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ACID PHOSPHATASE ACTIVITY AND ISOZYME PATTERN IN DEVELOPING VIVIPAROUS SEEDLING HYPOCOTYLS OF *RHIZOPHORA* SPECIES

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Acid phosphatase activity and isozyme pattern in *Rhizophora apiculata* and *R. mucronata* hypocotyls were examined during viviparous germination. Acid phosphatase activity was found to be higher in the stage I but the net decreasing trend from stage I to V was similar in both the species. The electrophoretic separation of acid phosphatase revealed the presence of four isozymes in *R. apiculata* and three in *R. mucronata*. In both the cases, isozyme pattern changed during growth and development.

Keywords : Acid phosphatase; Germination; Hypocotyls; Isozyme; *Rhizophora* sps.

Introduction

Acid phosphatase is one of the key enzymes which catalyses various biochemicals in regulating the cell metabolism and intended to modulate the level of inorganic phosphorus[1] particularly in carbohydrate metabolism[2]. Isozyme patterns are used as markers for studying the genetic variation among the plants with similar morphology[3].

Mangroves have acquired adaptative characteristics suitable for survival under saline environment and its importance has also been well demonstrated[4]. Vivipary is one of structural and functional adaptations and its physiology of germination is poorly understood and hence, to generate information on the enzymatic changes during germination of *Rhizophora sps.*, the present investigation has been undertaken. Results emerged out of this study, with special reference to acid phosphatase and isozyme pattern, in the developing viviparous seedling hypocotyls are presented and discussed.

Materials and Method

Seedlings of *Rhizophora mucronata* Lamk. and *R. apiculata* Bl. were collected from Pitchavaram mangrove forest. Based on the age, length and weight of the hypocotyls, seedlings were separated into five different stages (Table 1). Fresh hypocotyls were cleaned thoroughly and the crude enzyme extract was prepared[5]. To prevent the interference of phenolics with enzymes, seven per cent polyvinylpyrolidone (w/v) was included in the extraction medium[6]. Enzyme activity was assayed by using p- nitrophenyl phosphate as substrate and the liberated p-nitrophenyl was measured at 410 nm[7]. The protein content in the crude enzyme preparation was determined using Bradford reagent and bovine serum albumin as standard[8]. The enzyme preparation was subjected to electrophoresis on

seven per cent polyacrylamide gel at 4°C[9]. The gels were stained after lowering the pH from 8.5 to 5.0 as suggested by Sako and Stahmann[10] and the red colour bands developed after 30 min were observed.

Results and Discussion

The specific activity of acid phosphatase in the developing hypocotyls of *R. mucronata* and *R.apiculata* are shown in Figure 1. Significant decrease was observed in the level of enzyme activity during the progressive elongation of the hypocotyls in both the species. Between stage I and II, about 16 per cent decrease in enzyme activity was observed in *R. mucronata*, while it was 15 per cent in *R. apiculata*. Similar trend was noticed during course of development in both the species. However, the level of acid phosphatase activity was found always higher in *R. mucronata* than *R. apiculata*.

Electrophorotic separations showed four isozyme bands in *R. apiculata*. On the other hand, only three bands were observed in *R. mucronata* (Fig. 2). Isozyme No. 3 and 5 (Rm 0.37 and 0.91, respectively) were found to be common for all the stages in both the species of *Rhizohora*. Whilest, band No.2 (Rm 0.33) was stained only in *R. apiculata*. Isozymes No.1 and 4 (Rm 0.17 and 0.50) were faintly detected in *R. apiculata* and *R. mucronata*, respectively. Isozyme No.1 was distinguished from stage I to IV and it was not observed in stage V. Band No. 4 was stained during III and IV developmental stages.

Acid phosphatase activity was found to be decreased steadily in both the *Rhizophora* species with progressive development of hypocotyls. Higher level of activities in the early stages of growth may be due to the translocation of acid phosphatases either from endosperm or the mother plant directly. Translocation of macromolecules like carbohydrates into developing hypocotyls was well documented. Results obtained in this study corroborate with earlier findings[4].

Table 1 : Developmental stages of *Rhizophora mucronata* and *R. apiculata* during viviparous germination.

