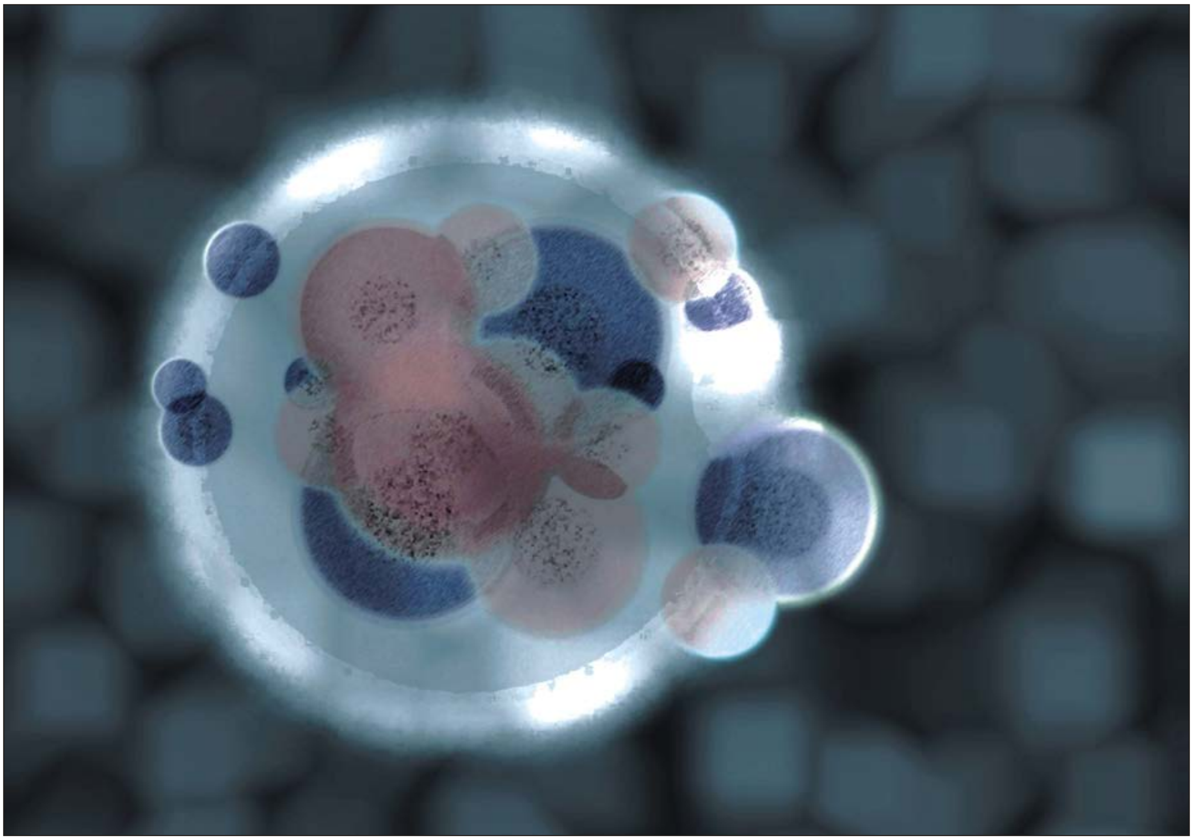

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# Effect of Temperature on Protein Profile of *Pisum sativum* L. Seeds During Germination

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**Abstract:** Abundance of protein content which does not change during temperature treatment is a characteristic feature of temperature tolerant seeds. The seeds after mechanical drying at 35, 40, 45 and 50°C did not show any difference in number of polypeptides between each other when germination was carried out in room temperature and cold condition. Additional polypeptides shown by SDS-PAGE in *Pisum sativum* seeds germinated under cold support the view of enhanced synthesis of housekeeping proteins during cold acclimation. This quality of pea seeds seems to be an ecological adaptation to growth at a wide range of temperature regime.

