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Mobilization of Seed Proteins in Germinating Seeds of *Chickpea (Cicer Arietinum* L.)

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Abstract: *The present study relating to the mobilization of stored protein in germinating chickpea seeds throws light on the understanding of changes in seed metabolism during germination. In chickpea the initial drop in the protein control clearly indicates the denovo production of metabolizing enzymes that break down protein which is required to be mobilized for the growth and development of embryo. Presence of proteins in radical and plumule indicate synthesis of new proteins.*

Keywords: *Chickpea, seed germination, protein mobilization.*

INTRODUCTION

Knowledge of the mature seed and its germination is of importance in the study of the seed formation. Embryogeny and germination are extensions of each other separated by a period of relative metabolic inactivity called quiescence, and are essentially different phase of the continuing process of embryo growth and development.

The nature of the pre-quiescence embryo bears very heavily on early germination behavior and its control, and answers to question to relating to germination behavior are increasingly being sought during the period of seed development.

Germination has been studied form a number of viewpoints. A large part of seed research in recent year has been devoted to understanding events of the normal germination process and seedling establishment. Storage organs (Cotyledons and Endosperm) in particular, have received considerable attention in relation to the breakdown of storage reserves and its control (Mayer and Mayber, 1982). Germination involves resumption of growth and development of the embryo and the transition of cells from a desiccated state having very low metabolic activity to a hydrated metabolically active one. Water uptake is a triphasic (Bewley and Black 1994; Muntz *et al.*, 2001) involving hydration in the first phase followed by a relatively long phase during which major metabolic changes accompanied by structural changes take place followed by a third phase of germination and further embryo growth.

Mobilization of seed proteins is generally associated with extractable endopeptidase activity (Ashton, 1976). However the difficulty has been is to establish that such enzymes are responsible to the breakdown of proteins. Numerous reports indicate the activity of increased activity of proteases with the breakdown of storage proteins associated with germination (Senyuk *et al.*, 1998; Rajeshwari and Ramakrishna Rao, 2002). The present study investigates the variation in total proteins during germination in chickpea seeds.

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MATERIALS AND METHODS

Chickpea seeds of the cultivar Pusa 256 were taken for study. About 100 seeds were put for germination in a 9 inch petry plate on a presoaked filter paper. Wetting of the filter paper was done every alternate day or as required with distilled water. The petry plates with the seeds were in incubated in B.O.D at 25 ± 1°C. On the basis of seed germination the stages of germination noted were: Dry seed (Stage 1), Fully imbibed seed (Stage 2) Radical emergence (Stage 3), Plumule emergence (Stage 4) and Fully germinated seedling (Stage 5).

Protein Extraction

Protein extraction was done at every stage of germination. The following procedure was adopted for protein extraction. One gram of seed was carefully weighed on a Mettler precision balance. The weighed seeds were made in to a paste in 5ml.phosphate buffer (PH 7.5) using mortar and pestle at room temperature. Extraction was done with three independent samples for all the stages under study. After centrifugation of the extract in a Remi centrifuge at 10,000 rmp for 15min, the pellet was collected and extracted twice with phosphate buffer and centrifuged. Supernatant collected from these steps was mixed to get a volume of 12.5 ml which was made up to 50 ml with phosphate buffer. Protein estimation at stages 3, 4, and 5 was done only for radical, plumule and cotyledon respectively.

Protein Estimation

The standard curve for protein estimation was prepared by using Bovine serum albumin (BSA) procured form SRL chemical. The Standard curve was prepared as follows. Stock solution form 1 mg/ml of BSA as prepared in distilled water. The stock solution was stored at –20°C. 10, 20, 40, 60, 80 and 100µl of BSA were pipetted out from the stock solution using Gilson micropipette in to 15 mm × 120 mm tubes. Each of these volumes was made up to 100µl with distilled water. To each of the tubes 5ml of coomassie blue G250 stain solution, that was prepared before hand, was added and the tubes were vortexed. 100µl of distilled water taken in an additional tube and was used as blank. Absorbance of sample was measured at 595 nm, in a Systronic make Spectrophotometer model 106, in glass cuvetts. Standard curve was plotted using this data.

Preparation of Coomassie Blue

100 mg of coomassie blue G250 stain (Sigma) was dissolved in 50ml of 95% ethanol. This solution was mixed with 100ml of 85% Phosphoric acid and made up to 1 liter with distilled water. The reagent was filtered through Whatman no. 1 filter paper and stored in amber bottle at room temperature. The regent was filtered before use.

