Effect of Growth Regulators on Lipase Activity of Peanut During Germination

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Abstract

Lipase activity is affected by the growth regulator treatments prior to germination of the seeds. The order of magnitude of this effect is as follows: Suc > MH ≃ AA > Sul > DW ≃ GA > control. The effect induced by the growth regulators is explained on the basis of interfering effect of the regulators with the functions of mitochondria. The lipase activity of all the seeds is found to increase with the period of germination reaching maximum values on different periods. The increase of lipase activity is not only dependent on the plant physiology, but other metabolic products such as ascorbic acid oxidase and amino acids produced during germination also play a great role on its production in oil-bearing seeds.

Introduction

During the last ten years the effect of chemical treatments on the physiology of plants and germinating power of seeds has been extensively studied. On account of different treatments, biochemical characteristics are influenced to a great extent.

Shabetai and Kamal (1) have studied the effect of hot air and chloropicrine treatment on lipase activity of Egyptian cotton seeds. They reported that dormant hot air treated seeds showed no enzyme activity at all even with activators, but on germination the development of the activity followed the same increasing trend in the treated and the untreated seeds. Rimon (2) has reported the effect of light, thiourea and Coumarin on the neutral and acid lipase of germinating lettuce seeds.

Black and Altschul (3) observed a 2.5 fold increase in the lipase activity on treatment of the distal halves of cotton seeds with gibberellic acid. They also reported that the development of GA-induced lipase activity could be completely inhibited with actinomycin-D or aflotoxin. Dieckhoff and Koch (4) noted that sulphonamides inhibit lipase activity.

Previous work (5) reported from these laboratories on lipase activity of γ-irradiated peanut indicated that higher dosage levels caused damage to the active centers. Increase in the lipase activity has been interpreted on the basis of other metabolites.

A little information is available on the effect of pre-sowing chemical treatment on the enzyme activity and its relation to metabolic by-products. Hence the present work has been undertaken to study the effect of growth regulators on lipase activity during germination and to search for a relation between lipase activity and the other activities or metabolites.

Experimental Procedures

Treatment and Germination

A special variety AK-12/24 MP of peanut (*Arachis hypogaea*, L.) was used for the work. The following growth regulators were used for the study. The concentration mentioned for each growth regulator is based on the preliminary experiments and on the literature, so as to have specific physiological changes in the plants: gibberellic acid (GA), 30 mg/100 ml; ascorbic acid (AA), 15 mg/100 ml; sucrose (Suc), 6%; maleic hydrazide (MH), 3×10^{-4} M; sulphanilamide (Sul), 1×10^{-2} M; and glass distilled water (DW).

In the case of each treatment, 30 ml of test solution were taken in a glass-stoppered conical flask containing 100 g of seeds. Two replications were preferred for each treatment. The content of the flask was shaken for about 5.5 hr on an electrically operated rotary shaker. During the shaking period the flasks were inverted periodically to have uniform soaking. The test solution was completely absorbed by the seeds during this period. The treated seeds in each case were allowed to dry at room temperature till they attained the original weight. The control seeds were given similar treatment with glass distilled water (DW) to verify the effect induced by the growth regulators alone.

The treated and the control seeds previously weighed individually, were germinated in sterilized sand, under laboratory environments at 28 ± 1 C. Distilled water was added daily in a measured quantity to the germinating seedlings. The periods of germination selected were: 0, 1, 3, 6, 10, 14 and 18 days.

At the end of each period four sets each consisting of eight seedlings from each treatment were removed from the sand, cleaned with cold distilled water and used for the extraction of the enzyme source.

Preparation and Estimation of Enzyme

The method of extraction of the enzyme source and that for the estimation of the lipase activity in the present work is essentially the same as that described by Patel et al. (5).

