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Short communication

Polyamine levels in the seagrass *Cymodocea nodosa*

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Abstract

The levels of the polyamines putrescine, spermidine and spermine were analysed in different tissues of nature-collected samples of the seagrass *Cymodocea nodosa*. The highest accumulation of polyamines was found in the apical section of the rhizome. The putrescine/spermine ratio was highest in the senescent tissue of the leaf. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: *Cymodocea nodosa*; Polyamine levels; Putrescine; Spermidine; Spermine

1. Introduction

Polyamines (PAs) are organic molecules with two or more aminic groups that act as polycations inside the cell (Evans and Malmberg, 1989; Flores, 1990). Polyamines can be bound to a variety of molecules and/or to cellular membranes; they are also found in the free state inside the cell (Torrigiani and Scoccianti, 1995). The diamine putrescine (put), and the PAs spermidine (spd) and spermine (spm) are common amines found in animals or plants. Their ubiquitous nature has been well reported before (Tabor and Tabor, 1984; Lee and Chu, 1992; Altman and Levin, 1993).

These molecules play an active metabolic role that has already been studied in terrestrial plants; they are involved in the regulation of plant growth and stress, they affect the pollen grain maturation and germination, and are also related to plant flowering (Smith, 1985; Evans and Malmberg, 1989; Harkess et al., 1992; Chibi et al., 1994; Rey et al., 1994; Das et al., 1995). Nevertheless, considering that PAs have effects similar to those of plant growth regulators (PGRs, Tiburcio et al., 1993), little is known about the incidence of PAs

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in seagrass. Indeed, only a few studies on the effect of PGRs over their development and growth have been published in recent years (Loquès et al., 1990; Bird and Jewett-Smith, 1994; Terrados, 1995; Balestri et al., 1998). In the present work, we analyse the distribution of endogenous PAs levels over different tissues of the seagrass *Cymodocea nodosa*.

2. Materials and methods

2.1. Plant material

Several plagiotropic sprigs of *Cymodocea nodosa* (Ucria) Arscherson were collected by SCUBA at a depth of 15 m from the same meadow at Gando Bay (Gran Canaria, Canary Islands) between the end of July and August 1998, and transported to the laboratory for an assessment of endogenous PA levels. Four types of plant parts were cleaned and tested: rhizomes, roots, blades and apical sections.

2.2. Extraction and dansylation of polyamines

The method followed was a modification of those of Biondi et al. (1993), and Gallardo et al. (1994).

C. nodosa tissue was ground in a small mortar with cold 5% trichloro-acetic acid (TCA) and centrifuged at $1500 \times g$ for 15 min. The supernatant containing free acid-soluble and bound acid-soluble PAs and the pellet with the bound acid-insoluble were separated. Half of the supernatant volume was kept frozen for the subsequent dansylation of the free PA fraction. The whole pellet, and 50 μl of the supernatant, were hydrolysed in separated sealed vials with 300 μl of 12 M HCl each for 20 h at 100°C to analyse the bound fraction. After hydrolization was completed, the samples were filtered, dried and redissolved in 300 μl of 5% TCA to be dansylated. In the dansylation process, 45 μl of the sample were mixed with 45 μl of a saturated solution of Na_2CO_3 and 90 μl of dansyl chloride (5 mg/ml acetone). The dansylation reaction lasted for 10 min at 70°C. Afterwards, 25 μl of an aqueous proline solution (100 mg/ml) was added to react for 30 min at darkness with the excess of dansyl chloride. When the reaction was completed 500 μl of toluene were mixed with the samples, well shaken and then left till the two phases (organic and aqueous) separated. The organic phase, containing the PAs, was then transferred to another vial, dried in a heat-speed vacuum and the residual dissolved in 600 μl of acetone.

2.3. Separation and quantification of the PAs

The dansyl-PAs were separated with TLC on 7.5 cm × 2.5 cm plates (Schleicher&Schuell, F1500/LS, 254). The mobile phase used was a chloroform/triethylamine 5:1 mixture (v/v). The chromatography lasted for about 10 min on closed recipients. The plates were revealed under UV light (254 nm), so the put (Rf 2.5 cm), spd (Rf 1 cm) and spm (Rf under the front) bands were visible by identification of bands with those of pure standards treated in the same way. Sigma provided all the standards. These bands were scraped off from the plate and redissolved with 800 μl of acetone. The samples were shaken and centrifuged

at 6000×*g* for 3 min, and quantified at 365 nm (excitation) and 510 nm (emission) with a high-resolution spectrofluorimeter (SFM 25, Kontron Instruments).

2.4. Statistics

The Mann–Whitney test provided with SPSS 6.1.3 (SPSS, Chicago, IL) was performed to detect differences in PA levels between the apical zone of the rhizome and the rest of the plant (leaves, roots and rhizome PA levels were set together). The Bonferroni's correction to the experimentwise error rate (5%) was applied to reduce the error rate in such a set of multiple comparisons.

