Metabolic Changes of Temperature -Treated Green Pea (*Pisum sativum* Linn.) and Soybean [*Glycine max* (L.) Merr.] Seeds During Germination

Thesis submitted to
the University of Calicut in partial fulfillment
of the requirements for the degree of
DOCTOR OF PHILOSOPHY
in
BOTANY

By

BEENA ANTO K.

Division of Plant Physiology and Biochemistry
DEPARTMENT OF BOTANY
UNIVERSITY OF CALICUT
KERALA

2008

DECLARATION

I hereby declare that the thesis entitled "**Metabolic Changes of Temperature - Treated Green Pea (*Pisum sativum* Linn.) and Soybean [*Glycine max* (L.) Merr.] Seeds During Germination**" submitted by me in partial fulfillment of the requirements for the degree of **Doctor of Philosophy in Botany**, to the University of Calicut, is an original research work carried out by me in the Department of Botany, University of Calicut and has not been submitted before for any other degree.

C.U. Campus **BEENA ANTO K.**
Date:

CERTIFICATE

This is to certify that the thesis entitled "**Metabolic Changes of Temperature - Treated Green Pea (*Pisum sativum* Linn.) and Soybean [*Glycine max* (L.) Merr.] Seeds During Germination**" submitted by **Ms. Beena Anto K.** in partial fulfillment of the requirements for the degree of **Doctor of Philosophy in Botany**, to the University of Calicut, is a bonafide record of research work undertaken by her in this Department under my supervision during the period 2001-2008 and that no part there of has been presented before for any other degree.

Dr. K.M. JAYARAM

CONTENTS

ACKNOWLEDGMENT

I would like to express my sincere thanks to **Dr. K.M. Jayaram**, Lecturer, Department of Botany, University of Calicut, for all the guidance and support provided throughout my research work.

I am greatly indebted to **Prof. Dr. Nabeesa Salim** for the constant encouragements, support, timely help, valuable guidance and suggestions given to me during this work. I express my deep sense of gratitude for her expert supervision of the work.

I owe my sincere thanks to **Prof. Dr. S. Nandakumar**,
Prof. Dr. P.V. Madhusoodanan, former Heads of the Department and
Prof. Dr. M. Sivadasan, the present Head, for providing me all the necessary facilities for carrying out this work successfully.

I extend my thanks to the faculty members, the supporting staff, the FIP research scholars and the students of the department of Botany, University of Calicut for their whole hearted support and help.

I specially acknowledge the active support and co-operation provided by my colleagues in the Division of Physiology and Biochemistry, **Ms. Vijayalakshmi K.M., Ms. Kochuthressiamma Andrews**, **Ms. Sheela Francis**, **Dr. Radha P.G.**, **Dr. Abis V. Cherusserry** and **Dr. Sheela S**. I am indebted to them for their help and valuable suggestions given to me to complete the work in time.

I express my sincere thanks to **Mr. Ratheesh Chandra P**., **Mr.Abdusalam K., Mr. Sanoj E**., and **Dr. Manju C. Nair**., **Dr. Jayasree** and all my well-wishers for their help, friendly approach and concern.

I extend my heartfelt thanks to the authorities of St. Joseph College, Irinjalakuda, for giving me the opportunity for doing research under FIP scheme.

I express my gratitude to the ministerial staff of my college for all the help and co-operation rendered to me.

I am also thankful to my colleagues of St. Joseph College Irinjalakuda, for their support and co-operation during the period. I sincerely acknowledge the love and concern of my own friends in the department of Botany.

I acknowledge the University Grants Commission for awarding me the fellowship under Xth plan.

I express my thanks to Mr. Rajesh, Mr. Vimesh and all the staff members of Bina Photostat, for their effort to prepare my thesis in time. And I sincerely thank all those who have helped me in one way or the other in this investigation.

I convey my gratitude to my husband **Mr. Jose Kaitharath**, my children, **Mithun** and **Manjusha,** my **parents** and other **family members** for their love, patience, support and encouragements in my academic life.

Above all I remember with gratitude, the grace of **God** for the successful completion of this work.

INTRODUCTION

Seeds are regarded as physiological enigma of the living world. Seed contains the embryo - the new plant in miniature - and is structurally and physiologically equipped for its role as a dispersal unit and is well provided with food reserves to sustain the growing seedling unit which eventually establishes itself as a self-sufficient, autotrophic organism.

Seeds have been classified into 2 categories, orthodox and recalcitrant, with regard to the post harvest behaviour such as desiccation tolerance/sensitivity, storability and germination behaviour (Roberts, 1973). The recalcitrant seeds cannot be dried below certain critical moisture content without reducing their viability/longevity (Roberts, 1973; Chin, 1988). Orthodox seeds can be dried to low moisture content without any damage and over a wide range of moisture contents there is a negative logarithmic relation between seed moisture content and longevity. Most of the orthodox seeds can be dried to approximately 5% moisture content or less on a wet weight basis.

Orthodox seeds of most plant species are shed from the parent plant with water content approximately below 15% of fresh weight and are air-dried or sun-dried before storage. The water content of seeds depends on the nature

of the major reserves they contain; starch rich seeds have higher water content than those which store lipids (Simon, 1984). Relative humidity and temperature are two important factors that influence seed longevity and these two are highly interdependent (Douglas, 1975; Woodstock *et al.*, 1985; Egli and Tekrony, 1996). Because of this interdependency, Harrington (1972) suggested two "rules of thumb" regarding the optimum conditions of relative humidity and temperature in seed longevity. According to the author, each 1% reduction in seed moisture doubles the longevity and each 5^0C reduction in seed temperature doubles the life span of the seed.

Even though post-harvest drying results in a significant reduction of moisture content, considerable quantity of moisture is retained in seeds. When the seeds are subjected to high temperature, progressive removal of water occurs. According to Bewley and Black (1994), bound water associated with macromolecules is lost, resulting in structural and functional deterioration of seeds. Higher temperature induces tighter packing of molecules and increases their structural disorder leading to loss of seed vigour and viability (Ahmad and Banaras, 1981; Cabrera and Boyd, 1988; Hanley *et al*., 2001; Thomas *et al*., 2003). Effects of high temperature on seeds, characterized by seed coat-imposed dormancy also have been demonstrated in many orthodox seeds in general and legumes in particular. It is also a known fact that high temperature treatment results in the breakage of dormancy (Bewley and Black, 1994; Copeland and McDonald, 1995; Bewley, 1997).

According to de Villalobos *et al*. (2002), high temperature treatment of *Prosopis caldenia* seeds for a short duration of five minutes resulted in a higher germination percentage. The seeds of certain genera of Fabaceae such as *Lupinus, Gastrolobium, Hippocrepis,* and *Cyclopia* showed a positive response towards high temperature treatment up to 120^0C for a short period of 5 minutes, inducing fast seedling growth and increased productivity (Hanley *et al*., 2001).

Mobilization of reserve materials in seeds during germination and seedling growth has been studied in many legume seeds (Khan, 1978; Bewley and Black, 1983; 1985; 1994; Mayer and Poljakoff-Mayber, 1989; Copeland and McDonald, 1995). Studies on reserve mobilization showed that various substances such as soluble sugars, insoluble polysaccharides, nucleic acids, soluble proteins and nitrogen move out of the cotyledons and are transferred to other parts of the growing embryo (Mayer and Poljakoff-Mayber, 1989).

The experimental plants selected for the present study are green pea *(Pisum sativum* L.) and soybean [*Glycine max* (L.) Merr.], belong to family Fabaceae. *Pisum sativum* is one of the domesticated plants with the widest range of uses in both agricultural and horticultural field. It is grown for its green seeds which are used as a vegetable and for canning. The dried seeds are used as food and fodder; and its green matter is an excellent component in forage mixtures. The pea contains up to 25% protein, 52% starch, and 6%

oils and also supplies adequate quantities of vitamins, particularly of the B group and minerals like potassium and phosphorus (Anonymous, 1969).

Pea plants require a cool, relatively humid climate and are grown at higher altitudes in tropics with temperature ranging from 7 to 30^0C and production is concentrated between the Tropics of Cancer and 50^0N latitude (Davies *et al*., 1985). The plant is distributed over a wide area, covering tropical, subtropical and temperate regions and the seeds are adapted to a wide range of fluctuations in climatic conditions (Makasheva, 1983). The pea seeds show hypogeal mode of germination. Peas are found to be an ideal material to study the reserve mobilization during germination since it is a large seeded legume. The cotyledons are bulky and conspicuous even after 7 days of seedling growth.

Soybean is the third largest oil seed crop in India (Bhatnagar and Tiwari, 1991). Due to its three dimensional utility as pulse, oil seed and vegetable, soybean is termed as 'miracle crop' of 20^{th} century (Kumar and Badiyala, 2005) and due to its high protein content (40-42%) it is known as "poorman's meat" (Anonymous, 1995). Many soybean products like soy flour, soy milk, soybean oil, soybean cake are very useful and popular in daily life.

Soybean is a sub-tropical plant. However, its cultivation extends to tropical and temperate regions; up to 52^oN latitude. It does not stand severe

winter or excessive heat. Soybean is a short day annual herb with erect or climbing stem, reaching a height of 1.5 to 6 ft. Soybean seed consists of 5.02 to 9.42% moisture content, 29.6 to 50.3% protein, 13.5 to 24.2% lipid and 14.07 to 23.88% starch (Anonymous, 1995). The composition varies according to the variety cultivated, and conditions of soil and climate.

Soybean seeds show epigeal type of germination. Cotyledons of legumes vary from flat photosynthetic organs that are in all respects functional leaves, to fleshy storage organs that have virtually no leaf-like characteristics (Anonymous, 1995). Soybean has cotyledons which occupy an intermediate position between these extremes as they are thick, fleshy structures which act initially as storage organs. Although they remain thick and show some expansion during and after germination, they eventually become photosynthetic.

The pea and soybean seeds of high viability are readily available throughout the year in most parts of the world. They are quiescent rather than dormant and germinate rapidly when placed under favourable conditions. There are no hard seed coats to be removed and no special conditions such as exposure to light or fluctuating temperature are required for germination.

A study conducted in this laboratory showed that *Pisum sativum* seeds which are protein rich retain their viability even at very high temperature of 70^0 C and above (Shereena and Nabeesa-Salim, 2006). The main objective of

the present investigation is a comparative study of germination behaviour in *Pisum sativum* and *Glycine max* seeds by treating the seeds at high temperature such as 50, 60 and 70^{o}C. Studies on pre-treatment of *G. max* seeds at high temperature are very scant. Although these two seeds are characterized by many similarities in their seed physiology, there are several dissimilarities also. For instance, seeds of *P. sativum* are starch and protein rich and show hypogeal germination while the *G. max* seeds are protein and lipid rich with epigeal mode of germination.

The present investigation intends to focus on the effect of high temperature on seed moisture content, viability, germination rate, seed vigour index, seedling vigour and rate of imbibition. Leachate analysis for estimating electrical conductivity, leakage of mineral ions like K^{+} and Ca^{++} and soluble sugars of the seeds of the two species forms another important aspect of the study. Reserve mobilization pattern is proposed to be investigated through biochemical analysis of important biomolecules like proteins, starch, total soluble sugars, total free amino acids and total lipids in dry seeds and in temperature treated seeds germinating -up to seven days- in etiolated condition. For the elucidation of temperature stress on seeds, estimation of proline as well as peroxidase activity and SDS-PAGE analysis of protein profile are included in the investigation. All the biochemical analyses are done by homogenizing the cotyledonary tissues of seeds and seedlings. In order to pinpoint the site of metabolic degradation and

mobilizations of biomolecules during the early phases of germination of the seeds of the two species, histochemical localization of metabolites like insoluble polysaccharides and total proteins are also carried out.

REVIEW OF LITERATURE

It is well known that physiological activity in seeds is affected by water content (Clegg, 1978; Rupley *et al.*, 1983; Vertucci *et al.*, 1985). Water binds with varying strengths at different water contexts, and therefore has different thermodynamic properties (Rupley *et al.*, 1983). Recognition of three water binding regions along with the idea that the type of water present determines physiological activity in relatively dry systems (Clegg, 1978; Vertucci and Leopold, 1984; Vertucci *et al.*, 1985) has led to the hypothesis of three levels of physiological activity associated with the three regions of bound water in seeds. At very low water contents (region 1), when water is tightly bound, very little activity is possible. At intermediate water contents (region-2), water is bound with intermediate strength and some simple enzymic systems are operable. Finally, at higher water contents (region – 3) water is bound weakly and integrated enzymic systems become functional. At 25^0C, there is a marked increase in the rate of oxygen uptake by soybean and pea seeds when their moisture content exceeded 24 and 26% respectively (Vertucci and Leopold, 1984; 1986).

Mature seeds have a moisture content of only 12 – 14% and can survive for a year or more. The moisture content of maturing and germinating

seeds varies greatly depending on their specific state of development or germination. For example, seed tissues undergo gradual loss of water during maturation to the quiescent state (Burris, 1973), but gain water during germination through the process of imbibition (McDonald *et al.*, 1988).

As orthodox pea seeds germinate, the tissues of the embryo become increasingly sensitive to dehydration (Reisdorph and Koster, 1999). During this transition period, the composition of the cells changes, both in chemistry and ultra structure. Di- and oligosaccharides are replaced by monosaccharides (Koster and Leopold, 1988) while dehydrins and other desiccation-related proteins disappear (Baker *et al*., 1995).

According to Bewley and Black (1994) dry seeds contain various types of water, occurring as tightly bound structural part of macromolecules, as thin film coated over the surface of molecule and/or as bridges over hydrophobic sites resulting in changes in the phase behaviour of membrane lipid. These authors pointed out that in the dry state, in the cytoplasm of desiccation tolerant seeds, water exists in a glassy (vitrified) form even at physiological temperatures and this vitrification stops or slows down all chemical reactions requiring molecular diffusion.

When the seeds are subjected to higher temperature, progressive removal of water occurs. Bound water, associated with macromolecules is lost first, resulting in structural and functional deterioration of seeds (Bewley and

Black, 1994). Higher temperature induces tighter packing of molecules and increases their structural disorder leading to loss of seed vigour and/or viability.

The final water content of dry seeds appears to be important in determining their survival over long periods of storage (Robert and Ellis, 1989). Several studies on the thermodynamics of hydration in dry seeds have documented the role of water binding as a component of desiccation tolerance. Different concepts have been used to characterize water properties in plant tissues. Water in seeds is found as free water and bound water. The term “bound water” is defined as water associated with the cell matrix. It is sufficiently structured so that its thermodynamic and/or motional properties differ from free and/or “bulk water” (Vertucci and Leopold, 1987). The bound water maintains the stability of macromolecules, membranes and functioning of multi-enzyme systems in desiccated orthodox seeds. The free water is necessary for the movements of molecules from one centre of metabolism to another. When dried, this free water is removed and loss of weight is expressed as moisture content of the seeds.

Moisture content reduction is initiated during maturation of seeds and orthodox seeds can be dried to low moisture content without any damage (Bewley and Black, 1985; 1994; Mayer and Poljakoff-Mayber, 1989; Baskin and Baskin, 2001).

The physical state of water in seeds determines the physiological manifestations connected with imbibition and germination. Saio *et al*. (1980) suggested that water content below 10% is desirable for long period storage; this is because most biological activities in the seeds cease when water content falls below this level (Ishida *et al*., 1987) and the stored food materials are consumed at a minimum level. On the other hand, the lowering of water content leads to serious imbibitional damage at germination. Due to the rapid uptake of water by the dry seeds up to a level of 2 to 3 times more than the dry weight of the seeds, some deterioration of tissues like imbibitional damage may occur. The imbibitional damage of soybean seeds depends on the mechanical destruction of cell membranes of the embryo and the cotyledon caused by swelling and expansion of constituents such as protein, starch etc. (Ishida *et al.*, 1988).

Membranes of dry seeds are in a disorganized, possibly non- lamellar, state and as such are inefficient barriers to movement of water and solutes (Simon, 1978). Conditions during the initial phase of seed imbibition, when the dehydrating membranes are becoming reorganized into a continuous bilayer configuration exhibiting efficient semipermeable properties, are critical to survival and successful germination. Excessive rates of water uptake before reorganization is accomplished, may lead to displacement of membrane components, deleterious mixing of cellular compounds and loss of cellular components through leakage into the aqueous medium (Simon, 1978).

The fundamental bilayer structure is dependent upon the interaction between the protein and lipid components of the membrane and the surrounding layer of water. Cell membranes have been regarded as the site of desiccation injury because the earliest symptom of injury is enhanced leakage of cytoplasmic solutes during dehydration (Simon, 1974).

Protein structure of seeds also is changed when most of the interstitial water is removed during drying (Rupley *et al.*, 1983). According to Crowe and Crowe (1992) the membrane phospholipid component forms a gel phase during maturation drying.

Simon (1974) has pointed out that the dry seed should be expected to have porous membranes due to the absence of sufficient water to maintain a hydrophilic/hydrophobic orientation of the lipids in membranes. The author has presented evidence of a rapid leakage of solutes out of pea seeds during the first 10 minutes of imbibition, presumably because of the poor structural arrangement of membranes.

Imbibition is an essential primary process for seed germination and it involves absorption of water by cell walls and protoplasmic macromolecules i.e., protein and polysaccharides wherein water molecules are ‘held’ by electrostatic forces (Noggle and Fritz, 1986). The imbibition of water converts the seed from a quiescent and dormant body with very low respiratory rate into a dynamic organism, active in respiration, biosynthesis and capable of

growth (Mayer and Poljakoff – Mayber, 1963). The water potential (ψ) of the dry seed is so low that it can take up very rapidly the liquid water with which it comes into contact (Koller and Hadas, 1982).

The orthodox seeds are air-dried at the time they are shed, with water content below 15% of the fresh weight (Koller and Hadas, 1982). Increase in cellular volume during initial hydration of seed tissues is both rapid and pronounced. In lima bean, for example, there is an estimated 400% increase in seed volume during early imbibition for approximately 1 hour in warm water (Woodstock and Pollock, 1965).

