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ACCUMULATION OF FLAVONOIDS IN TWO DIFFERENT PHENOTYPES OF THE TROPICAL SEAGRASS *THALASSODENDRON CILIATUM* (FORSKÅL) DEN HARTOG

Udo MARGNA and Tiit PÄRNIK

Institute of Experimental Biology, Estonian Academy of Sciences, Instituudi Rd. 11, EE-3051 Harku, Harjumaa, Estonia

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FLAVONOIDIDE SISALDUS TROOPILISE MEREROHU *THALASSODENDRON CILIATUM* (FORSKÅL) DEN HARTOG KAHES ERINEVAS FENOTÜÜBIS. Udo MARGNA, Tiit PÄRNIK

НАКОПЛЕНИЕ ФЛАВОНОИДОВ В ДВУХ РАЗЛИЧНЫХ ФЕНОТИПАХ ТРОПИЧЕСКОЙ МОРСКОЙ ТРАВЫ *THALASSODENDRON CILIATUM* (FORSKÅL) DEN HARTOG. Удо МАРГНА, Тийт ПЯРНИК

Key words: *Thalassodendron ciliatum*, flavonoids.

INTRODUCTION

During the 1989 Indian Ocean expedition of the research vessel *Akademik Aleksandr Nesmeyanov* photosynthetic activity of the tropical seagrass *Thalassodendron ciliatum* (Forskål) den Hartog (Den Hartog, 1970) was measured. Marked biochemical and physiological differences between individuals growing in deep and shallow water habitats were established in these studies (Pärnik et al., 1992). The differences were in good accord with the observation that the seagrass blades from shallow water were intensely coloured with red pigments, probably anthocyanins, while there was no red pigmentation in individuals growing in deep water. It prompted us to examine flavonoids of these two *Thalassodendron* phenotypes in some more detail taking advantage of the fact that a collection of suitable samples botanized during the expedition was available in this laboratory.

EXPERIMENTAL

The leaves of *Thalassodendron ciliatum* used for analyses were taken from the dried herbarium samples collected from different habitats within a 0.25 sq. km ocean territory of the coastal waters of Seychelles near the Island of Desroche in February 1989. The dried material was ground into powder and exhaustively extracted with hot 75% ethanol. The resulting alcoholic extracts were reduced at room temperature and

then chromatographed two-dimensionally in FN-11 paper sheets using a mixture of *n*-butanol—acetic acid—water, 6 : 1 : 2 (I) and 10% acetic acid (II) as developing solvents. The spots of flavonoids in the developed chromatograms were located under UV-light, characterized by their fluorescence, and thereafter eluted with 75% ethanol for UV-absorption spectra measurements and quantitative assay. The eluted flavonoids were tentatively identified by comparing their UV-absorption characteristics with the absorption spectra of known flavonoids (Markham, 1982). The quantitative content of the flavonoids (expressing it in rutin equivalents) was determined by a routine spectrophotometric technique (Margna & Margna, 1969).

RESULTS

Paper chromatographic study of the material demonstrated that the leaves of *T. ciliatum* contained two major flavonoids and at least three minor compounds of similar nature. The compounds showed dark-purple fluorescence in UV-light. When exposed to ammonia vapour, the three minor components had a yellowish colour while the two major pigments changed into bright yellow. These colour changes suggest that all five are probably flavonol glycosides (Markham, 1982). The two major flavonoids had similar UV-absorption spectra in 95% ethanol which were characterized, similarly to a variety of known glycosides of quercetin, by two distinct absorption maxima at 255—260 nm (Band II) and 362—364 nm (Band I), a small supplementary maximum near 300 nm, and a vague shoulder near 270 nm. Sodium acetate caused no shifts in absorption maxima but sodium acetate with borate led in both compounds to a small 3—4 nm bathochromic shift in Band II and a 20 nm similar shift in Band I.These shifts show that a free orthodi—OH—group is present in the B-ring of flavonoid nucleus suggesting that the likely aglycone of both flavonol glycosides should be quercetin (Markham, 1982).

Leaf samples taken from different *T. ciliatum* plants differed in their quantitative levels of flavonols, depending on whether the plants came from shallow or deep water (Table). The leaves of shallow (0.5—1 m) water individuals, which remain periodically uncovered with water at low tide, contained markedly lower amounts of the two major glycosides (only those two could be quantified after paper chromatography) than the leaves of deep (32—33 m) water plants. In one out of the four leaf samples from shallow water plants available for analyses, the content of flavonol glycosides was 10-fold lower than in the leaves of deep water plants. Moreover, in two other samples of shallow water leaves, the level of both glycosides remained below the limits of accurate measurements. Plants from shallow water showed low levels of flavonols in spite of the fact that they were growing under favourable light conditions and were able to produce large amounts of anthocyanins, a group of related compounds of flavonoid origin.

The content of major flavonol glycosides in the leaves of *Thalassodendron ciliatum* grown in coastal waters of Seychelles (in rutin equivalents, mg per g dry weight)

Depth of water, plant sample	Flavonol No. 1	Flavonol No. 2
0.5—1 m		
No. 1	0.63	0.89
No. 2	Low level, not measurable	
No. 3	3.59	6.18
No. 4	Low level, not measurable	
32—33 m		
No. 5	7.65	12.0
No. 6	6.02	6.98
No. 7	6.30	9.48

DISCUSSION

The fact that high levels of flavonol glycosides were found to occur in the poorly-illuminated deep water plants of *T. ciliatum* rather than in its well-illuminated individuals from shallow water is really unexpected and does not correlate with the present knowledge on flavonoid physiology. Plants exposed to high levels of irradiance typically accumulate much higher amounts of flavonoids than it can be observed at low light intensities (McClure, 1975). It seems therefore that leaves of *T. ciliatum*, by some reason, respond differently to light.

When attempting to find an explanation to this "anomaly", one should take into consideration that the production of flavonoids and other phenolic compounds is directly dependent on the supply of their primary precursor L-phenylalanine synthesized via the shikimic acid pathway (Margna, 1977; Маргна, 1990) and the latter process is functionally related to the oxidative transformation of glucose through the pentose phosphate pathway. It may well be that under low irradiance conditions leading to a sharply reduced photosynthetic activity of the seagrass in deep water habitats (Pärnik et al., 1992), that plant compensates for its energy need by increasing the activity of the oxidative pentose phosphate pathway. A concomitant increase both in the functional activity of the shikimic acid pathway and in the production of L-phenylalanine may then create favourable conditions for an elevated formation of flavonoids.

A surplus of L-phenylalanine leading to an increased synthesis of flavonoids in deep water individuals of *T. ciliatum* could also arise from suppressed growth of plants under less favourable nutrition conditions in deep water habitats. Resulting from reduced utilization of amino acids for growth needs, higher amounts of L-phenylalanine could become available for the production of secondary compounds as shown under comparable conditions in the brown alga *Fucus vesiculosus* (Ilvessalo & Tuomi, 1989).

A possibility exists also that high levels of flavonols characteristic of deep water plants of the seagrass result from a shift in the distribution of L-phenylalanine as the common substrate between parallel routes of biosynthesis of separate flavonoids. Provided that the pool size of L-phenylalanine shows little variance in plants from different habitats, the leaves of well-illuminated shallow water plants can predominantly use its reserves for producing anthocyanins. In contrast, in leaves of plants not sufficiently illuminated to allow formation of anthocyanins the same substrate is channelled into flavonols.

In any event, the observed differences in the accumulation of flavonoids in *T. ciliatum* leaves seem to be rather intriguing making a closer study of the cell metabolism of this widely distributed tropical seagrass highly desirable.

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