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Chilling Tolerance in *Pisum sativum* L. Seeds: An Ecological Adaptation

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Abstract: *Pisum sativum* L. plants are well-adapted to grow in the areas with cool and humid climatic conditions of tropics, subtropics and temperate regions with temperature ranging from 7-30°C. These plants grow as inter annual, can tolerate frost to -20°C in the seedling stage. In *Pisum sativum* seeds the heat shock given at 45 and 50°C resulted in seedling growth retardation at room temperature but the heat shock was ameliorated by chilling treatment since in all experimentals (treatments at 35, 40, 45 and 50°C) seedlings exhibited uniform proliferated growth in cold condition. This quality of *Pisum sativum* seeds seems to be an ecological adaptation to growth at a wide range of temperature regime.

Key words: Chilling, ecology, germination, heat shock, pea

INTRODUCTION

Pisum sativum seeds are highly viable and readily germinating and they rapidly germinate under favourable environmental condition (Mahler *et al*., 1988). Pea plants require a cool, relatively humid climate and are grown at higher altitudes in tropics with temperatures from 7 to 30°C and production is concentrated between the Tropics of Cancer and 50°N (Davies *et al*., 1985). As a winter annual, pea tolerates frost to -2°C in the seedling stage, although top growth may be affected at -6°C. Winter hardy peas can withstand -10°C and with snow cover protection, tolerance can be increased to -40°C and the optimum temperature levels for the vegetative and reproductive periods of peas were reported to be 21 and 16 and 10°C (day and night), respectively (Slinkard *et al*., 1994). Since *P. sativum* seeds are well adapted to wide variations in the environmental temperature an attempt is made in the present investigation to elucidate the physiological and biochemical aspects of temperature-treated seeds during germination at room temperature (30±2°C) and under chilled condition (Refrigerator 3±1°C).

MATERIALS AND METHODS

Garden pea or green pea (*Pisum sativum* L. cv. Bonni villa) seeds were purchased from National Seed Corporation, Thiruvananthapuram, Kerala, India and the study was conducted at Physiology and Biochemistry Division, Department of Botany, University of Calicut, Kerala, India in 2005. Healthy and good quality seeds were selected by hand picking method and deformed seeds with broken test were discarded. The seed samples were tested for viability using tetrazolium test. Healthy seeds were selected and made into five lots. Among them four lots were kept separately in hot air oven at 35, 40, 45 and 50°C for 7 days and after 7 days, seeds were taken for germination studies. The fifth lot is treated as control. Germination studies, at room temperature (30±2°C) and in the refrigerator (3±1°C), of control and the seed treated at 35, 40, 45 and 50°C were carried out in sterilized Petri dishes lined with Whatman No.1 filter paper.

Embryonic axis of control seeds and treatments germinated at the room temperature and in the refrigerator were collected. The samples were fixed in Carnoy's fluid for 2 h and were dehydrated through alcohol- TBA series and embedded in paraffin wax. Using a rotary microtome the individual blocks were cut at 11 μ thickness. The deparaffinised sections were hydrated through alcohol series and were immersed in Harris hematoxylin for 30 min (Harris, 1900). The stained sections were washed in water and dehydrated through alcohol series. The clearing was carried out in xylene and was mounted in DPX mountant.

The photographs of the stained sections were taken using Nikon Model Eclipse E-500 Image analyzer.

RESULTS

Considerable difference in morphological character between control and treatments was observed during germination. When the germination was conducted at room temperature the seedlings of control seeds and treatment at 35°C did not show any difference (Fig. 1b). The seedling of treatment at 40°C was slightly shorter in size (Fig. 1c). The seedlings of treatment at 45 and 50°C were showed stunted growth short and stout radicle

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(Fig. 1d and e). Germination rate and seedling growth in refrigerator condition were very slow compared to that of room temperature but showed profuse root growth in the later stages of germination. The morphological character between control and treatment was similar when germinated in refrigerator.

When the germination was conducted at room temperature the root apex after one day of germination showed the characters of a typical root (Fig. 2a) consisting of root cap and differentiating provascular strands. The control seeds and the treatment showed many differences between them. In the case of seeds

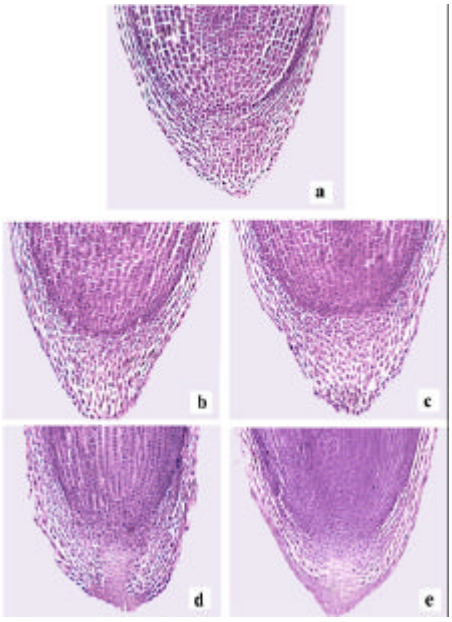

Fig. 1: Radicle apex of *Pisum sativum* L. seedling after germination in room temperature, a) Control seeds b) Seeds treated at 35°C, c) Seeds treated at 40°C, d) Seeds treated at 45°C and e) Seeds treated at 50°C

