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PROTEIN CONTENT AND PROTEOLYTIC ACTIVITY IN THE HYPOCOTYLS OF *RHIZOPHORA* SPECIES DURING VIVIPAROUS GERMINATION

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ABSTRACT

Protein content, level of protease (E.C. 3.4.2.2) activity and electrophoretic pattern of proteins were studied in the developing hypocotyls of *Rhizophora mucronata* and *R. apiculata*. Total protein content was increased, while the level of protease activity decreased exponentially during viviparous germination. Higher amount of protein and protease activity were recorded in *R. apiculata* than *R. mucronata*. A negative correlation was quadratically existing between protein and protease activity with the age of the hypocotyls. Higher amount of protein content in the mature hypocotyls is an adaptive mechanism to such an environment. The electrophoretic separation of proteins showed a marked variation in the profile between the *Rhizophora* species and also the pattern changed during the course of germination.

Key words: Germination, Hypocotyls, Protease activity, *Rhizophora* sp. Vivipary.

INTRODUCTION

Protein metabolism has been associated with the adaptation of plants to environmental changes and stress (McCown *et al* 1969). During germination, the storage proteins are hydrolysed to free amino acids to enable protein synthesis in actively growing parts of a seedling (Tully & Beevers 1978; Segundo *et al* 1990). These proteolytic events are mediated by a system of proteases and peptidases with varying substrate specific activities during germination (Baumgartner & Chrispeels 1977). Depletion of protein content associated with an increase in the level of protease activity was reported in germinating seeds of *Pisum sativum* (Basha & Beevers 1975), *Vigna radiata* (Kern & Chrispeels 1978) and *Ricinus communis* (Tully & Beevers 1978).

As the cells in growing parts of a seedling mature, their enzymes pattern changes and this has been ascribed to changes in relative amounts of the various proteins (Steward *et al* 1965). The changing pattern of the proteins revealed the genetic as well as developmental expression in course of time and the electrophoresis is perhaps

potential and most useful tool to observe a spectrum of proteins. The qualitative changes in relation to seedling development of *Pisum sativum* was reported earlier (Harris & Chrispeels 1975).

The present investigation deals with the qualitative and quantitative changes of proteins in relation to proteolytic enzymes in the hypocotyls of viviparous seedlings of two *Rhizophora* species.

MATERIALS AND METHODS

Seedlings of *Rhizophora mucronata* Lamk. and *R. apiculata* Bl. were collected from Pitchavaram mangrove forest, Tamil Nadu. Fresh hypocotyls were categorised into five developmental phases, based on their length and biomass and used after a thorough distilled water wash as reported earlier (Gunasekar *et al* 1992; Gunasekar & Parimala 1993).

Total proteins were extracted by precipitating with trichloro acetic acid (20%; w/v) and then the precipitate was dissolved in 0.1 N NaOH. The amount of total protein content was estimated by using the method of Lowry *et al* (1951). Bovine serum albumin was the standard.

The protease activity was determined by the method of Prisco *et al* (1975). The crude enzyme preparation was made by using 0.1 M sodium phosphate buffer, pH 7.6 containing NaCl (1%; w/v) and PVP-40 (7%; w/v) as extraction medium. All operations were carried out at 4°C except the reaction. The reaction was performed at 28±2°C using casein solution (1% in 0.1 M sodium phosphate buffer, pH 6.0; w/v) as substrate. The liberated amino nitrogen by the activity of protease was determined and was expressed by the equivalent of glycine. The results were subjected to statistical analysis.

Proteins were separated on polyacrylamide gels (7%) as essentially followed the method of Davis (1964) and the relative mobility (Rm) of each protein fraction was calculated against the bromophenol front.

RESULTS

Protein content increased steadily from the phase of protrusion of the hypocotyls from seed coat (phase I) until detached (phase V) from the mother plant after maturity (Fig. 1a). The increase was found to be exponential in *R. apiculata*, while linear in *R. mucronata*. A marked difference in the protein content was observed between the two *Rhizophora* species throughout the developmental period, that the *R. apiculata* accumulates higher amount of protein than *R. mucronata*.

The enzyme protease activity decreased exponentially in the hypocotyls of *Rhizophora* during viviparous germination (Fig. 1b). Higher amount of activity was recorded in the initial phases of both the *Rhizophora* species and started declining until maturity of the hypocotyls. *R. apiculata* exibited higher level of protease activity than *R. mucronata* in all developmental phases of the hypocotyls. A negative correlation existed between the total protein content and the level of protease activity with the age

Table 1 Relative mobility (Rm) of electrophoretically separated proteins from developing hypocotyls of *Rhizophora mucronata* and *R. apiculata*.

Fraction (Band) number	Rm value	Developmental phases									
		R. apiculata					*R. mucronata*				
		I	II	III	IV	V	I	II	III	IV	V
1	0.02	-	-	-	-	-	+	+	+	+	+
2	0.16	-	-	+	-	-	-	-	-	-	+
3	0.28	-	-	-	+	-	-	-	-	-	-
4	0.31	+	+	+	+	+	+	+	+	+	+
5	0.36	-	-	-	+	-	-	-	-	-	-
6	0.41	+	+	+	+	+	+	+	+	+	+
7	0.47	-	-	-	-	-	+	+	+	+	+
8	0.48	-	-	+	-	-	-	-	-	-	-
9	0.53	+	+	+	+	+	+	+	+	+	+
10	0.60	-	-	+	+	-	-	-	-	+	+
11	0.70	-	-	+	-	-	+	+	+	+	+
12	0.80	-	-	+	-	-	-	-	+	-	-