3. Results

3.1. Endogenous PAs levels

The most remarkable result is the high levels of PAs in the bound acid-soluble fraction (Table 1). The levels of bound acid-insoluble put are greater than those of the free fraction, but this changes, except for the spm of the leaves, with the other two diamines where the free spd and free spm amounts are greater. But these differences are smaller than when comparing the bound acid-soluble fraction with the other two fractions. Among tissues, the distribution of PAs was quite similar.

The highest amounts of free PAs were found in the apical zone of the rhizome whilst the root tissue had the lowest amounts of PAs. The apical section had four times the amount of put and spd of leaves and rhizome, and eight times the amount of roots. This pattern changed for the spm levels, 10 times the amount measured for leaf and root was found in the apical section, and only three times as much as in the rhizome.

For the bound acid-soluble and acid-insoluble PAs, again the apical meristem had, on average, for the three PAs, approximately a factor 10 as much as roots and rhizomes, but only three times the amount of leaves.

The apical section of the rhizome always had a significantly higher amount of PAs when compared with the rest of the plant. This result was the same for every PA on each PA fraction (Table 1).

4. Discussion

PAs are related to a number of development events that take place in higher plants, and a high cell-division activity is well correlated with the presence of significant amounts of endogenous PAs (Smith, 1985). Although there are no references about PAs in other seagrasses, the values reported for *C. nodosa* in this work fall within the range of those cited for terrestrial plants (Egea-Cortines et al., 1993; Gallardo et al., 1994; Rey et al., 1994; Pedroso et al., 1997). The highest amounts of PAs (free and bound fraction) are found in the apical section of the rhizome, where the highest growing activity for *C. nodosa*

Table 1
Free and bound polyamine levels (nm g^{-1} FW) in samples from different plant parts of *Cymodocea nodosa*[a]

	Free[b,e]			Bound-soluble[c,e]			Bound-insoluble[d,e]		
	put	spd	spm	put	spd	spm	put	spd	spm
Leaf	403±11	5.7±0.2	0.8±0.1	9250±784	17±0.07	24±2.45	2541±215	1.44±0.07	3.24±0.29
Root	199±8	2.5±0.1	1.03±0.15	2184±113	5.82±0.07	5.29±0.05	622±33	0.48±0.07	0.78±0.05
Rhizome	402±20	4.9±0.2	3.33±0.54	3522±136	6.1±0.68	5.64±0.05	1014±41	0.48±0.68	0.83±0.05
Apical zone	1583±147[f]	19±1.4[f]	9.17±0.49[f]	25886±488[f]	35±2.05[f]	63±2.99[f]	7530±136[f]	3.29±0.14[f]	8.43±0.39[f]

[a] Experimentwise error rate is 0.05. Comparison error rate is experimentwise error rate/number of comparisons (Bonferroni's correction). Data are mean±s.e. from 4–10 replicates.
[b] Free acid-soluble PAs.
[c] Free acid-soluble plus bound acid-soluble PAs.
[d] Bound acid-insoluble PAs.
[e] Put, putrescine; spd, spermidine; spm, spermine.
[f] Significant differences between apical zone and the rest of the plant ($p<0.0056$).

is expected. Cells in constant division and differentiation were found within the apical meristem (results not shown). Our low levels in roots are in accordance with Larkum et al. (1989).

The absolute endogenous values of PAs given for a particular plant show little information about their physiological role since there is a wide range of PAs levels found in current literature. Other authors have already used the ratios between PAs to understand their role in the development of plant tissues (Rey et al., 1994). If we consider the put/spd and put/spm ratios among tissues, they remain steady in both the free and bounded fractions, except for the free put/spm ratio on the leaf tissue, which was three times higher than in other tissues. This is a relative decrease of free spm in *C. nodosa* leaves, shown in the increase of the put/spm and spd/spm ratios. The diamine spm has been identified as an anti-senescence-inducing factor (Galston et al., 1997), thus, this accumulation of put relative to spm could be the factor promoting the senescence of the leaf. At the time of the year when the samples were collected (August), the meadow generally starts to lose leaves after reaching its biggest area. Previously, Rey et al. (1994) reported this accumulation of put in plant tissue as an indicator of senescence. The endogenous bound-PAs ratios give little information as the physiological function of this fraction is unknown (Slocum and Galston, 1985).

In conclusion, we report the presence of PAs in *C. nodosa*, a common Mediterranean seagrass, to be within the range for terrestrial plants, and their differential distribution among tissues. We found the highest levels in the meristematic tissue of the rhizome, where the greatest growing activity occurs. A possible senescence process identified in the leaf tissue is correlated with a relative accumulation of free put.

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