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LIPASE ACTIVITY IN COTTON SEEDLINGS

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A survey of the literature revealed that only a limited amount of information was available concerning the lipase of cotton seeds and seedlings (Bamann and Ullmann, 1940; Olcott and Fontaine, 1941b; Ramakrishnan and Nevgi, 1951). Since more detailed information on this enzyme was required in connection with another study which was in progress, it became necessary to determine certain characteristics of this enzyme in the cotton plant.

Material and Methods

Cotton seeds delinted with acid were germinated in rolled paper toweling in darkness for various periods of time; the seed coats were then removed from the seedlings, and the seedlings were dried in a vacuum desiccator over sulfuric acid at room temperature. Dehydration was normally complete in three to four days, after which the seedlings or seedling fractions were ground in a mortar. The meal was extracted with diethyl ether in a Soxhlet apparatus for 24 hours, after which it was reground to pass a 100-mesh screen. Embryos from ungerminated seeds were ground and extracted with ether in a similar manner. Seeds of Stoneville 2B variety of *Gossypium hirsutum* were utilized throughout this investigation.

The lipase activities of the preparations were assayed by a modification of the method of Olcott and Fontaine (1941b). To weighed samples of the cotton preparations in 50 ml. Erlenmeyer flasks were added 1 ml. of cottonseed oil (Wesson oil), 3 ml. of distilled water, and 1 ml. of pH 6.8 phosphate buffer solution (0.1M). The flasks were stoppered and shaken on a mechanical shaker for 16 hours at 30 $\pm$ 1°C. At the end of this time 15 ml. of isopropanol-petroleum ether (2:1) mixture were added to each flask and the contents titrated with 0.1N sodium hydroxide, using phenolphthalein as the indicator. The percentage of Wesson oil hydrolyzed was used as an index of enzyme activity. Appropriate blanks were run with all experiments. Forty milligrams of desiccator-dried preparations of whole cotton seedlings were used in all instances, unless otherwise indicated. This material contained approximately 5.7% water; thus these samples represented only about 37.7 mg. of oven-dried preparation.

In the assays the oil was added to the preparations before the aqueous material, for it was observed that at pH 6.8 this procedure consistently resulted in slightly higher percentages of oil hydrolyzed.

Data and Discussion

It was found in this study that enzyme activity of preparations from different seed lots tended to vary considerably. Also it was noted that upon storage, the preparations

lost some of their activity. These factors, at least in part, account for differences that may be observed in the maximum activity indicated for the various experiments. It was observed, however, when experiments were repeated with different enzyme preparations, that though the levels of activity varied, the trends were essentially the same. This is the reason why in certain instances the data presented represent mean values while in others the data from a single representative experiment are given.

Seedling Growth and the Effect of Seedling Age on Lipase Activity.—As the seeds germinated and the seedlings continued their growth, it was observed that the oil content and percentage dry weight decreased, whereas the free fatty acid content and lipase activity increased (Table 1, Fig. 1). The apparent increase in oil content on the second day cannot be explained, although it was observed to occur consistently in this investigation. It may have been that, with germination, certain oil components became extractable which prior to germination were not. This apparent increase in oil content could not be accounted for through loss in dry weight of the seedling.

The lipase activity of the seedlings increased for about four days after which time there appeared to be little change (Fig. 1). This stabilization of lipase activity is also indicated in the reduction in the rate of free fatty acid production in the cotton seedlings after the fourth day (Table 1). It is not known whether or not the development of the large amounts of free fatty acids accounted for the lack of any additional increase in lipase activity.

