The Effect Of Some Respiratory Inhibitors On The Lipase Activity Of Germinating Peanuts (*Arachis hypogea*)

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Abstract

Peanut (*Arachis hypogea*) seeds were treated with varying concentrations of KCN, KNO_3 and Urea (0.01 M and 0.05 M) prior to germination. Lipase activities of germinating seeds were affected by chemical treatment. The effect induced by the chemicals is explained on the basis of interfering effect with the function of the organelles. The lipase activity of all the seeds had maximum activity on the 6th day of germination.

Key word: Peanut, lipase, chemical treatment.

Introduction

During the last twenty 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. The effect of growth regulators on lipase activity of some plants during germination have been studied (Patel et al, 1965). They reported that lipase activities are affected to a great extent by growth regulators prior to germination of the seeds and that the increase in lipase activity is not only dependent on the plant physiology, but on other metabolic products such as ascorbic acid and amino acids produced during germination. A little information is available on the effect of respiratory inhibitors as pre-sowing chemicals treatment during germination (Njoku et al., 1995).

Hence in the present work we undertake to study the effect of some respiratory inhibitors on lipase activity during germination of peanuts, and to complement existing knowledge on the effect of chemical treatment on this economic oil bearing seed.

Materials and Method

Treatment and germination: A common specie of peanut (*Arachis hypogea*) found in the Eastern parts of Nigeria, and cultivated extensively in Abakiliki, parts of Ebonyi state, Nigeria was used for the study. The following respiratory inhibitors and chemicals were also employed for the study: Potassium cyanide, Urea and Potassium nitrate. The concentrations mentioned for each respiratory inhibitor is based on preliminary experiments carried out in our laboratory as well as information from literature (Patel et al., 1965), so as to have specific physiological changes in the plants.

Treatment of plant materials: In the case of each treatment 30 ml 0.01 M potassium cyanide, urea and potassium nitrate and 0.05 M potassium cyanide, urea and potassium nitrate, and glass distilled water were taken in a glass stoppered conical flask containing 100 g of the seeds. Three replications were preferred for each treatment. The content of the flask was shaken for about 5½ hr on an electrically operated rotary shaker. During the shaking period the flasks were inverted periodically to have uniform soaking. The test solutions were completely absorbed by the seeds in each case during the period.

The treated seeds 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 respiratory inhibitors alone. The treated and the control seeds previously weighed individually were germinated in a wet clean cheese cloth under laboratory environment at 28 ± 1 °C. In order to study the effect of light on lipase, control seeds were also planted in the dark. The periods of germination selected were 0, 1, 2, 3, 6, 10 and 14 days. At the end of each period six seedlings from each treatment were removed, cleaned with cold distilled water and used for the extraction of the enzyme.

Preparation of crude enzyme: The acetone powder (crude enzyme) was prepared according to the method of Wetter (1957) as modified by Patel et al. 1965). The cleaned seedlings were crushed with cold acetone in a mortar for about 5 minutes. The homogenates were filtered in the

