*J. Phytol. Res.* 16(2) : 235-238, 2003

# BIOCHEMICAL CHANGES IN GERMINATING SEEDS OF GRAM *(CICER ARIETINUM)*

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The changes in total lipid, carbohydrate, protein contents and protease activity were studied in germinating seeds of gram up to 7th day. The total lipid content decreased with onset of germination, while carbohydrate and protein contents increased although the increase in carbohydrate content is not regular. The highest protease activity was observed on 2nd day of germination.

***Keywords :*** Carbohydrate; *Cicer arietinum*; Germination, Lipid; Protein.

## Introduction

In most of the seeds, the food reserves are stored in the form of fat. During the maturation of seeds the fat componenets increase in comparison to other components of the seeds as more engergy can be stored in the form of fat as it exist in a highly reduced state. Massive breakdown of fat reserves and their conversion to carbohydrate occurs during the germination of fat storing seeds[1]. Glyoxalate cycle has been shown to be resonsible for conversion of fats into carbohydrates in a variety of seeds[2,3]. The rate of conversion of fat to carbohydrate can be correlated with changes in isocitrate lyase activity during germination[1]. The total lipid concentration of the germinating seeds decrease with increase in number of days of germination. It has been observed that the triglycerides and fatty acids decreases during germination. Abrahamsen and Sudia[4] have suggested that oil depletion in germinating seeds which is determined by the activity of the glyoxalate cycle, may be dependent upon oil-carbohydrate equilibrium. The changes in total carbohydrate content during germination is characteristic of each type of seeds. It has been observed that the increase in carbohydrate content is not regular. In germinating seeds of flax and mustard the total soluble carbohydrate decreases starting from the onset of germination till 4th day[1]. The changes in total protein content during germination is significant. The amount increase with germination up to 7th day. In *Acacia laeta L.* seeds protein undergoes modification on 3rd day of germination[5]. The changes in protease activity of germinating seeds is interesting. The highest enzyme activity was observed on 2nd day. From 5th day onwards no change in enzyme activity was observed.

The present investigation was undertaken to study the changes in neutral lipids, carbohydrate content, protein content and protease activity. The above parameters were assayed in germinating seeds up to 7th day of germination as the germination was found to be completed with development of leaves on this day.

## Material and Method

*Materials :* Gram seeds were purchased from market. The sources of the reagents used were, Sodium carbonate anhydrous, TCA (Central Drug House (P) Ltd. Bombay), Acetic acid glacial, Phenol (E. Merk (india) Ltd.), Hexane (Qualigens Fine Chemicals, Bombay), Ether (Alembic chemical Works Co., Ltd. Baroda), BSA (Sigma Chemical Co.), Glucose, Conc. $H_2SO_4$ (BDH, India) and Casein (ICN Pharmaceuticals). All the reagents were of analytical grade.

*Seed germination :* The healthy gram seeds (70 gm per day of germination) were selected and soaked in water for 12 hrs. before the germination started. After soaking, the seeds were spread on sand previously sterilized, above a plate and allowed to germinate at room temperature from 1 to 7 days. Water was given thrice a day for each sample. After each day of germination the seeds were taken out carefully and were washed thoroughly in running water followeed by several

washes with glass double distilled water.

*Preparation of Crude Extracts :* From each sample 70 gms (wet weight of the germinated seed) were taken, crude homogenates were prepared in 150 ml of 0.2 M phosphate buffer pH 7.4. The homogenates were filtered through several layers of cheese cloth and then through a fine filter paper. The residues were kept in fridge for lipid extraction. The filtrates were centrifuged at 5000 x g for 60 min. The supernatants thus collected were analysed for carbohydrate, protein content and protease activity.

*Neutral lipid extraction :* The residues by the filtrtion of the homogenates were taken for neutral lipid extraction. 30 gms (wet weight) of the residues was taken in the Sauxlet apparatus. The lipids were extracted using Hexane (60-70) as solvent. The solvent was allowed to circulate for 30 min by keeping the apparatus in boiling water bath. After extracting the lipids, the solvent i.e. Hexane, was removed under reduced pressure. Added 0.5 ml of chloroform to each sample of the lipid and the samples were analysed for the lipid contents by thin layer chromatography (TLC).

*Thin Layer Chromatography of extracted lipids :* The silica gel was used to prepare TLC plates. The gel (80 gms) was dissolved in 200 ml of distilled water and applied on the TLC plate with an applicator.

After activating the plates for 1 hr at 90°C in incubator, the samples were applied carefully with the help of syringe. The TLC plates were developed in 80 : 20 Hexane : Ether mixture. After the plates were kept for 10 min to dry after development and kept in iodine vapour chamber for the non-specific localization of lipid.

*Determination of Protein, carbohydrate contents and Protease activity :*

1. The total carbohydrate contents were estimated by Phenol-Sulphuric acid method of Dubois *et al*[6].
2. The total protein contents of each sample were determined by the method of Lowery *et al.*[7]
3. The protease activity in each sample was determined by taking 0.2 ml of seed extract in 0.8 ml of 0.2 M phosphate buffer pH 7.4 and 1 ml of 2% casein solution. This reaction mixture was incubated at 30°C for 15 min. After incubation the reaction was stopped by adding 1 ml of 20% TCA. The product was measured in terms of TCA soluble peptides, formed as a result of protease activity, by Lowry *et al.* procedure[7].