These data appear to be in agree-

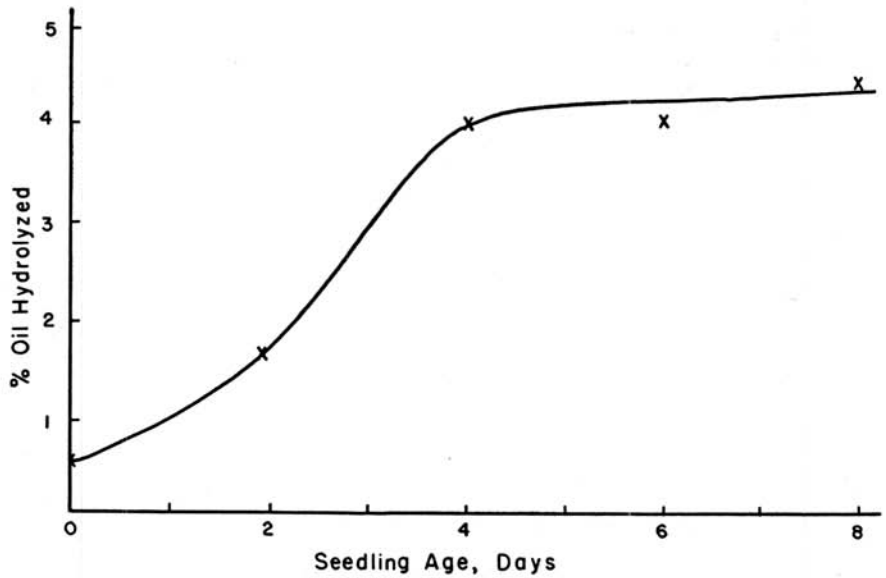

FIG. 1.—Effect of age on lipase activity in cotton seedlings; points represent means of three experiments.

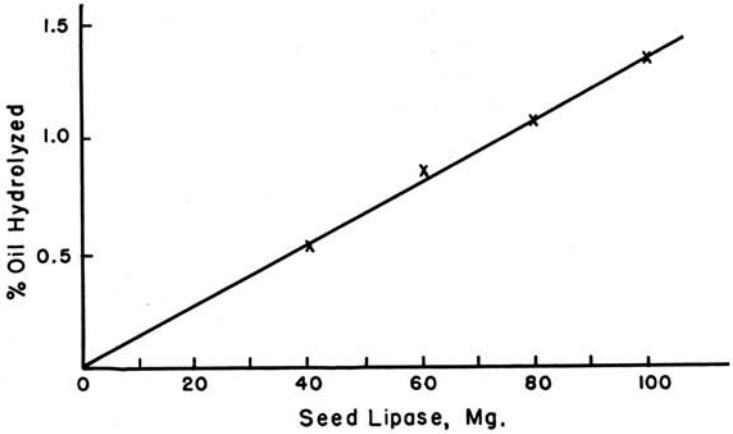

FIG. 2.—Lipase activity of ungerminated cotton seed.

ment with those of Olcott and Fontaine (1941a, 1941b) except that these workers were unable to demonstrate lipolytic activity in the preparations from dormant seeds. Although in this investigation the lipase activity of dormant seeds was very low, it was observed in six separate experiments. Because of the lack of agreement between the data of the above workers and those of the present study, an experiment was performed in which the concentration of the enzyme was varied (Fig. 2). These data indicated that the reaction velocity was essentially directly proportional to the enzyme concentration. This was assumed to be indicative of enzymatic hydrolysis of the substrate, for a similar reaction velocity was observed for cotton seedlings (Fig. 3). The possibility exists, of course, that acidic substances were merely leaching out

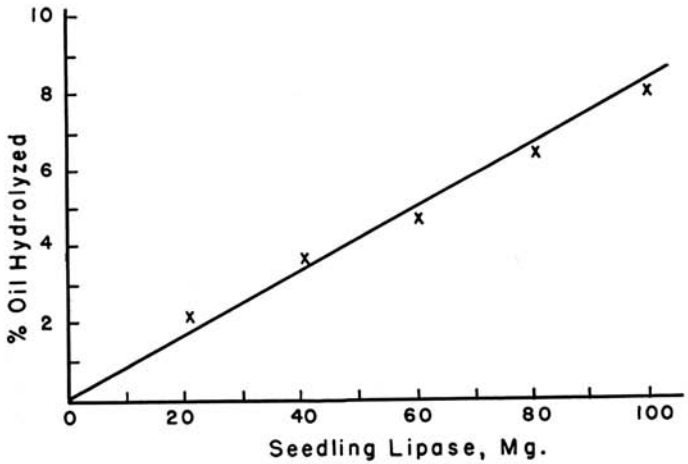

FIG. 3.—Lipase activity of 10-day-old cotton seedlings, assayed at 22° C. for 4 hours.

of the enzyme preparation and that with increased amounts of preparation more leachable acids were present. This seems to be only a remote possibility, however, for blanks in which the enzyme had been destroyed were run with all preparations. Bamann and Ullmann (1940) using a different assay method from that indicated here observed slight lipase activity in dormant seeds of another cotton species, *G. herbaceum*. Ramakrishnan and Nevgi (1951) also working with *G. herbaceum*, and utilizing an assay method very similar to that used by the author, reported relatively high lipase activity in dormant seeds.

