial stress conditions of short days, a situation in which a particular photosynthetic mechanism known as "Crassulacean Acid Metabolism" (CAM) is induced. The photosynthetic process "CAM" is a mechanism developed in plants in the course of evolution as a mean of adaptation to extreme arid climates and is characterized by a nocturnal accumulation of malic acid, and hence by a tendency to a prolonged acidification of the cytoplasm. Osmond (1978) has reviewed the environmental and biochemical context of CAM induction. In his review, two distinct CAM induction processes are defined. The onset of photosynthetic CAM process, as a result of an external stress condition such as light or drought defines the plant as "facultative CAM", as opposed to "obligate CAM" where the CAM process develops in relation to internal conditions such as leaf age. Even though CAM metabolism is a very important physiological process which allows plants to survive in extremely dry conditions, its activity in relation to polyamines has only been studied in facultative CAM plants. The aim of this study was therefore that of better understanding the role of these molecules in plant physiology by examining changes in PA during the growth and development of the obligate CAM plant *Opuntia Ficus indica* under natural growth conditions. *Opuntia F. indica* is extremely resistant to aridity and is cultivated in more than twenty countries of the world with a very high index of productivity. The common factor in all the countries where the plant grows is the degree of aridity.

This study is in fact part of an on-going research on the mechanisms of adaptation by plants to arid environment.

Materials and methods

Polyamines products used for HPLC analyses (putrescine, spermidine, spermine, cadaverine and 1,8-diaminooctane), as well as dansylchloride, were from Sigma (St. Louis, Mo, USA). All other chemicals were of the best purity available.

Cladodes of different ages of *Opuntia ficus indica* cv "Gialla" were sampled from a commercial plantation near Cagliari (Sardinia, Italy), in May 1993. Sampling was done in triplicate from three homogenous plants. Cladodes of the following ages were sampled: less than 7 days, 7 days, 14 days, 21 days, 30 days, 45 days, one year. Age of cladodes was based on a previous study carried out in 1992 where growth rate of cladodes from bud emergence up to maturity was followed up (data not shown). Sampling was done in the morning and in the evening (at 07:00 and at 17:00) in relation to maximum (morning) and minimum (evening) CAM activity. Cylindrical samples across a cladode were collected using a cork borer for the following analysis: polyamines, pH and dry weight. Samples for PA and pH analysis were cut into two equal halves, immediately weighed, and frozen in liquid nitrogen until the arrival at the laboratory. Samples were then transfered at $-20°$ C until analysis. Cladode samples collected for dry weight analysis were weighed in the field and then kept at 4° C until the arrival at the laboratory. Dry weight% of samples was determined by drying samples in an oven at 70° C until no weight change was observed. pH values were determined using a pH meter.

Free polyamine extraction, dansylation and HPLC determination were based on a modification of the method described by Stefanelli et al. (1986). Frozen samples were

homogenized in 9 volumes of cold 0.5 M $HClO_4$, extracted for 1 h at 4° C and then centrifuged at 30,000 g for 30 min. The supernatant was used for the dansylation procedure; dansyl-polyamines were separated with a reverse phase C_{18} adsorbosphere 5 mm, 250 × 4.6 mm ID column (Alltech Ass. Inc., Deerfield, IL, USA) protected by a Nova-Pak C_{18} precolumn (Waters Millipore Corp., Milford, MA, USA). An Waters 600 E system with a fluorescence detector set at 300–360 nm excitation and 400 nm emission wavelenghts was used. Data are expressed in nmoles of PA/gram fresh weight.

Results

CAM activity, evaluated in terms of diurnal changes in pH value, was detected when young cladodes attained 30 days of age. Such diurnal variation in pH value was not present in < 30 day old cladodes. (Fig. 1). This is in accordance with our previous study (Fig. 2) where correlation between the onset of CAM and cladode's age was also observed.

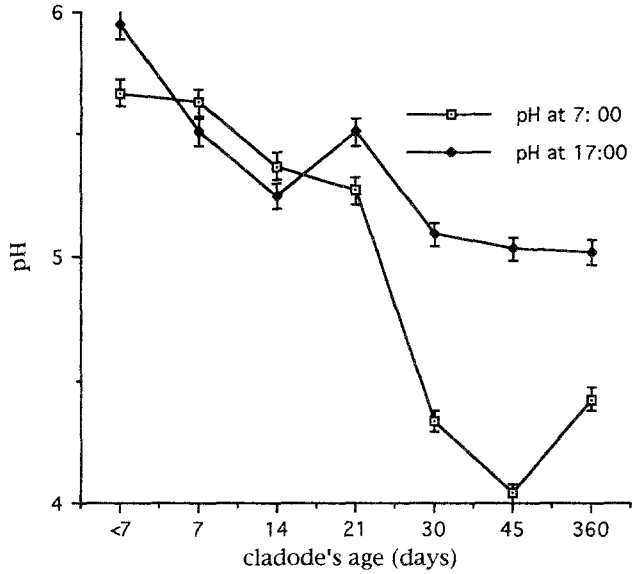

Fig. 1. pH values in cladodes of various age (in days) determined at 7:00 and 17:00

