J. Phytol. Res. 18(1) : 19-25, 2005

VARIABILITY IN THE ACTIVITY OF THE MAJOR LIGNIFYING ENZYMES IN DIFFERENT ECOLOGICAL GROUPS

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The activity of major enzymes of lignin synthesis, phenylalanine ammonia lyase (PAL), cinnamyl alcohol NADPH dehydrogenase (CAD) and peroxidase in the leaf tissues of plants of different ecological groups viz., mangroves, mesophytes and hydrophytes were examined. Remarkable variation in enzyme activity was observed in taxa between the ecological groups indicating their specificity towards their growing habitat. In mangroves the three enzymes showed a significant level of activity. CAD the indicator of lignin synthesis, showed high level of activity in mesophytes than in hydrophytes indicates the gravitational stress nature of the enzyme. Meanwhile the low profile of CAD activity in hydrophytes reveals the affinity of the group towards aqueous environment. The end product lignin content corroborates the assay data in both the mesophytes and hydrophytes. In mangroves an ambiguous correlation was observed between CAD activity and lignin content ie., high CAD activity low lignin suggesting that CAD possibly exhibits a physiological suppression due to the saline habitat.

Keywords : Hydrophytes; Mangroves; Mesophytes; Lignin; Stress.

Introduction

Lignin is a principal structural component of cell wall in higher terrestrial plants. As an abundant natural polymer in plant system the origin, distribution and synthesis of lignin has been a serious concern in plant research. Although this biopolymer plays a crucial role in plant growth, environmental factors have a positive impact on its synthesis along with the genetic signal[1,2]. It has allowed the evolution of plants capable of surviving in relatively arid environments[3]. The biochemistry of lignin synthesis is a complex process involving the action of several enzymes of phenylpropanoid pathway as well as of lignin biosynthesis branching enzymes. The three major enzymes involved in lignin synthesis are phenylalanine ammonia lyase (PAL), cinnamyl alcohol NADPH dehydrogenase (CAD) and peroxidase (POD)[4]. As the first enzyme of phenyl propanoid metabolism, PAL is one of the most extensively studied enzymes catalyzing the transamination of ammonia from L-phenyl alanine to form cinnamic acid, a precursor of lignin biosynthesis[5]. CAD has been considered to be an indicator of lignin biosynthesis because of the specific role in the reduction of hydroxyl cinnamaldehyde to hydroxyl cinnamyl alcohol[6]. Due to its crucial role in lignification CAD is a potential target enzyme in molecular biology for modulating the quality and quantity of wood in plants[7]. The polymerization of Cinnamyl alcohol to lignin is initiated by the oxidation of phenolic hydroxyl groups catalyzed by peroxidase (POD).The evidence in support of the involvement of this enzyme POD in lignin synthesis is also extensive[8,9]. Thus the role of these enzymes PAL, CAD, POD in lignin synthesis has been convinced based on correlation with the lignification process. Ecologically the plants are categorized in to different groups viz., mangroves, mesophytes and hydrophytes based on histomorphic and ecophysiological features. The present study has been an attempt to trace the phylogeny of lignification on different plant taxa based on their habitat.

Materials and Methods

The whole study was focused on the mangrove vegetation of Ayiramthengu, an interior mangrove patch located in the coastal belt of Arabian sea of Kollam district. Tender and mature leaves were used for the whole study. The mangrove members focused are *Lumnitzera racemosa, Avicennia alba, Acanthus ilicifolius, Bruguiera cylindrica, Rhizophora apiculata, Excoecaria agallocha*. As control, selected plants from mesophytes: *Murraya exotica, Tecoma stans, Hamelia pattens, Quassia amara, Ixora coccinea, Bauhinia accuminata*; hydrophytes: *Eichhornia crassipes, Nymphaea stellata, Limnophila heterophylla, Limnanthemum cristatum, Hydrilla verticillata, Pistia stratiotes* were used. Chemicals: cinnamic acid, caffeic acid, ferulic acid, sinapic acid, coumaric acid, hydroxy benzoic acid, chlorogenic acid, gallic acid, dehydroconiferyl alcohol polymerizate and vanillic acid were obtained from Sigma Chemical Co; St. Louis, MO, USA. Tris-HCl, mercaptoethanol, polyethylene glycol,

Table 1. Major phenolic acid in leaf samples of mangroves involved in lignin synthesis.