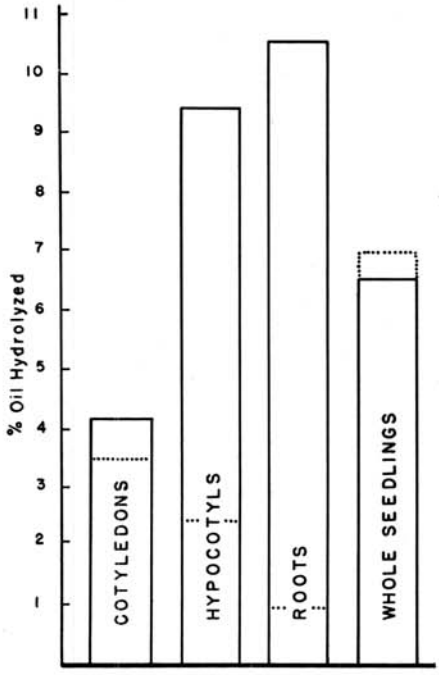

FIG. 4.—Lipase activity of various fractions of ten-day-old cotton seedlings; levels represented by solid lines are activities in 40-mg. samples; levels represented by dotted lines are activities in one seedling, mean of two experiments.

Lipase Activity of Seedling Fractions.—In the experiments represented in Figure 4, ten-day-old seedlings were fractionated into cotyledons, hypocotyls, and roots before drying. On a unit dry weight basis, the roots were observed to exhibit the greatest activity of the various fractions (Fig. 4). The activity of the cotyledons was only 39.2 and 43.7% and that of the whole seedlings 61.6 and 68.8% of that shown by the roots and hypocotyls, respectively.

On the other hand, consideration of the total lipase activity of the various tissues of an individual seedling showed the greatest amount of lipase to be in the cotyledons. It was calculated that only 1.2 pairs of cotyledons were required to make the 40 mg. assay sample whereas 11.1 roots and 4.0 hypocotyls were needed. Therefore, on a total activity basis, an individual root contained only 25.6% and a hypocotyl 64.5% of the activity found in the cotyledons (Fig. 4). Since a dried ten-day-old seedling in these experiments weighed approximately 47.5 mg., the total activity of a single seedling was in excess of the 40 mg. assay sample of whole seedling preparation (Fig. 4). It is interesting to note that values for the hypocotyl remained intermediate between those for the cotyledons and root, whether activity calculations were made on a per unit dry weight basis or on a per hypocotyl basis.

Effect of pH on Lipase Activity.—In this study the preparations of cotton seedling lipase were most active in the pH range 6.1 to 7.0. The data indicated in Figure 5 were typical of several experiments in which

TABLE 1.—Changes Occurring During the Growth of Cotton Seedlings in the Dark.

Number of days	Hypocotyl-root length[1] mm.	Dry weight[2] %	Oil content[2] %	Free fatty acids of oil[3] %
0	3.3	100.0	37.1	0.8
2	10.8	42.5	39.3	8.9
4	39.7	19.8	34.9	33.2
5	51.7	14.6	27.4	29.0
6	69.9	11.8	20.5	40.9
7	82.2	10.7	16.3	43.0
9	88.8	9.6	17.1	50.5

[1] Mean of 100 measurements.
[2] Based on seedlings dried in a vacuum desiccator over sulfuric acid.
[3] Expressed as oleic acid.

the effect of pH on hydrolytic activity of lipase was determined. The optimal pH reported here is in fair agreement with that reported by Olcott and Fontaine (1941b). Bamann and Ullmann (1940) indicated a slightly higher pH (8.5-10.5) as being optimum in their experiments with *G. herbaceum* seed. As pointed out by Olcott and Fontaine, however, the optimal pH tends to shift to the alkaline range when calcium is add-

