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Variations in the esterase activity during the germination period of *Jatropha curcas* seeds

T. Subramani[1*], K.C. Manjunath[1], K.S. Chandrashekharaiah[2], N. Ramachandra Swamy[1] and K.R. Siddalinga Murthy[1]

[1] Department of Biochemistry, Central College Campus, Bangalore University, Bangalore 560001, Karnataka, India
[2] Department of Biotechnology, PES Institute of Technology, BSK III Stage, Hosakerahalli, Bangalore 560085, Karnataka, India

Abstract
Germination brings out the synthesis or activation of enzymes responsible for the degradation of seeds reserves. Among these enzymes, esterases are involved in the metabolic processes of germination and maturation of plants. They are constitutively expressed in seeds during germination to release the reserve materials for the growing embryo. In the present study, total protein content and esterase activity was monitored in germinating *Jatropha curcas* seeds. The esterase activity and specific activity observed were 9.07 µmoles/min/gm and 0.09258 IU/mg, respectively. Electophoretic analysis for esterase activity showed thirteen bands of esterases, among these 8 esterolytic bands were major and remaining were minor bands. The protein content and esterase activity decreased on 2nd, 4th, 5th and 8th day of seed germination and activity increased on 3rd, 6th, 7th day of germination. Similarly esterase activity increased on 7th day and decreased on 8, 9 and 10th day in the shoot tissue.

Keywords: Esterase, *Jatropha curcas*, Germination, Endosperm.

INTRODUCTION

Esterases are a group of hydrolytic enzymes that catalyze the hydrolysis of various types of esters and are widely distributed in nature. They occur in multiple molecular forms and exhibit broad substrate and inhibitor specificity. The functions of esterases are multifaceted. They are involved in fruit ripening, abscission, cell expansion, somatic embryogenesis, stomatal movements, reproduction as well as detoxification of xenobiotics. Esterases also play a significant role in the metabolism and subsequent detoxification of many agrochemicals and pharmaceuticals [1, 2]. In particular, carboxylesterases hydrolyze pyrethroids [3-5] and bind stoichiometrically to organophosphates [6, 7]. Esterases are also important in the metabolism of a number of a therapeutics [8], including the cholesterol-lowering drug, lovastatin [9], the narcotic analgesic meperdine (Demerol) [10], cocaine and heroin [11].

Jatropha curcas is drought resistant, oil yielding plant, belonging to the family Euphorbiaceae and is a common plant in most arid areas of Asia, South America and Africa. The plant is a common shrub of 3 – 6 meter and medicinally the leaves are used as remedy against rheumatism and jaundice. The esterase enzyme activities increase with germination to release the reserve materials for the growing embryo [12]. The seeds are rich sources of oil and the oil obtained from the seeds is used up in the soap industry and also as source of energy [13]. The oil after transesterification can be used as substitute for diesel and the cake obtained after pressing seed can also be used as an animal feed stock or for biogas production [14, 15]. The *Jatropha* plant has attained economical importance due to these applications in areas with extreme climates and soil conditions because of its extraordinary high drought resistance [16]. Since *Jatropha* constitutes one of the potential sources of various phytochemicals and esterases might be involved in transesterification, detoxification and insecticide or pesticide scavenging activity. The present work was undertaken to study the variation in esterase activity and protein content during germination of *Jatropha curcas* seeds.

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*Corresponding Author

T. Subramani
Department of Biochemistry, Central College Campus,
Bangalore University, Bangalore 560001, Karnataka, India

Tel: +91 9632410585
Email: subbuccc11@gmail.com

MATERIALS AND METHODS

Materials

The seeds of *Jatropha curcas* were collected from Upparahally, Kolar District, Karnataka, India.

Germination study

The germination studies were carried out for 10 days. The endosperm was collected after every 24 hours interval and the shoot tissue were collected on 6th day of the germination. 10% enzyme extract was prepared and protein and esterase activity were determined as described below.

Preparation of acetone powder and enzyme extract

The acetone powder (10%) was prepared by blending soaked and dehulled seeds of *Jatropha* in chilled acetone for 1 -3 minutes, it was then filtered by suction pump, dried at 37°C and stored at 4°C until further use. A 10% extract of the acetone powder of *Jatropha* seeds was extracted using 50 mM sodium phosphate buffer pH 7.0

by stirring over a magnetic stirrer for 1 hr at 4^{0}C. The extract was then centrifuged at 10,000 rpm for 20 min at 4^{0}C. The supernatant was collected and used for qualitative and quantitative analysis of total protein and esterase activity.

Protein assay

The protein content in the enzyme extract and fraction was determined by the method of Lowry [17] using bovine serum albumin as standard.

Esterase assay

Esterase activity was monitored quantitatively according to the method of Gomori [18] with modification by VanAsperen [19]. A typical assay mixture contained 5 ml of 0.3 mM substrate solution (a stock solution of 30 mM 1-naphthyl acetate was prepared in acetone and diluted to 100 fold with 0.05 M sodium phosphate buffer, pH 7.0) and 1 ml of suitably diluted enzyme extract. The reaction mixture was incubated for 15 minutes at 27^{0}C and the reaction was arrested by the addition of 1 ml DBLS reagent (2 parts of 1% diazo blue B and 5 parts of 5% sodium lauryl sulphate). The reaction mixture was allowed to stand for 30 minutes and the intensity of the color formed was measured at 600 nm. A calibration curve was prepared using 1-naphthol.