Basin mangroves	Caffeicacid (μ g/g)	Cinnamic acid (μ g/g)	Ferulic acid (μ g/g)
Excoecaria	80.8	484	4.2
Bruguiera	12.87	32	6
Acanthus	2.3	13.4	8.2
Avicennia	9.2	80	1.2
Rhizophora	2	3.3	5
Lumnitzera	0.86	78	0.02

Table 2. Activity profile of PAL, CAD and POD enzymes associated with lignification and lignin content in mangroves. Values are as mean ± SE of 15 replicates in each enzyme of six species.

Basin Mangroves	PAL	CAD	POD	Lignin	Lignin: CAD
Excoecaria	4.5	3.9	4.6	2.5	64.41
Bruguiera	3.5	3.1	5.9	1.6	51.6
Acanthus	2.8	2.5	4.2	1.2	48.0
Avicennia	3.6	4.1	4.4	1.4	34.15
Lumnitzera	2.9	3.3	3.9	0.91	27.58
Rhizophora	3.2	3.7	6.1	1.8	48.65
F-Ratio	13.8**	17.2**	29.6**	12.2**	59.6**
SE	0.144	0.123	0.104	0.091	22.34

Table 3. Activity profile of PAL, CAD and POD enzymes associated with lignification and lignin content in mesophytes. Values are as mean ± SE of 15 replicates in each enzyme of six species.

Mesophytes	PAL	CAD	POD	Lignin	Lignin: CAD
Murraya	3.0	4.9	2.6	4.7	95.92
Ixora	3.5	5.7	3.0	5.3	92.98
Quassia	3.6	3.9	2.9	3.2	84.21
Tecoma	3.7	4.9	3.4	4.6	93.87
Hamelia	3.2	5.2	1.9	4.9	94.23
Bauhinia	3.5	5.3	2.7	5.0	94.33
F-Ratio	13.7**	34.71**	16.62**	32.2**	91.6**
SE	0.144	0.123	0.104	0.091	22.34

Table 4. Activity profiles of PAL, CAD and POD enzymes associated with lignification and lignin content in hydrophytes. Values are as mean ± SE of 15 replicates in each enzyme of six species.

Hydrophytes	PAL	CAD	POD	Lignin	Lignin: CAD
Limnophila	1.3	0.55	2.2	0.52	95.55
Hydrilla	0.8	0.6	1.6	0.49	81.66
Pistia	1.0	0.5	1.9	0.46	92.00
Nymphaea	1.9	1.4	2.6	1.2	85.7
Limnanthemum	1.2	0.6	2.0	0.55	91.67
Eichhornia	1.5	0.9	1.9	0.81	90.00
F-Ratio	6.62**	1.61	15.22**	32.2**	91.6**
SE	0.144	0.123	0.104	0.091	22.34