Free PA levels were examined in young and mature cladodes where the onset and the full activity of CAM was respectively observed. In all the age group analysed, the most represented PA was put, followed by spd and spm. Cad was detectable only sporadically and in cladodes of all ages. Levels of put, spd and spm varied depending on cladode's age.

In cladodes up to 30 days of age, put level increased with age and was higher in the evening (Fig. 3). When cladodes attained 45 days of age, however, put level in the morning was 2 times higher than in the evening; pH values vary accordingly, as they were low in the morning and high in the evening (Figs. 1 and 3). In one year old cladodes, put concentration was 10 times lower than in the young ones and it's level did not vary between morning and evening (Fig. 3).

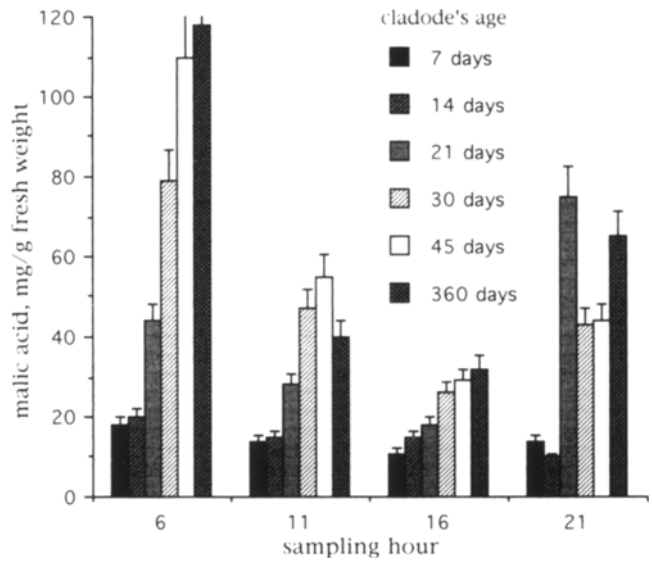

Fig. 2. Malic acid content in cladodes of various age (in days) measured at different hours during the day: 6:00, 11:00, 16:00, 21:00. Malic acid concentration was determined by titration

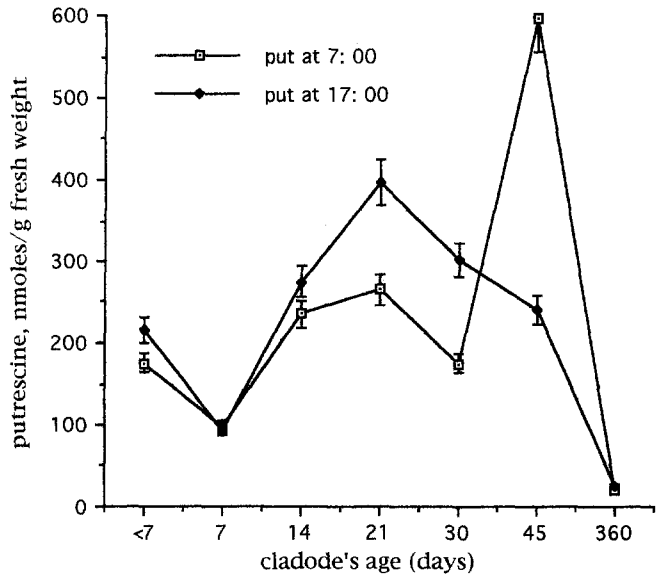

Fig. 3. Putrescine levels in the morning (at 7.00) and evening (at 17.00) in cladodes of various age (days)

Spd, the second most represented PA, followed the same trend as that of put as its level decreased in relation to cladode's age. Spd levels were higher in the morning for cladodes <14 days of age. This trend however reversed when cladodes attained 14 days of age with higher (50%) levels in the evening as compared to morning values (Fig. 4). With the increase in age (21–30 day old cladodes), spd levels were not appreciably different between morning and eve-