RESULTS AND DISCUSSION

Seeds of the chickpea (*Cicer arietinum* L.) cultivar Pusa 256 species that were put for germination showed the stages of soaked seed (Day-1), radicle emergence (Day-3), plumule emergence (Day-5) and complete seedling (Day -6). The absorbance and total protein content of germinating chickpea seeds at different stages is presented in Table 1 and 2 respectively. From an initial protein content of 6 mg/g in the dry seeds there is a gradual decrease in the total protein as the seed germinates. The imbibed seeds had a protein content of 4.4 mg/g. At the radical emergence the protein level decreases to 4.2 mg/gm with radical containing a larger proportion (2.4 mg/g) of protein and with the emergence of plumule protein level decreased to 4mg/gm with plumule having 1.8 mg/g protein.

Table 1
Absorbance measurement for the total protein in chickpea seeds

S. No.	*Stage (Day)**		*Absorbance*+
1.	Dry Seeds	(0)	0.23
2.	Soaked Seeds	(1)	0.17
3.	Seed with Radicle	(3)	0.16
4.	Radicle only	(3)	0.09
5.	Seed with Plumle	(5)	0.15
6.	Plumule only	(5)	0.07
7.	Seed with Radical and Plumule	(6)	0.14
8.	Seed without Radicale and Pulumule	(6)	0.09

*Figures in parentheses indicate Days of Germination

+The Data is an average of three independent readings.

Table 2
Variation for total protein in germinating chickpea seeds

S. No.	Stage (Day)*		Total Protein+ (mg/g)
1.	Dry Seeds	(0)	6.0
2.	Soaked Seeds	(1)	4.4
3.	Seed with Radicle	(3)	4.2
4.	Radicle only	(3)	2.4
5.	Seed with Plumle	(5)	4.0
6.	Plumule only	(5)	1.8
7.	Seed with Radical and Plumule	(6)	3.6
8.	Seed without Radicale and Pulumule	(6)	2.4

*Figures in parentheses indicate Days of Germination

+The Data is an average of three independent readings.

At stage 5 *i.e.* in a fully germinated seedling the protein content is 3.6 mg/g. It is interesting to note that even after germinated seed, a total protein of 3.6 mg/g with the cotyledons having a significant amount of protein (2.4 mg/g).

The present study relating to the mobilization of stored protein in germinating seeds reaffirms the understanding of changes in seed metabolism during germination. In chickpea the initial drop in the protein control clearly indicates the de novo production of metabolizing enzymes that break down protein which is required to be mobilized for the growth and development of embryo. Presence of protein in radical and plumule indicate the synthesis of new proteins. The role of seeds in the plant life cycle requires that they store materials which can be made available to support the rarely growth and development of the embryo (Rachna Srivastava *et al*., 2006), and to aid establishment of the seedling. The reserves are stored commonly as polymers including protein, lipid, and carbohydrate (starch or various cell wall polymers), and in addition compounds like phytin and calcium oxalate (Muntz *et al*., 2001). All of these reserves occur in discrete cellular organelles (Shewry *et al*., 1995). In the present study, it has been observed that stored protein is broken down in to readily usable metabolites for the growth and development of the embryo. Associated with these compositional changes there are striking structural changes in protein bodies. In *Cicer* sps. protein bodies initially swell to above twice their size during hydration. Further increase in size is associated with the commencement of rapid protein hydrolysis and is due to coalescence of protein bodies. During the initial swelling which occurs usually over 1-1½ days, protein body contents become more diffuse and with protein hydrolysis the contents disappear, usually after 3-5 days.

The bodies become vacuole which coalesces to form larger vacuoles and, ultimately, often one large central vacuole. The original unit membrane of the protein body becomes the tonoplast of the cell although presumably its nature could change in the process (Senyuk *et al*., 1998). This may be the probable cause of decrease in protein content. This clearly indicates that there is a rapid breakdown of proteins in chickpea. However there is increase in protein content of radical and plumule indicating de novo synthesis of proteins in these parts. Nandi *et al*., (1996) reported that the higher concentration of cytokinins mobilize greater reserves to the embryonic axis in developing seeds of lupine. Though not much studies have been done at physiological and molecular level, studies in wheat using microarray suggest that in germinating seeds several new mRNAs are synthesized de novo and are responsible for many proteins whose function is still to be ascertained (Wilson *et al*., 2005) which can be collaborated here too and may require further investigations.

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