According to Obendorf and Hobbs (1970) soybean seeds with water content below 13% suffered seriously from imbibitional damage while in those above 17% there was little damage. Obendorf and Hobbs (1970) and Phillips and Youngman (1971) reported that raising the initial moisture content of seeds could protect them from the damage associated with rapid water imbibition.

Waggoner and Parlange (1976) have shown that water entry proceeded at similar rates for living and dead pea seeds and they concluded that there is an initial period of rapid water uptake, which lasts for 30 minutes. Pollock (1969) showed that seeds which started imbibition at higher moisture levels leaked less and produced more vigourous seedlings.

Many studies have attempted to define the physiological responses of the soybean seed to imbibition. Parrish and Leopold (1977) found that the initial stage of imbibition i.e. within 10 minutes, culminated in a wetting of the seed coat and the release of adsorbed gas. Webster and Leopold (1977) and Bramlage *et al.* (1978) showed that proliferation and reorganization of the membrane system such as plasma membrane, endoplasmic reticulum and mitochondria start as early as 20 minutes after the onset of rehydration.

According to Powell and Matthews (1978), the imbibition is a temperature-dependent process and the testa or seed coat has a marked degree of control over the rate of water uptake. Seed size, nature of testa, seed viability and vigour, membrane permeability, chemical composition of the seed tissues etc. play very important roles in imbibitional water uptake.

According to Bewley (1997) the germination commences with the uptake of water by the dry seed and is completed when the radicle protrude to outside. During germination, storage reserves are hydrolyzed and the products are used by the axis for the synthesis of protoplasm, structural components and subsequent growth. The initial energy required for the metabolic processes and growth is also derived from these storage reserves. Although all the three types of storage reserves occur in most seeds, the relative concentrations of each vary considerably among various species.

The effect of temperature in the range of 40-85^{0}C on the permeability

and germinability of the hard seeds of the pioneer tree *Rhus javanica* with a fire syndrome was studied by Washitani (1988). The temperature, effective for the removal of water impermeable coat dormancy of the seed was 55±7.4^{0}C. The most favourable temperature regimes among those tested were in the range of 65-75^{0}C for durations of 30-120 minutes, which frequently occur on denuded ground during the midday hour of clear spring or summer days. The seeds become permeable and germinable after exposure to high temperatures of 50-70^{0}C for appropriate length of time, depending on the temperatures (Washitani and Takenaka, 1986). The top soil temperature of bare ground often rises to around 65^{0}C during mid day hours on clear spring or summer days in temperate Japan (Washitani and Sacki, 1984). A considerable proportion of seeds of the *Rhus javanica* (Washitani, 1988) should be able to become permeable while retaining their viability under the temperature conditions which the seeds can encounter on the soil surface in open sites or during fires i.e. several hours of exposure to temperatures around 60^{0}C or brief exposure to much higher temperatures.

The seeds of certain genera of Fabaceae such as *Lupinus*, *Gastrolobium*, *Hippocrepis* and *Cydopia* can withstand high temperatures up to 120^{0}C for a short period of 5 minutes, and these seeds showed sound germination percentage (Hanley *et al.*, 2001). A similar study of high temperature treatment for short duration of about 5 minutes on *Prosopis*

caldenea showed a higher germination percentage (de Villalobos *et al.*, 2002). Thomas *et al.* (2003) reported that the heat shock of 50^0C resulted in stimulated seed germination of *Dracophyllum secundum*, *Sprengelia monticola*, *Gahnia sieberiana* and *Kunzea ambigua* from soil seed bank. Stupnikova *et al*. (2006) reported that in pea seeds mitochondria play central roles in allowing plants to adapt to extreme temperatures.

According to Ellis *et al.* (1990) the *Pisum sativum* seeds with 14.8% moisture content showed 94% germination but on slow drying of seeds to 3.7% moisture content on a wet weight basis at ambient temperature, germination percentage was declined to 50%. Soaking the seeds in water for 24 hours at 20^0C resulted in increased germination loss.

Khan *et al.* (1973) reported that heat stress at 50-70^0C had a stimulatory effect on seedling emergence and subsequent performance of cotton plants, but higher temperatures caused thermal injury. Cabrera and Boyd (1988) evaluated the effect of temperature in the range of 50 to 70^0C and moisture content of 7.5 to 14.5% on germination of gin-run cottonseed. They found that heat stress reduced the viability and vigour at 70^0C or higher. Faster field emergence was reported by heat stress of 70^0C (Khan *et al.*, 1973) and 60^0C (Ahmad and Banaras, 1981). However, according to those authors while the final germination percentage remained unaffected by heat stress, the root and shoot length was significantly influenced by temperature.

Dry storage at high temperatures, however, reduced seed moisture content and seeds generally became germinable, but there were species specific differences in the duration of the dry storage period required to overcome dormancy (Hilhorst, 1995). In general, high temperature and/or high humidity are major environmental factors causing weathering damage. Measurement of leakage of cations from damaged seeds and electron-microscopy indicated that weathering causes disruption to membranes by changes in lipid and protein bodies, a loss of ribosome and impaired respiratory activity (Woodstock *et al.,* 1985).

Viability of a seed is its ability to germinate under favourable conditions, provided it is non-dormant, and if it is dormant, the dormancy is removed by appropriate means (Roberts, 1972). Environmental factors and storage conditions have a critical effect on viability of seeds. The major factors influencing retention of viability are 1) temperature 2) moisture content and 3) oxygen pressure. In general, the viability period of a seed is increased by decreasing these three factors (Roberts, 1972).

The vigour of dry seed is reduced and their viability is lost as they age in storage (Maguire, 1977). Abdul-Baki (1969) lists 4 categories of biochemical changes associated with reduction in seed vigour: 1) a decline in metabolic activity and its manifestations like reduced respiration, slower seedling growth and lower germination, 2) an increase in the total activity of

certain enzymes like phytase, protease, phosphatases, 3) a decrease in the activity of others, chiefly respiratory enzymes such as catalases, peroxidases, dehydrogenases, cytochrome oxidases, glutamic acid decarboxylase and 4) an increase in membrane permeability and there upon greater leakage of sugars, amino acids and inorganic solutes from the seeds. According to Gelmond *et al.* (1978) the seed vigour means a high rate of the overall biological activities of the seed, resulting in a high yield performance. They measured and predicted seed vigour according to the rate of root emergence of germination or field emergence. Vigour represents the potential ability of the seed to yield the maximum plant product at the earliest time under variable environmental field conditions.

Seed vigour has been negatively correlated with the exudation of electrolytes from seeds soaked in distilled water for 24 hours (Matthews and Bradnock, 1968). Seed vigour is closely related to seed longevity as seeds of low vigour generally have shorter potential longevity than high-vigour seeds (Ellis and Roberts, 1981; Sanhewe and Ellis, 1996). The rate at which seeds lose vigour during storage is affected by environmental factors such as temperature, moisture and O_2/CO_2 concentrations (Harrington, 1972; Villiers, 1973; Douglas, 1975).

Water content of the seeds plays a significant role in retaining viability. A higher physiological quality with respect to germination and vigour

indicates greater probability of having a seed lot with good performance under a wide range of environmental conditions (Egli and Tekrony, 1996).

Seedling vigour is the ability which enables a seedling to grow rapidly after germination (Heydecker, 1972). Seedling growth is used as a measurement of seed vigour (Gelmond *et al.,* 1978). The quality of seed material is influenced by seed technological parameters like germinating capacity, seedling vigour and seedling emergence index (Singh and Afria, 1985).

Germination and vigour are the highest when seed is at its maximum dry weight, a stage known as physiological maturity in most crops (Tekrony and Egli, 1997). During deterioration, vigour is the first component of seed quality which is lost followed by a loss of germination capacity and viability (Trawatha *et al.,* 1995). Temperatures > 30^0C during soybean seed development and maturation are known to reduce seed vigour (Spears *et al.,* 1997).

Viability is tested in terms of percentage of germination. Seed viability denotes the degree to which a seed is alive, metabolically active and possesses enzymes necessary for catalyzing metabolic reactions needed for germination and seedling growth (Basara *et al.,* 2002).

According to Simon and Raja-Harun (1972), Matthews and Rogerson (1976) and Pandey (1988), the evaluation of potassium leakage produced

results comparable to that of the electrical conductivity test. The physiological potential of pea (*Pisum sativum*) seeds can be identified through difference in the quantity of potassium ions released during the first 20 minutes of imbibition. Simon and Raja-Harun (1972) observed a close relationship between electrical conductivity and the amount of potassium leakage from pea (*Pisum sativum*) and cotton (*Gossypium hirsutum*) seeds respectively.

Simon (1984) reported that when dry seeds of pea are placed in water, leakage of potassium is very fast at the start of imbibition and about 10% of this element is lost by 10 to 20 minutes. Potassium is the main inorganic ion leached by seeds during imbibition, followed by sodium and calcium (Loomis and Smith, 1980; Powel, 1986; Granquist, 1987; Queiroga and Parra, 1989; Lee and Karunanithy, 1990; Wood, 1990). There is a strong relationship between the release of these ions during seed imbibition and membrane integrity.

Woodstock *et al.* (1985) showed degenerative changes in cell membranes of cotton (*Gossypium hirsutum* L.) seeds and emphasized that the intensity of potassium and calcium leachate was a more efficient indicator of seed physiological quality than the evaluation of the total amount of ions released by seeds as found in the electrical conductivity test. Those authors concluded that potassium and calcium ions released were better indices for

vigour evaluation than total electrolytes. According to Dias *et al. (*1996) soybean seed potential or vigour can be assessed by analyzing the quantity of potassium ions released during 90 minutes of imbibition.

The conductivity of the seed soaking water or leachate has been used as a standard method for testing seed vigour in the handbooks of the International Seed Testing Association (ISTA, 1987) and AOSA (1983). The leakage of intracellular substances from stressed seeds, including cations, aminoacids, phytohormones and proteins is closely correlated with seed vigour. Poor membrane structure and leaky cells are usually associated with deterioration and low seed vigour (AOSA, 1983).

The testa of legume seeds protects the embryo from massive cellular rupture and the leakage of intracellular substances during imbibition (Duke and Kakefuda, 1981; Duke *et al.*, 1983). The leakage of various intracellular substances from imbibing legume seeds is negatively associated with seed vigour (Bramlage *et al.*, 1979; Yaklich *et al.,* 1979). Leachates may reflect a general deterioration of seed tissues, which results in loss of seed vigour. Electrical conductivity and soluble sugar content were high in the leachate of aged seeds (Parrish and Leopold, 1978).

In soybean cotyledons, membranes were reported to exhibit a rate-limiting function in the case of solute leakage, but not in water uptake (Leopold, 1980). The bulky nature of the soybean cotyledons may have

marked changes in the water uptake. According to Murphy and Noland (1982) the membrane involvement in solute leakage resulted in significant increase in measurable leakage both in radish seeds and excised pine embryos due to heat damage. In the case of solute leakage, similar increases were obtained with heat-killed soybean cotyledons (Leopold, 1980).

The factors which affect the leakage of intra-cellular substances from legume seeds during imbibition include: 1. Testa integrity (Simon, 1974; Powell and Matthews, 1978; Duke and Kakefuda, 1981; Tulley *et al.*, 1981; Duke *et al.*, 1983), 2. Seed moisture content (Hobbs and Obendorf, 1972; Simon and Wiebe, 1975; Parrish and Leopold, 1977), 3. Temperature (Hobbs and Obendorf, 1972; Bramlage *et al.*, 1978; Leopold, 1980; Tully *et al.*, 1981; Duke *et al.*, 1983; Marbach and Mayer, 1985), 4. Water potential (Knypl *et al.*, 1980; Woodstock and Taylorson, 1981; Duke *et al.*, 1983) and 5. Seed ageing (Parrish and Leopold, 1978; Schoettle and Leopold, 1984).

The electrical conductivity test has been recognized in the literature and by seed technologists as one of the best tests for the evaluation of soybean seed vigour (Abdul-Baki and Anderson, 1970; Yaklich *et al.*, 1979; Loeffler *et al.*, 1988). The extent of the leakage of inorganic ions, sugar, organic acid, and protein and amino acid molecules during seed imbibition is promoted by the passive diffusion of low molecular weight solutes and by the leachate of macromolecules through the cellular rupture (Duke *et al.*, 1983). The AOSA

(1983) recommended that the conductivity of soybean seeds should be measured after 24 hours of imbibition in distilled water. Lee *et al.* (1995) suggested a relationship of viability to leaching of sugars from leek, onion and cabbage seeds. The sugar content of dry seeds was unchanged by seed aging due to the temperature treatment at 45^0C, but the leachate collected at 24 hour soaking showed an increase in the total sugar along with the decreased seed germinability in all seeds. According to the authors, non-germinable cabbage seeds leaked the greatest amount sugars compared to viable lots.

Proteins are reported to have the ability to protect intracellular components during desiccation. Their mechanism of action might be analogues to that proposed for the prevention or repair of aggregates of denatured proteins by some heat-shock proteins (Pelham, 1986). Proteins could also act less directly in controlling the water binding characteristics of the seed. Like sugars, specific proteins are known to accumulate during late seed maturation (Goldberg *et al.*, 1989; Bewley and Marcus, 1990; Kermode, 1990) and some of these proteins accumulate during drought stress of vegetative tissue (Mundy and Chua, 1988; Cammue *et al.*, 1989). A role for this class of “late embryogenesis accumulating” (LEA) (Dure *et al.*, 1989) or maturation proteins (Rosenberg and Rinnie, 1988) in protecting against desiccation – induced damage has been proposed (Baker *et al.*, 1988; Dure *et al.*, 1989) and studied in barley (Bartels *et al.*, 1988) and in soybean (Blackman *et al.*, 1991).

Tri-and tetra saccharides such as raffinose and stachyose often occur in considerable quantities in dry seeds of many plant species (Amuti and Pollard, 1977). They have been implicated by correlation as adaptive agents for desiccation tolerance during seed development and germination (Koster and Leopold, 1988; Chen and Burris, 1990; Leprince *et al*., 1990). Many cultivars of soybean (*Glycine max*) accumulate high levels of the raffinose series of oligosaccharides, particularly stachyose, in addition to sucrose (Saravitz *et al*., 1987; Koster and Leopold, 1988; Lowell and Kuo, 1989; Dey, 1990).

The raffinose series of oligosaccharides seem to play acquisition of desiccation tolerance in maturing soybean seeds, yellow lupinus seeds, maize and other seeds (Koster and Leopold, 1988; Blackman *et al*., 1992; Horbowicz and Obendorf, 1994). Sugars are observed to stabilize macromolecular structures including membranes (Crowe and Crowe, 1992; Hoekstra *et al*., 1992; Leprince *et al*., 1993) and enzymes (Carpenter *et al*., 1987; Colaco *et al*., 1992; Hottiger *et al*., 1994) during desiccation. Some experiments indicate that accumulation of raffinose series of oligosaccharides during seed formation prolongs storability of seeds (Bernal-Lugo *et al*., 1993; Horbowicz and Obendorf, 1994).

According to Gorecki *et al.* (1997) mature yellow lupinus seeds accumulate 10.9% of their dry mass as stachyose, verbascose and raffinose and 1.5% as sucrose. Raffinose series of oligosaccharides seem to play the

role of desiccation tolerance in maturing soybean seeds (Koster and Leopold, 1988, Blackman *et al*., 1992; Horbowicz and Obendorf, 1994).

Koster and Leopold (1988) have analyzed changes in sugar contents in seeds after the start of imbibition and attempted to correlate them with desiccation tolerance. Loss of desiccation tolerance in the various seed parts coincided with an increase in glucose and fructose levels and the complete loss of stachyose. The amount of sucrose, the major sugar, was still very high. i.e., decreasing contents of oligosaccharides and increasing contents of monosaccharides coincide with loss of desiccation tolerance. Oligosaccharides together with sucrose have a role in the protection of dry membranes, by promoting the vitrification of water. They also facilitate the stabilization of lipids and proteins in cell membranes by binding the oligosaccharides to phospholipids forming hydrogen bonding (Koster and Leopold, 1988). According to Bewley and Black (1994), disaccharide sucrose and oligosaccharides, raffinose and stachyose, are abundant in desiccation tolerant seeds. In soybean seeds, it has been found that there is a conversion of previously existing monosaccharide to sucrose and/or oligosaccharide during desiccation and these metabolites are formed from the breakdown of starch and there is a steep drop in starch content coinciding with the acquisition of desiccation resistance and these sugars facilitate stabilization of protein and lipids in the cell membranes during higher temperature treatment/desiccation period (Blackman *et al*., 1992).

Sugars not only preserve membrane integrity in dry organisms, but also afford protection to proteins (Darbyshire, 1974). The role of soluble carbohydrates during storage in the deterioration of seed vigour and the loss of germinability was well studied by Bernal-Lugo and Leopold (1992) in maize seeds. According to these authors, the decline in seed vigour was associated with the decline in several soluble carbohydrates. The protective effects of sugars during desiccation also include protein stabilization. For example, the activity of three enzymes, glucoamylase, cellulase, and glucose oxidase, which contained carbohydrate residues stabilizing their tertiary structures, was not affected by dehydration (Darbyshire, 1974).

Mobilization of the stored reserves in seeds during germination and seedling growth has been studied in many legume seeds (Khan, 1978; Bewley and Black, 1983; 1985; 1994; Mayer and Poljakoff-mayber, 1989; Copeland and McDonald, 1995). Almost all seeds store their nitrogen in the form of protein deposited within the storage tissue.

Bhandari and Chitralekha (1984) carried out histochemical studies on dry and germinating seeds of *Brassica campestris* and noted that during germination the protein bodies of the seeds were broken down to provide free amino acids to the growing seedlings. Plenty of lipid droplets were also present in the mature seeds as reserve materials.

Characteristics of seed storage proteins have been systematically

described by Bewley and Black (1985). According to these authors, seed storage proteins are usually deposited within special cellular organelles called protein bodies, which range in diameter from 0.1- 2.5nm and are surrounded, at least during development, by a single membrane. Protein bodies are spherical or oval organelles that contain storage protein and other substances bound by a single limiting membrane. They vary in internal structure and size. During germination, the protein bodies and reserve proteins disappear from the cotyledon and there is an accumulation of protein in the developing axis.

