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Properties of lipase from Pearl millet seedling

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Abstract: In this study, lipase activity was assayed in four days old seedlings of Pearl millet by using olive oil emulsion as substrate. Lipase activity was found to be optimum at pH 5.5 and 8.5 with acetate and Tris - HCl buffer at 30˚C. The pH stability was found in the range of 7.5-8.5. Lipase activity was fairly stable up to 70˚C and completely lost at 100˚C within 30 minutes. Lipase activity was slightly increased in the presence of $CoCl_2$ and decreased by the addition of $MnCl_2$, $ZnCl_2$, $CaCl_2$, Triton X-100, and Tween 80. Pearl millet seedlings found most specific towards almond oil.

Key words: Lipase, Pearl millet, enzyme, *Pennisetum glaucum* L.
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INTRODUCTION

Lipase (triacylglycerol acylhydrolase, E.C.3.1.1.3) is the enzyme catalyzing the stepwise breakdown of the triacylglycerol into diacylglycerol, monoacylglycerol, glycerol and free fatty acids[1,2]. Lipases are probably rate controlling during germination and the activity of the lipase is high during germination[3]. In addition to their importance for proper growth of seedlings, plant lipases are enzymes that play an important role in various agro-industrial processes[4]. Lipase from origins has been found to have numerous applications in the food, cosmetics, detergents, leather, textile and pharmaceutical industries[5-10]. Due to the growing demand, lipase applications have been the driving force in lipase research during the last few years. In some plants lipases are not well known.

Pearl millet (*Pennisetum glaucum* L.) is largely grown for grain and fodder purpose under hot, dry conditions on infertile soils of low water holding capacity where other crops generally fail. Pearl millet as a food crop is limited to the developing countries in Asia, and particularly in Africa. It is estimated that over 95% of pearl millet production is used as food, the reminder being divided between animal and poultry feed (7%), other uses (seed, bakery products, snacks, etc.,) and waste. Total metabolizable energy of pearl millet is similar to corn. Pearl millet contains more calories than wheat, probably because of it's higher oil content of 4.2% which is 50% polyunsaturated. Research concerning the lipase of pearl millet has not been reported previously. The present research work was carriedout for the determination of lipase from the crude extract of germinating seedlings of pearl millet.

MATERIALS AND METHODS

The study was conducted at Department of Plant Physiology and Biochemistry, Sindh Agriculture University Tando Jam Pakistan. The pearl millet seeds were purchased from authorized seed dealers of Tando Allahyar Sindh (Pakistan). All reagents used were of analytical grade.

Plant material

The pearl millet seeds were washed with distilled water. The seeds were hydroprimed for 2 hours at room temperature and allow to germinate in plastic trays layered with, muslin cloth at 25˚C ± 1˚C in an incubator. The seeds were germinated up to 1, 2, 4, and 6 days. The seeds were harvested at required time period and washed with distilled water. The harvested seedlings were allow to dry and dry seedlings were defatted with diethyl ether at low temperature and residues were stored in a vacuum desiccators until it was used.

Preparation of soluble lipase from the defatted seedlings

The defatted seedlings were ground in 0.1M citrate buffer (pH 7.0) and centrifuge for 15 minutes at 7000 rpm.

Preparation of substrate emulsion

The olive oil substrate emulsion was prepared according to the method[11].

Measurement of hydrolysis activity of lipase

Lipase activity was measured by the olive oil gum arabic emulsion method[12]. A unit of lipase activity was defined as the amount of enzyme required to release one micromole of free fatty acids per hour under the assay conditions.

Protein assay

Protein was estimated according to method[13] using bovine serum albumin as standard.

pH and pH stability

The pH optimum was determined by performing activity assay in the ranges of pH 3-10.5 (acetate, citrate, tris-HCl and glycine-NaOH buffer). The pH stability of lipase enzyme was checked with different buffers (universal, citrate, tris-HCl and glycine-NaOH buffer) from 3-10.5. The desired buffer was reacted with enzyme for 15 minutes at 30˚C. The remaining activities were assayed by standard method using olive oil emulsion as substrate.