**Key words:** Drying, germination, pea, *Pisum sativum*, protein, seed

## INTRODUCTION

Several metabolic changes occur in seeds during post-harvest drying. These changes involve the appearance of important metabolites that are believed to have functional significance with respect to the protection of seed tissues against rigors of desiccation. They are sugars, oligosaccharides and some specific proteins (Bewley and Black, 1994). Late-Embryogenesis-Abundant (LEA) proteins which are hydrophilic due to their specific amino acid composition and stability at high temperature do not get denatured at high temperature. Their ability to attract water molecules maintains water enriched local environment inside the seed and they are thought to play an important role in protection against desiccation (Bewley, 1997). Germination commences with the uptake of water by the dry seed and is completed when the radicle protrude to outside. During the early phase of imbibition most of the water taken up is presumably used to re-hydrate desiccated organic molecules, such as those of nucleic acids and proteins. Later, free water begins to accumulate in cytoplasmic vacuoles and it is at this stage that the cells appear to become susceptible to drying (Sutcliffe and Pate, 1977). Several studies on seed germination in *Pisum sativum* are available pertaining to the effect of moisture content on germination and longevity (Ellis *et al.*, 1990), leachate constituents and conductivity during imbibition (Powell and Matthews, 1978), soaking injury and seed quality (Prusinski and Borowska, 1996) and temperature effect on germination (Munro *et al.*, 2004). *P. sativum* seeds are highly viable and rapidly germinate under favorable environmental condition (Mahler *et al.*, 1988). The ecological survey of pea plants shows that they require a cool, relatively humid climate (Davies *et al.*, 1985). In the present study, seed protein composition was studied, in an attempt to determine the wide range temperature adaptability of pea seeds.

## MATERIALS AND METHODS

The research was done in Physiology and Biochemistry division, Department of Botany, University of Calicut, Kerala, India in 2005.

The *Pisum sativum* L. var. Bonne villa seeds were dried under temperature ranging from 35, to 50°C at an interval of 5°C for 7 days in hot air oven. The seeds after treatment were sterilized by washing in 0.1% (w/v) mercuric chloride. After rinsing in sterilized double distilled water, the seeds were placed in Petri dishes lined with whatman No.1 moist filter paper. The germination under dark was carried out in room temperature (30±2°C) and in cold (3±1°C).

Protein extracts were prepared from the randomly selected seedlings. The homogenate was prepared in 0.05 M ice cold pH 7.5 sodium phosphate buffer. The resulting mixture was precipitated with 10% (w/v) TCA and centrifuged at 20,000 g in cold centrifuge. The precipitate was washed with 80% (v/v) acetone and re-suspended in 0.1 N NaOH. The protein was quantified by the method of Lowry *et al.* (1951). SDS-PAGE was done following the method of Laemmli (1970) using 12% mini gel. After electrophoresis the gel was stained with 0.1% Coomassie brilliant blue and the bands were compared with known molecular weight marker protein (PMW-M) procured from GENEI chemical company.

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## RESULTS AND DISCUSSION

The protein profile of the control seeds and the treatment at 35, 40, 45 and 50°C did not show any difference in number of polypeptides between each other when germination was carried out in room temperature and cold condition (Fig. 1). But when a comparison was made between the seeds germinated in room temperature and cold condition there was considerable increase in soluble protein quantity (Fig. 2) and number of polypeptides (Fig. 1c) in the case of protein profile of the seeds geminated in the cold condition. Also the protein bands were thicker in seeds treated at 40, 45 and 50°C on germination under cold condition when compared with the control and treatment at 35°C. These polypeptides cannot be considered as proteins produced to withstand chilling stress because according to Guy (1990) a number of enzymes and proteins are produced/activated during cold acclimation and all of them are not HSPs.

A number of enzymes show shift in isozymic composition upon exposure to low temperature and numerous electrophoretic studies have shown both qualitative and quantitative differences in the protein content between non-acclimated and cold acclimated tissues. This apparent increase in protein synthetic capacity of *P. sativum* seeds during germination under cold (Fig. 1 and Fig. 2) supports the idea that the synthesis of housekeeping proteins were continued to be synthesized during low temperature acclimation (Laroche and Hopkins, 1987). Recently Munro *et al*. (2004) suggested that *Pisum sativum* is a chilling tolerant plant with respect to respiration rate, lipid peroxidation and ubiquinone content distribution. According to these authors, antioxidant system might have protected both total ubiquinone and membrane lipids in pea seedlings owing to their chilling tolerance.

The protein quantity was slightly higher in the case of seeds treated at 40, 45 and 50°C on germination in cold and also the bands were thicker when compared with the control seeds and the treatment at 35°C. This result was in coincidence with chilling resistance observed in cucumber seeds (Lafuente *et al*., 1991). In cucumber the pretreatment with higher temperature induces the production of heat shock proteins and in the presence of these the plants were resistant to chilling. But in the case of *P. sativum* no additional bands are observed. Previously, we have shown that in *Pisum sativum* seeds, the heat treatment given at 45 and 50°C resulted in seedling growth retardation at room temperature but the heat shock was ameliorated on germination in cold condition (Shereena and Nabeesa, 2006).