Staining and Destaining of proteins

The proteins were stained on polyacrylamide gels, according to the method of Davis [20] using 0.5% solution of coomassie brilliant blue R-250 in 25% methanol and 7.5% acetic acid in water for 1 hour and was destained in 25% methanol and 7.5% acetic acid in water for overnight.

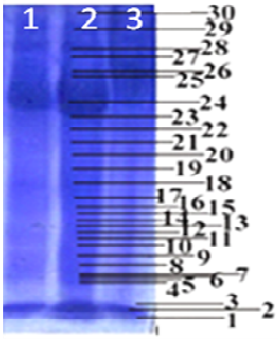
Staining for esterase activity

Esterase activity on polyacrylamide gels was detected essentially according to the method of Hunter and Markert [21]. Fast-blue RR was used for simultaneous coupling with 1-naphthol moiety released upon hydrolysis of 1-naphthyl acetate. The gels were removed from plates and stained for esterase activity using 100 ml of 0.05 M phosphate buffer pH 7.0 containing 40 mg of fast-blue RR and 20 mg of 1-naphthyl acetate (dissolved in 2 ml of acetone) for 20 minutes at room temperature.

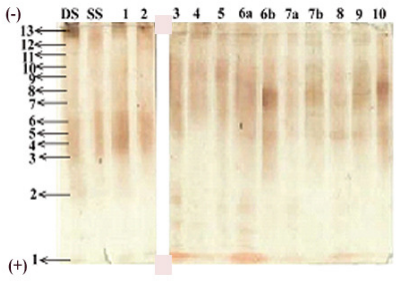
RESULTS AND DISCUSSION

A major concern of investigators interested in cellular differentiation is to discern the biochemical steps that accompany the development of cells, which includes continuous synthesis and / or degradation of specific enzymes and structural proteins. Two principal alterations encountered in most organisms are qualitative and quantitative changes in proteins (enzymatic activity) within their cells. The appearance of new or increased enzyme activity in a developing organism may results from either the *de novo* synthesis of the enzyme molecule or the activation of a pre-existing enzyme precursor.

10% acetone powder extract of *Jatropha* seeds was dissolved using 50 mM sodium phosphate buffer pH 7.0 by stirring over a magnetic stirrer for 1 hr and centrifuged at 10,000 rpm for 20 min at 4^{0}C. The supernatant was collected and used for qualitative and quantitative analysis of total protein and esterase activity. The esterase activity and specific activity for the extract was 9.07 µmoles/min/gm and 0.09258 IU/mg, respectively. There are 30 protein bands of which, there were 9 major bands (band no.1, 2, 3, 9, 12, 21, 24, 28 and 29) and remaining were minor bands (Fig.1). Electophoretic analysis for esterase activity showed thirteen bands of esterases (Fig.2). Among these, 8 esterolytic bands were major bands (band no 1, 3, 6, 7, 8, 11, 12 and 13) and remaining were minor bands. The esterase activity decreased on 2nd , 4th, 5th and 8th day of seed germination and activity increased on 3rd , 6th, 7th day of germination. Similarly esterase activity increased on 7th day and decreased on 8th, 9th and 10th day in the shoot tissue. Changes in the total esterase activity and total soluble proteins was quantitatively determined for the germinated samples and the results were as shown in Fig.3a and b. Similar observation was noticed during the germination of the seeds of Saguaro cactus *Carnegina gigantean* [22], suggested that the appearance of new esterolytic activity at a particular stage of germination could be either due to the activation of a pre-existing enzymes or de novo synthesis. The disappearance of existing bands and appearance of new bands are also reflection of shifts in the activities of specific esterase isozymes [23]. The increased activities and intensified bands of these enzymes may directly or indirectly be involved in the formation of specific biomolecules which act as precursors for the synthesis of essential components required for the developing tissue. Further, these enzymes being hydrolytic in nature may provide the necessary energy required for a growing shoot by mobilizing energy from the esters or fatty acid esters [24]. Multiple forms of esterases and their differential expression during embryogenesis and organogenesis suggested their important role in several physiological processes [25].



Lane 1: Dry seed; 2: Soaked seed; 3: Germinated seed of Jatropha curcas.
Fig 1. Electrophoretic profile of proteins from the seeds of *Jatropha curcas*



Lane: DS-Dry seeds, SS-Soaked seeds; Lane: 1-5, 6a and 7a endosperm esterase activity; Lane: 6b, 7b, 8, 9 and 10 shoot esterase activity.
Fig 2. Zymogram profile showing 1 to 10th day of esterase activity from the endosperm and shoot tissue of Jatropha curcas seeds.

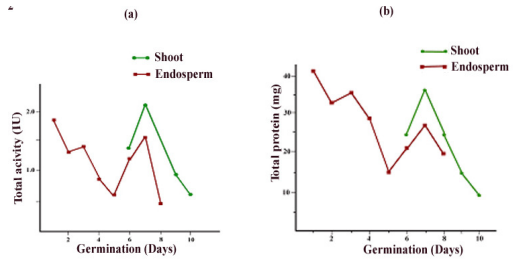


Fig 3. Changes in the total proteins and esterase activity during germination a) Esterolytic activity b) Total protein

CONCLUSION

The esterolytic activity during the germination period showed 8 major bands (band no 1, 3, 6, 7, 8, 11, 12 and 13) on all the days of germination, while the minor bands gradually disappeared up to fifth days of germination, but subsequently reappeared in the following days. The esterase zymogram of sixth, seventh and tenth day shoot tissue showed similar intensity like major bands of endosperms. Total protein content and esterase activity varied during the germinating period in *Jatropha curcas* seeds.

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