Lipase Assay

Freshly extracted peanut oil was used as a source of glyceride. One gram of oil was taken in a glass-stoppered Erlenmeyer flask and 5 ml of M/15 phosphate buffer ($Na_2HPO_4 + KH_2PO_4$) having pH 8 and 2 ml distilled water were added. The contents were stirred for 5 min for thorough mixing, then 0.100 g of the test material was added with vigorous stirring. Hydrolysis was carried out for 24 hr at about 28 ± 1 C. During hydrolysis the contents of the flask were shaken continuously on a rotary shaker. At the end of hydrolysis 10 ml of neutral 1:1 ethanol-ether mixture were added. The liberated fatty acids were titrated against 0.10 M NaOH. The determination of the blank was also carried out with the only difference that the test material was added after 24 hr. Lipase activity has been expressed in terms of ml of 0.10 M NaOH after subtraction of the blank. From the weight of the total test material, the activity per gram of the original seeds was calculated. The results are shown in **Figure 1.**

TABLE I

Lipase Activity of Groundnut

Treatments	GA	AA	Suc	MH	Sul	DW	Control
Periods[a]	10	6	6	14	14	10	10
Activity[b]	12.9	13.0	12.0	15.5	14.1	13.8	15.2

[a] Period of germination in days for maximum activity.
[b] Maximum activity for the specified period, expressed in terms of ml of 0.1 M NaOH.

Results

The results on lipase assay of two replications and amongst the four sets of each treatment showed a relative error of 5%. The results of Table I and those in Figure 1 are the average of the four sets involved for each treatment. It is noted (Fig. 1) that before germination, the lipase activity of the control seeds is slightly higher than that of the treated seeds. Further it is noted that the lipase activity is affected to an appreciable extent by chemical treatments under investigation.

Lipase activity of the control seeds and the seeds treated with GA and DW has been found to decrease on the first day of germination. Thereafter, it gradually increased reaching a maximum value in each case on the 10th day of germination. Then there was a decline in the activity.

The treatments of seeds with AA, Suc, MH and Sul resulted in gradual increase in lipase activity on germination of these seeds, the maximum being reached at different periods of germination (Table I). The highest lipase activity observed at different periods of germination is found to vary in the following trend: MH seeds $\simeq$ Control seeds $>$ Sul seeds $\simeq$ DW seeds $>$ AA seeds $\simeq$ GA seeds $>$ Suc seeds.

Discussion

Results of the present investigation indicated that the chemical treatments prior to germination have pronounced effect on the lipase activity. The activity of the control seeds dropped from 10 to a low level of 4.3 when the seeds were treated with Suc even before germination. The order of magnitude of this effect was as follows: Suc treatment $>$ MH $\simeq$ AA $>$ Sul $>$ DW $\simeq$ GA $>$ Control. The initial effect of the growth regulators could be explained on the basis that these growth regulators also interfere with the functions of mitochondria as pointed out by other investigators (5,6).

The activity of the control seeds and the seeds treated with GA, and DW decreased to a certain level on the first day of germination. In cotton seeds similar decrease in activity has also been noted by the other investigators (1). Previous work (5) on the lipase activity of γ-irradiated peanut showed a similar decrease in activity during early stages of germination. The rate of change of activity could be ascribed to varied effect of growth regulators on the plant-cells including vacuoles which consist of water solution of inorganic anions and cations as well as sugars and organic acids. Gula (7) reported that dehydroascorbic acid considerably raised lipase activity. It may be proposed that higher activity of AA treated seeds may be due to the partial conversion of ascorbic acid into dehydroascorbic acid. From the third day onwards the lipase activity of the control seeds and the seeds treated with GA and DW was found to increase gradually reaching a maximum value in each case on the 10th day of germination. However, the activity of GA treated seeds for this period was lower than that of the control or DW treated seeds. In

