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**SHORT COMMUNICATION**

# PROTEASE FROM GERMINATING MOTH BEAN SEEDS

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**The protease activity in the crude extracts of germinating moth bean (*Vigna aconitifolia* Jacq.) seeds was investigated. The activity was measured at different pH upto 48 hours of germination. The maximum protease activity was found in 12 hours imbibed seeds and it declined up to 48 hours of germination. The protease activity was enhanced by cysteine, mercaptoethanol, EDTA and thiourea. Increase in activity with these thiol specific activators suggested that this protease possessed cysteine at its active site or near to it. Enzyme inhibition with iodoacetic acid also supported this phenomenon. Enzyme was also inhibited with bivalent cations, viz. $Zn^{2+}$,$Cu^{2+}$,$Mg^{2+}$ and $Ca^{2+}$.This inhibition suggested that enzyme does not belong to metalloprotease class and did not require bivalent cations for the degradation of storage protein.**

***Key words*** **: Activators, inhibitors, protease, thiols**

Germination enhances the nutritive value of legumes. The vitamin C content, not found in dry grain but present in appreciable amount in sprouted grains. During sprouting other changes of nutritional significance take place. Soaking followed by sprouting is associated with changes similar to those in fermentation. The nutrient content of foods improved without any additional cost, a most economical way of improving the diet.

During germination seed proteins are hydrolyzed by proteases into peptides and amino acids, which are translocated to the growing embryo. Amino acids are used in the synthesis of enzymes, proteins, hormones, purines and pyrimidines bases. The maximum rate of hydrolysis of the storage proteins coincides with the maximum rate of growth of the seedlings. The enzymes for hydrolysis of proteins are present in the germinating cotyledons. The proteases readily digest the globulin present in mungbean cotyledons to smaller polypeptides (Yamaoka *et al*. 1990). Protease synthesis is not always delayed until germination. As the seed accumulates storage proteins, some plant produce proteolytic enzymes that will actively degrade stored proteins later in seedling growth as in the case with some asparaginyl endopeptidases (Botteri *et al.* 1996 and Senyuk *et al.* 1998).

The investigation was carried out with moth bean seeds (*Vigna aconitifolia* Jacq.) cv. RMO-40 (short duration and drought tolerant cultivar) which was collected from Agricultural Research Station, Mandor (Jodhpur), RAU Bikaner. Moth bean (*Vigna aconitifolia* Jacq.) seeds were surface sterilized with 0.1per cent mercuric chloride solution. Sterilized seeds were soaked for 12 hours in double distilled water and kept for germination on moist filter paper. Samples were collected after 6 and 12 hours soaking and at 18, 24, 36 and 48 hours of germination. Samples were homogenized with 0.1 M citrate buffer (pH 3.0 and 5.5) and 0.1 M Tris buffer (pH 8.1) for the protease activity.Protease activity was assayed by Wrint (1974) method and water soluble protein was determined by folin ciocalteau reagent

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(Lowry 1951).The protease was characterized by observing the effects of activators and inhibitors on its activity.

The pattern of protease activity in moth bean during germination expressed an fresh weight basis is given in (Fig. 1). The protease activity was observed maximum at pH 5.5 followed by pH 3.0 and minimum at pH 8.0. The activity increased with increase in germination period and was maximum in 12 hours imbibed seeds and declined thereafter. The activity was found maximum at pH 5.5 in 12 h imbibed seeds and minimum in 48 hours seeds at pH 8.0 (Fig. 1). Similar trend was observed in specific activity (Fig. 2).