Amorphous protein bodies lacking inclusions are characteristic of the Leguminosae (Guillermond, 1941) and present a common ultrastructrual appearance in several species. Investigations on *Pisum sativum* (Varner and Schidlovsky, 1963; Bain and Mercer, 1966), *Phaseolus vulgaris* (Opik, 1966) and *Glycine max* (Tombs, 1967) reveal many amorphous, membrane-bound protein bodies in the cytoplasm of the cotyledonary cells during the early stages of germination.

It is generally agreed that the storage protein is hydrolyzed to amino acids by proteolytic enzymes. The amino acids may remain in the storage tissue, but most are translocated to the developing axis tissues. They are used for the synthesis of various enzymes and structural proteins. Some of the amino acids may also undergo deamination. These products may then be used for the synthesis of non-nitrogen containing compounds or further

metabolized to yield energy. The hydrolytic enzyme, proteinase, exhibits a steady increase in activity with accelerated aging and hence plays a key role in seed deterioration. A reduced protein synthesis was observed in soybean and pea seeds with aging (Gidrol *et al.*, 1988). Kalpana and Rao (1997) observed that the reduction in the protein content was accompanied by a rise in the level of amino acids, indicating the degradation of protein during the process.

According to Shutov and Vaintraub (1987) during germination proteins undergo quantitative changes of modifications. Many storage proteins become more soluble (Tully and Beevers, 1978) and susceptible to the action of more endogenous proteases which are *de novo* synthesized or translocated during germination (Shutov and Vaintraub, 1987). *De novo* synthesis of proteins is reported in many seeds during germination (Bewley and Black, 1983; Wilson, 1987; Torrent *et al.*, 1989). According to Quail (1979), the major storage proteins, which constitute 50-70% of the total extractable protein, can be histochemically localized in germinating seeds.

Protein body degradation apparently starts either within the protein mass or at the periphery of the protein mass. The internal type of degradation suggests that the proteolytic enzymes originate within the proteinaceous mass where as peripheral degradation suggests that the proteolytic enzymes are associated with the limiting membrane or originate outside the protein body.

Protein degradation plays a major role in controlling the amounts of many key enzymes involved in maintaining cellular homeostasis and regulating differentiation, growth and adaptation to new environmental conditions (Vierstra, 1987). The digestion of protein bodies may begin at the surface of the matrix in the form of numerous peripheral vacuoles which increase in size and fuse with one another, resulting in a large vacuole. In *Pisum sativum* cotyledons, the protein bodies clumped together or vacuolated, indicating their internal breakdown of the protein body during germination (Ashton, 1976). In *Pisum arvense* cotyledon, protein bodies swell and fuse to form large aggregate bodies, which then disintegrate (Smith and Flinn, 1967).

In peanut (*Arachis hypogea*) cotyledons, protein bodies swell and develop cavities, some assume a loose spongy structure and aggregate in the centre of the cell and then disintegrate into fragments, which disappear as germination proceeds (Bagley *et al.*, 1963). Protein bodies first swell to a maximum size, then decrease in volume, become vacuolated, and then swell again after protein and phytin are mostly depleted (Smith and Flinn, 1967). After 24 hours the protein bodies are reduced to vacuoles, which then become a large central vacuole. In pea embryo radicles it is observed that the protein contents are degraded, leaving vacuoles. In *Glycine max* cotyledons, protein bodies become more granular and the limiting membrane disappears during germination. The protein bodies eventually become irregular and sometimes coalesce into a single mass (Tombs, 1967).

The breakdown of the reserve proteins of seeds during germination is well documented. Altschul *et al.* (1961) and Bagley *et al. (*1963) have shown that in *Vicia faba,* the globulins appear to be degraded to amino acids before being incorporated into new proteins. Such proteolysis, if it occurred within the protein-body membrane, would greatly increase the osmotic and imbibition pressure of the vacuolar contents and could account for the swelling and coalescence observed.

Protein degradation is resulted in the production of amino acids (Bagley *et al.,* 1963). Dry seeds contain very little free amino acids. The growth of the embryo in the germinating seed is dependent on the supply of amino acids for its protein synthesis. The amino acid pool increases during germination. The main source of these amino acids is the storage protein. Mobilization of cotyledonary reserve protein is a major event during the germination of legume seeds. The amino acids generated during proteolysis of reserve proteins are translocated to and utilized by the embryonic axis. The interconversions of amino acids through transamination reaction were also possible in the developing axis, because the newly synthesized seedling proteins may be different from the storage proteins (Bagley *et al.*, 1963).

Amino acid formation and transformation therefore constitute an important aspect of the metabolism of germinating seeds (Lea and Joy, 1983). In contrast to the more balanced composition of average cytoplasmic proteins

in the seedlings, the seed proteins often have a great predominance of a few amino acids. Over a half of protein in legumes such as pea or soybean amino acids are in the form of glutamic acid, aspartic acid, arginine and leucine (Sosulski and Holt, 1980).

According to Bewley and Black (1983), variations between species are considerable. In pea it was found that the major 'Nitrogen carrier' was a non-protein amino acid homoserine. Only very little aspartate or glutamate is transported to the axis. During the germination of cottonseed, the total amino acid pool of cotyledons showed a five-fold increase on the third day (Capdevila and Dure, 1977). Collins and Wilson (1975) suggested that amino acids rapidly provide ketoacids to allow establishment of respiratory cycles in the early stages of germination and to sugar synthesis in gluconeogenesis.

Starch is the most common carbohydrate found in seeds. Considerable references are available on the starch-mobilizing metabolism in legumes (Bewley and Black, 1983; 1985; Mayer and Poljakoff-Mayber, 1989; Copeland and McDonald, 1995). During germination, the starch molecules are degraded in the cotyledons and the products are translocated to the developing axis. Hydrolysis of reserves in intact legume cotyledon commences after emergence and elongation of the radicle. In garden pea, 35 – 40% of the seed dry weight is attributable to starch (Bewley and Black, 1985). The depletion of starchy reserve from the cotyledon on pea is biphasic,

showing an initial slow rate that lasts for 5-6 days being followed by rapid decline. The free sugar and dextrin released by starch hydrolysis are rapidly translocated to the growing axis. Mayer and Poljakoff-Mayber (1989) suggested that most of the enzymes involved in the breakdown and interconversion of carbohydrates become active during germination and are synthesized *de novo.*

Marbach and Mayer (1976) and Garcia-Luis and Guardiola (1978) reported that starch metabolism and starch mobilization during germination varies from plant to plant and sugars are not accumulated in the cotyledons. Webster and Leopold (1977) and Adams *et al.* (1980) suggested the appearance of new small starch granules in the cotyledon parenchyma cells of *Glycine max* during germination. The appearance of starch grains and increased starch content in the cotyledons of soybean seeds during germination may be attributed to gluconeogenesis from lipid reserves. Acyl Co-A of lipid origin is used for carbohydrate synthesis *via* glyoxylate cycle during germination in fat rich seeds (Beevers, 1980). Sucrose is generated through intermediate function of hexose phosphate by reversal of glycolytic reactions. The sucrose synthesized in the cotyledons is transported into phloem or converted into starch and stored in the plastids. Starch synthesis during germination, presumably from lipids, has been reported in jojoba (Moreau and Huang, 1977) in soybean cotyledons (Adams *et al.,* 1980) and castor bean endosperm (Reibach and Benedict, 1982).

In contrast to the large size of starch grains formed during seed development, grains synthesized during germination are smaller having the mean volume 20μm^2 and 50μm^2 in *Phaseolus* and *Vicia* respectively are found grouped together around the nucleus (Smith, 1974). The production of these small granules may represent a mechanism for secondary carbohydrate storage as in epigeal species cotyledons take on a leaf like role in the metabolism of germination.

Soluble sugars are utilized early in germination, possible as an immediate energy source (Adams *et al.*, 1980). A decrease in dry weight of cotyledons is an indication that food reserves are being utilized by growing embryonic axis (Adam *et al.*, 1980; Harris *et al.*, 1986). Swain and Dekker (1966) have pointed out that in germinating pea cotyledons, where a rapid increase in starch phosphorylase activity was observed during the first eight days of germination, the role of this enzyme could be the provision of glucose-1-phosphate needed for the formation of UDP-glucose for use in the synthesis of sucrose which is then transported to the embryonic axis. During germination of seeds of *Voandzeia subterranea*, known as Bambarra ground nut which is a tropical underground pulse like groundnut, the starch content in the cotyledons fell progressively from 51.8% to 6.8% within 10 days (Umezurike and Numfor, 1979). According to these authors, in Bambarra groundnut, during germination period, amylase activity in the cotyledons

increased during the first four days and declined thereafter. At the period of decline in amylase activity, the cotyledons contained a high level of soluble carbohydrates, which consisted predominantly of maltose and malto-oligosaccharides and some glucose. Amuti and Pollard (1977) also reported the increase in the activity of α-amylase in the cotyledons of Bambarra groundnut during germination.

Smith and Flinn (1967) made histochemical analysis of the cotyledon of *Pisum arvense* during germination and found that initially starch is present in all the cells except those of the epidermis and possibly the potential conducting cells of the procambium. It is present in large quantities in the storage parenchyma, particularly in the inner zone. The hypodermis and procambium contain only very small grains, which disappear within the first three days. The pattern of degradation of starch in the storage parenchyma begins at the periphery of the cotyledon and proceeds in a wave towards the centre. During the first seven days, the outer storage cells become depleted of their starch and after 12 days no starch remains in the central core. During the first two days small starch grains, mostly in the size range 3 to 15μ, appear in the storage cells. They become aggregated around the nucleus; they have disappeared from the outer region of the cotyledon within 5 days and from the central core within 7 days.

Briarty and Pearce (1982) reported the formation of a new family of

starch granules during germination of *Vicia faba* and *Phaseolus vulgaris*. In contrast to the granules formed during seed development, these were very much smaller. Such synthesis appeared to be a fairly common phenomenon in germinating legume cotyledons, and the production of these small granules may represent a mechanism for secondary carbohydrate storage - perhaps more significant in epigeal species where the cotyledons take on a leaf like role.

According to Issa *et al.* (1994) during germination of cowpea, the seed starch and non-reducing sugars were decreased and were followed by an increase in reducing sugars. Sucrose content was highest after 2 days of germination and protein mobilization was slightly increased.

Changes in the soluble carbohydrate contents could contribute to both the decline in both vigour and germinability of the seeds. It is known that soluble carbohydrates generally decline with seed aging (Petruzelli and Taranto, 1989) and this decline might result in limited availability of respiratory substrates for germination (Edje and Burris, 1970). Continuous decrease in sucrose had been observed in pea during its germination (Monerri *et al*., 1986). It is a widespread phenomenon that reserve carbohydrates, in the course of germination, undergo solubilization (Bewley and Black, 1983; Simon, 1984). Changes in total sugar are brought about by changes in its components which undergo active metabolism.

Priestley and Leopold (1983) reported that in control soybean seeds the percentage of lipids in dry state is 20. The lipid content in the seeds decreased with storage time, due to the lipid oxidation in the seeds caused by the intensification of the deterioration process, as soybean seeds quickly lose their physiological quality under inadequate temperatures and relative air humidity.

Becker *et al.* (1978) observed that the cotyledonary metabolism during cucumber germination is characterized initially by gluconeogenic utilization of stored fat through the glyoxalate cycle. Priestly and Leopold (1983) found that storage for 44 months under natural conditions caused a sharp decline in the soybean seed vigour and viability which was associated with a decrease in the proportion of poly unsaturated fatty acids. Hildebrand (1992) concluded that the lipid peroxidation process leads to a reduction in the unsaturated fatty acid content in seeds.

The heat shock proteins (HSPs) are believed to play a role in the acquisition of thermo-tolerance (Vierling, 1991; Howarth and Ougham, 1993). Ubiquitin is also a heat-inducible protein and is believed to play a major role in tagging proteins for proteolysis (Hershko and Ciechanover, 1992; Vierstra, 1993; Munro *et al*., 2004). Ferguson *et al.* (1994) have suggested that ubiquitin may also have a protective function as postulated for HSPs. Synthesis of HSPs at normal growth temperatures has also been reported in *Salix* (Valhala *et al.*, 1990) and was suggested to be an ecological

adaptation to climates that exhibit large air temperature fluctuations (50 – 60^{0}C). Seasonal accumulation of dehydrin, a stress-related protein, has been reported in poplar and other species of woody plants (Wisniewski *et al.*, 1996). Gumilevskaya *et al.* (1996) reported the synthesis of high molecular weight and low molecular weight heat shock proteins in pea seeds on elevating the temperature to 38 – 40^{0}C for 2 – 4 hours from 28^{0}C in which the seeds have been germinated.

In higher plants, HSPs are generally induced by a short exposure to a temperature of 38-40^{0}C (Iba, 2002). Cucumber seed cotyledons on exposure to 42^{0}C for 6 hours were induced to produce five HSPs with molecular mass of 25, 38, 50, 70 and 80 KDa and upon treatment of 25^{0}C for 4 days, the synthesis of three proteins with molecular mass of 14, 17 and 43 KDa were reduced (Lafuente *et al.*, 1991).

Seedlings of soybean, pea, sunflower, wheat, rice, maize and pearl millet have been reported to show the accumulation of heat shock proteins to significant levels after three hours of heat shock but the proteins were characterized by considerable heterogeneity in molecular weight, isoelectric point, stainability and radiolabel incorporation (Mansfield and Key, 1987).

According to Helm *et al*. (1993) in *Pisum sativum* and *Glycine max* low molecular weight heat shock proteins of PsHSP 22.7 and GmHSP 22.0 respectively are synthesized after 3 hours of heat stress at 37^{0}C and these two

HSPs are observed in higher molecular weight structures with apparent masses between 80 and 240 KDa. DeRocher *et al*. (1991) showed that the accumulation of HSP with molecular weight 18.1KDa and immuno-detected protein was proportional to the severity of the heat stress.

The seeds of sorghum have been reported to synthesis heat shock protein after temperature treatments and the seedlings have shown maximal thermo tolerance after the full development of HSPs. But to withstand the lethal temperature, the seeds required a treatment with lower temperature so as to induce HSP for tolerating the effect of high temperature (Howarth and Skot, 1994).

The abiotic stress such as high level of temperature and radiation, salinity, drought etc. may cause the generation of oxidative stress (Foyer and Noctor, 2000). The oxidative stress is characterized by the over production of highly active oxygen species (AOS), represented predominantly by superoxide anion $(O_2)^-$, hydrogen peroxide (H_2O_2), hydroxyl radical ($^{\bullet}OH$) and singlet oxygen ($^1O_2$). Plants have defensive mechanisms and utilize several biochemical strategies to avoid damage caused by AOS. Plant enzymatic defences include antioxidative enzymes such as peroxidases, superoxide dismutase and catalase that promote the scavenging of AOS (Hernandez *et al.,* 2001). Peroxidase is widely distributed in all higher plants and protects cells against the destructive influence of H_2O_2 by catalyzing its decomposition through oxidation into O_2 and H_2O (Dionisio-Sese and Tobita, 1998; Sudhakar *et al.,* 2001). Active oxygen species and the degree of damage depend on the balance between the formation of an AOS and its removal by the antioxidative scavenging systems (Hernandez and Almansa, 2002). The enzyme peroxidases are hemoproteins that catalyze the oxidation of a substrate and the reduction of H_2O_2. Plant peroxidases are typically glycoprotein of 30 to 60 KDa (van Huystee, 1987). These enzymes may participate in many processes of plant growth and defense (Gaspar *et al.,* 1982; Greppin *et al.,* 1986). Plants contain many isoenzymes of peroxidases which show variation in their isoelectric point (pI). Both cationic and anionic peroxidases have been described.

Buttery and Buzzell (1968) classified the peroxidase enzymes into two broad categories based on their activity in the seed coat of soybean (*Glycine max*) as low activity peroxidase and high activity peroxidase enzyme. It is assumed that the peroxidase enzymes in soybean seed coat are important in the process of lignifications and the resulting hardness and permeability, characteristic of the seed tissues (Egley *et al.,* 1983; La Beller *et al.*, 1986; Riquelme and Cardemil, 1993). It has been suggested that soybean seed coat peroxidase enzyme is involved in extensin polymerization, lignification or suberization (Gillikin and Graham, 1991).

According to Wise and Naylor (1987), temperature stress is an environmental factor that may cause oxidative injury. Aerobic organisms have evolved protective scavenging or antioxidant defense systems, both enzymatic and non-enzymatic. The former are based on enzymes capable of removing the free radicals and oxy-intermediates. Peroxidase, catalase and superoxide dismutase play a major role in protecting cells from oxidative damage (Scandalios *et al*., 1980; Zheng and van Huystee, 1992). Apart from their role in scavenging systems, peroxidases are involved in a number of processes like lignification, inactivation of catalytic proteins, anthocyanin degradation and catabolism of plant growth regulators (van Huystee, 1987). Resistance to environmentally induced oxidative stress has been shown to be associated with high levels of peroxidases (Tsang *et al.,* 1991; Zheng and van Huystee, 1992; Scandalios, 1993). This antioxidant enzyme had a major role in the

scavenging of free radicals which are responsible for membrane deterioration in aging tissues (Kellogg and Fridowitch, 1975). Univalent reduction of molecular oxygen to O_2 and H_2O_2 during aging of tissues, senescence of flower petals and fruit ripening is a natural phenomenon to protect the biological system (Fridowitch, 1989). High peroxidase activity is thought to scavenge the hydrogen peroxide evolved during the process of ripening of mango (Reddy and Srivastava, 2003). It has been noted that hydrogen peroxide is consumed by peroxidases, which are present in many plants (Saunders *et al.*, 1964). The role of oxygen species and antioxidant systems in the acquisition of desiccation tolerance was reviewed (Bailly, 2004). With the progress of aging of the tissues or ripening of fruits, the activities of this enzyme decreased which may be due to disintegration of enzyme proteins.

The exposure of *Amaranthus* seeds to elevated temperatures and NaCl salinity causes significant accumulation of reactive oxygen species such as hydrogen peroxide and both these forms of abiotic stress were related to significant reduction of antioxidative efficiency of enzymes like peroxidases (Bhattacharjee and Mukherjee, 2006). According to these authors, abiotic stresses like heat and salinity during early germination may result in the induction of oxidative stress in germinating tissues, which increases the vulnerability of newly assembled membrane systems to oxidative damage.