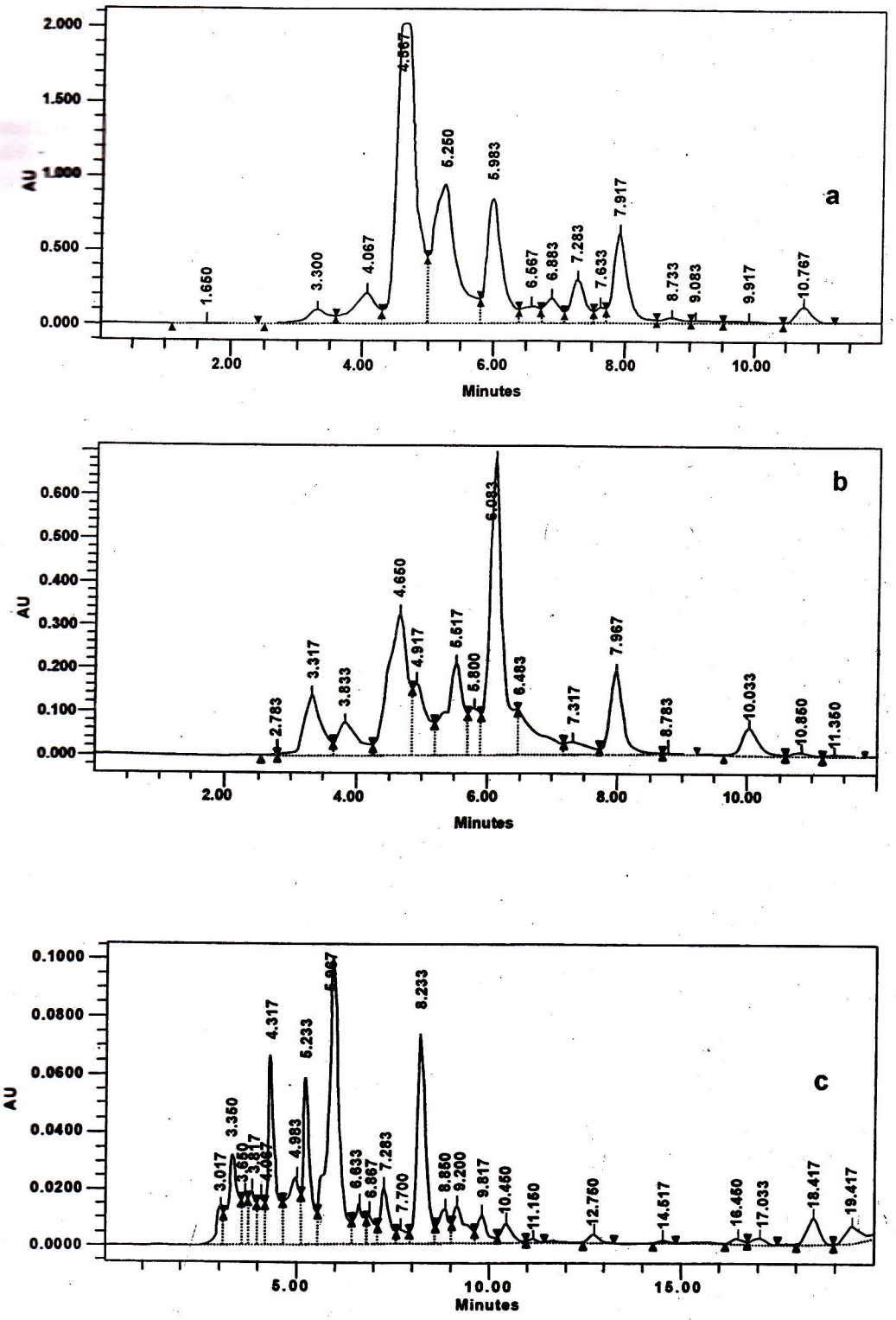

Fig. 1. a, b & c. RP-HPLC chromatogram showing the phenolic acids in the leaf samples of *Excoecaria agallocha* (a), *Lumnitzera racemosa* (b) and *Avicennia alba (c)*