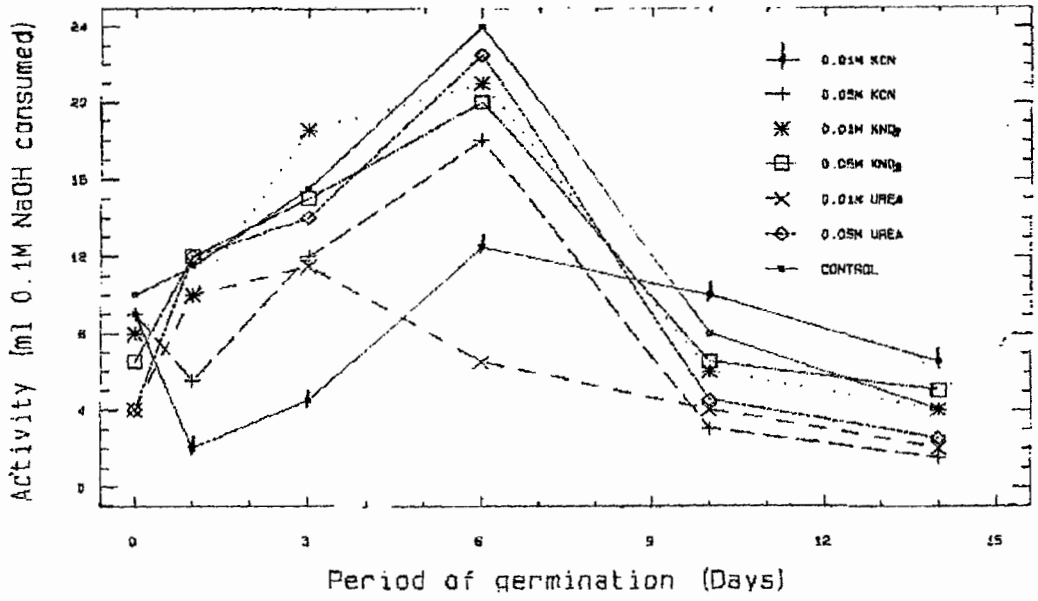

Fig. 1: Effect of growth inhibitors on the lipase activity during germination

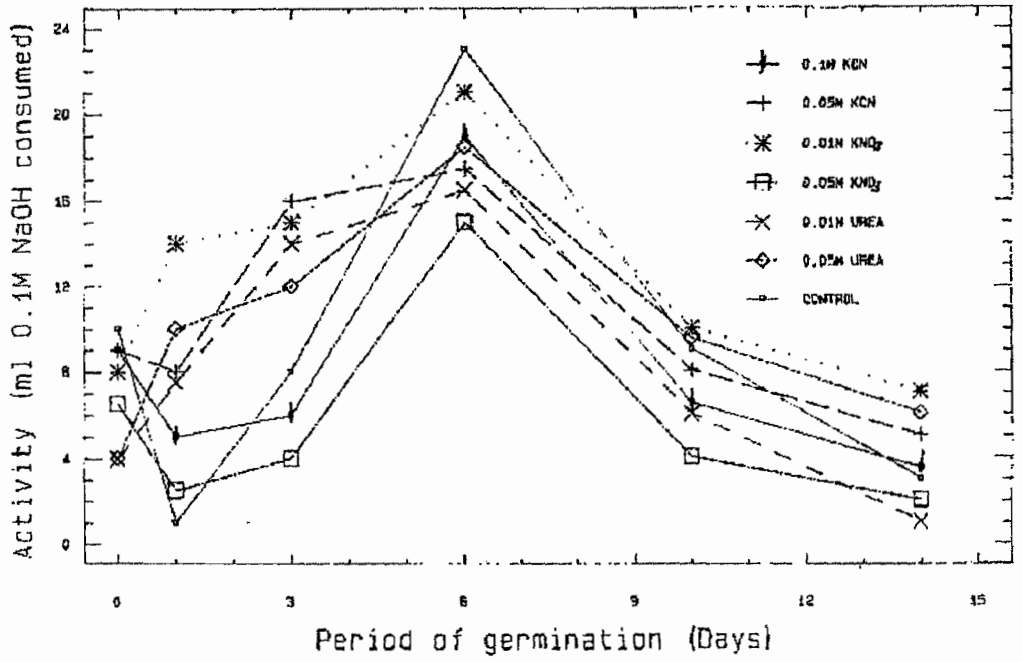

Fig. 2: Effect of growth inhibitors on the lipase activity during germination using corn oil as substrate (dark)

cold and washed free of fat with the cold acetone. The resulting finely crushed acetone powder was dried in a vacuum desiccator, weighed and stored cold until ready for assay. The above procedures were carried out in a cold room.

Measurement of lipolysis: Fresh corn oil was used as substrate. One gramme of oil was taken in a glass stoppered flask and 5 ml of 0.1 M phosphate buffer (Na_2HPO_4 + KH_2PO_4) pH 8.0 and 2 ml distilled water were added. The contents were thoroughly mixed by stirring for 5 minutes. Then 0.1 g of the enzyme was added and mixed by vigorous shaking. Hydrolysis was carried out for 24 hours at 28 ± 1 °C. During the hydrolysis, the contents of the flask were shaken continuously on a water bath shaker. At the end of the hydrolysis, 10 ml neutral 1:1 v/v ethanol – ether mixture was added. The liberated fatty acid was titrated with 0.1 M NaOH using ethanolic phenolphthalein as indicator. A blank was set up using boiled enzyme.

Enzyme unit: Lipase activity was expressed in units, where 1 unit is equivalent to 1 ml of 0.1 M NaOH required to neutralize the fatty acid under the described assay condition. From the weight of the total materials, the activity per gram of the original seed was calculated.

Results

The results of the determination of lipase activity are shown in figures 1 and 2. From the result, there

was a decrease in activity on the first day in seeds treated with KCN in both the light and dark conditions, while for urea and KNO_3, there was an increase in activity.

In general, the maximum activities were recorded on the 6^{th} day in all the treatments. The lipase activities of the control seeds were higher than those of the treated seeds. The highest lipase activity observed at different periods of germination is found to vary in the following trend in both light and dark treatment. Control > KNO > Urea > KCN.

Discussion

The results of the present investigation indicates that chemical treatment prior to germination have pronounced effect on the lipase activity of peanut seeds. The activity of the control seeds dropped from 10.0 units to a low level of 4.0 units when urea was used for treatment of seeds even before germination in both light and dark conditions, so also did KCN and KNO_3. The initial effect of the chemicals could be explained on the basis that these chemicals also interfere with the functions of the organelles especially the mitochondria as reported by earlier investigators (Patel et al., 1965). The actual effect of these chemicals on the metabolism of the plants is not clearly understood, however some speculations may possibly explain the observed effects.

The inhibitory effects noticed with some of the chemicals – potassium cyanide may be explained based on the role of this chemical on the cytochrome oxidase of the respiratory chain and inhibition on the oxidative phosphorylation pathway, as this may result in the poor development of the seedling as was observed during the course of the experiment, and hence on the other physiological aspects of the plant as well as enzyme synthesis and possibly regulation. On the other hand, the slight activatory role observed by urea and KNO_3 on the enzyme activity when compared to KCN, might equally be explained on the role these compounds play as nitrogen sources for normal growth of plant tissues (Dotta, 1968). This may equally explain the normal growth rate observed in the seedlings during the course of the experiment, and hence the possible normal metabolic activities suspected in the plants treated with these chemicals.

It is evident from this study, that lipase activities are affected to a great extent by these compounds. The actual mechanism is equally not clearly understood, but since these compounds play important role in both the nutrition and metabolism of the plants, as well as in hormonal activities especially the auxins, which have been found to affect the lipase enzyme (Salisbury and Ross, 1992). Further studies need to be carried out to then check the effect of plant hormones on the lipase activities of germinating oil-bearing seeds.

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