During water deficit in the environment, many organisms accumulate a

range of amino acids to a greater or lesser degree in different organisms, especially, proline. Accumulation of proline upon dehydration due to water deficit or increasing osmotic pressure has been recorded to occur in the plants which are exposed to high temperatures (Chu *et al.*, 1974). When intact plants are subjected to NaCl stress (1.0 MPa) for 48 hours, there is an increase of 537% in the concentration of proline than in the normal plants. According to the authors, the proline accumulation in response to high temperature could be due to a disturbance in tissue water status, comparable to that observed during simple water deficit.

Proline accumulation is a common metabolic response of higher plants to water deficits and salinity stress. Proline protects membranes and proteins against the adverse effects of high concentrations of inorganic ions and temperature extremes. The level of free proline was found to increase up to 50 fold in many living organisms under various stressful conditions. Alia *et al*. (2001) suggested that proline accumulation might be involved in the protection of these organisms against singlet oxygen induced damages. As a cyclic secondary amine, proline has a low ionization potential and can therefore act as a quencher of singlet oxygen, either chemically by forming products as super oxide or peroxides or physically by inter-system crossing via spin-orbit coupling. The accumulation of proline in a wide variety of both halophytes and non-halophytes when subjected to various stresses and the role of proline in adaptive responses has been reviewed (Aspinall and Paleg,

1981). Moftah and Michel (1987) reported more accumulation of proline in salt susceptible soybean cv. Bragg as compared to semi-tolerant cv. Ransom. There are reports that proline is helpful in drought tolerance (Narayan and Misra, 1989). It appears that proline may play a role in maintaining the cell membrane stability which is desirable for maintaining the cell metabolic processes under drought stress. Gupta *et al.* (1994) suggested that cell membrane stability under stress was more in genotypes with higher proline, showing that proline may also be helpful in maintaining the cell membrane integrity under stress.

Free proline has been suggested as a metabolic measure of drought, and is suggested to play an important role as an organic osmolyte (Heldt, 1999). Teixeira *et al*. (2003) suggested that the expression of glutamine synthetase genes in salt stressed potato plants (cv. Desiree) might cause the production of the osmoprotectant proline and the maintenance of high photorespiratory levels to minimize oxidative stress.

MATERIALS AND METHODS

3.1 Plant Material

Seeds of pea (*Pisum sativum* L.) and soybean [*Glycine max* (L.) Merr.] were selected for the present study. The pea seeds c.v. Boneville were procured from the National Seed Corporation, Ltd. Coimbatore and soybean seeds c.v. SL. 525 from the Plant Breeding Division of Punjab Agricultural University, Ludhiana. From the seed lots, seeds having uniform size, colour and shape with intact seed coats were selected by hand picking.

3.2 Temperature Treatment

From the selected seed lot of pea and soybean seeds, 250 g each of seeds were kept for ten days in a hot air oven, at 50°C. This type of temperature treatments were given to separate pea and soybean seed lots at 60°C and 70°C for 10 days. The treated seeds were subjected to imbibition and germination studies, leachate analysis and biochemical estimation and histochemical localization of various metabolites. The fresh, healthy, untreated seeds were used as the control. The control and temperature treated pea and soybean seeds were surface sterilized with 0.1% (w/v) aqueous solution of mercuric chloride for one minute and then washed thoroughly and

soaked in distilled water for 12 hours. After 12 hours of imbibition, the seeds were taken out of the water and kept for germination in sterilized Petri dishes of 9 cm diameter and 1.5 cm height lined with moistened filter paper (Whatman No. 1). Germination studies were conducted by keeping the Petri dishes in darkroom and daily count of germinated seeds was taken up to seven days.

3.3 Physiological Studies

3.3.1 Germination

Three replicates of 30 seeds each of treated and untreated or control seeds were placed in sterilized Petri dishes and covered with lid plates which also lined with moistened filter paper. Petri dishes were watered as required to replace evaporation losses. Radicle emergence of 1 mm was scored as germinated and germination percentage was calculated by using the formula.

$$\text{Germination Percentage} = \frac{\text{Number of seeds germinated}}{\text{Total number of seeds taken for Germination studies}} \times 100$$

3.3.2 Moisture Content

Thirty seeds from each of the treatment and control were taken and brought to room temperature by keeping in a desiccator, and fresh weight of these seeds was determined by using electronic balance (Shimadzu AX 120).

Then the weighed seeds were kept in a hot air oven at 100°C for one hour and kept at 60°C until constant weight was obtained. Percentage of moisture content was calculated, as explained by ISTA (1985).

$$\text{Moisture content percentage} = \frac{\text{Fresh weight of the seeds - Dry weight}}{\text{Fresh weight of the seeds}} \times 100$$

3.3.3 Seed Vigour Index (SVI)

Thirty seeds each in triplicate from the control and temperature treated seed lot were sampled and sown in garden pots filled with garden soil, sand and dry powdered cow dung mixed in 2:1:1 ratio. Daily count of germinated seeds was taken and percentage of germination was calculated. The seed vigour index was calculated according to Copeland and McDonald (1995) using the formula given below:

$$\text{SVI} = \frac{\text{Number of seeds germinated on first count}}{\text{days of the count}} + \ldots + \ldots + \ldots + \frac{\text{Number of seeds germinated on last count}}{\text{days of the last count}}$$

3.3.4 Seedling Vigour

Vigour of seedlings of control and temperature treated pea and soybean was calculated using biomass method. Seedlings after 7 days of germination

were uprooted carefully and washed in running tap water to remove the sand particles. The seedlings with cotyledon were blotted and weighed using electronic balance. After noting the initial weight, the seedlings were kept in hot air oven at 100°C for one hour and transferred to and kept in 60°C until constant weight was obtained. The seedling vigour was expressed as biomass per seedling.

3.3.5 Imbibition Studies

Control and temperature treated pea and soybean seeds were kept in Petri dishes filled with double distilled water. Imbibition percentage was calculated by counting the unimbibed seeds from the total number of seeds.

3.3.6 Leachate Analysis

3.3.6.1 Collection of Leachate

One gram each of temperature treated and control seeds of pea and soybean were soaked in 40 ml double distilled water taken in beakers for 12 hours. From each beaker, the leachate was collected with care into labelled boiling tubes. Each beaker of seeds was rinsed with water and the total volume of leachate was made up to 50 ml. This leachate was used to analyse,

1. the quantity of minerals like K and Ca,

2. electrical conductivity and

3. estimation of total sugar.

3.3.6.2 Mineral ion concentration in Leachate

Concentration of mineral ions, potassium and calcium in pea and soybean seeds was estimated by Flame photometry method for potassium and calcium ions and expressed in μg g-1 seeds. The estimation of potassium and calcium was conducted by using the Systronics Flame Photometer (Model 128). The leachate was sprayed as a fine mist into a non-luminous flame which becomes coloured according to the characteristic emission of elements, emission of potassium at 768 nm and that of calcium at 420nm.

3.3.6.3 Electrical Conductivity

Electrical conductivity is a measure of ion concentration in leachate. The cumulative conductivity of the leachate was noted using a conductivity meter (Toshinwal TCM 15, auto ranging conductivity and TDS meter) and the electrical conductivity was expressed as μ mhos cm^{-1} g^{-1} of seeds. The leachate samples were centrifuged to remove coarse particles and the conductance was measured. Conductivity of deionised water was found out first and was water having very low electrical conductivity was found out first and was subtracted from the conductivity values of the leachates of treated seeds. Since the conductance changes with temperature, the control and sample measurements were all carried out at the same temperature.

3.3.6.4 Estimation of Sugar in Leachate

Fifty ml of the leachate, collected after 12 hour of imbibition was used for the estimation of total sugar content.

To 5.0 ml volume of the leachate, an equal volume of 80% ethanol was added and centrifuged, so as to sediment the particles if any. The supernatant was decanted to evaporating dishes and was kept in boiling water bath to make it dry. The residue was eluted in 3.0 ml of redistilled water and collected in sterilized and labelled test tubes, for the estimation of total sugar.

Estimation of total sugar was done according to the method of Montgomery (1957). To 1.0 ml of the supernatant solution 0.1 ml 80% (w/v) phenol was added and shaken well. Five ml concentrated sulphuric acid was added to the tubes quickly from a burette and allowed to cool. The optical density of solution was measured colorimetrically (No. 4 green filter) at 540 nm using Photochem Colorimeter. Glucose procured from Merck Company was used as standard.

3.4 Biochemical Studies

3.4.1 Sampling for Biochemical Analysis

Samples for the biochemical estimation of various metabolites were collected on first, second, third, fourth, fifth, sixth and seventh day of germination of temperature treated and control seeds of pea and soybean.

Temperature treated and control pea and soybean seeds before placing for germination were considered as zero or control seeds. Seed coats were removed and the seeds were powdered nicely and were used for biochemical estimation of various metabolites. From first day onwards, the biochemical estimation of metabolised in the cotyledons and axes was done separately. The cotyledons and axes of a few seeds were cut into small pieces and pooled for sampling. Biochemical estimation of total protein, buffer soluble protein, electrophoretic separation of protein by SDS-PAGE, assay of peroxidase activity, total free amino acids, proline, starch, total sugar, individual sugar by HPLC method and total lipid were done.

3.4.2 Dry weight determination

For the quantitative analyses of metabolites, the dry weight of tissue was calculated as explained by ISTA (1985). One gram of fresh tissue was taken and the weighted tissue was kept in hot air oven at 100°C for 1 hour and then at 60°C until constant weight was obtained. Dry weight percentage was calculated using the formula,

$$\frac{\text{Dry weight}}{\text{Fresh weight}} \times 100$$

3.4.3 Estimation of Total Protein

The colorimetric method of Lowry *et al*. (1951) was followed to

estimate the total protein. From the finely chopped pooled samples 150 mg tissue were weighed and homogenised in cold, double distilled water using mortar and pestle and the homogenate was collected. A known volume from the homogenate was pipetted and mixed with equal volume of cold 10% (w/v) trichloroacetic acid and kept for flocculation for thirty minutes in an ice-bath. The protein precipitate was collected by centrifugation for 10 minutes and the supernatant was decanted off. The residue was washed twice with cold 2% (w/v) trichloroacetic acid, followed by washing with 80% (v/v) acetone. The precipitate obtained after centrifugation was digested in known volume of 0.1 N NaOH and heated in a boiling water bath for five minutes. The resulting suspension was clarified by centrifugation. To 1.0 ml of supernatant 5.0 ml of alkaline copper reagent was added and shaken well. After 10 minutes, 0.5 ml of 1N Folin-Ciocalteu Phenol reagent was added and immediately shaken well and kept undisturbed for 30 minutes. The optical density was read at 700 nm using Genesis - 20 spectrophotometer. Bovine Serum Albumin fraction V, (BSA) procured from Merck Chemical Company was used as standard.

3.4.3.1 Estimation of Soluble Protein

The method of Lowry *et al.* (1951) was followed to estimate the soluble protein. One hundred and fifty milligram of the tissue was ground in 50 mM (7.5 pH) Tris buffer using chilled mortar and pestle. The suspension was centrifuged and the supernatant was collected, measured the volume and

used for the analysis of soluble protein. A known volume (2.0 ml) from the supernatant was pipetted and mixed with equal volume of cold 10% (w/v) trichloroacetic acid and the precipitate was allowed to flocculate for 30 minutes in an ice-bath. The protein precipitate was collected by centrifugation for 10 minutes and the supernatant was decanted off. The residue was washed twice with cold 2% (w/v) trichloroacetic acid, followed by washing with 80% (v/v) acetone. The precipitate obtained after centrifugation was digested in a known volume (2ml) of 0.1 N NaOH by heating in boiling water bath for 5 minutes. The resulting suspension was clarified by centrifugation and to 1.0 ml of the supernatant 5.0 ml of alkaline copper reagent was added and shaken well. After 10 minutes, 0.5 ml 1N Folin-Ciocalteu Phenol reagent was added, immediately shaken well and kept for 30 minutes. The optical density was read at 700 nm using Genesis-20 spectrophotometer. Bovine Serum Albumin (BSA) procured from Merck Chemical Company was used as standard.

3.4.3.2 Electrophoretic Separation of Protein

Protein profile of the control and temperature treated pea and soybean seeds were prepared by SDS-PAGE. From the finely chopped pooled samples 200 mg were weighed and homogenised in Tris-HCl buffer of pH 8.4 under ice-cold condition using a clean mortar and pestle. The homogenate was centrifuged at 16,000 x g for 20 minutes at 4^{o}C, using Plastocraft model ROTA R4RV/FM refrigerated centrifuge.

SDS-PAGE was performed in a BIORAD Mini Gel Electrophoretic system according to the method of Laemmli (1970), using 10% separating gel and 2.5% stacking gel. Reservoir buffer used was Tris-Glycine of pH 8.4. 30 µl samples each were loaded to the well using sample mixture prepared by 2% bromophenol blue (10 µl), 10% sucrose (10 µl), 2% SDS (10 µl).

Composition for the preparation of separating gel (12%)

30% Acrylamide/8% Bis Acrylamide -6 ml.

4 x Tris.HCl (pH 8.8) - 3.75 ml.

10% SDS-3.75 ml.

Redistilled water – 5.25 ml.

10% (w/v) ammonium per sulphate- 0.05ml.

TEMED-0.01ml.

Composition for the preparation of stacking gel (4%)

30% Acrylamide/8% Bis Acrylamide – 0.65ml.

4 x Tris.HCl (pH 6.8) - 1.25 ml.

10% SDS-1.25 ml.

Redistilled water – 3.05 ml.

10% (w/v) ammonium per sulphate- 0.025ml.

TEMED- 0.05ml.

After connecting to the power pack, care was taken to note that the tracking dye reached to the bottom of the gel just above the level of sealing agar. The gels were stained with coomassie brilliant blue R-250 and destained in a mixture of 10% methanol, 10% acetic acid and deionised water. The gel was kept in 7% acetic acid solution, photographed in Gel Doc (BIO-RAD) and the bands were compared with known molecular weight marker protein.

3.4.4 Determination of Guaiacol Peroxidase Activity

The enzyme activity was determined according to the procedure given by Abeles and Biles (1991). The peroxidase enzyme was extracted from the seed samples of pea and soybean, control and temperature treated dry seeds as well as from the cotyledons of germinated seeds, sampled daily up to seventh day of germination.

The chopped cotyledons of seedlings as well as the powder of dry seeds were homogenized in 10 ml Tris buffer (50 μM with pH 7.5), using a clean mortar and pestle. The homogenate was centrifuged at 16,000xg at 0^{o}C for 15 minutes in a Kubota KR 20000 T refrigerated centrifuge. The supernatant was collected and 2.0 ml of 10% Trichloroacetic acid was added to 2.0 ml of supernatant and kept for flocculation in an ice-bath for one hour, for the estimation of soluble protein, in order to determine the specific activity of the enzyme.

For the enzyme assay, 0.3 ml of the above mentioned supernatant solution (from which 2.0 ml solution was taken for protein estimation) was used in triplicates. For each 0.3 ml sample, 3.0 ml reaction mixture was prepared by taking 1.5 ml 100 μM Phosphate buffer (pH 6.0), 0.3 ml of 10μM, guaiacol 0.6 ml water and 0.3 ml H_2O_2. All the ingredients except H_2O_2 were added and mixed well in a test tube. Finally the H_2O_2 was added to initiate the enzyme activity. Immediately after the addition of H_2O_2, the mixture was transferred into the cuvette and the activity was 01measured at 470 nm by direct spectrophotometry using Shimadzu UV-1601 UV-visible spectrophotometer. The blank was prepared by adding 0.3 ml H_2O to the reaction mixture instead of enzyme extract. The changes in absorbance were recorded at 30 second interval for 3 minutes such as 30, 60, 90, 120, 150 and 180 seconds. Enzyme activity was determined by subtracting the initial absorbance from the absorbance at 180^{th} second. The unit activity of peroxidase enzyme was expressed as the change in absorbance per minute per gram fresh weight tissue. The enzyme activity was also expressed as specific activity, which is the activity per mg g^{-1} protein.

3.4.5 Estimation of Total Free Amino Acids

Total free amino acids of cotyledons and axes of pea and soybean seeds were estimated colorimetrically according to the method of Lee and

Takahashi (1966).

3.4.5.1 Extraction

From the finely chopped pooled samples, 0.15 g tissue was homogenized in 80% (v/v) alcohol using a clean glass mortar and pestle. The homogenate was transferred to round bottomed flask and refluxed over a steam bath for 2 hours. The flasks were cooled, contents transferred to centrifuge tubes and centrifuged and the supernatant was collected. The residue was re-extracted with 80% alcohol and after each centrifugation the supernatant was combined with original extracts. The combined supernatant was then evaporated to dryness over a boiling water bath in china dishes. The residue obtained was eluted with 3.0 ml of 10% isopropyl alcohol and was used for the estimation of total free amino acids.

3.4.5.2 Estimation

To 0.2 ml of the sample 3.8 ml of nin-hydrin-citrate-glycerol mixture was added. The reaction mixture was prepared by mixing 1.0 ml 1% ninhydrin solution in 0.5 M citrate buffer (pH 5.5), 2.4 ml glycerol and 0.4 ml of 0.5 M citrate buffer. After shaking well, the whole mixture was heated in a boiling water bath for 12 minutes and cooled to room temperature, by keeping in tap water. Within one hour, the optical density of the resultant solution was measured at 570 nm using Genesis - 20 spectrophotometer. Glycine was used as the standard.

3.4.6 Estimation of Proline

Proline content in the temperature treated and control pea and soybean seed sample was estimated according to the method of Bates *et al*. (1973).