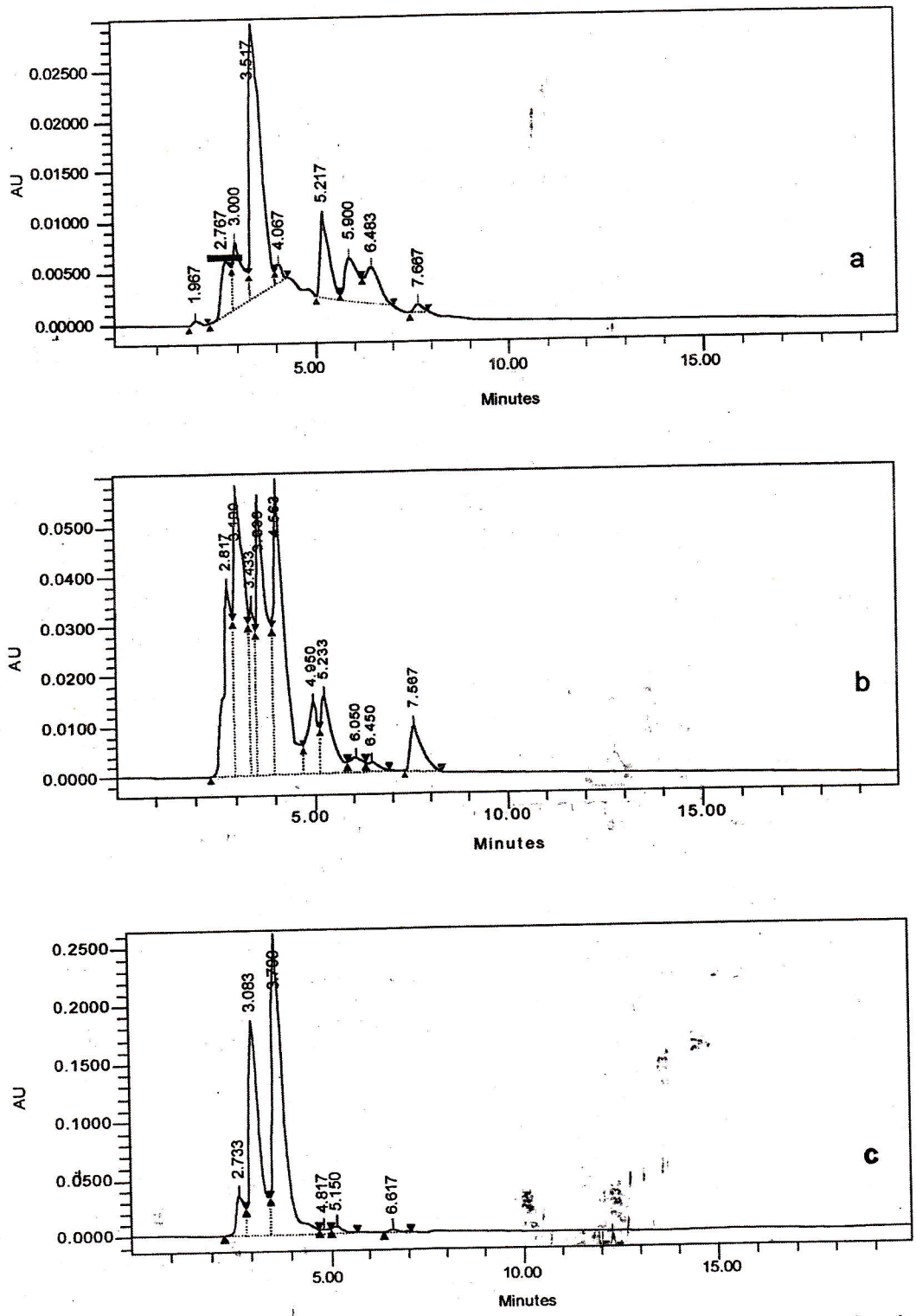

Fig. 2. a, b & c. RP-HPLC chromatogram showing the phenolic acids in the leaf samples of *Acanthus ilicifolius* (a), *Bruguiera cylindrica* (b) and *Rhizophora apiculata* (c)

phenylalanine, cinnamaldehyde, NADPH, guaiacol, hydrogen peroxide, were obtained from SD Fine Chemicals, India; other chemicals used were of highest purity.

Isolation and assay of lignin synthesis enzymes: Phenylalanine ammonia lyase (PAL), cinnamyl alcohol NADPH dehydrogenase (CAD) and peroxidase (POD) were extracted from leaf tissues and assayed[10-12]. Lignin was extracted and quantified spectrometrically at 280 nm using dehydroconiferyl alcohol polymerizate as standard[13]. Total phenol was estimated by the method of Mayr *et al*[14]. Phenolic constituents from the leaf tissues were fractionated by RP-HPLC following the method of Beta *et al.*[15] and quantified by using appropriate standards. Protein content was determined by Bradford method[16]. The data was analyzed by students't' test followed by ANOVA and the level of significance was expressed as $P< 0.01$.