+ or - indicate the presence or absence of protein fraction

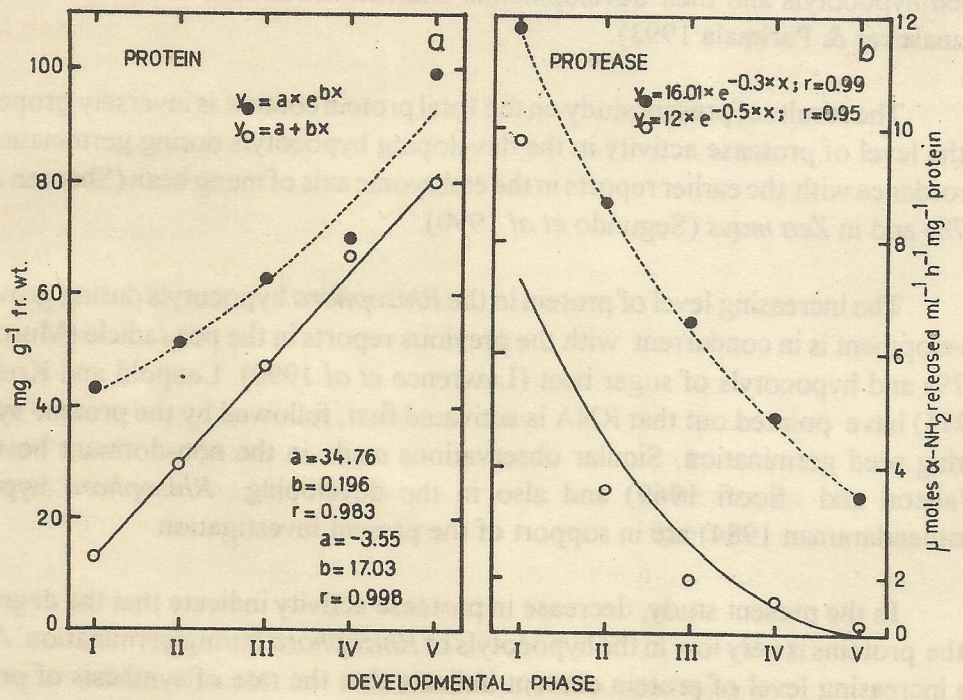

Fig. 1 Changes in a) total protein content and b) protease activity in the developing hypocotyls of *Rhizophora mucronata* (○——○) and *R. apiculata* (●— — —●) during viviparous germination.

of the hypcotyls. The relationship was found to be quadratic for both the species with the r value of 0.98 ($y=154.80 + - 20.38x + 0.94x^2$) for *R. apiculata*; y = 109.60

+ $-30.90x+2.15x^2$ for *R. mucronata*, both were significant at 1% probability.

The electrophoretic characterization of total proteins showed distinct variation between the two species (Table 1). The protein profile was found to be changed in the developing hypocotyls during viviparous germination. Protein fractions with Rm values of 0.31, 0.41 and 0.53 were stained in both the species, while 0.02 and 0.47 were detected only in *R. mucronata*. Here again, bands with Rm 0.16 was observed in phase III of *R.apiculata*, while this band appeared in the final phase of *R. mucronata*. In general, the developmental expression of the protein profile of the hypocotyls found to be changed during elongation and clearly distinct from one species to another.

DISCUSSION

Rhizophora exhibits a true vivipary (Pannier & Pannier 1975), where the propagating organ is the seedling (Juncosa 1982). *Rhizophora* seedling has prominent green hypocotyls and their developmental characteristics have been reported earlier (Gunasekar & Parimala 1993).

The results of present study on the total protein content is inversely proportional to the level of protease activity in the developing hypocotyls during germination, is in accordance with the earlier reports in the embryonic axis of mung bean (Sheoran & Garg 1978) and in *Zea mays* (Segundo *et al* 1990).

The increasing level of protein in the *Rhizophora* hypocotyls during growth and development is in concurrent with the previous reports in the pea radicle (Murray *et al* 1979) and hypocotyls of sugar beet (Lawrence *et al* 1990). Leopold and Kreidmann (1975) have pointed out that RNA is activated first, followed by the protein synthesis during seed germination. Similar observations made in the non-dormant bean seeds (Walston and Soofi 1969) and also in the developing *Rhizophora* hypocotyls (Kothandaraman 1984) are in support of the present investigation.

In the present study, decrease in protease activity indicate that the degradation of the proteins is very low in the hypocotyls of *Rhizophora* during germination. As such, the increasing level of protein content indicate that the rate of synthesis of protein is higher than its degradation. Apart from this, the basic requirements might have been fulfilled by the translocation of amino acids from the mother plant, tubular cotyledon or endosperm. Translocation of macromolecules have also been noticed (Pannier & Pannier 1975; Bhosale & Shinde 1983).

The higher amount of protein in the mature hypocotyls may be an adaptive mechanism for the specialized environment of *Rhizophora*. Hence, assumption is that the mature hypocotyls stored excess proteins like the seeds of other angiosperms and the reserve proteins may be further utlized at the time of seedling erection/rooting after detachment from the mother plant. Similar observation was made in the case of starch in the developing hypocotyls of *Rhizophora* (Gunasekar 1994).

The electrophoretic variation between the *Rhizophora* species was found to be distinct and it may be used as a marker system to identify these two closely related species. Earlier reports revealed that the protein profile changed during germination and early seedling growth (Segundo *et al* 1990) which coincide with the present report. The protein fractions 1, 4, 6, 7 and 9 obtained may be due to genetic expression, which determine the species status of *Rhizophora* and the other fractions due to developmental expression. The fluctuation in the banding pattern may be essentially due to continuous synthesis of protein during hypocotyls development. This can be conceived from the fact that isozymes fluctuations are controlled by independent alterations either in the rate of constant degradation or of synthesis (Scandalios 1974).

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