Stages	*R. apiculata*			*R. Mucronata*		
	Length (cm)	Weight (gm)	Age (days)	Length (cm)	Weight (gm)	Age (days)
I	3 - 4	1 - 2	15 - 20	2 - 3	1 - 2	15 - 20
II	13 - 14	12- 13	40 - 45	12 - 13	10 - 11	35 - 40
III	25 - 26	22 - 23	65 - 70	23 - 24	21 - 22	60 - 65
IV	36 - 37	34 - 35	120 - 125	33 - 34	34 - 35	90 - 95
V	47 - 48	49 - 50	> 160	43 - 44	45 - 46	> 125

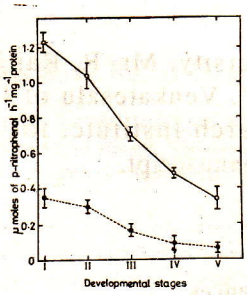

Fig-1

Fig. 1. Changes in the level of acid phosphatase activity in the various developmental stages of the hypocotyls of *R. mucronata* (0 — 0) and *R. apiculata* (●---●). *Values are mean of three replicates expressed in equivalents of p- nitrophenol and the vertical bars represent S.D.*

Fig. 2. The mobilities of the multiple forms of acid phosphatase detected with alpha naphthyl phosphate as described in Materials and Method.

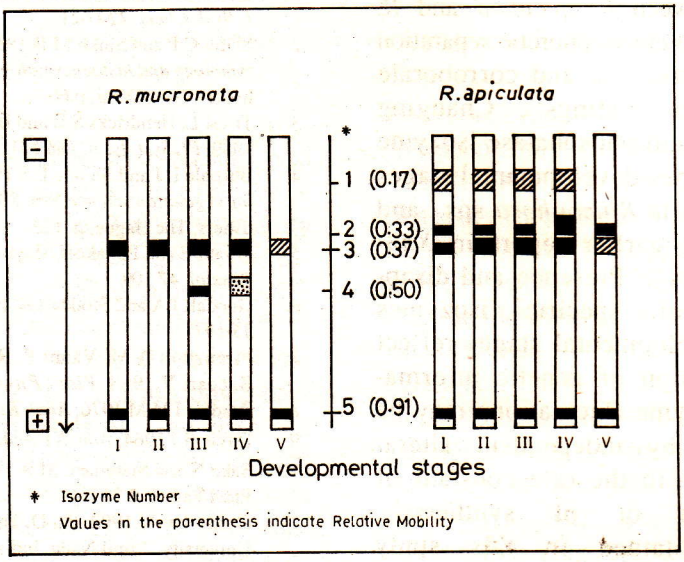

Fig-2

The enzyme activity declined with progressive development of *Rhizophora* seedlings and this may be due to the lower levels of starch phosphorylase and ribonuclease[11].

It has been reported that acid phosphatase catalyses various substrates including hydrolysis of phosphorylated sugars and liberates inorganic pyrophosphate[12]. The liberated inorganic pyrophosphate may be utilized by starch phosphorylase to enable phosphorylation of glucose. It is quite interesting to note that the acid phosphatase as well as starch phosphorylase decreased with subsequent accumulation of starch in the elongating hypocotyls of *Rhizophora*. As phosphorylated glucose is being the major substrate for glycolytic and pentose phosphate pathways, its level should increase during elongation. In the present study, phosphorylated sugars may be translo-

cated from the mother plant to developing hypocotyls and this leads to the reduction in the enzyme activity.

Electrophoretic characteristics of the enzyme has been used as a marker to identify the genetic variation of morphologically similar plant species[13]. Results obtained in this study showed distinct difference between *R apiculata* and *R. mucronata* in electrophoretic separation of acid phosphatase and corroborate with earlier findings[14]. Changing pattern of acid phosphatase isozyme during various developmental stages was evident in *Rhizophora* sps., and support the earlier report in *Xanthium* leaves[15]. Presence and disappearance of specific isozymes during developmental stages reflect the expression of genetic information[16]. Isozyme fluctuations may be controlled by independent alterations either in the rate constant of degradation or of synthesis[17]. Results obtained in this study revealed that fluctuations in isozyme expression is only due to constant degradation and not because of synthesis.

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