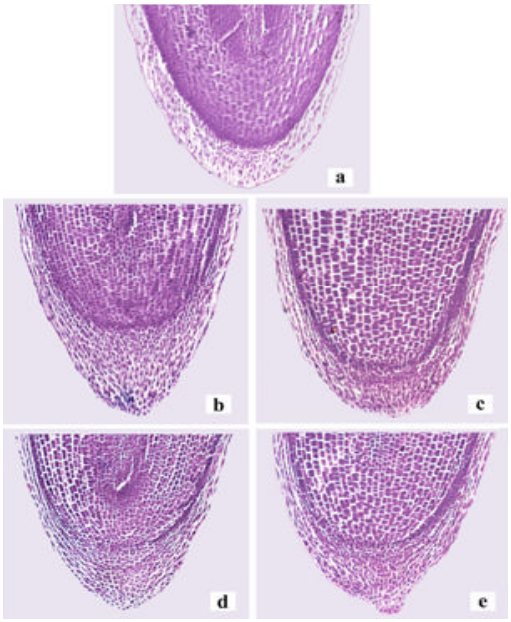

Fig. 2: Radicle apex of *Pisum sativum* L. seeds during germination in cold, a) Control seeds, b) Seeds treated at 35°C, c) Seeds treated at 40°C, d) Seeds treated at 45°C and f) Seeds treated at 50°C

treated at 45 and 50°C (Fig. 1d,e) the cap cells showed the cell wall breakage of feeble staining in the meristematic region was very distinct. Differentiation of provascular strands also were not clear and intense staining masked cellular structure.

In the root apex after germination in refrigerator an important observation was the absence of any breakage in the root apex of seeds treated at 45 and 50°C compared to seedling germinated at room temperature. There was no significant difference between the control and the treatments (Fig. 2a-e).

DISCUSSION

Many hypotheses have been offered to explain the cellular events of chilling injury and a phase transition of membrane is still thought to be the initial step in a chain of events that results in chilling injury (Nishida and Murata, 1996). In chilling sensitive plants, radicle growth is an indication of chilling stress and the chilling sensitivity is shown by the incremental decrease in subsequent elongation of cucumber (*Cucumis sativus*) radicle (Rab and Saltviet, 1996). Eventhough the slow growth rate of radicle was shown by *Pisum sativum* seeds during early stages of germination, profuse root development occurred as seedling growth advanced and hence the inhibitory effect of high temperature (45 and 50°C) was not expressed. Mangrich and Saltviet (2000) reported that cucumber radicle exposed to chilling temperature for 6-7 days resulted in severe injury and the radicle failed to elongate. These observations are similar to the finding of Jennings and Saltviet (1994) who reported that irreversible chilling injury occurs in cucumber seedling after 4 days at 25°C. According to Bochicchio *et al.* (1991) maize seeds absorbed water very slowly and hence imbibitional injury is independent of chilling temperature and seeds experienced the lowest inrush of water into, showed highest radicle growth.

According to Mangrich and Saltviet (2000) cucumber seeds exposed to heat shock of 40°C for 4-12 min increased chilling tolerance such that 4 days of chilling caused only 30% decrease in radicle growth compared to 66% for seedling which are not heat shocked. The ability of heat shock to ameliorate the effect of chilling on subsequent radicle elongation was affected by the severity of chilling. In *Pisum sativum* seeds the heat shock given at 45 and 50°C resulted in seedling growth retardation at room temperature but the heat shock was ameliorated by chilling treatment since in all experimentals (treatments at 35, 40, 45 and 50°C) seedlings exhibited uniform proliferated growth in cold condition

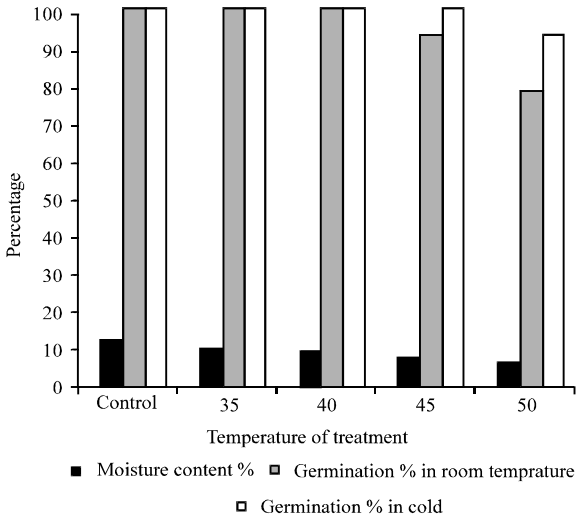

Fig. 3: Effect of temperature treatment on germination and moisture content in *Pisum sativum* seeds

(Fig. 1 and 2). This quality of *Pisum sativum* seeds seems to be an ecological adaptation to growth at a wide range of temperature regime as suggested by Davies *et al.* (1985). Hence *P. sativum* seeds are chilling tolerant since chilling sensitivity restricts wide geographical distribution of plants because temperature variation is significant at different geographic regions.

According to Markowski (1998) chilling injury can be prevented if seeds are exposed to chilling in a more hydrated state, moisture content being more than 10% or after a first imbibition period at warm temperature. The present study in *P. sativum* seeds shows imbibitional injury is prevented or ameliorated even when the moisture content is less than 10% (about 6.5%) during germination in refrigerator (Fig. 3). 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. Pea seedlings under chilled condition showed no difference in the rate of carbon loss from cotyledon, carbon gain in the embryonic axis or total carbon loss of the whole seedlings measured by dry weight between control and chilled plants. During low temperature treatment lipid peroxidation was measured by malondialdehyde (MDA) accumulation and was not increased in comparison with control seedlings and total ubiquinone content was not decreased. According to these authors, antioxidant system might have protected both total ubiquinone and membrane lipids in pea seedlings owing to their chilling tolerance.

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