Thermostability

Thermostability was determined by pre-incubation of enzyme solution at various temperatures ranging from 30-100˚C at pH 8.5 for 15 minutes and then remaining activity was checked by standard method as described above.

Effect of metal ions and chemical reagents

The effect of chemical reagents (5mM) on lipase activity was investigated by standard assay method.

RESULTS AND DISCUSSION

The effect of germination on lipase activity of crude enzyme preparation of pearl millet seedlings during incubation with various time periods was increased up to 120 min. and slowly decrease up to 180 min. and then sharply decline (Figure 1). It was observed that the rate of enzymatic reaction increased with the increase of enzyme concentration up to 10% and then declined. The result is similar with the findings of Dahot *et al.,* and Khan *et al.*[14,15]. The decline in enzymatic rate of reaction after certain time period, may be suggested due to product inhibition[16] or side product of the reaction, which inhibits the enzyme or the effect of heat on the tertiary structure of the enzyme[17], or enzyme inactivation due to prolonged incubation[18] or presence of other enzyme in crude sample could also not be ruled out.

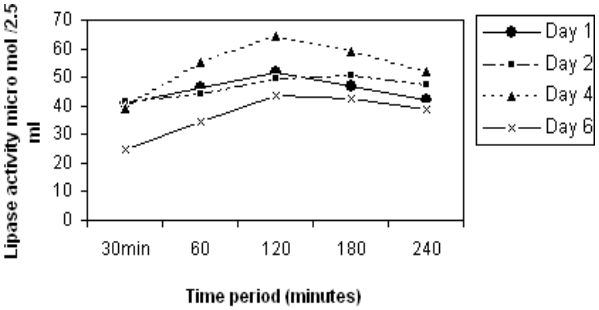

Figure 1: Effect of time period on lipase activity of Pearl millet seedlings.

The effect of enzyme concentration on the rate of reaction was carried out with different concentrations of enzyme sample ranging from 2-12% as shown in Figure 2. It is similar to lipase of *Carrisa carandas* fruit, Cowpea seedlings, which also showed highest activity at 10% enzyme concentration[19,20] .

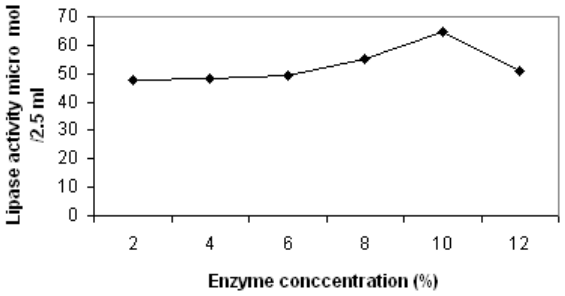

Figure 2: Effect of enzyme concentration on lipase activity of Pearl millet seedlings.

The effect of substrate concentration on the lipase activity of pearl millet seedlings was investigated using different concentration of olive oil emulsion ranging from 2-12% as shown in Figure 3. The rate of enzymatic reaction increased with the increase of substrate concentration up to 10% and then declined. It is similar to lipase of *Cajanus cajan L. (matri), Ceasalpinia bonducella L.,* Cowpea seedlings which also showed maximum activity at 10% substrate concentration [14,15,20,21]. The declination in the rate of reaction at higher concentration of substrate might be due to the change of enzyme substrate concentration ratio or enzyme inhibited by the excess of substrate concentration or change of physiochemical characteristic[15].

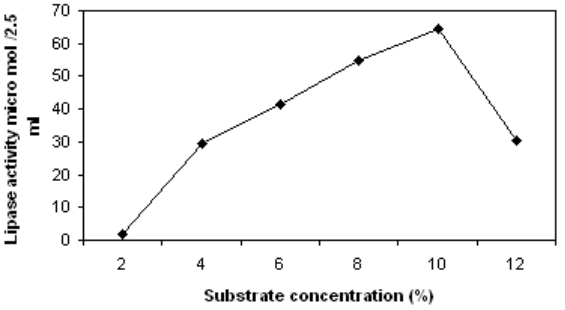

Figure 3: Effect of substrate concentration on lipase activity of Pearl millet seedlings.

