Behaviour of Lipase Activity of the Gamma-Irradiated Groundnut During Germination

G. M. PATEL, D. N. VYAS and K. C. PATEL, Department of Chemistry, Sardar Vallabhbhai Vidyapeeth (University), Vallabhvidyanagar, Gujarat State, India

Abstract

Lipase activity of gamma-irradiated groundnut has been reported. The dosage levels: 10, 30, 50, 70, 90 and 120 Kiloroentgen (kr) units have different effects on lipase. Radiation levels of 50 kr units and above induced damage to the active centers. Lipase activity was found to decrease during the initial stages of germination, and to increase in later stages. After reaching maximum value, the activity decreased. The lipase of control seeds doubled during germination in light. Increase in irradiated seeds was about 1.5-fold. Maximum activity for seeds treated under different conditions was attained at different periods of germination. Plant growth and the behaviour of lipase has been explained on the basis of other metabolic factors such as: ascorbic acid oxidase, amino acids and free fatty acids liberated during germination, and their mutual effects.

Introduction

LIPASE ACTIVITY plays a major role in liberation of fatty acids from the glycerides (1). It has been further shown that the lipase activity of oil-bearing seeds increases during the course of germination. Wetter (2) for example, has noted that rapeseed possessing very little activity in dormant condition, showed a remarkable increase (100-fold) in lipase activity after 3 days of germination. The activity was found to decrease later in the germination period. Similar observations have been made by Shabetai and Kamal (3), working with cotton seeds. They found that the enzyme activity increased gradually up to the fourth day of germination and, thereafter, it diminished gradually. Torzhinskaya (4) has observed that the lipase activity of cornseed increased during germination, with a corresponding decrease in percent of fat. The lipase activity of oats reached its maximum value within a short period (8 hr) of germination and then it suddenly decreased (5). In case of groundnut, Ramakrishnan (6) has pointed out that there was a quantitative increase in the lipase activity up to the third stage (appearance of 8 leaves) of germination, and then a decrease.

Thus it can be perceived that lipase activity increases gradually during germination in certain oil-bearing seeds. In some seeds it increases suddenly in the initial stages of germination then drops off rapidly. Little information is available on the effect of ionizing radiation on the lipase activity of oil-bearing seeds. The present investigation is part of a program for study of the changes in the glyceride composition of the fat, of the lipase, amylase, ascorbase activities, and of vitamin C, free amino acids and carbohydrate contents during germination of groundnut seeds which are irradiated to different dosage levels of γ-radiation.

Experimental

Irradiation and Germination of Groundnut (Arachis Hypogaea L.) Seeds

Seeds utilized for the present investigation were of AK-12/24 M.P. variety obtained from Junagarh Research Farm (Gujarat State, India). Gamma radiation was applied from a Co^{60}-source located at the Atomic Energy Establishment—Trombay, Bombay (India). Dosages of 10, 30, 50, 70, 90 and 120 kr units were used in the gamma radiation experiment. Control and irradiated seeds were weighed individually and planted in chemically purified and sterilized sand. Germination was carried out under laboratory environment at constant temp of 25 ± 1C. Distilled water was added daily in a measured quantity to the germinating seedlings. In order to study changes in lipase activity by light, control seeds were also planted in darkness. The periods of germination selected for the present work were: 0, 1, 3, 6, 12, 20 and 30 days. No nutrients were added during germination. At the end of each period, some of the germinating seedlings were removed from the sand, cleaned with cold distilled water and used for the extraction of the enzymic activity.

Preparation of Enzyme

The cleaned seedlings were crushed with cold acetone in a mortar and pestle for about 10 min. The homogenates were filtered in the cold and washed with cold acetone until free of oil. The filtrate was utilized for the study of fat content and its glyceride composition. The residual, finely crushed plant materials were dried in a vacuum desiccator, weighed, and stored cold until ready for assay. Ungerminated groundnut cakes were prepared by the same method. It was observed that extraction with ether was unsuitable, as a prolonged period of extraction with ether diminishes the enzyme activity. Hence acetone, because of its dehydrating property, was used for extraction in the present work.