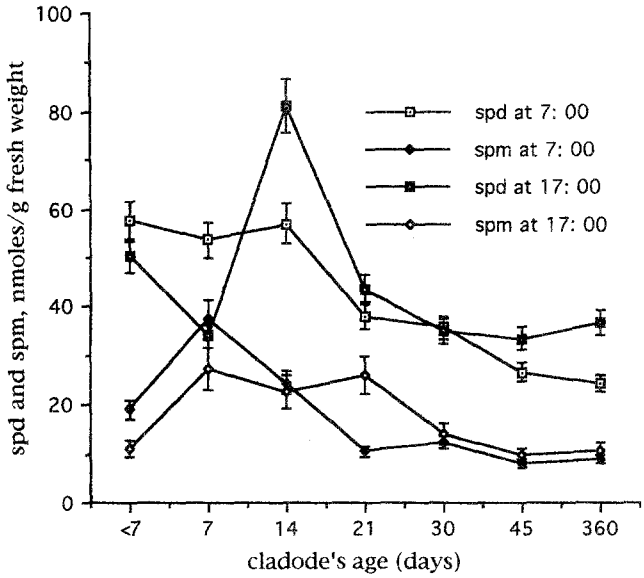

Fig. 4. Spermidine and spermine levels in the morning (at 7.00) and evening (at 17.00) in cladodes of various age (days)

ning. Cladodes of 45 days of age or more, had levels higher in the evening (40%) than in the morning.

Spm concentration decreased with age with higher levels in young cladodes and lower levels in older cladodes (Fig. 4) and no clear variation in its levels was seen between morning and evening. Cad was not detectable in all age groups and did not follow any trend in relation to age or hour of sampling. Dry weight was 50% higher in younger cladodes (up to 30 days of age) than in older cladodes, while there was no variation in dry weight between morning and evening (data not shown).

Discussion

Polyamines have been associated to various physiological and biochemical processes in plants. In this study we have examined the onset of CAM activity during the growth of *Opuntia F. indica* cladodes and the relationship between changes in PA levels and CAM activity. The data presented clearly demonstrate that CAM machinery in this species functions when cladodes attain one month of age. Acevedo et al. (1983), observed that onset of CAM activity in *Opuntia F. indica* was related to the age of young cladodes under natural environmental conditions in Chile. Their observation indicated that two week old cladodes exhibited both C-3 and CAM type photosynthesis having the stomata open both during the day and night, while four weeks old cladodes exhibited full CAM activity by completely closing the stomata during day-time and opening them at night. It appears therefore that, under natural field conditions, CAM photosynthetic mechanism in *Opuntia F. indica* is age dependent.

Moreover, the data presented clearly demonstrate that total free PA levels are highest in young cladodes where cell division is most active. In mature one

year old cladodes, where cladode growth rate is very low, total PA levels were lowest.

In facultative CAM plants, put is synthesized along with accumulation of malate into the cytoplasm, and thus would serve to buffer cellular pH (Morel et al., 1980). Put and to a lesser extent spd accumulate in higher plants in response to various stresses as well (Weinstein et al., 1986; Young et al., 1983). In our case, during the onset of CAM in young cladodes (30–45 days old), put level correlated positively with tissue acidity. In fact, put level in 45 days old cladodes was 100% higher in the morning (when tissue acidity is also very high), in comparison to the evening (Figs. 1 and 3). When CAM activity is fully in function, however, there was no appreciable diurnal change in put level. In fact, in mature one year old cladodes, put level did not correlate to CAM activity. In our case therefore, put level increases in parallel with tissue acidity only during the onset of CAM in young cladodes.

Another interesting obsevation in this study was the fact that spd level correlated negatively with CAM activity, with highest levels in the evening when tissue acidity was lowest. Such negative correlation actually starts during the onset of CAM (45 days old cladodes) and can be found in mature one year old cladodes as well (Fig. 4). Previous reports have indicated that spd accumulation contributed significantly to maintain cell integrity (Guye et al., 1986; Smith, 1982). In mature mung bean it was observed that spd was always found in the bound form, while put was mainly present in the free form (Goldberg et al., 1984). The increase in spd level we observed in mature cladodes may be due therefore to a shift in the ratio of bound to free spd and/or to a possible stimulation of spd synthase. When one year old olive plants were exposed to salt stress, spd and spm levels increased while put level was higher in the control plants (Tattini et al., 1993). A low temperature treatment in different cultivars of Citrus also activated the accumulation of spd, while no variation in put level was observed (Kushad et al., 1987). PA being essential for growth and development in plants, their function may vary according to the species and degree of tolerance to stress by different plants.

As far as our study is concerned, it would be essential to determine free and bound PA levels, as well as enzymes of their metabolism, during the onset of CAM activity in young cladodes and during a full day cycle in mature cladodes, where CAM mechanism is already in function. Such observations could give a much clearer view on the involvement of PA during the onset and activity of the CAM machinery in obligatory CAM plants.

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