One hundred and fifty milligram fresh tissues were weighed out from the finely chopped pooled samples and homogenised in 10 ml of 3% (w/v) aqueous sulfosalicylic acid using a clear glass mortar and pestle. The homogenate was transferred to centrifuge tubes and centrifuged for 10 minutes and the supernatant was collected. From the supernatant solution, 2 ml aliquot was taken in triplicates and equal volumes of glacial acetic acid and were added and mixed well. The tubes with mixture were heated in a boiling water bath for one hour and then the reaction was terminated by placing the tubes in an ice-bath. For colour development, 4.0 ml of toluene was added to the reaction mixture and stirred well for 20-30 seconds. Then the coloured toluene layer was separated and brought to room temperature. The colour intensity of the solution was measured at 520 nm using toluene as reagent blank in Photochem colorimeter. L. Proline was used as the standard.

3.4.7 Estimation of Starch

The method of Pucher *et al.* (1948) described by Whelan (1955) was used to estimate the starch in seed samples. From the chopped and thoroughly mixed samples, one hundred and fifty mg tissue was weighed and homogenized using a glass mortar and pestle in double distilled water and

heated to break amyloplast. After cooling to room temperature, 5.0 ml of 30% (v/v) perchloric acid was added and mixed well. The homogenate was then centrifuged for 10 minutes and the supernatant was collected. The residue was again homogenized and re-extracted 4 times with 30% perchloric acid, centrifuged and the supernatant was collected. The final volume of combined supernatant was noted. To known volume of extract an equal volume of freshly prepared iodine-potassium-iodide reagent was added and kept for 20 minutes and centrifuged for 10 minutes. The supernatant was decanted off. The precipitate was then washed with alcoholic sodium chloride to remove excess iodine reagent. After centrifugation the blue precipitate was treated with alcoholic sodium hydroxide till the blue colour was discharged and then centrifuged to remove the supernatant. The residue was again washed with alcoholic sodium chloride. The precipitate was dissolved in a known volume of 10% (v/v) sulphuric acid by heating in a boiling water bath and centrifuged for 10 minutes.

Estimation of starch was done according to Montgomery (1957). To 1.0 ml of the aliquot 0.1 ml 80% (w/v) phenol was added and shaken well. Five ml of concentrated sulphuric acid was added quickly from a burette and allowed to cool. The optical density of the solution was measured at 540 nm (No. 4 green filter) using Photochem Colorimeter. Soluble starch was used as standard.

3.4.8 Estimation of Total Sugars

Total sugars of cotyledons and axes of temperature treated and control seeds were estimated according to the method of Montgomery (1957). One hundred milligram tissue was weighed and homogenized in 10 ml 80% (v/v) alcohol, using a clean mortar and pestle. The homogenate was collected into centrifuge tubes and boiled for 10 minutes in boiling water bath. After centrifuging this solution for 10 minutes, the supernatant was collected into a test tube. The residue was re-extracted with 80% alcohol 4 times and the supernatant was pooled together and poured into clean and sterilized china dish. After evaporating the solution the residue was eluted in 10 ml double distilled water. Suitable aliquots were taken and final volume was made up to 1.0 ml with double distilled water and one drop of 80% phenol was added and shaken well. Five ml of concentrated sulphuric acid was added quickly from a burette and allowed to cool. The optical density of the resultant solution was measured at 540 nm using Photochem Colorimeter. Glucose procured from Merck Chemical Company was used as standard.

3.4.8.1 HPLC analysis of sugar

The tissue for HPLC analysis was prepared by the protocol of Gorecki *et al.* (1997). One hundred and fifty milligram tissue of temperature treated and control seed samples were weighed and homogenized in 80% ethanol and refluxed for 2 hours at 100^{o}C. After cooling down to room temperature the

refluxed mixture was centrifuged at 16,000 x g for 20 minutes. The supernatant was collected and the residue was re-extracted with 80% ethanol, refluxed and centrifuged and the combined supernatants were evaporated to dryness in a hot air oven at 60°C. The residue was dissolved in 4.0 ml of 80% ethyl alcohol and collected in a clean, sterilized 10 ml vials for further analysis, by HPLC. The HPLC system consisted of Waters u Bondpak - NH_2 column, waters 600 pump, 7725 Rheodyne, 7725 injector, waters 2414 Refractive index detector. The mobile phase was Acetonitrile: water - 70:30 at a rate of 1.0 ml per minute. Sugars were detected by water 2414 Refractive index detector, considering the sensitivity as 4. Sugars were identified by comparison of retention time with known standards (Sigma). Saccharides were quantified by comparison of the peak areas of the samples with those of standard solutions.

3.4.9 Estimation of Total Lipid

Total lipid in the temperature treated and control seed samples were estimated gravimetrically. Seeds/cotyledon tissues were chopped, randomized and weighed and kept at 60°C in hot air oven for 4 hours to make it dry. These dried tissues were powdered and homogenized in chilled diethyl ether, using a mortar and pestle. The homogenates were centrifuged and the supernatant was collected in pre-weighed china dishes. The residue was re-extracted 5 times with chilled diethyl ether and the pooled supernatant in

the china dishes were kept in a hot air oven at 60°C. The china dishes with dried samples were kept in a desiccator for bringing it into room temperature and then the weight of the china dishes was noted. The difference in weight gave the weight of total lipid in the tissue. The total lipid present in one gram dry tissue was calculated.

3.5 Histochemical Studies

Histochemical studies include the localization of total proteins and insoluble polysaccharides in the cotyledons of temperature treated and control seeds/seedlings.

3.5.1 Sampling and Tissue Preparation

Sample collection was made on first, second and seventh day of germination. After decoating, the cotyledons were fixed in FAA, dehydrated through alcohol-TBA series, infiltrated and embedded in paraffin wax (Johansen, 1940). Using a rotary microtome (LEICA, model RM 2125RT) the individual blocks were cut at 10 µ thickness and the sections were deparaffinised, hydrated and stained for localization of total proteins and insoluble polysaccharides.

3.5.2 Histochemical Staining

3.5.2.1 Histochemical Localization of Total Proteins

For the histochemical localization of total proteins, the method proposed by Mazia *et al.* (1953) described by Berlyn and Miksche (1976), was followed and mercuric bromophenol blue was used for staining the total protein.

3.5.2.1.1 Preparation of Mercuric Bromophenol Blue Stain

The mercuric bromophenol blue stain was prepared by dissolving 10 grams of mercuric chloride and 100 mg of bromophenol blue were dissolved in 100 ml of distilled water.

3.5.2.1.2 Staining Procedure

The deparaffinised and hydrated sections were placed in mercuric bromophenol blue solutions for 5 minutes. Then the sections were rinsed for 20 minutes in 0.5% acetic acid. The sections were treated in Sorensen's buffer at pH 6.5 for 3 minutes to form blue colouration. After dehydrations through alcohol series and clearing in xylene, the sections were mounted in DPX.

3.5.2.2 Histochemical Localization of Insoluble Polysaccharides

The localization of insoluble polysaccharides was done according to the method of Berlyn and Miksche (1976) using Periodic Acid Schiff's (PAS) reagent.

3.5.2.2.1 Preparation of Schiff's Reagent

For the preparation of Schiff's reagent, 500 mg of basic fuchsin and 500 mg of Potassium meta bisulphite were dissolved in 100 ml of 0.15 N HCl. The mixture was shaken continuously for 3 hours, using a magnetic stirrer to convert the dye to fuchsin sulphurous acid. Three hundred mg of activated charcoal was added and the mixture was shaken for 5 minutes and filtered. The stain was extremely light sensitive and therefore the bottle was covered with black paper and stored in refrigerator.

3.5.2.2.2 Staining Procedure

The deparaffinised and hydrated sections were placed in 0.5% (w/v) periodic solutions prepared in distilled water at a temperature of 24^{o}C for 15 minutes, and washed thoroughly in running water. Then the slides were stained with Schiff's reagent for 15 minutes at 4^{o}C in refrigerator. The sections were rinsed in water and placed in 2% sodium bisulphite for 2 minutes and washed in running tap water for 5 minutes. The slides were dehydrated through alcohol series and cleared in xylene and mounted in DPX.

Histochemical observations were done by using Nikon microscope (ECLIPSE- E 400) and photomicrographs were taken at 40x with the Nikon Digital Camera (DXM 1200F), attached with Digital Image Analyser.

3.6 Statistical Analysis

All the analyses described were carried out in 6 replicates and the values expressed as mean± standard deviation and standard error. Test of significance was done using Fischer's 't' test. Values of $P<0.05$ were taken as statistically significant.

RESULTS

4.1 Physiological Studies

4.1.1 Germination Percentage

Control seeds of *Pisum sativum* and *Glycine max* exhibited cent percent germination (Table 1, Fig. 1). All the seeds subjected to various temperature treatments showed a rapid decline in the percentage of germination. The seeds of *P. sativum*, treated at 50ºC exhibited a significant reduction in germination percentage and the reduction was very rapid in the case of seeds treated with 60 and 70º C.

The same pattern of reduction was observed in *G. max* seeds also. However, the percentage of germination of temperature-treated seeds was comparatively higher in *G. max* seeds than that of *P. sativum.*

4.1.2 Moisture Content (MC) Percentage

The percentage of moisture content was the maximum in control seeds of *P. sativum*, which showed a reduction in temperature-treated seeds (Table 1, Fig. 2). In seeds, which were given 50ºC temperature treatment, the decrease was slightly rapid and those treated at 60 and 70ºC exhibited a

gradual but significant decrease ($P<0.01$). The moisture content percentage of seeds treated at 70ºC was only half of that of the control seeds.

In *G. max* also the maximum MC percentage was seen in control seeds and the temperature-treated seeds exhibited similar pattern of reduction as that of *P. sativum* seeds (Table 1, Fig 2). Between the 2 species of seeds, the highest MC was observed in *G. max*.

4.1.3 Seed Vigour Index (SVI)

Control seeds of *P. sativum* showed the maximum SVI and the values were found to decrease significantly with temperature treatments (Table 2, Fig. 3). In seeds treated at 50ºC, the decrease was slightly gradual but in 60ºC and 70ºC treated seeds the decrease was very rapid. The control seeds of *G. max* also exhibited maximum SVI and a similar pattern of decrease was noted in temperature-treated seeds as that of *P. sativum*.

4.1.4 Seedling Vigour

Seedling vigour was observed to be the maximum in control seedlings of both *P. sativum* and *G. max* (Table 3, Fig. 4). A gradual but significant ($P<0.01$) reduction in seedling vigour was noted in all the treatments of 50, 60 and 70ºC, in both *P. sativum* and *Glycine max*. But in 70^0C temperature-treated seeds of *P. sativum*, the decrease was more rapid than that of *G. max*.

4.1.5 Imbibition Studies

In *P. sativum*, the control as well as 50ºC treated seeds showed cent percent imbibition after 24 hours (Table 4, Fig. 5). In seeds treated at 60 and 70ºC, a reduction in imbibition percentage was noticed and hard seed formation was found to occur in very low percentage after 24 hours. Hard seed formation was not observed in control and 50ºC treated seeds. Higher percentage of hard seeds was seen in 70ºC treated *P. sativum* seeds. In control as well as all the temperature-treated seeds of *Glycine max*, imbibition rate was cent percent and no hard seeds were noted.

4.1.6 Leachate Analysis - Mineral ion concentration

4.1.6.1 K^+ ion concentration

K^+ ion concentration in the leachate of control seeds of *P. sativum* was the minimum as compared to that of temperature-treated seeds (Table 5, Fig. 6). In 50^0C treated seeds, the K^+ ion concentration was lesser than that in other treatments and the maximum K^+ ion concentration was observed in seeds which were given 70^0C treatment.

The K^+ ion concentration in the leachate of control and 50^0C treated seeds of *G. max* was more or less identical as that of *P. sativum*. But in 60^0C and 70^0C treated seeds of *G. max*, a slight reduction in K^+ ion concentration was noticed in the leachate compared to that of *P. sativum* seeds.

4.1.6.2 Ca^{++} ion concentration

The concentration of Ca^{++} ions was found to be very less than that of K^{+} ions, in the leachates of both *P. sativum* and *G. max* seeds (Table 5, Fig.7). In control seeds of both the species, the concentration was very less and a rapid increase was found to occur in temperature-treated seeds. In 50^{0}C treated seeds of *G. max*, the increase was significant. In *P. sativum*, 60^{0}C and 70^{0}C treated seeds exhibited only a slight increase in Ca^{++} ion concentration in the leachates. But in *G. max* seeds, a rapid increase was noted in Ca^{++} ions in the leachate of 60 and 70^{0}C treated seeds. Temperature-treated seeds of *G.max* exhibited an increased quantity of Ca^{++} ion concentration when compared to that of *P. sativum.*

4.1.6.3 Electrical conductivity

The electrical conductivity of leachates collected after 12 hours of imbibition of *P. sativum* seeds was very less and was found to increase with temperature treatments (Table 6, Fig. 8). In 50^{0}C treated seeds the increase was gradual, but in 60^{0}C treated seeds a three-fold increase in conductivity was noted than that of control seeds. The electrical conductivity was observed to be the maximum in the leachate of 70^{0}C treated seeds.

The same trend of increase in leachate conductivity was observed in *G. max* seeds and the values were greater in the control and treated seeds than that of *P. sativum*. In 50^{0}C treated seeds of *G. max*, the leachate conductivity

values were nearly double the value of the control, but such an increase was not observed in *P. sativum* seeds. In seeds treated at 60 and 70°C the increase in the values was gradual.

4.1.6.4 Estimation of Total sugar

It was observed that the total sugar content in the leachate of control seeds of *P. sativum* collected after 12 hours of imbibition was comparatively low and increased gradually with treatments at higher temperatures (Table 7, Fig. 9). Maximum sugar content was detected in the leachate of 70^0C treated seeds. The increase in total sugar content in the leachates of temperature-treated seeds was gradual.

In the leachate of the control seeds *G. max*, the total sugar content was very low compared to that of the control seeds of *P. sativum.* The same pattern of increase in sugar content was noticed in the leachate of temperature-treated seeds of *G. max* as that of *P. sativum*. But the increase was comparatively lesser than that in *P. sativum*. Maximum sugar content was noted in the leachates of 70^0C treated seeds of both the species

4.2 Biochemical Studies

4.2.1 Dry weight Determination

The dry matter of *P. sativum* seeds showed a gradual increase with temperature treatment and attained the maximum in the 70^0C treated seeds

(Table 8, Fig. 10A). On germination, the amount of dry matter of cotyledons showed a gradual decrease up to seven days of seedling growth. The axes of the control as well as the temperature-treated seedlings showed the same pattern of decrease in dry matter distribution (Table 8, Fig. 10 A and B).

The dry matter percentage of *G. max* seeds showed the same pattern of increase, with the temperature treatments and attained the maximum value in the 70^0C treated seeds than that of the other treatments and control (Table 8, Fig. 10 C). The dry matter distribution in the axes and cotyledons of *G. max* seedlings exhibited the same pattern of decrease up to 7 days of growth as that of *P. sativum* seedlings (Table 8, Fig. 10 C and D).

4.2.2 Total protein

The total protein content was very high in the dry control seeds of *P. sativum* (Table 9, Fig. 11A and B). The total protein content in the axes and cotyledons of germinating seeds was estimated separately. In the axis, the total protein content was very low (1%) initially, and was found to increase significantly throughout the period of study (Fig. 11). But the cotyledons exhibited high protein content (15%) in the initial stage and then showed an insignificant decline up to 4 days and became significant thereafter. In the axis of temperature-treated seeds of *P. sativum*, an increase in the protein content was noticed during germination/seedling growth.

The cotyledons of seeds treated at 50, 60 and 70 ^{o}C showed a gradual

decline in the protein content up to 4 days and thereafter exhibited a slightly rapid and significant ($P < 0.05$) decrease as that of control seeds/seedlings (Table 9, Fig. 11A).

The seeds of *G. max* also showed the similar pattern of protein distribution during the temperature treatments as that of *P. sativum.* The control as well as temperature-treated seeds at 50°C, 60°C and 70°C showed very high protein content in approximately identical quantities (Table 9, Fig. 11C and D).

On the 1st day of germination, the protein content was very low in the axes of control seeds of *G. max*, which increased in the subsequent stages of germination (Table 9, Fig. 11C). The axes of temperature-treated seeds also exhibited less protein content on the 1st day of germination but it was slightly greater in 70°C treated seeds. Similar pattern of increase in protein content was noticed in the axes of temperature-treated seeds during the subsequent seven days of germination. The protein content in the cotyledons of control and temperature-treated seeds of *G. max* showed a gradual reduction during seven days of seedling growth. In the case of 70°C treated seeds, the maximum reduction was observed in the seven day old seedling.

4.2.2.1 SDS - PAGE Protein Profile

One-dimensional SDS-PAGE showed only very slight changes in the protein profile of temperature-treated seeds of *Pisum sativum* compared to control seeds (Fig. 12 A). The gel of control seeds of *P. sativum* exhibited 7 bands between molecular weight 239.20 KDa to 16.68 KDa. In temperature-treated seeds, all the 6 bands were seen distributed within the same range of molecular weight.

During germination of control seeds, on 3rd day, the cotyledons showed seven bands but the specific bands present in the dry state were replaced by new bands with low molecular weight proteins (Fig. 12 B). Two new bands appeared at 43.6 KDa both in 60^{0}C and 70^{0}C treated seeds. The number and colour intensity of bands were found to decrease and was very prominent in the seeds treated at 70^{0}C.

The protein profile of control seeds of *G. max* exhibited 12 bands of protein in between the molecular weight 241.32 KDa and 4.41 KDa. Not much variation in the protein bands was observed in the temperature-treated seeds compared to control (Fig. 13 A).

After 3 days of germination of *G. max* control seeds, the cotyledons exhibited only 8 bands ranging between 94.40 KDa to 3.00KDa (Fig. 13B). The high molecular weight proteins present in dry seeds were not observed in the cotyledons of three days old seedlings.

In 50^0C treated seeds, protein bands having the same molecular weight as in control seeds were seen. But seeds treated at 60^0C and 70^0C showed slight variation in the protein profile. The protein band of molecular weight 94.40 KDa was not seen in the cotyledons of 60 and 70^0C treated seeds.

4.2.3 Guaiacol Peroxidase Activity

Specific activity of peroxidase was measured minimum in dry control seeds of *P. sativum* and was found to increase significantly in the cotyledons of one day old seedlings (Table 10, Fig. 14 A). No significant change in peroxidase activity in the cotyledons of seedlings was noticed up to 4 days. But in 5 days old seedlings, a significant increase ($P<0.01$) was recorded and thereafter no significant change was seen throughout the period of study.