Method

The method adopted for the estimation of the lipase activity in the present work is essentially the same as that described by Wetter (2).

Commercial groundnut oil was used as a source of glyceride. For assay purpose, 2 ml of 22% $CaCl_2 \cdot 6H_2O$ followed by 10 ml of NH_4Cl/NH_4OH buffer ($pH = 8.7$), were added to approx 0.5 g oil in a beaker (7,8). The contents of the beaker were stirred for about 5 min in order to have thorough mixing. Then 0.100 g of the test material was added with vigorous stirring and the time was recorded as zero. Hydrolysis was carried out at 25C for 1 hr. The pH was maintained at 8.7 by the addition of 0.1 N NaOH from a microburette with mild stirring. Lipase activity has been expressed in terms of ml of 0.1 N NaOH con-

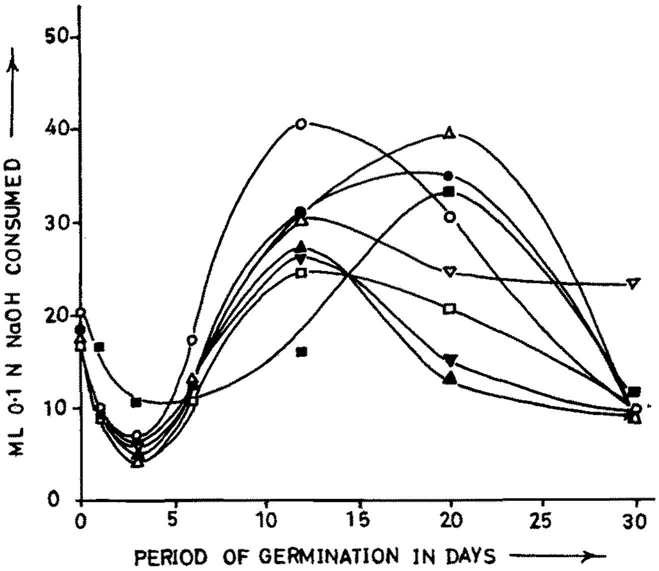

FIG. 1. The behaviour of lipase activity with the period of germination. Radiation doses:—■ dark; ○ control; ● 10 Kr.; △ 30 Kr.; ▽ 50 Kr.; ▲ 70 Kr.; ▼ 90 Kr.; □ 120 Kr.

sumed. From this and the weight of the total sample the activity per gram of the original seed was calculated.

Results

Seeds planted in dark grew faster than those grown in light. Irradiated seeds showed a comparatively poor rate of germination with increase in radiation dose as compared to control seeds grown in light. The increase in lipase activity of the seeds grown in the dark was poor in comparison to that of seeds grown in light. Results of the determinations of lipase activity are shown graphically in Figure 1 and Figure 2. Figure 1 indicates the change in activity with period of germination, while Figure 2 shows the variation of activity with different dosage level of γ-radiation. It should be noted (Fig. 1) that the activity present in both irradiated and nonirradiated seeds decreased in the initial stages of germination. This decrease in activity