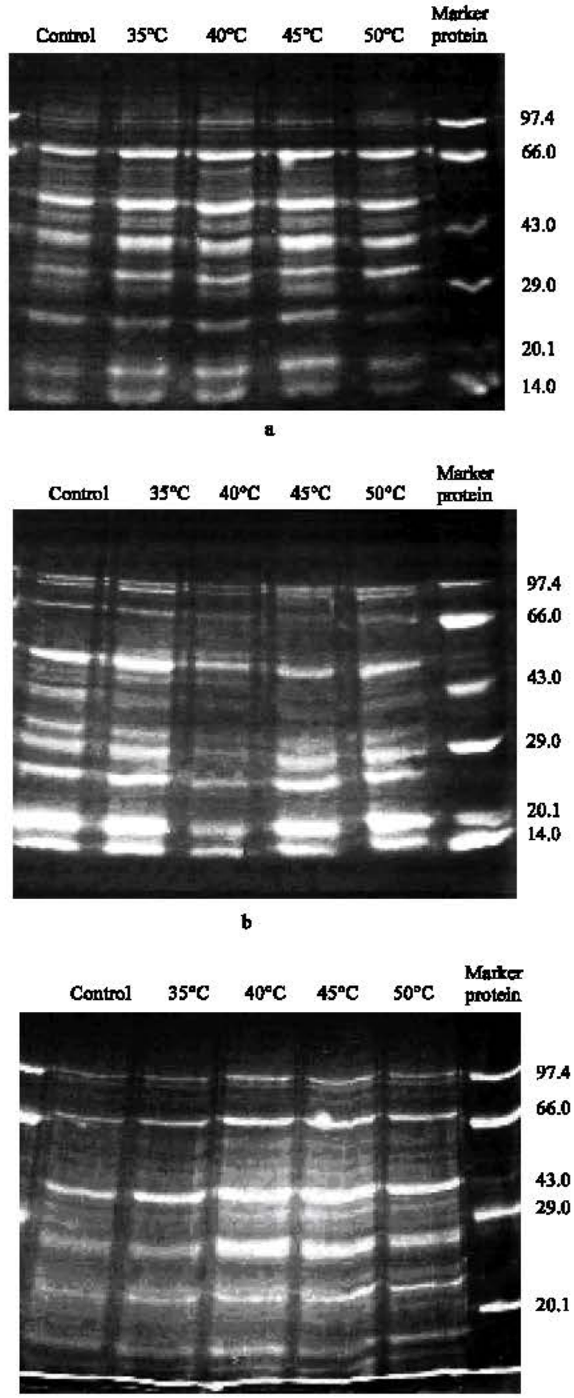


Fig. 1: SDS-PAGE of soluble protein in *Pisum sativum* L. seeds. Each well was loaded with 10 µg of protein a) Dry seeds, b) Seeds germinated in room temperature and c) Seeds germinated in cold

These results in comparison with the observations of Munro *et al*. (2004), the pea seeds are having the

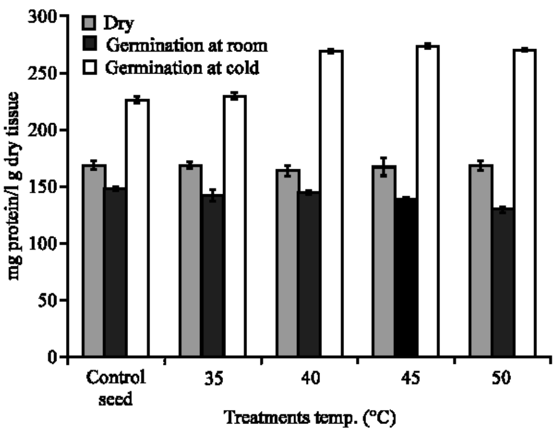


Fig. 2: Soluble protein from *Pisum sativum* L. seeds during germination under different condition

ecological adaptation to grow in cold condition. Along with these observations and the wide variability in growth region ranging from -20 to 30°C (Slinkard *et al.*, 1994) it is tempting to say that the *Pisum sativum* seeds are well adapted to grow in cold condition.

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