Results and Discussion

Very little information is available regarding the lignin formation in mangroves based on their unique habitat. The total phenol of leaf samples exhibits remarkable variation between the taxa based on their habit. The semi terrestrial member *Excoecaria agallocha* and aquatic member *Avicennia alba* showed higher amount of than the other mangroves. The members *Lumnitzera racemosa, Acanthus ilicifolius* and *Bruguiera cylindrica* expressed a low profile of phenol at the range of 24 - 26 mg/g tissue. The waxing and waning pattern of phenols in mangroves was further investigated by fractionating the phenols by reverse phase high performance liquid chromatography (RP - HPLC). The figures 1a,b & c and 2a, b & c represent the HPLC chromatogram of phenolic extracts of leaf tissues of *Excoecaria agallocha, Lumnitzera racemosa, Avicennia alba, Acanthus ilicifolius, Rhizophora apiculata, Bruguiera cylindrica.* Phenolic acids such as cinnamic acid, caffeic acid, ferulic acid, sinapic acid, coumaric acid, hydroxy benzoic acid, chlorogenic acid, gallic acid and vanillic acid were used as standards for detecting the compounds. It is evident from the figures that phenolic extracts of leaf samples contain the peaks of most of the standards indicating the functional compartmentation of phenolic acids during plant growth (Table 1). The involvement of major phenolic acids viz. cinnamic, caffeic and ferulic acids in lignin synthesis can be interpreted effectively from the data. Higher amount of lignin was observed in *Excoecaria agallocha* than in other members can be correlated with the increased level of cinnamic and caffeic acids, the precursors of lignin synthesis. Meanwhile in members like *Lumnitzera racemosa, Acanthus ilicifolius* and *Bruguiera cylindrica* a negative correlation was observed between the phenolic acids and lignin content suggesting the minimal involvement of phenolic acids for the conversion to lignin. It is also interesting to note that in these members the enzyme CAD showed an increased level of activity, which again confirms the functional suppresion of the marker enzyme CAD from lignification. In control plants i.e., mesophytes a positive correlation can be observed between the CAD and lignin content indicating the specificity of the enzyme in lignification.

To ascertain the role of the three major enzymes PAL, CAD and POD in lignin synthesis can be checked by assaying the enzymes in mangroves, mesophytes and hydrophytes. The three enzymes showed a moderate level of activity in all the members of mangroves (Table 2). The pattern of enzyme activity in mesophytic members showed a profound difference from that of mangroves suggesting the terrestrial habitat of the group (Table 3). The significant increase in the activity of CAD, the indicator enzyme of lignin synthesis than PAL and POD reveals the affinity of the members towards land habitat. In contrary to the above groups the hydrophytes showed a low profile of CAD activity reflects the passive attitude of the members towards the terrestrial adaptation (Table 4). The statistical comparison of the enzymes activity between the plant members of each group was found significant at 1%level. The differential nature of the activity of the enzymes noticed in mesophytes and hydrophytes strongly suggest the inclination of the taxa towards their specific habitat. It further indicates the preferential action of the enzyme CAD towards the production of lignin based on the habitat of the groups. Being the initial enzyme of phenylpropanoid metabolism, the presence of PAL, in plant tissue becomes an indispensable part for the synthesis of phytoalexin, lignin and phenolics[5].The high profile of PAL activity in mangroves suggests the active phase of the trans - cinnamic acid formation the precursor of all secondary metabolites. Similarly the enzyme POD was involved in multi functions like the normal balance, ethylene biosynthesis, membrane integrity, respiration control, metabolic control of ripening and senescence of fruits, preparing responds to wounding, auxin metabolism and also in defense mechanism apart from the oxidative polymerization of Cinnamyl alcohol to lignin[17]. However the higher level of POD activity in mangroves than in mesophytes needs further clarification for establishing the physiological role of the enzyme. Apart from the normal functions of POD it is possible to explain the high level of POD activity in mangroves may be for scavenging the H_2O_2 the superoxide radical from the cell system for its survival and this also provides indirect evidence of the anoxia or hypoxia habitat of the ecological group. Blokhina *et al.*[18,19] had studied the response of anoxic stress towards hydrogen peroxide (H_2O_2) formation in plants like Rice, Wheat and Iris. On the other hand, the presence of CAD and its variation in members of different habitat needs