**Results and Discussion**

The total triglycerides and fatty acids decrease in amount from the onset of germination up to the 6th day, as compared with the sizes of the spots observed.

A non-linear change in total carbohydrate content was observed during germination (Table 1). A sharp increase in the carbohydrate content was observed on the first day with a sudden fall on the second day. While from the 3rd day of germination up to the 7th day, a regular increase was observed except that there is small decrease in amount on 6th day.

Changes in total protein contents in the

**Table 1.** Change occurring during germination.

| Days of Germination | Carbohydrate (mg/ml of extract) | Protein (mg/ml of extract) | Protease activity mg of product/ min/ml of extract |
|---|---|---|---|
| 0 | 5.76 | 3.96 | 0.240 |
| 1 | 9.90 | 4.40 | 0.355 |
| 2 | 4.44 | 4.86 | 0.505 |
| 3 | 4.68 | 5.46 | 0.450 |
| 4 | 6.38 | 6.18 | 0.405 |
| 5 | 9.08 | 6.84 | 0.490 |
| 6 | 8.36 | 7.08 | 0.490 |
| 7 | 10.56 | 9.92 | 0.490 |

different phases of the germination were also studied (Table 1). The gradual incrase in the protein contnet was found from zero day of germination up to 7th day.

The pattern of protease activity change is interesting. The maximum protease activity was observed on 2nd day of germination, however any significant change in the protease activity of germinated seeds from 4th day onwards was not observed (Table 1).

In germinating seeds of gram, a significant change in total neutral lipid has been observed. The total triacylglycerides and fatty acids decreases in amount with germination. The decrease in amount of triacylglycerides and fatty acids may be because these components are metabolized to realese energy for germination or fat components are converted to carbohydrates[1]. In *Corylus colurna*, the fat content of mature seeds has been shown to be 49.7% of dry matter, which decrease after 21 days of germination by 80%. Fat mobilization was most intensive between 4th and 7th day, when 50% of the fats were mobilized. The changes in the content of fatty acids are similar. The reserve lipids contain mainly oleic (74.5%), linoleic (16.5%), palmitic (6.0%) and stearic acid (2.5%). In later stages of germination multiple unsaturated (C 18 : 3) and long chained fatty acids (C 20 and C 20 : 1)[8]. In germinating flax and mustard seeds, the fall in triglyceride concentration was observed after the first day, a marked decrease was observed in total lipid only after the second day of germination. In later stages the fall in total lipid and triglycerides was almost identical[1]. During the germination of *Madicago sativa* the amount of triglycerides decreases while that of diacylglycerides and monoacylglycerides increases. The fatty acid component of triglycerides did not change but that of diacylglycerides and monoacylglycerides showed an increase in linoleic and linolenic acids and a decrease in palmitic and oleic acids. The content of free fatty acids remained fairly constant but the composition of fatty acids shifted to higher unsaturation[9].

The increase in total carbohydrate content during germination is due to conversion of fat to carbohydrates. In most of the fat storing seeds, fat reserves break down and are converted to carbohydrates through glyoxalate cycle. During germination, Isocitrate lyase activity increases initially and after reaching a maximum the activity decreases. In flax and mustard the peak of Isocitrate lyase activity has been shown on 4th day in either seed but compared to flax the activity was remarkably low in mustard[1]. In dormant seeds of *Corylus colurna*, no or only very small quantities of starch, glucose and sucrose were present. During germination, up to the 15th day starch content increases and it remains constant till the 18th day[8]. During the germination of cowpea seeds there was a gradual decrease in oligosaccharides while monosaccharides increases[10]. A decrease in total carbohydrate contents is observed on 2nd day of germination indicating the oxidation of carbohydrate to provide instant energy.

The increase in total protein content is almost relatively linear except a sharp increase on the 7th day. In *Acacia laeta* L. seeds there was initial increase in protein content on the 3rd day of germination followed by a decline throughout the germination period[5]. Similar to our observation an increase in protein content of cowpea seeds has also been found during germination[10]. The transition of the seeds from a dormant to a non-dormant state in *Echinochloa crus-galli* (L) *Beauv* has been shown to be associated with the synthesis of specific proteins and a decrease in content of others in the plasma membrane[11].

The increase of protease activity observed on the 2nd and 5th day may be because of the involvement of two different proteases being activated on different days. Some studies have shown a change in peptidase activity with the course of germination of the seeds. In *Lodgepole pine* seeds megagametophytes and embryonic axes contain two classes of peptidases :

aminopeptidases and carboxypeptidases. Cell free extract studies showed that carboxypeptidase activity was very low in mature seed megagametophytes and embryonic axes but increased rapidly in these tissues after ridicule emergence. However maximum rates of carboxypeptidase activity occured only when 70% of the megagametophyte storage protein reserve had been depleted, 5 days after inhibition. Thus it was unlikely that carboxypeptidase played a significant role in the rapid mobilization of megagametophyte storage proteins. Mature seed megagametophyte contain high levels of amino peptidase activity. Following germination this level of activity was almost completed, 5 days after inhibition. Thus, aminopeptidase are involved in the hydrolysis of megagametophyte storage proteins in the germinated *Lodgepole pine* seed[12].

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