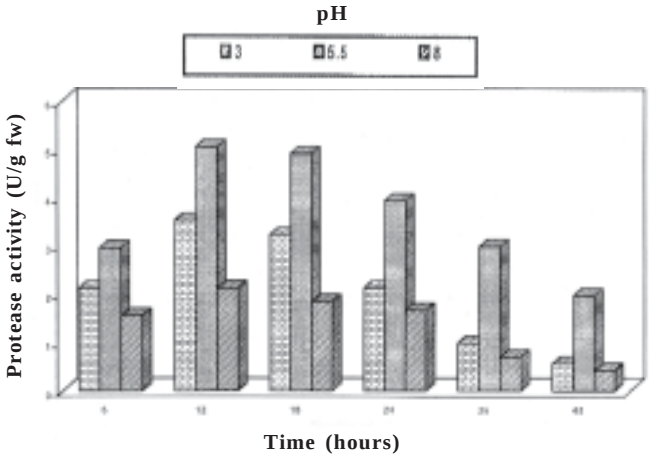


**Fig. 1. Pattern of protease activity (expressed on fw basis) in moth bean during germination**

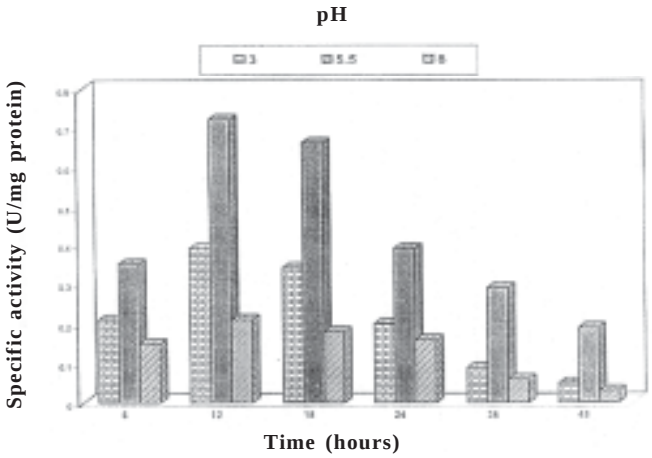


**Fig. 2. Pattern of protease specific activity in moth bean during germination**

The variation in level of proteolytic activity in seeds during germination suggests that there may be more than one ways to regulate proteolysis in the seeds during the breakdown of storage proteins (Ryan 1973). During germination there was a progressive increase in free amino acid contents, which may be used in new protein synthesis in developing seedlings. The profile of protein content changed considerably in the early stage of germination, however, it decreased at later stages of germination (Ismail 2003). Soaking for 12 hour and germination for different period [18, 24, 36 and 48 hours], contributed in reducing the phytic acid and polyphenol content. Soaking (12 h) brought about an improvement in protein digestibility. Progressively an increase in protein digestibility was also noticed with an increase in germination period in cowpea [Preet and Punia 2000].

Cysteine, mercaptoethanol, thiourea and EDTA stimulated the protease activity, whereas, Iodoacetic acid and bivalent cations ($Cu^{2+}$, $Mg^{2+}$, $Zn^{2+}$ and $Ca^{2+}$)act as inhibitors of protease. The effect of activators and inhibitors on protease activity is shown in Table 1. Maximum activity was observed with cysteine (182.09% activation) at 4 mM concentration followed by thiourea, EDTA and mercaptoethanol at 20, 2 and 20 mM concentrations respectively. Thiourea, EDTA and mercaptoethanol showed 181.09, 129.85 and 91.54% activation in protease activity. Iodoacetic acid inhibited the protease activity by 72.13% at 0.3 mM concentration as compare to control. Bivalent cations also act as inhibitors of protease. $Mg^{2+}$(12.5 mM) and $Cu^{2+}$(15 mM) caused maximum inhibition about 75.87% and 75.62% respectively. $Zn^{2+}$ (3 mM) and $Cu^{2+}$ (15 mM) caused 58.28 and 9.23% inhibition of protease activity respectively.

The stimulation of protease activity in the presence of cysteine and mercaptoethanol indicated that this may be acid protease, which possessed cysteine at its active site or near to it. Similar findings were observed by Umar (1992) in Cajanus cajan seeds. The cysteine of protease might got oxidized *in vitro* and resumed its activity by the addition of thiol group. Cysteine proteinases play a major role in protein turn over, degradation, and possibly even enzyme activation in germinating barley (Susan *et al.* 1988). The activation of protease also observed with cysteine and mercaptoethanol in buckwheat seeds (Iordon and Belozerskii 1976), in *Ervatamia heyneana*

**Table 1.** Effects of various activators and inhibitors on protease activity in moth bean seeds

| Chemical | Concentration (mM) | Enzyme activity (u/g fw) | Relative activity (%) | Increase in activity (%) |
|---|---|---|---|---|
| Control | 0.00 | 4.02 | 100 | 00.00 |
| Cysteine | 4.00 | 11.34 | 282.09 | 182.09 |
| Thiourea | 20.00 | 11.30 | 281.09 | 181.09 |
| EDTA | 2.00 | 9.24 | 229.85 | 129.85 |
| Mercaptoethanol | 20.00 | 4.70 | 191.64 | 91.54 |
| Iodoacetic acid | 0.3 | 1.12 | 27.87 | –72.13 |
| $CaSO_4$ | 15.0 | 3.65 | 90.77 | –9.23 |
| $ZnSO_4$ | 3.0 | 1.68 | 41.72 | –58.28 |
| $CUSO_4$ | 15 | 0.98 | 24.38 | –75.62 |
| $MgSO_4$ | 12.5 | 0.97 | 24.13 | –75.87 |

(Patel and Jagannadham 1999) and in germinating cotyledons of soybean (Asano 2001).

Results showed that the protease which was activated by thiol specific activators, viz. EDTA, thiourea and mercaptoethanol possessed a thiol group or a sulphydryl group at its active site. This protease was called thiol protease (Iordan and Belozerskii 1976). Similar findings were observed during germination in *Vigna mungo* seeds and enzyme designated as SH-EP [Sulphydryl-Endopeptidase], degraded the seed storage protein in protein storage vacuoles (Okamoto 1995).

Inhibition with iodoacetic acid supported this assumption that the presence of a thiol group in protein structure of enzyme, which is not a simple functional group, but constitute a part of an interactive system at the active site (Csoma and Polgar 1984). Similar results were found in sarcocarp of bead tree fruits (Kaneda *et al*. 1994), in *Ervatamia heyneana* (Patel and Jagannadham 1999) and in *Cajanus cajan* seeds (Umar 1992). The inhibition of protease activity with metal ions indicated that this is not a metallo- protease because it did not required metal ions for its activity. Similar findings were observed in germinating pea where $Pb^{2+}$ and $Cd^{2+}$ act as inhibitors (Bansal *et al*. 2001). The protease enzyme inhibition with iodoacetic acid and with HgCl was also observed in germinating mungbean seeds (Yamaoka *et al.* 1990). These all findings suggested that this was not a metalloprotease.

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