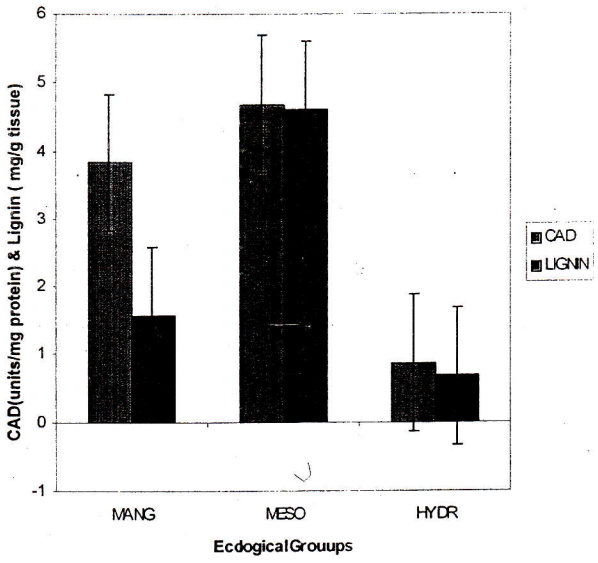

Fig. 3. Pattern of activity of the major lignifying enzymes such as PAL, CAD and POD in mangroves, mesophytes and hydrophytes.

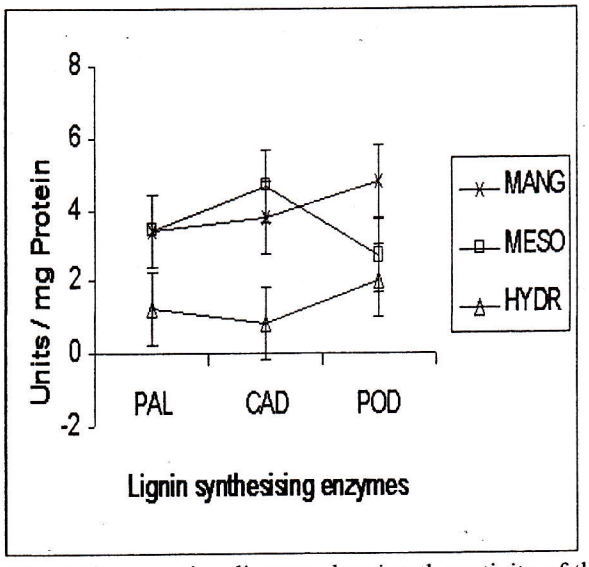

Fig. 4. Comparative diagram showing the activity of the marker enzyme of lignin synthesis CAD and the end product lignin in mangroves, mesophytes and hydrophytes.

physiological attention[11,20]. The moderate level of CAD activity in mangroves clearly points to the semi terrestrial adaptation of the members and this hypothesis is further supported by the differential action of CAD in other ecological groups i.e., high in mesophytes and low in hydrophytes. This physiological affinity of the enzyme CAD towards terrestrial adaptation by forming lignin unambiguously prove the influence of environmental stimulus towards the action of the enzyme based on their habitat[21-22]. Moreover the three enzymes express three different patterns in their activity (Fig. 3). In mangroves the enzymes expressed the activity more or less uniformly whereas in the mesophytes the graph appears 'triangular' with a sharp peak for CAD. In hydrophytes the CAD show a decline in its activity. Thus from the data it can be interpreted as the three enzymes PAL, CAD and POD displayed its functional diversity based on their habitat. The ratio of CAD: lignin was found significance at 1% level. The mean activity of the enzyme CAD was compared with the average lignin content in each group (Fig. 4). The lignin content showed a reciprocal relationship with the activity of the enzyme CAD in all the groups except in mangroves ie., high CAD activity and low lignin content suggesting the suppression of the enzyme under the *in vivo* condition in saline habitat[23]. The low CAD activity and lignin level in hydrophytes revealed the poor mechanical strength required by the plants for their survival in water. The unique activity of the enzymes in mangroves unlike the pattern observed in mesophytes and hydrophytes indicates that these groups of plants are so flexible in their nature that can be either varied to terrestrial / aquatic habitat by activation or suppression of the CAD enzyme. Thus it is possible to suggest that the CAD enzyme plays a crucial role in determining the adaptability of taxa either to aquatic or terrestrial habitat.

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