Seeds treated at 50^0C exhibited a significant ($P < 0.05$) increase in peroxidase activity than that of control seeds. A significant increase in peroxidase activity was observed in the cotyledons of one day old seedlings and no significant changes were observed in the two succeeding days. Thereafter, the activity was found to increase in the cotyledons of 4, 5, 6 and 7 days old seedlings and the increase was significant ($P < 0.05$) on 5^{th} day. Similar pattern of peroxidase activity was observed in the cotyledons of 60 and 70^0C treated seeds. Among the different temperature treatments, cotyledons of 70^0C treated seeds exhibited the highest peroxidase activity.

In *G. max*, the specific activity of peroxidase of *control* seeds was very

low on zero days and was found to increase significantly on the first day of germination (Table 10, Fig. 14 B). No significant change in peroxidase activity was noticed in the cotyledons of two days old seedlings, but a continuous and significant increase was seen afterwards.

The temperature-treated seeds showed an increase in peroxidase activity. The cotyledons of *G. max* seeds treated at 50^0C exhibited an identical trend as that of control seeds. But in 60^0C treated seeds the peroxidase activity was found to increase up to 3 days old seedlings and thereafter the changes were similar to that of 50^0C treated seeds. The peroxidase activity in the cotyledons of seedlings of 70^0C treated seeds showed a significant increase throughout the period of study. Of the various treatments, high peroxidase activity was observed in 70^0C treated seeds/seedlings of *G. max*.

4.2.4 Total Free Amino Acids

The control seeds of *Pisum sativum* were found to possess only minimum amount of total free amino acids (Table 11, Fig. 15 A). Somewhat increased amount was observed in all the temperature-treated seeds at dry stage and those treated at 70^0C showed the maximum free amino acid content.

During germination of the control seeds *P. sativum*, a slight but significant increase in the quantity of total free amino acids was observed on

the first and second days. But on the third day, total free amino acid content twice as that on the 2nd day and in the subsequent intervals there was a gradual increase in total free amino acid content. In all the temperature-treated seeds, the same trend of increase was noticed throughout the period of study. Seedlings of 70^0C treated seeds exhibited the maximum total free amino acid content than the other treatments and control.

In *G. max* also, the control seeds showed minimum free amino acids in the dry state. The temperature-treated dry seeds exhibited a gradual increase in total free amino acids, especially in 50^0C and 60^0C treatments and maximum total free amino acid content was shown by 70^0C treated seeds (Table 11, Fig. 15 B).

During germination of the control seeds of *G. max*, an insignificant increase in free amino acid content was observed initially, but the increase became significant thereafter. The same pattern of increase was seen in all the temperature-treated seeds. Maximum total free amino acid content was found to occur in the seedlings of 70^0C treated seeds. Of the two species, *P. sativum* seeds exhibited higher quantity of total free amino acids than that of *G. max.*

4.2.5 Proline

In the control seeds of *P. sativum* and *G. max*, proline content was very low and was found to increase significantly with temperature treatments (Table 12, Fig. 16 A and C). Of the two seeds studied, 70^0C treated seeds of *P. sativum* exhibited the maximum proline content, which was double the value of control seeds.

During germination, the proline content in the axis and cotyledons was estimated separately and a significant difference was noted (Table 12, Fig.14 A to D). In the seedlings of control seeds of *P. sativum*, an increase in the proline content was observed in both the axis and cotyledons throughout the period of investigation. The increase was slightly greater in the axis than in the cotyledons. The same trend was observed in the seedlings of temperature-treated seeds also. In the axis of seedlings of 50^0C treatment, the increase was slightly higher in all the stages of germination than that in the control seedlings. However, in the cotyledons, the proline content was slightly lesser than that in the axis but greater than that of control seedlings. The same pattern of increase was observed in the axis and cotyledons of the seedlings of 60^0C and 70^0C treatments. Among the various treatments, maximum proline content was estimated in the axis and the cotyledon of 70^0C treated seeds on the 7^{th} day of germination.

The proline content in the axis of one day old seedlings of *G. max*

control seeds showed a rapid increase than that of *P. sativum* and thereafter the increase was gradual and significant. In the cotyledons also, the same pattern of increase was noticed but on 7th day, the proline content was slightly greater than that in the axis. The axes and cotyledons of one day old seedlings of 50^{0}C treated *G. max* seeds also exhibited a rapid increase in proline content. The increase in the subsequent intervals was gradual in both the axes and cotyledons and the cotyledons exhibited higher proline content than the axis on 7th day. This pattern of increase in proline content was found to occur in 60^{0}C and 70^{0}C treated seedlings. However, on 7th day, the cotyledons of 70^{0}C treated seedlings exhibited almost the same proline content as that of axis.

4.2.6 Starch

Control seeds of *P. sativum* possessed high starch content that was found to decrease with temperature treatment (Table 13, Fig. 17A). During germination, starch content in the axis and cotyledons was estimated separately, which was much lesser in the axes than in the cotyledons. The starch content in the axis exhibited a gradual increase from first day up to 4 days old seedlings (Table 13, Fig. 17B). On the 5th day, a rapid and significant increase in starch content in the axis was noticed and thereafter the increase was gradual. Similar trend of increase was observed in the axis of the seedlings of 50, 60 and 70^{0}C temperature-treated seeds. The axis of

seedlings of all the temperature-treated seeds exhibited a reduction in starch content on the 7th day as compared to that of the control, and the starch content was very low in the axis of 70^{0}C treated seeds as compared to other treatments and control seeds.

Cotyledons of seedlings of *P. sativum* showed higher starch content than in the axis and the maximum amount was observed in the cotyledons of seedlings of control seeds (Table 13, Fig. 17 B). The starch content in the cotyledon was found to decline with temperature treatments. During seedling growth, a gradual decrease in the starch content was observed gradually up to 4 days, but on 5th and 7th day the decline became significant ($P < 0.01$). The cotyledons of seedlings of seeds treated at temperatures such as 50, 60 and 70^{0}C exhibited the same pattern of decrease of starch content as that of control seeds. On the 7th day of germination, the cotyledons of seedlings of 70^{0}C treated seeds exhibited minimum starch content compared to other treatments and control.

Starch content in the control seeds of *G. max* was comparatively high, but was lesser than that of *P. sativum* seeds (Table 13, Fig. 17 A and C). The temperature treatments given to *G. max* seeds did not show much difference in starch content though there was a tendency to decrease slightly in 60 and 70^{0}C treated seeds. The starch content was found to be less in the axis of *G. max* seeds than the cotyledons during the first four days of growth and a

gradual increase in the starch content was observed in the control seedlings up to 4 days which showed a rapid increase on the 5th day (Table 13, Fig. 17 C and D). The increase was gradual from sixth day onwards. In the axis of seedlings of temperature-treated seeds, a decrease in starch content was observed on the first day of germination than that of control seedlings. Further increase in starch content in the axis was more or less identical to that occurred in control seedlings. The starch content was the minimum in the axis of 70^{0}C treated seeds of *G. max* as compared to other treatments and control seeds.

Cotyledons of seedlings of control and temperature-treated seeds of *G. max* showed higher starch content than the axes (Table 13, Fig. 17 C and D). In the cotyledons of control seeds, the starch content showed a gradual reduction up to four days of germination and the decrease became rapid and significant thereafter. The same pattern of change in starch content was observed in the seedlings of temperature-treated seeds and the starch content in the cotyledon was the minimum in the 7th day old seedlings. (Fig. 17 C).

4.2.7 Total Soluble Sugars

The control seeds of *P. sativum,* exhibited the maximum total soluble sugar content (Table 14, Fig. 18 A). Temperature treatment, like 50^{0}C did not show much difference in the amount of total soluble sugar content, but a slight and significant decrease was observed in 60 and 70^{0}C treated seeds. A

rapid decrease in the amount of total soluble sugars was noticed immediately after the commencement of germination of control seeds, and in one-day-old seedlings very small quantity of sugar was estimated in the cotyledons. Thereafter, a gradual increase in total soluble sugar content was seen up to 5 days and from 6th day, a rapid reduction was noticed.

The same trend was observed in the cotyledons of 50, 60 and 70^{0}C temperature-treated seeds of *Pisum sativum* during germination. In 50^{0}C treated seeds, the total soluble sugar content was more or less identical to that of control seeds. This was clearly seen throughout the stages of germination. In 60 and 70^{0}C treated seeds also, the total soluble sugar content was more or less in equal quantities as in control and 50^{0}C treated seeds during germination.

In the control seeds of *G. max*, the quantity of total soluble sugar was almost the same as observed in *P. sativum* seeds. Temperature-treated seeds exhibited a slight but insignificant increase in total sugar content and in 70^{0}C treated seeds, the total soluble sugar content was found to be the maximum compared to other temperature treated and control seeds (Table 14, Fig. 18 B). On the first day of germination of control seeds of *G. max*, a rapid decrease in total soluble sugar content was observed, but the value of sugar was much greater than that observed in one day old seedlings of *P. sativum.* On second day of germination, there was a decrease in the quantity of total

sugar in the cotyledon of seedlings of control seeds of *G. max* than that of the previous day. A further increase was observed on the 3rd day but afterwards the value decreased continuously up to the 5th day. On the 6th day, a slight increase in sugar content was observed which showed a decrease on 7th day. The same pattern of change in total soluble sugar content was found to occur in the cotyledons of seeds of 50, 60 and 70^{0}C treated seeds.

4.2.8 HPLC profile of sugars

In the analysis of individual sugars by HPLC method, the amount of glucose and fructose was observed to be very small in *P. sativum* control seeds, but in 60 and 70^{0}C treated seeds; these sugars were not detected (Table 15). The quantity of rhamnose present in control seeds was found to increase with temperature treatment. The amount of maltose was comparatively low compared to rhamnose and was greater than that of glucose and fructose, which was found to decrease with temperature treatment. The sucrose content was seen to be the maximum in control seeds of *P. sativum* and a gradual reduction was noticed with temperature treatments (Table 15). The presence of raffinose was also noted in the control seeds of *P. sativum* and the value decreased with temperature treatments. The sugar stachyose showed a significant reduction in quantity in seeds treated at 60^{0}C, which gradually decreased in 70 ^{0}C treated seeds. Verbascose also followed the same trend as that of raffinose and a gradual reduction was observed in temperature-treated

seeds. Of the various sugars detected, the amount of sucrose was found to be the maximum while that of glucose and fructose, the minimum. The total sugar content was high in control seeds, which decreased with temperature treatments.

Glucose was present in the cotyledons of 3 days old seedlings of *P. sativum*, in considerably high quantity than that of control dry seeds. A considerable amount of glucose was detected in the cotyledons of 60^0C treated seeds (Table 16). But in 70^0C treatment, glucose content was not detected in the seedlings. Fructose was found to be present in higher amounts than glucose and a reduction in the value was noticed in 60^0C treated seeds. This sugar also was not detected in the cotyledons of 70^0C treated seedlings. The sugar rhamnose was higher than glucose and fructose in control seeds. But in the cotyledons of seedlings of 60^0C treated seeds, a considerable increase in rhamnose content was noticed. However, in 70^0C treated seeds, a rapid increase was observed. Maltose was not detected in control and 70^0C treated seeds but was present in the cotyledon of 60^0C treated seedlings. The maximum detected sugar was sucrose in the cotyledons of control seedlings, but in 60^0C treated seedlings a slight reduction was noticed (Table 16). In 70^0 C treated seeds, the decrease was not so much, as that of 60^0C treated seeds. Raffinose content was found to decrease in the cotyledons of 60^0 C and 70^0C treated seedlings than that of the control. The amount of stachyose sugar also showed a gradual reduction in the case of temperature-treated seeds

as compared to control seeds. The verbascose sugar also followed the same trend of reduction in the quantity in temperature-treated seeds compared to control. In the cotyledons of seedlings of 60^0C treated seeds an unknown sugar was detected.

The amount of glucose present in dry seeds of *G. max* was the minimum in control and found decreased in quantity by temperature treatments (Table 17). A slight decrease in the quantity of glucose was noted in 70^0C treated seeds than 60^0C treated seeds. The amount of fructose was almost the same in control and 60^0C treated seeds, but there was a sharp decrease in the level of fructose in 70^0C treated seeds. The amount of rhamnose sugar was gradually increased in 60^0C and 70^0C treated seeds. Maltose sugar was totally absent in the samples of *G. max* seeds. The sucrose content was greater than other sugars and was found decreased in the temperature-treated seed samples. Seeds treated at 70^0C showed minimum sucrose content. Raffinose was present in *G. max* control seeds and there was a sharp decline in the quantity in 70^0C treated seeds compared to control and 60^0C treated seeds. Stachyose was also found to decrease in temperature-treated seeds than in control. Verbascose became reduced to very low amount in seeds treated at 70^0C.

The cotyledon of three day old seedlings showed the presence of glucose in the control as well as temperature-treated seedlings and the

quantity was the same in 60 and 70^{0}C treatments, which was greater than that of the control seeds (Table 18). The fructose also showed the same pattern of distribution and both the treatments showed the same quantity, which was greater than control seedlings. Rhamnose was present in the cotyledons of control seedlings and showed an increase in the cotyledons of temperature-treated seedlings. Maltose was not detected in the seedlings of *G. max* as in dry seeds. Of the various sugars detected, sucrose was found to be the maximum in the cotyledons of three days old seedlings of *G. max* control seeds and not much difference was noticed between the control and the temperature-treated seedlings (Table 18). Raffinose sugar was also detected in the cotyledons of the seedlings of control seeds of *G. max.* The amount of raffinose was high in control seeds which showed a gradual decrease in the temperature-treated seeds. Stachyose and verbascose occurred in comparatively lesser quantities than that of raffinose and both the sugars showed a trend of decrease by temperature treatments.

The quantity of raffinose family of oligosaccharides such as raffinose, stachyose and verbascose present in the dry seeds of pea and soybean and in the cotyledons of seedlings on 3rd day of germination was compared. It was found that the total amount is higher in soybean dry seeds than in pea. A sharp decline in raffinose family of oligosaccharides was observed in *G. max* seeds treated at 70 ^{0}C, but such a sharp decline was not observed in *P. sativum* seeds treated at 70 ^{0}C (Fig. 19 A and B). Seeds of both the species

exhibited the same trend of decrease during germination.

4.2.9 Total Lipid

In the control seeds of *P. sativum* the lipid content was only 4% which increased insignificantly to 5% during germination (Table 19, Fig. 22 A). The value reached the maximum in seven day old seedlings. The 50^0C treated seeds exhibited a rapid decrease in lipid content and a slight increase was observed on the first day of germination with a slight decrease on 2nd day. After two days, no significant change in lipid content was noticed in germinating seeds of *P. sativum*. Seeds treated at 60^0C exhibited a slight decrease in lipid content than those treated at 50^0C and no significant change in the value was noticed throughout the period of study. In 70^0C treated seeds, lipid content was comparatively low but a negligible increase was observed on the first day of germination and no significant change was noticed in the subsequent days.

The lipid content was very high, about 30%, in control seeds of *G. max* and an increase was found to occur immediately on the commencement of germination (Table 19, Fig. 22 B). From the 2nd day of germination onwards, the lipid content decreased continuously up to 5 days. Thereafter, a slight increase was seen on the 6th and 7th day. In 50^0C treated seeds, the lipid content was more or less equal to that of control seeds. The change in the lipid content during germination in 50^0C treated *G. max* seeds was also

identical to that of control seeds. A considerable decrease in lipid content was observed in 60^0C treated seeds. A similar pattern of change in lipid content during germination was observed in 50 and 70^oC treated seeds. In 60 and 70^0C treated seeds, the lipid content was higher on the 7^{th} day of germination than that occurred in the dry control seeds.

4.3 Histochemical Studies

4.3.1 Localization of Protein

4.3.1.1 Dry Seeds

4.3.1.1.1 *Pisum sativum*

Cross section of the cotyledon of control seeds of *Pisum sativum* showed spherical shaped cells, compactly arranged with very small intercellular spaces (Fig. 23 A). A blue stained mass was seen in the central region of the cells. Some hyaline bodies were seen embedded in the blue stained mass.

The spherical cells of cotyledon of *P. sativum* seeds treated at 50^oC were filled with blue coloured mass, almost in the centre of the cell lumen (Fig. 23 B). Walls of the cells were found broken in some areas of 50^oC treated seeds. The blue stained protein masses in the cortical cells were unevenly distributed. Some of the cells were composed of very less protein masses than the remaining cells. Unstained, large bodies were found

embedded in the blue coloured mass present in the centre of the cell lumen.

In the sections of cotyledons of seeds treated at 60^0C, the cell walls were found broken, resulting in the loss of the intact nature of the cells (Fig. 23 C). The staining intensity of the protein mass in the cells was lesser in 60^0C treated seeds than in the previous stages. The cross section of cotyledons of *P. sativum* seeds treated at 70^oC showed breakage of cell walls in many places than in 60^0C treated seeds (Fig. 23 D). The staining intensity of protein mass was less compared to the other treatments and the control.

4.3.1.1.2 *Glycine max*

Cross section of cotyledons of the control seeds of *G. max* showed elongated cells arranged compactly. The cell walls were intact and the cells were filled with deeply stained blue masses (Fig. 23 E). In 50^oC treated seeds, no difference was observed in the nature and arrangement of cotyledonary cells (Fig. 23 F). But the staining intensity was found decreased when compared to that of the control seeds.

The walls of cell were found broken at some regions in the section of cotyledons of the seeds treated at 60^0C (Fig. 23 G) and a slight decrease in the staining intensity of the protein masses was noticed. In 70^0C treated seed samples, the cell wall breakage was seen more frequently than in 60^0C treated seeds (Fig. 23 H). The staining intensity was lesser compared to the other treatments and the control seeds. Certain cells were found devoid of any

coloured mass and appeared empty.