The effect of pH on lipase activity of pearl millet seedlings was determined in the range of 3.0 to 10.5 using acetate buffer, citrate buffer, Tris-HCl buffer and glycine-NaOH buffer at 30°C as shown in Figure 4. Lipase activity was found to be optimum at pH 5.5 and 8.5 with acetate and Tris - HCl buffer. The result is in agreement with Zhong *et al,* [22] which was 5.5 in

corn seed. Theimer and Rosnitschek[23], Ejedegba *et al*, [24] and Yesiloglu and Baskurt[25], reported the same results pH 8.5 in rapeseed, coconut seeds and almond seed. Above and below optimum pH activity was declined. It may be pointed out that on the basis of optimum pH pearl millet seedlings lipase belongs to category of acidic lipase with acetate buffer and alkaline lipase with Tris-HCl buffer.

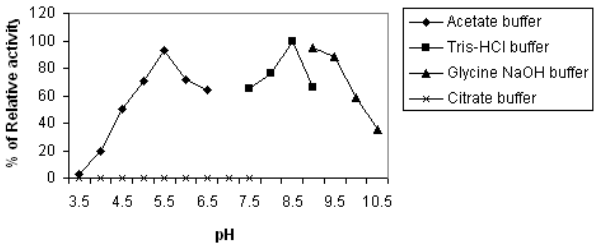

Figure 4: Effect of pH on lipase activity of Pearl millet seedlings.

The effect of pH on stability of the lipase was determined in the presence of various buffer solution of different pH value. Lipase activity of pearl millet seedlings was found stable over the pH range of 7.5-8.5 (Figure 5). These results are in agreement with Pahoja and Dahot[20]. All enzymes have an optimum pH range for their activities, which is often very narrow. The optimum pH does not depend only the nature and ionic strength of the buffer; in general it also depends on the temperature and substrate concentration[26,27].

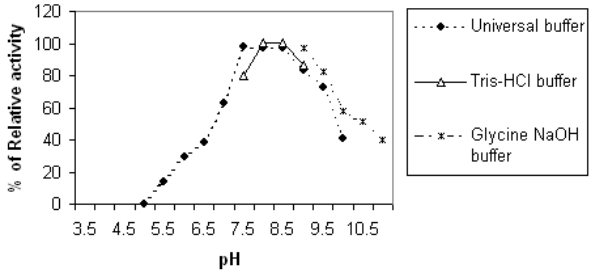

Figure 5: Effect of pH stability on lipase activity of Pearl millet seedlings.

A definite optimum pH for enzyme activity is usually observed because, like other proteins, enzymes have a number of ionisable groups so that pH changes may modify the conformation of the enzyme, the binding of the substrate and the catalytic activity of the groups present on the active site of the enzyme. It also means that pH affects the enzyme stability and loss of enzyme activity depends on the length of time when the enzyme has been maintained at the unfavorable pH such that the optimum operational pH is generally a compromise between the effects on enzyme activity and enzyme stability[28]. At points corresponding to pH 7.5 and 8.5, the activity was measured with different buffers. In each case, the results obtained almost overlapped, indicating that, as expected, a change of buffer made no difference.

The temperature profile indicates that the optimum temperature was observed at 30°C and then declined with the increased of temperature (Figure 6).

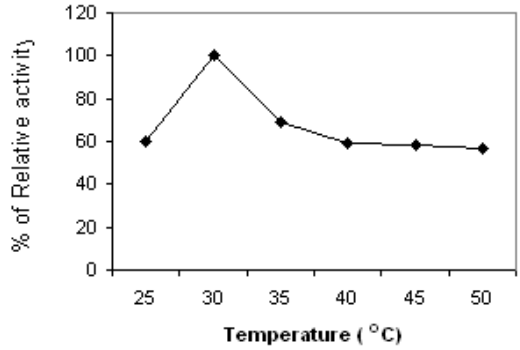

Figure 6: Effect of temperature on lipase activity of Pearl millet seedlings.