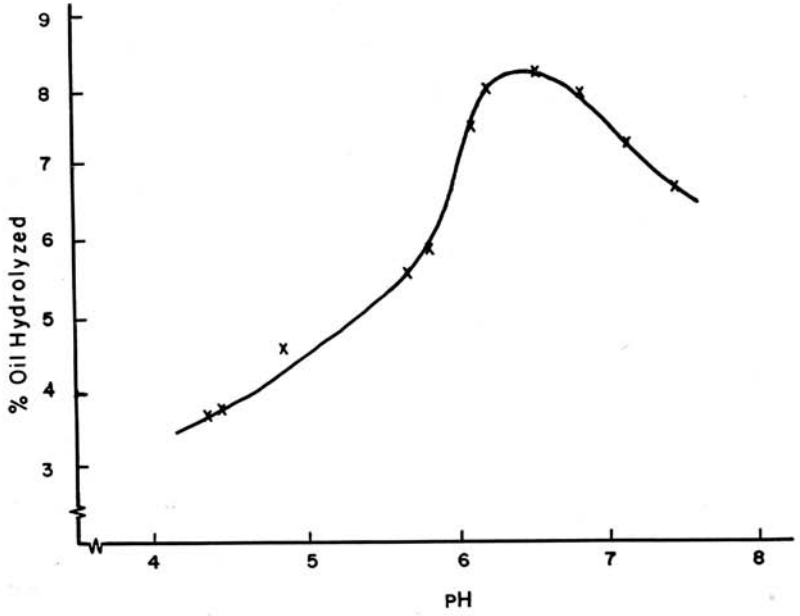

FIG. 5.—Effect of pH on lipase activity in cotton seedlings; acetate and phosphate buffers utilized.

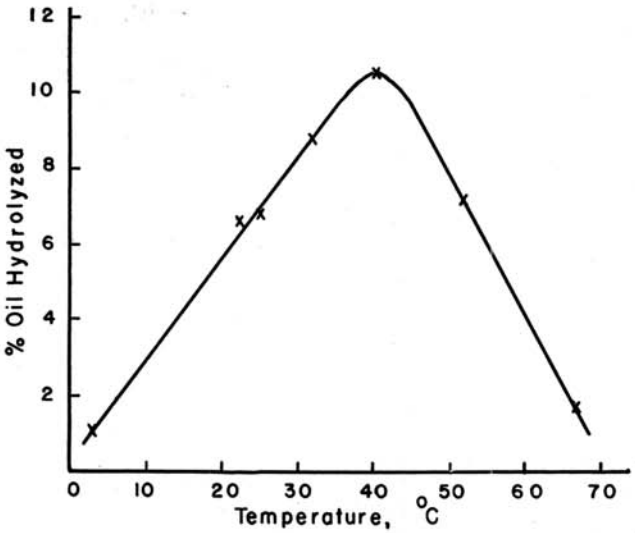

FIG. 6.—Effect of various temperatures on lipase activity in cotton seedlings; lipase preparation from four-day-old seedlings.

ed to the assay mixture. Several authors (Bonner, 1950; Ramakrishnan and Nevgi, 1951; Singer and Hofstee, 1948) have reported an optimal pH for lipase activity in cotton seed in the same range as that for castor bean seeds (pH 4.7-5.0). I feel that at least one of these reports (Singer and Hofstee, 1948) is based on a misinterpretation of the data of Bamann and Ullmann (1940). Since Bamann and Ullmann pointed out that the optimal pH for castor bean lipase was 4.7 when obtained from ungerminated ripe seeds, as opposed to 6.8 when prepared from germinating seeds, it was thought perhaps this might explain the variance between the author's results with seedlings and those of Ramakrishnan and Nevgi (1951) with ungerminated seeds. A check of the effect of pH on ungerminated cotton seed lipase, however, failed to reveal any significant difference in pH optima between seeds and seedlings.

Preliminary experiments seemed to indicate that the decreased lipase activity at acid and alkaline pH's was the result of enzyme destruction rather than a direct effect of pH on enzyme activity.

Optimal Temperature for Lipase Activity.—Although the majority of the lipase assays in this investigation were performed at 30°C., this was found not to be the optimal temperature for its action. As shown in Figure 6, the optimum appeared to be nearer to 40°C.

ACKNOWLEDGMENTS

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SUMMARY

Stoneville 2B cotton, *Gossypium hirsutum*, was germinated and grown in the dark; the lipase activity of the seedlings at various ages was studied. It was observed that as the seedlings became older the free fatty acid content and lipase activity increased, whereas the oil content and percentage dry weight decreased. The lipase activity increased to a maximum at about four days, after which it remained relatively constant. Slight activity was observed in dormant seeds. The highest lipase activity on a dry weight basis was observed in the roots. On a per seedling basis, the greatest total amount of lipase was in the cotyledons. The lipase preparations from whole seedlings were found to be most active in the pH range 6.1 to 7.0. Optimal temperature for lipase activity in cotton seedlings was approximately 40°C.

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