4.3.1.2 First day of germination

4.3.1.2.1 *Pisum sativum*

On the first day of germination, the size of the cells of the cotyledons of *P. sativum* seeds was found increased than of the dry control seeds. Blue stained masses were found almost filling the lumen of the cells (Fig. 24 A). Large hyaline bodies were found distributed in the blue stained mass. In seeds treated at 50^0C all the cells were almost filled with blue coloured masses on the first day of germination, as in the control seeds (Fig. 24 B). Large unstained grains were seen embedded in the blue mass.

In 60^0C treated seeds, on the first day of germination, the size of the cells of the cotyledon was found increased than that of the dry control seeds (Fig. 24 C). The intact nature of the cell wall was found lost at many places and wall breakage also was observed. The staining intensity of the blue stained mass became decreased in this sample. Cell wall breakage was very clear in the cells of cotyledon of 70^0C treated seeds and spherical nature of the cells became inconspicuous (Fig. 24 D). The staining intensity also decreased and the volume of the mass present in each cell showed much depletion.

4.3.1.2.2 *Glycine max*

On the first day of germination, the cotyledonary cells of *G.max*

control seeds exhibited tightly packed elongated cells which were filled with deep blue coloured masses. (Fig. 24 E). A slight decrease in the staining intensity was observed in the cotyledonary cells of 50^0C treated seeds of *G. max*, on the first day of germination (Fig. 24 F). The cell wall remained intact as in the case of seedlings of control seeds.

In the cotyledons of 60^0C treated seeds of *G. max* some of the cells were found broken on the first day of germination (Fig. 24 G). The staining intensity of proteins was found decreased in this treatment than in the previous treatment and the control seeds. The cotyledonary cells of the seeds treated at 70^0C exhibited broken cell walls in more areas than in 60^0C treatment (Fig. 24 H). The staining intensity for protein was found decreased very much. The volume of the protein mass was also found decreased at this stage.

4.3.1.3 Second day of germination

4.3.1.3.1 *Pisum sativum*

On the second day of germination, the cotyledonary cells of *P. sativum* control seeds showed a slight increase in size compared to that of dry control seeds (Fig. 25 A). The cell wall was intact and the cells were filled with deep blue stained masses. Some unstained bodies were seen embedded in the protein masses. The cells of the cotyledons of 50^oC treated seeds were observed to be intact and were filled with blue stained masses on the second

day of germination (Fig. 25 B). The staining intensity of the mass showed no significant change as in the control seeds.

On the second day of germination, the protein masses were restricted to certain cells in the cortical region the cotyledons of 60°C treated seeds (Fig. 25 C). The staining intensity for proteins was found decreased than in the previous stages. Unstained bodies of various shapes and sizes were embedded in the blue coloured mass. The cell wall breakage was observed very clearly. The cotyledonary cells of seeds treated at 70°C showed a decrease in the staining intensity than in the other treatments and control (Fig. 25 D). Cell wall disruption was clearly seen at this interval. Unstained grains of various shapes and sizes were embedded within the blue coloured mass.

4.3.1.3.2 *Glycine max*

On the second day of germination, the cross section of cotyledons of control seeds of *G. max* showed tightly packed elongated cells filled with deeply stained blue granular mass. These masses were found at the peripheral part of the cells. Vacuoles were clearly seen in this sample (Fig. 25 E). No change in the compact nature of the cotyledonary cells was noticed in 50^0C treated seeds on second day of germination (Fig. 25 F). The staining intensity of the protein mass showed a reduction compared to the cotyledonary cells of the control seeds. In certain places the cell walls showed breakage.

In 60^0C treated seeds, the cell wall of cotyledon showed breakage on

the second day of germination (Fig. 25 G). The staining intensity for proteins showed considerable reduction than that in the previous stages. Broken cell walls were found in 70^0C treated seeds on the second day of germination. The staining intensity for protein masses was feeble than that in the other treatments and the control (Fig. 25 H). Certain cells were devoid of blue stained masses.

4.3.1.4 Seventh day of germination

4.3.1.4.1 *Pisum sativum*

Cotyledonary cells of the control seeds of *P. sativum* exhibited enlarged size on the seventh day of germination when compared to previous stages (Fig. 26 A). Cell wall breakage was clearly seen at this stage. The cells were filled with deep blue stained masses. Certain hyaline bodies were embedded in the stained mass. The cells of the cotyledon of the 50^oC treated seeds also were found filled with blue stained masses (Fig. 26 B). The staining intensity for proteins was similar to those of the control seeds.

In 60^oC treated seed, cell wall breakage was observed to occur. The cells were filled with blue stained masses but with a lesser staining intensity than that in the 50^oC treatments (Fig. 26 C). Unstained bodies were seen distributed in the blue coloured mass. Broken cell walls were seen in much more areas in 70^oC treated seeds than in the other treatments and the control (Fig. 26 D). Feebly stained blue masses were seen in the cells of the

cotyledon on the 7th day of germination of 70^{0}C treated *P. sativum* seeds. Unstained grains of various shapes and sizes were embedded within the blue coloured mass.

4.3.1.4.2 *Glycine max*

On the 7th day of germination the cross section of cotyledon of the control seeds of *G. max* was found to be composed of tightly packed elongated cells. The cells were filled with deep blue stained masses (Fig. 26 E). Temperature treatment at 50^{0}C did not show any change in the compact nature of cotyledonary cells (Fig. 26 F). Each cell was filled with deep blue coloured masses as in control seedling. In certain areas of the section the cell walls showed breakage.

In 60^{0}C treated seeds, cell wall breakage was visible in more places than in 50^{0}C treated seeds (Fig. 26 G). The staining intensity for proteins showed a reduction in the cells than the previous stages. The cell wall breakage was very clear in 70^{0}C treated seeds on the 7th day of germination (Fig. 26 H). The staining intensity showed a considerable reduction than that of the 50 and 60^{0}C treated seeds. Certain cells were devoid of any blue coloured mass.

4.3.2 Localization of Insoluble Polysaccharides

4.3.2.1 Dry Seeds

4.3.2.1.1 *Pisum sativum*

The cross section of cotyledon of control seeds of *P. sativum*, stained with PAS, showed magenta coloured cell walls (Fig. 27 A). The cells contained magenta coloured starch grains of various size and shape, the staining intensity being very deep. Starch grains present in the peripheral cells were smaller and inconspicuous in size. The three-fourth part of each cell lumen was completely filled with starch grains and each starch grain was composed of a deeply stained region at the centre, called hilum. The cells of the cotyledon of 50^0C treated seeds of *P. sativum* were filled with magenta coloured starch grains (Fig. 27 B). The grains were stained intensely and were closely packed with deeply stained hilum at the centre as seen in the control seeds. Cell wall breakage was observed in some areas of the section of cotyledons. No difference in the number of starch grains was observed in 50^0C treated seeds.

On the seventh day of germination in 60^0C treated seeds, the wall of the cortical cells of the cotyledons was not found to be intact in certain areas (Fig. 27 C). All the cells were filled with varying number of magenta coloured starch grains of different size and shape, all with a prominent hilum at the centre. Smaller grains were found aggregated almost in the center of the cell lumen. The number of starch grains was found reduced in 60^0C treated seeds as compared to seeds treated at 50^0C and control. In seeds

treated at 70^0 almost all the cells showed breakage of cell walls on the 7^{th} day of germination (Fig. 27 D). The size of the starch grain was found reduced than that of other treatments and control. The staining intensity of starch grains was also found reduced in 70^0C treated seeds. Most of the grains were distributed towards the peripheral region of the cells. All the grains were with prominent hilum at the centre.

4.3.2.1.2 *Glycine max*

In the cross section of the cotyledons of control seeds of *G. max*, compactly arranged elongated cells were seen with magenta coloured cell wall (Fig. 27 E). The cells were found filled with magenta coloured granular mass of starch.

Numerous small sized granules were seen in each cell. In 50^0C treated seeds, the staining intensity of the cell wall and starch granules were more or less identical to that of the control seeds (Fig. 27 F).

G. max seeds treated at 60^0C exhibited a decrease in the staining intensity of both the cell wall and starch grains (Fig. 27 G). The size of starch grains was approximately the same as those of the 50^0C treated seeds and the control. At some regions, the wall of cells was found broken. Cell wall breakage was clearly seen in 70^0C treated seeds (Fig. 27 H). The staining intensity of the cell wall and the starch grains was very less compared to the other treatments and control.

4.3.2.2 First day of germination

4.3.2.2.1 *Pisum sativum*

In the control seeds of *P. sativum*, cells in the cortical region of cotyledons showed compactly arranged cells and each cell was filled with magenta coloured starch grains. The grains were arranged at the centre of the cells. The shape of the starch grain remains unchanged as in dry control seeds. The size of starch grains was found increased on the first day of germination (Fig. 28 A). No significant change in the distribution and staining intensity of starch grains was observed on the first day of germination of 50^0C treated seeds (Fig. 28 B). The size of starch grains was found increased in 50^0C treated seeds than in the dry control seeds. The cell wall breakage was observed in some regions.

The 60^0C treated seeds also did not show much difference in the distribution and staining intensity of starch grains on the first day of germination (Fig. 28 C). But a reduction in the size of the grains was noticed at this stage of germination. The staining intensity of the starch grains was found reduced in 70^0C treated seeds on the first day of germination than in the other treatments and control (Fig. 28 D). A reduction in the size of starch grains was also observed in 70^0C treated seeds on first day of germination. Cell wall breakage was very clear at this stage of germination. The shape of the grains was more or less the same as in the previous treatment and control.

4.3.2.2.2 *Glycine max*

On the first day of germination the cell wall of the cotyledon of *G. max* control seeds was intensely stained with PAS (Fig. 28 E). The central portion of the elongated cells of cotyledon was almost occupied by the magenta coloured mass of starch granules. The size of the starch grains was remaining unchanged as in dry control seeds. The nature of distribution and staining intensity of starch grains in 50^0C treated seeds on the first day of germination was more or less identical to that of control seeds (Fig. 28 F). Certain cells looked empty but majority of cells were stained intensely.

The cotyledonary cells of 60^0C treated seeds of *G. max* on the first day of germination exhibited a reduction in staining intensity compared to 50^0C treated seeds (Fig. 28 G). A further reduction in staining intensity was observed on the first day of germination in 70^0C treated *G. max* seeds (Fig. 28 H).

4.3.2.3 Second day of germination

4.3.2.3.1 *Pisum sativum*

The cotyledon cells at the cortical region were found compactly arranged on the second day of germination of the control seeds and each cell was filled with magenta coloured starch grains of various size and shapes (Fig. 29 A). Very small sized starch grains were also found intermingled with the large ones. The hilum region was clearly seen in the magenta coloured starch grains. The starch granules in the cells of 50^0C treated seeds showed a slight reduction in staining intensity on the second day of germination (Fig. 29 B). The number and size of starch grains was found reduced on the second day of germination as compared to that of the previous stages. The shape of starch grains was identical to that of the control seeds.

The intact nature of cells was lost in some parts of the section of the cotyledons of 60^0C treated seeds on the second day of germination (Fig. 29 C). The number and staining intensity of the starch showed a decrease. No change in the shape of starch grains was observed at this stage. On the second day of germination, cell wall rupture was clearly seen in 70^0C treated seeds (Fig. 29 D). The staining intensity showed a reduction than in the other treatments and control. The size and number of starch grains became reduced.

4.3.2.3.2 *Glycine max*

The cell wall of the cotyledon of *G. max* control seeds was intensely stained with PAS on the second day of germination (Fig. 29 E). The central portion of the elongated cells of cotyledon was almost occupied with the magenta coloured starch grains, which were very small in appearance. In each cell a large number of such grains were seen. In 50^0C treated seeds also the staining intensity was more or less identical to that in the control seeds. The cells were filled with numerous magenta coloured starch grains. Certain cells were found empty but majority of the cells were occupied with starch granules (Fig. 29 F).

On the second day of germination, cell wall breakage was seen in some cells of the 60^0C treated seeds (Fig. 29 G). The starch grains were dispersed uniformly in the lumen of the cells. The staining intensity of the starch grains was found decreased on the second day of germination of 60^0C treated seeds than those of the previous stages. The cotyledonary cells of *G. max* seeds treated at 70^0C, on second day of germination exhibited cell wall rupture in most of the cells (Fig. 29 H). A reduction in staining intensity was observed at this stage than the other treatments and the control. No change in the size and shape of starch grains was observed.

4.3.2.4 Seventh day of germination

4.3.2.4.1 *Pisum sativum*

The cortical cells of the cotyledon of *P. sativum* control seeds on the seventh day of germination exhibited compactly arranged nature and each cell was filled with many starch grains of various size and shape (Fig. 30 A).

Large and small grains were observed in each cell. The grains were intensely stained and in each grain the hilum portion was clearly seen. The grains of different shapes and sizes were seen intermingled. The intensity of starch granules was noted to be reduced in each cell of 50^0C treated seeds on the seventh day of germination than those in the control seeds (Fig.30 B). The size and number of starch grains were also found reduced as compared to control seeds. The shape of starch grains remained unchanged as in control seeds.

On the seventh day of germination, the intact nature of cells was found lost in some parts of the cotyledons of seeds treated at 60^0C (Fig.30 C). The intensity and number of starch grains showed considerable reduction than those of the previous stages. The shape of the starch grains was identical as those of the previous stages. In 70^0C treated seeds of *P. sativum*, cell wall breakage was seen in more parts of the cotyledons on the seventh day of germination, (Fig.30 D). The staining intensity showed slight decrease than the other treatments and control. A reduction in the number of starch grains

was also observed. No change in the shape of the starch grains was noticed as in the other treatments and control.

4.3.2.4.2 *Glycine max*

The elongated cells of the cotyledon of the control seeds of *G. max* were compactly arranged and were intensely stained with PAS (Fig.30 E). The central portion of the elongated cells was almost occupied with the magenta coloured starch grains. The size of the starch grains was very small. Each cell contained numerous grains. On the seventh day of germination, the staining intensity of 50^0C treated seeds was more or less the same as in the control seeds (Fig.30 F).

The staining intensity of starch grains in the cotyledon cells of 60^0C treated *G. max* seeds was found reduced on the seventh day of germination (Fig.30 G). The small starch grains were evenly distributed in the lumen of some of the cells and certain cells were empty. Cell wall breakage was seen in some cells. The cotyledon cells of *Glycine max* seeds, treated at 70^0C, exhibited cell wall breakage very clearly on the seventh day of germination (Fig.30 H). The staining intensity was much reduced than the other treatments and control.

DISCUSSION

Although dry seeds tolerate a number of diverse environmental conditions, almost all of them are vulnerable to high temperature stress. Stress tolerance of seeds is manifested mainly due to the quiescent tissue that has limited metabolism. Air-drying/sun-drying influences the quality of seeds and is considered as one of the important seed technological parameters. Seed germination capacity, seedling vigour and seedling emergence index are significantly influenced by high temperature (Singh and Afria, 1985; Copeland and McDonald, 1995; Bedell, 1998; Baskin and Baskin, 2001).

Seeds of *Pisum sativum* and *Glycine max* are orthodox and the seed lots used in the present study exhibited cent percent germination up to the moisture content percentage 12.8 and 13.9 respectively (Table 1, Fig. 1). When the seeds were subjected to temperature treatments at 50, 60 and 70^{o}C for 10 days continuously, the germination percentage was decreased with a concomitant reduction in the moisture content. As a consequence of high temperature treatment, reduction in germination has been reported in *Solanum nigrum* seeds (del Monte and Tarquis, 1997) and in *Raphanus sativum* (Meng *et al.,* 2003). Similarly high temperature treatment for a short duration of 5 minutes on *Prosopis*, *Lupinus* and *Cyclopia* showed a higher percentage of

germination (Hanley *et al*., 2001; de Villalobos *et al*., 2002).

The longevity of seeds is affected by the reduction in moisture content below a critical value and the estimates of critical moisture content vary considerably among species (Ellis *et al*., 1988; 1989; 1990; Sanhewe and Ellis, 1996). According to these authors, the critical moisture content values of selected species range from 1.95% to 6.04%, i.e., 1.95% in *Arachis hypogea*, 2.04% in *Helianthus annus* (Ellis *et al*., 1988) and 5.59% in *Vigna unguiculata* and 6.04% in *Vigna radiata* (Ellis *et al*., 1989). Ellis *et al*. (1990) reported that the critical moisture content of *Pisum sativum* seeds is 6.2%, where as in *Glycine max* it is 3.3%. However, in the present study, critical water content level which determines the seed viability range cannot be proposed for *P. sativum* and *G. max* seeds, because during continuous drying, moisture content is gradually decreased and some seeds treated at 70^0C remained viable. In *P. sativum* and *G. max* seeds, loss of viability is inversely proportional to temperature treatments as has already been reported by various authors (Ellis *et al*., 1988; 1989; 1990; Sanhewe and Ellis, 1996). This is also confirmed by the present author that temperature treatment results in reduced germination with a concomitant reduction in moisture content.

According to Ellis and Roberts (1982) and Ellis *et al*. (1990), the proportion of pea seeds germinated normally in standard germination tests declined progressively from 94 to 50% when the seeds were dried from 14.8

to 3.7% moisture content at ambient temperatures. Roberts (1973) opined that orthodox seeds can be dried to low moisture contents without damaging the embryo and their longevity increases with decrease in moisture content during storage over a wide range of conditions. Contradictory to this view, drying at high temperature leads to significant reduction of moisture content and concomitant loss of viability in pea and soybean seeds, plausibly due to the continuous treatment for 10 days at 50^0C to 70^0C (Table 1, Fig. 2).

For the maintenance of cent percent germination, 12.8% and 13.9% of moisture content are found to be essential in pea and soybean seeds respectively. According to the terminology of seed water content proposed by various authors (Bewley and Black, 1994; Copeland and McDonald, 1995), the free water, which is held by capillary force, escapes during maturation drying or post harvest drying of seed storage. The free water is necessary for the movements of molecules from one site of metabolism to another. When dried, this free water is removed and loss of weight is expressed as moisture content of the seeds. When the moisture content is reduced to 7.3% in pea and 8.7% in soybean at 60^0C, the germination is reduced to 33% and 40% respectively and this reduction in germination is directly proportional to the temperature. It can be presumed that during this temperature treatment, the tissues lose the water molecules adsorbed to the molecules of cell membranes. This is in consonance with the views of Bewley and Black (1994), who suggested that the adsorbed water or “bound water” is loosely held by

bonding to macromolecules. This water is believed to occur as tightly held to ionic groups of biomolecules such as amino or carboxyl groups and exist as a monolayer around macromolecules, which is essential for viability. However, Bewley and Black (1994) suggested that the concept of "bound water" is losing popularity and according to them this refers to water which has virtually no mobility, being associated with macromolecular surfaces, and is sufficiently structured so that its thermodynamic properties differ from those of free water. According to these authors, there exist 3 types of bound water in seeds: type I, which is absorbed tightly on to macromolecules through ionic bonding; type II, which has glassy characteristics and occurs as thin film of water that coats the surface of macromolecules and type III, which forms bridges over hydrophobic sites and the pressure of which results in changes in the phase behaviour of membrane lipids.