It is similar to lipase of *Cajanus cajan* L. (matri), Caissa carandas fruit, Ceasalpinia bonducella L., Africa bean seeds and cowpea seedlings which was also 30°C[14,15,19,20,21,29]. The lipase activity is fairly stable up to 60°C and retains 87% activity, whereas, 28% lipase activity was remaining up to 100°C within 15 minutes (Figure 7). Increasing temperature has two separate effects on enzyme, which operate simultaneously; one is increasing the catalytic activity, the other is destruction of the enzyme, producing a continuous fall in the concentration of the active enzyme, which is considered to be due to protein denaturation[30].

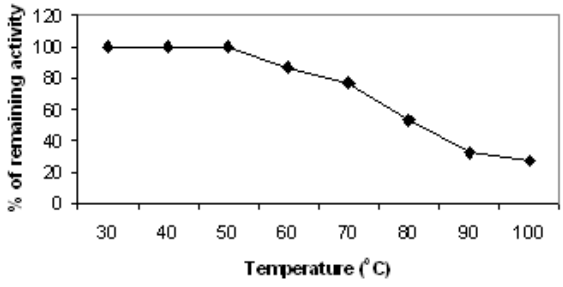

Figure 7: Effect of heat stability on lipase activity of Pearl millet seedlings.

The effects of various metal ions and reagents on lipase activity of pearl millet seedlings are shown in table 1. The lipase activity was slightly increased with the addition of $CoCl_2$. While the rate of reaction is decreased by the addition of $MnCl_2$, $ZnCl_2$, $CaCl_2$, Triton X-100, Tween 80, EDTA, sodium dodecyl sulphate, o-phenanthroline, mercaptoethanol, silver

nitrate and mercuric nitrate. Similar results are reported in Carissa carandas fruit by Mala and Dahot[19], in *Caesalpinia bonducela* L. seeds, by Pahoja *et al.*,[21] and in cowpea seedlings, by Pahoja and Dahot,[20]. The slightly increase in the rate of reaction in the presence of $CoCl_2$ may be due to the presence of already optimum concentration of Co^{+2} in the crude sample of pearl millet seedlings. The inhibition with metal chelating agents may be suggested due to metal dependent enzyme.

Table 1: Effect of various metal ions/reagents in 5mM concentration on lipase activity of pearl millet seedlings.

Reagent added	Relative (%)	Activation/[Inhibition] (%)
Control	100	
Sodium deoxycholate	90.55	9.45
Sodium dodecyl sulphate	88.97	11.03
Mercuric nitrate	62.20	37.8
Silver nitrate	79.52	20.48
o-phenanthroline	91.33	8.67
EDTA	86.61	13.39
Cysteine	85.03	14.97
cobalt chloride	102.36	2.36
Mercaptoethanol	62.99	37.01
Calcium chloride	81.10	18.9
Manganese chloride	68.50	31.5
Zinc chloride	81.10	18.9
Triton X-100	65.35	34.65
Tween 80	86.61	13.39

The lipase activity of pearl millet seedlings against different substrates was mostly specific towards almond oil (Figure 8).

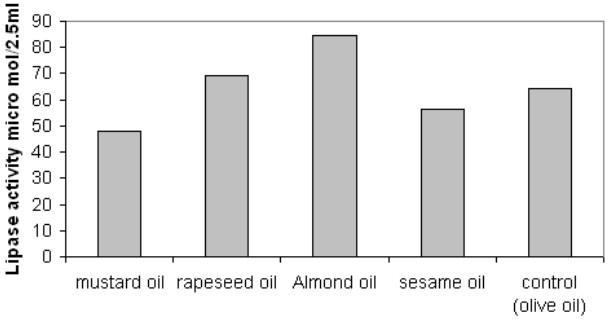

Figure 6: Effect of substrate specificity on lipase activity of Pearl millet seedlings.

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