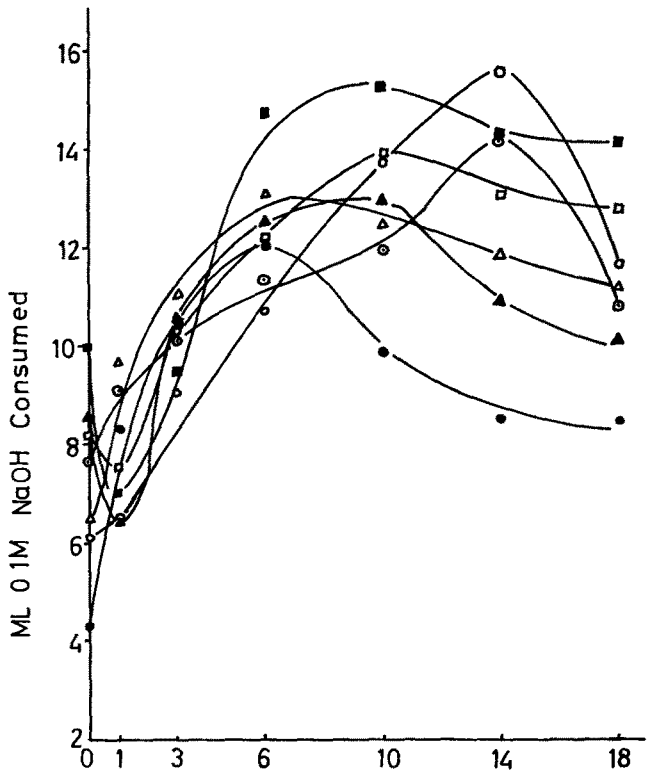

FIG. 1. Variation in lipase activity with the period of germination. Treatments: ▲ GA, △ AA, ● SUC, ○ MH, ⊙ SUL, □ DW, ■ Control.

general the GA treated seeds were found to have low level of the lipase activity, a high level of amylase and ascorbase activities and also high amount of reducing sugars and free amino acids with almost the same amount of the neutral oil utilization as compared to the corresponding quantities (8) of the DW treated seeds for the same period of germination. The proportions of these metabolic products allow us to interpret that the energy necessary for the growth of GA seedlings is not mainly derived from fat metabolism but also through other processes and hence lipase activity is low.

The lipase activity of the control seeds was higher than that of other seeds. This is because there is no adverse effect of the soaking and drying process on the function of mitochondria. The higher lipase activity may be due to the more active metabolic state of the control seedlings. The higher lipase activity is responsible for the rapid utilization of the fat. The rate of fat metabolism in the control seedlings was faster than that of the seeds treated with the growth regulators and DW (8).

The lipase activity of the AA and Suc treated seeds attained maximum values on the 6th day of germination. These values are less than those of the control and DW treated seeds and almost equal to that of GA treated seeds. The moisture content of the seeds treated with Suc was lower than that of the AA treated seeds. The study on plant physiology indicated an inhibiting effect of Suc on the growth. Ascorbic acid oxidase (AAO) activity of the Sul treated seeds was higher than that of the AA treated seeds (8). This might result in higher proportion of dehydroascorbic acid (9) in the Suc treated seeds than AA treated seeds. Thus lower value of lipase activity at maxima may be due to the inhibiting effect of Suc but the early attainment of maxima may be due to high proportion of dehydroascorbic acid on the 6th day of germination. In AA treated seeds, the

higher percentage of moisture, the remarkable growth and low AAO activity compared to Suc treated seeds (8) have resulted in high value of lipase activity at the maxima on the 6th day.

The seeds treated with MH and Sul showed a regular increase in lipase activity and attained maximum values on the 14th day of germination. MH and Sul are considered to be inhibitors because of anti-auxin nature. MH and Sul may change the auxin level in these seeds during germination and thereby affect the function of mitochondrial activity. The effect induced by these regulators is not severe because of their low concentration employed in the present work. The effects induced are similar to those induced by lower dosage levels (10–30 kr) of γ-rays as observed in the previous work (5). It may be assumed that the damage or dormancy of the active centers caused by MH and Sul is so little that as germination proceeded the dormant centers got reactivated due to the effects of other metabolic products formed during the growth. The amino acids have been reported to increase the stability of the lipase enzyme (10). The biochemical activity like AAO affects the production of dehydroascorbic acid (9) during the growth of seedling. The dehydroascorbic acid is reported to raise the lipase activity (7). The maximum value of lipase activity obtained in these seeds on the 14th day may be due to the resulant effect of such factors.

In all these treatments with growth regulators, the lipase activity is found to diminish after reaching maximum value. This is the general characteristics observed also in other oil bearing seeds (1,5,11,12).

Even though there is a marked divergence in the behavior of the lipase of AA treated seeds from that of the control and GA treated seeds, root length and total height of the plants do not show appreciable variation. The lipase of AA and Suc treated seeds shows similar characteristics even though their physiological changes are remarkable (8). Hence the observations of the present work confirm the views (5) that plant physiology might not be only criteria for the increase of lipase activity and vice versa. Other metabolic products produced during germination may also be playing a great role in the production of the lipase in oil bearing seeds.

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