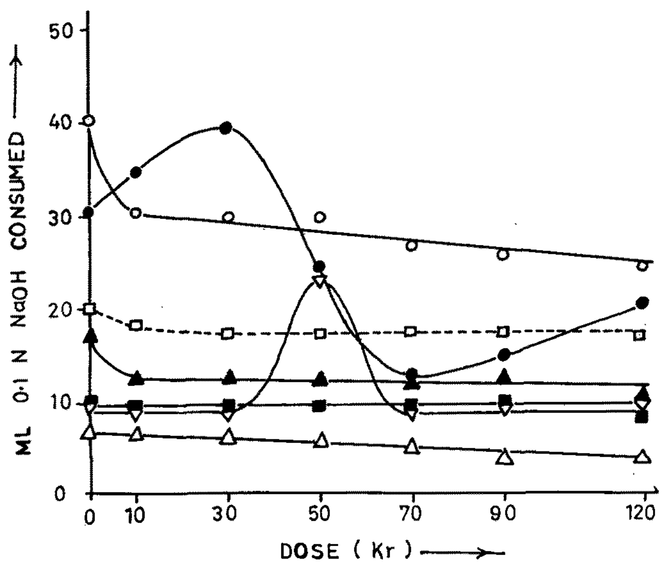

FIG. 2. The behaviour of lipase activity with various gamma doses: □ 0-day; ■ 1-day; △ 3-days; ▲ 6-days; ○ 12-days; ● 20-days; 30-days.

was observed up to the third day of germination; thereafter there was an increase in lipase, with maximums being reached (Table I) at different periods of germination for the several treatments.

The activity of the control seeds attained maximum value on the 12th day of germination and was higher than that obtained for irradiated seeds germinated for the same period. Further, the seeds irradiated to 10 kr units, showed maximum lipase content on 16th day of germination, but variation in activity between the 12- and 20-day periods is not great. Other irradiated seeds, except for 30 kr dosage level, attained maximum activity on the 12th day. Seeds grown in dark or irradiated at 30 kr units reach maximum lipase activity on 20th day of germination. The lipase activity at maxima observed on 12-days germination has the following trend: control seeds > 10 kr seeds > 50 kr seeds > 70 kr seeds > 90 kr seeds > 120 kr seeds. Maximum activity of the seeds (20-day germination) irradiated to 30 kr dosage level was greater than that of seeds grown in darkness.

From Figure 2 it may be noted that before germination the lipase activity of control seeds is slightly higher than that of irradiated seeds, and that it is not greatly affected by the increase in dosage levels. This is also true for the 1-, 3- and 6-day periods. In case of 12-day germination, the activity of the control seeds is higher than that of irradiated seeds and there is a regular decrease in lipase with dosage level. It is also noted that the lipase of all the seeds on 12th day of germination > that of zero day > that of sixth day > that of one day > that of third day of germination. This type of regularity in behaviour is not marked with the germination period of 20 and 30 days. In both these cases lipase attained maximum value for a definite dosage level (30 and 50 kr units, respectively) and thereafter there was a decrease in the activity.

Discussion

The present studies suggest that the lipase activity of dormant seeds is definitely affected by radiation. This might be ascribed to the fact that the radiation interferes with the functions of the mitochondria (9). The effect of radiation becomes more pronounced during the period of germination. This is visualized from the rate of production of lipase.

The activity in control as well as in irradiated seeds decreases to a certain level during the early stages of germination, in contradiction to the observation made by a previous investigator (6) who has shown that there was a gradual increase in the lipase on germination of groundnut. This is explained by the fact that the previous worker selected the stages of germination in terms of physiological aspects, i.e., the appearance of number of leaves. According to him the appearance of 2-leaves is the second stage of germination. In the present investigation the leaves appear after 8-day germination, and at that stage the activity is gradually found to increase. Thus the previous worker seems to have missed the observation on behaviour at intermediate stages. In cotton seeds similar decreases in activity have also been observed by other investigators (3). This decrease in activity in the initial stages of germination might be explained as follows: In the initial stages of germination cell division does not occur; hence the number of mitochondriae may remain the same. Other chemical processes may lead to the increase in weight of the cake but not in activity. Hence, due to the increase in weight of the cake, the activity is found to decrease proportionately.

After the third day of germination the lipase once again increases in all the cases. The rate of increase in lipase of seeds grown in dark is low in comparison to that of all other seeds. Of course the growth of the seeds planted in dark is faster than that of other seeds grown in light. This behaviour is in agreement with the observation made by Wetter (2). The faster production of lipase in seedlings grown in the light may result from the more active metabolic state of the plant grown in light. This suggests that growth might not be the only factor affecting the activity.

The rate of increase in activity for the seeds grown in light is different and is definitely affected by the radiation. This is illustrated by the rates at which control and irradiated seeds attain maximum value. In case of control seeds, the maximum activity is attained on the 12th day of germination, in light. This is also the case with the dosage levels of 50, 70, 90 and 120 kr units. However, it may be noted that the activity of control seeds at maximum is higher than that of irradiated seeds under the same conditions. This may be interpreted by assuming that the active centers of control seeds, whatever number initially present, are undisturbed and can show a characteristic increase in the rate; while in case of γ-irradiated seeds there may have occurred some sort of radiation damage to the active centers. This view is supported by the work of Meisel (9), who observed that irradiation damaged the nucleus and interfered with the functions of the protoplasmic structure, including the mitochondria, which responded differently to irradiation.

Further, in case of 10 and 30 kr dosage levels, the lipase activity reaches its maximum value on 16th and 20th day of germination, respectively. The value of the lipase of control seeds and that of seeds irradiated to 30 kr unit, at their respective maxima, is practically the same. There is a little difference in the value of activity in case of seeds exposed to 10 kr unit. Here it may be assumed that the radiation-damage of the active centers was slight and that as germination proceeded the interfering effects of irradiation on mitochondrial activity became less pronounced. This could explain why there was a time lapse in attaining maximum value of lipase in the above-mentioned seeds. The activity after reaching maximum value diminished gradually in all the treatments. This is in agreement with the observations made by the previous workers (2,3,6) on different oil-bearing seeds.

The behaviour of the lipase with radiation for a given period of germination is indicated in Figure 2, which shows that the activity of control seeds germinated in light increases by 2-fold over that of dormant seeds. For irradiated seeds the increase is about 1.5-fold.

As said before, plant growth might not be the only criteria for the increase of lipase, and vice versa. In general the growth of irradiated seeds was poor (only epicotyls are developed), and seeds irradiated to higher dosage levels, i.e., 70 kr unit and above did not grow at all. Even in that case lipase activity increased. This might be interpreted as follows: in the initial stages of germination certain amount of activity may be needed by the seedling for normal growth and other metabolic features. If this is not available the growth may be inhibited. Since lipase is affected by irradiation, as mentioned previously, the growth of irradiated seeds is poor. Further, with the increase in period of germination, other metabolic factors play a part in producing the lipase activity. Thus lipase activity of the irradiated seedlings might increase but not at the usual time. Hence this increase in lipase at the later stages is ineffective for promoting the growth of seedling. Thus it may be that over and above plant growth, other metabolic factors such as: ascorbic acid (10), ascorbic acid oxidase (AAO), amylase, carbohydrates and free amino acids liberated during germination are also operative. They may also play a role in the production of lipase activity. Amino acids increase the stability of the enzyme (11). It has been observed that there was an increase in free amino acids during germination. Other factors also show corresponding increases (13) and reach maximum values after different periods of germination of these seeds. Thus it is assumed that these factors have their mutual effect on each other and are responsible for the observed increase in lipase activity of irradiated seeds.

TABLE I

Lipase Activity of Groundnut [a]

Kr units, irradiation	0 (Dark)	0 (Light)	10	30	50	70	90	120
Period [b]	20	12	16	20	12	12	12	12

[a] Except for "O" (Dark) irradiation treatment, all seeds were grown in optimum light condition.
[b] Period of germination in days for maximum activity.

The abnormal behaviour of lipase observed in case of seeds irradiated to 30 and 50 kr units, is explained on the following ground. The AAO and free fatty acids (ffa) of these irradiated seeds at their specific period of germination are found to attain maximum values. Further the increase in AAO is ultimately responsible for the corresponding increase in dehydroascorbic acid. It has been mentioned by Gula (12) that dehydroascorbic acid considerably raised the lipase activity. Thus the increase in AAO and ffa of the above-mentioned seeds may be responsible for abnormal increase in lipase activity.

Hence, in general, the assumption regarding the mutual effects of all the factors mentioned above is strengthened by the observations made in the present investigation. Further work for confirmation of the above views on the behaviour of lipase activity of other oil-bearing seeds having various degree of unsaturation is in progress.

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