The reduction of moisture content below 6.4 and 6.8% respectively in pea and soybean seeds, when treated at 70^0C, resulting in the loss of viability presumably reveals the loss of all the three types of water as suggested by Bewley and Black (1994). However, temperature treatment at 50^0C showed above 60% germination, which may be due to the loss of free water alone. The metabolic activities of the seeds at this temperature treatment could be maintained almost normal even in the absence of free water. Since the rate of viability loss in *P. sativum* and *G. max* seeds as a response to higher temperature treatment and to the rate of water loss is gradual and more or less

continuous, the concepts of “bound” water cannot be directly correlated to the reduction of moisture content in these seeds at any particular temperature. It seems that the entire “bound water” or type III of “bound water” is not completely removed at 70^{0}C because viability is still retained at least 10% in pea and 17% in soybean. Supporting these physiological changes, it is also seen that the cellular structure in general and protein and starch content distribution in particular are not adversely affected by high temperature up to 70^{0}C (Fig. 23 to 30).

Bewley and Black (1994) suggested that water content of seeds exists in a glassy (vitrified) state even at physiological temperatures and it stops or slows down chemical reactions, assures stability and quiescence and prevents interactions of cell components. In vitro studies have shown that vitrification of water retards or prevent denaturation of proteins including enzymes (Bruni and Leopold, 1991). So, in the present study, it can be presumed that even the vitrified water in *P. sativum* and *G. max* seeds is being slowly removed by temperatures higher than 50^{0}C, resulting in gradual loss of viability.

Viability of the two legume seeds, *P. sativum* and *G. max* is inversely proportional to temperature and above 50^{0}C; the germination percentage is below 50%. The germination percentage is declined to 10% from 33% when the temperature was raised from 60^{0}C to 70^{0}C. The reason for the viability of seeds being maintained at high temperatures like 70^{0}C may be at least partial

maintenance of vitrification or glass formation of water within the cytoplasm, which is the potential mechanism to avoid crystallization of proteins and solutes present in the cytoplasm as suggested by Bruni and Leopold (1991). Williams and Leopold (1989) explained the glassy nature of water in corn embryos as a liquid with the viscosity of a solid and its formation from a liquid involves no chemical and physical change in the solution. The major function of the glassy state in the dry seeds is its contribution to the stability of the seed components during storage and thus to the survival during desiccation (Leopold *et al*., 1994).

Seed vigour index is an important parameter that gets affected by high temperature treatments. Maximum seed vigour index was observed in control seeds of *P. sativum* and *G. max* and a reduction was found to occur in temperature-treated seeds (Table 2, Fig. 3). According to Nichols and Heydecker (1968), the rate or speed of germination is considered as the criteria for seed vigour index determination. The time taken for imbibition/germination is also very important. In the present study, when the seeds were dried gradually, more time was taken for imbibition as well as germination and this was reflected in the reduced values of seed vigour index. The germination is found delayed in both the seed samples subjected to temperature treatment and soybean exhibited more seed vigour in samples treated at 70^0C than those of pea seeds. The decline of seed vigour index in *P. sativum* and *G. max* seeds is closely correlated with the loss of moisture

content which is the determinant factor of seed quality. This observation is in conformity with the view of Fu *et al.* (1994), who suggested that seed vigour index is an accurate measure for testing quality of seeds.

A close correlation between moisture content, germination percentage and seed vigour index was observed in both the legume seeds studied. The temperature treatments made the seeds more desiccated, resulting in reduction of seed vigour index, germination percentage and moisture content percentage throughout the period of study. According to various authors (Harrington 1972; Villiers, 1973; Douglas, 1975), seed vigour is affected by various environmental factors, such as temperature, moisture content and concentrations of oxygen and carbon dioxide.

In the present study, soybean seeds showed higher resistance to temperature stress with a higher germination percentage and seed vigour than the pea seeds. The oil-rich nature of soybean seeds can be correlated to these qualities (Table 1 and 2). This observation is in agreement with the view of Pixton (1967), and according to the author, oil content influences seed water relations and is a major determinant of equilibrium and relative humidity, which in turn are related to water potential. Roberts and Ellis (1989) and Ellis *et al.* (1989) reported the evidence for a common response of seed longevity to moisture content in oily and non-oily seeds.

The effect of temperature treatment on pea and soybean seeds reflected

on the rate of germination and growth of seedlings up to seven days showed that the seedling vigour was gradually decreased proportional to the increase in temperature (Table 3, Fig. 4). In pea, reduction in seedling vigour was more prominent, indicating more adverse effect of high temperature compared to soybean seeds. Khan *et al*. (1973) reported that, heat stress at 50 to 70^{0}C had a stimulatory effect on seedling emergence and subsequent performance of cotton plants, but higher temperatures caused thermal injury. However, the stimulatory effect of temperature treatment on seedling growth was not shown by pea or soybean seeds treated with high temperature.

An important observation in pea seeds was that, the percentage of hard seeds treated at 60^{0}C was 3.3% and at 70^{0}C, the percentage was increased to 6.6% (Table 4, Fig. 5). But soybean seeds showed no hardness in any of the temperature treatments. Even when conditions are apparently favourable for germination, high temperature treatment induced dormancy. Ellis and Roberts (1982) suggested that hard-seeded condition is an important consequence of desiccation. According to those authors, legume seeds which fail to imbibe water in 18-24 hours are called “Hard seed”. Hard seeded nature has been attributed to both genetic factors and environmental conditions such as soil fertility, photoperiod, relative humidity and temperature (Rolston, 1978; Bewley and Black, 1983; Mayer and Poljakoff-Mayber, 1989). So the difference in the distribution of hard seeded condition between pea and soybean seeds treated to similar higher temperature regimes is presumably

due to the genetic factors.

Hard seeds achieve and maintain a very low percentage of moisture, despite wide fluctuations in the moisture contents of the surrounding air. Mai-Hong *et al*. (2003) observed that in seeds of *Peltophorum pterocarpum*, a tree legume, the hard-seededness was induced when seeds were dried to about 15% moisture content. Storage of seeds for extended period of time at high temperature and humidity resulted in the occurrence of increased hardness in most legumes.

In legume seeds like pea, storage brings about hardening of the seed coat by oxidation of the phenolic compounds present in the testa (van Staden *et al.,* 1989). According to those authors, the oxidation of phenolic compounds blocks the small pores in the seed coat and thereby makes the seeds impermeable to water. Therefore a large percentage of hard seeds were seen in stored samples for a long period. However, in the present study, instead of prolonged storage, high temperature treatment results in enhanced oxidation of phenolics of the testa of pea seeds. In *Glycine max* seeds, there is no sign of decolourisation or browning of the seed coat and there occurred no hard seeds and all the seeds imbibed readily and uniformly when soaked in water.

The molecular basis of resistance to heat stress found in plants, reacting towards temperature variations is by altering the metabolic rates,

protein turn-over, osmolytes, membrane function and gene expression (Smallwood and Bowles, 2002; Sung *et al*., 2003). All these processes require energy and therefore depend on the competence and stability of mitochondria. Stupnikova *et al.* (2006) worked out this problem and obtained evidence from pea seeds that mitochondria played a central role in allowing plants to adapt to extreme temperatures. In the life cycle of higher plants, seeds must complete the crucial task of protecting the embryo and driving it towards the establishment of a new generation. The majority of higher plant seeds are desiccation tolerant, a complex trait that has contributed to the evolutionary success of angiosperms. Desiccation tolerant seeds are in fact anhydrobiotes and certainly represent the most stress-tolerant stage of plants.

Analysis of mineral ion leakages during imbibition of pea and soybean seeds is another aspect of the present study. The temperature treated seeds of pea and soybean undergo extreme desiccation and lose cell wall integrity so that the ion leakage occurs in profuse quantities. From the data (Table 5, Fig. 6 and 7) it is clear that the higher the temperature used to treat the seeds, the more the ion leakage. Even then, seeds could maintain the viability to some extent. The temperature treatment made both the legume seeds leakier than the control seeds during imbibition and the electrical conductivity was directly proportional to the increase in the temperature treatments. The maximum electrical conductivity was observed in the leachates of both pea and soybean seeds treated at 70^{o}C (Table 6, Fig. 8). Soybean seeds exhibited

high rate of solute leakage than pea seeds and this observation in the soybean seeds is revealing a link between leakage of ions during imbibition and the type of seed reserves. According to Lott *et al*. (1991), when dry seeds are soaked in water, the loss of elements is a general phenomenon that applies to both lipid rich and starch rich seeds and the leakage of elements do not appear to be closely related to the main type of organic reserve materials present. The same authors noticed that soybean seeds which contain starch, protein as well as lipids, leaked potassium in amounts similar to that of the lipid rich endosperm of castor beans.

In pea and soybean, seed vigour index values are found negatively correlated with electrical conductivity of the seed leachates as, reported as by Matthews and Bradnock (1968) in pea seeds. Studying the electrical conductivity test for pea seeds Matthews and Powell (1981) presented a range of electrical conductivity values for the classification of seed lots depending on the perspective of their utilization potential of viability. According to those authors, the lower electrical conductivity of seed leachate indicates better membrane stability. Membranes of dry seeds are in a disorganized, possibly non-lamellar state and are inefficient barriers to the movement of water and solutes (Matthews and Bradnock, 1968; Crowe and Crowe, 1992). During the initial phase of seed imbibtion, the hydrating membranes are becoming reorganized into a continuous bilayer configuration exhibiting efficient semi permeable properties which are critical to the survival and

successful germination. This reorganized nature of the membranes will be lost while high temperature/heat stress is given. According to Murphy and Noland (1982), the membrane involvement in solute leakage in radish seeds and excised pine embryos is due to heat damage. In the case of solute leakage, similar increases were obtained with heat killed soybean cotyledons (Leopold, 1980). Electrolyte leakage from tissues could be used to indicate the effectiveness of membranes as barriers to solute diffusion (Berjak *et al.*, 1993; Reisdorph and Koster, 1999). The loss of viability is accompanied by an increase in membrane permeability (Senaratna and McKersie, 1983).

Greater leakage of electrolytes from over dried and mechanically damaged seeds suggested that initial water content of the embryo in *Brassica* seeds (Taylor and Prusinski, 1990) and testa in pea seeds (Powell and Matthews, 1979) determine the result of conductivity test. In the present study, the temperature treated seeds of pea and soybean showed a decrease in moisture content, high solute leakage and electrical conductivity. These results are in conformation with the views that the initial water content in the seed determines the electrical conductivity of leachates (Simon and Mathavan, 1986). Leakage of solutes through testa has been used as a rapid method to assess seed quality.

A semipermeable layer in the seed coat of many species restricts the leakage of relatively large molecules and thus confounds the relationship

between leakage and seed quality (Taylor *et al*., 1993). Simon and Raja-Harun (1972) observed a close relationship between electrical conductivity and the amount of potassium leakage from pea (*Pisum sativum*) and cotton (*Gossypium hirsutum*) seeds respectively. The electrical conductivity of leachate of seeds as a result of imbibition is mainly due to leakage of ions including of K^+, Ca^{++} etc. In the present study, minimum quantities of potassium and calcium ions were found in leachates of control seeds and an increase was noted in both the seeds with temperature treatments (Table 5, Fig. 6 and 7). Matthews and Rogerson (1976) suggested that the physiological potential of pea (*Pisum sativum*) seed lots can be identified through differences in the quantity of potassium released during the first 20 minutes of imbibition. According to Dias *et al.* (1996), in soybean, potassium leaching after 90 minutes of imbibition was significantly and negatively correlated with germination. Those authors suggested that the K^+ ion leakage is a strong indicator to express the seed vigour, especially in the case of soybean seeds.

Rate of solute leakage is considered as a parameter of imbibitional injury and viability (Doijode, 1988; Bruggink *et al.*, 1991). Cortes and Spaeth (1993) investigated the origin and characterization of electrolyte loss as a result of aging of seeds by compartmental analysis of potassium efflux in submerged *Pisum sativum* embryos during imbibition and suggested that aged seeds showed increased amount of potassium since the potassium ions were

available for efflux from both compartments. Woodstock *et al.* (1985) showed degenerative changes in cell membranes of cotton seed (*Gossypium hirsutum*) and emphasized that the intensity of potassium and calcium leachate was a more efficient indicator of seed physiological quality than the evaluation of the total amount of ions released by seeds as found in the electrical conductivity tests. The quantity of K^+ ions present in the leachate of pea seeds both in the control as well as treated seeds is more or less similar to that of soybean. This observation can be directly correlated to the difference in the types of seed reserves of pea (protein rich) and soybean (protein and oil rich).

Leakage of Ca^{++} ions during imbibition of temperature-treated seeds of pea is more, compared to that of the control. Effect of temperature treatment on pea seeds has already been correlated to a significant accumulation of γ amino butyric acid (GABA), which is a non-protein amino acid derived from glutamic acid reaction (Shereena, 2005). According to Snedden *et al.* (1995), the synthesis of GABA in heat stressed plant tissues from glutamate is catalyzed by the enzyme glutamate decarboxylase, which is activated by the calcium containing regulatory protein calmodulin. It can be presumed that in pea seeds calcium ions are involved in the synthesis of calmodulin for the enhanced synthesis of GABA, which is a characteristic feature of temperature stress, and hence Ca^{++} ions are not leaked out during imbibition.

On the other hand, in soybean seeds, significant amount of Ca^{++} ions are leaked out during imbibition in temperature-treated seeds, particularly in the seeds treated at 60^0C and 70^0C (Table 5, Fig. 7). According to Saure (2005) calcium is known to stabilize membranes and hence abundant leakage of Ca^{++} ions from temperature-treated seeds during imbibition reveals the loss of membrane stability. Abundant leakage of Ca^{++} ions from temperature-treated seeds of soybean is directly related to the loss of seed viability. Buchanan *et al.* (2000) suggested that diffusion of Ca^{++} ions in plant cells are slow and the impediments to Ca^{++} ion diffusion include uptake into the organelles, vacuoles etc. However, temperature treatment enhanced the leaching of Ca^{++} ions either due to the increased diffusion rate or lack of accumulation in the vacuoles as a mechanism of temperature acclimation as opined by Buchanan *et al*. (2000) and Saure (2005).

The increase in sugar content in the leachate was gradual in the temperature-treated seeds and maximum content was noted in seeds treated at 70^0C and minimum in the control seeds of both the plants (Table 7, Fig. 9). The sugar leakage in the leachate of soybean seeds exceeded quantitatively that of pea seeds. Leaching of sugar by seeds has been associated with the loss of viability and used as an index for predicting the viability of rape seeds (Takayanagi and Murakami, 1968). The quantity of sugar leached out from the seeds during imbibition was found to be proportional to the quality and viability of both the types of seeds (Table 7, Fig. 9).

Reserve mobilization during germination of control and temperature-treated seeds of pea and soybean was studied by analyzing the distribution pattern of dry matter and various metabolites in the cotyledons as well as embryonic axis, which develop into the seedling. An increase in the dry weight was observed in the axis of *P. sativum* and *G. max* of both the control and temperature-treated seeds during germination with a corresponding decrease in the cotyledons (Table 8, Fig. 10 A to D). Similar results have been reported in pea seeds in which a loss of storage protein in the cotyledons and a gain in the axis during germination resulting in an increase in the dry weight of the axis (Bewley and Black, 1983; 1994; Callis, 1995). A decrease in dry weight of cotyledon is an indication that reserves are being utilized by the growing embryonic axis (Adams *et al*., 1980; Harris *et al.*, 1986). Garcia-Luis and Guardiola (1975) observed that the distribution of cotyledonary reserve materials to the developing shoot and root depends on the relative growth of the two organs in dark grown and light grown *P. sativum* seedlings. Dark grown seedlings accumulated more transported reserves in the shoot than in the light grown seedlings. But in the present study where germination was carried out only in the dark, enhanced reserve mobilization has occurred and this is in agreement with the view of Garcia-Luis and Guardiola (1975). During germination and subsequent seedling growth of seeds, starch, soluble sugars and protein contents were degraded and utilized to support the growth of the embryonic axis (Abrahamsen and Sudia, 1966; Adams *et al*., 1980;

Harris *et al*., 1986).

In the present study, the protein content was at its maximum in the dry state of control and temperature-treated seeds of both pea and soybean (Table 9, Fig. 11 A to D). On germination, the axis exhibited a gradual increase in protein content where as the cotyledon showed a gradual decrease. During seed germination, proteins and non protein nitrogenous compounds are degraded in the cotyledon and are translocated to the developing axis (Chin *et al*., 1972; Bewley and Black, 1983; 1994; Callis, 1995; Shewry *et al*., 1995). An overall decrease in protein levels, particularly storage proteins, is an integral part of germination of protein rich seeds and has been recorded in many species such as *Cicer arietinum* (Sharma, 1988), *Macrotyloma uniflorum* (Karunagaran and Rao, 1990) and *Vicia sativa* (Misra and Kar, 1990).

Protein content of axes and cotyledons in *P. sativum* did not show any significant variations in temperature-treated seeds compared to the control (Table 9, Fig. 11 C and D). But as growth proceeded, a proportionate increase in protein content was noted in the embryonic axis of both the control and the experimentals. A gradual degradation of proteins was clearly observed in the cotyledons of control and temperature-treated seeds of pea and soybean. Similar observations have already been made earlier in soybean seeds treated at 40^0C (Key *et al*., 1981) and in maize treated at 41^0C (Riley,

1981). Late-Embryogenesis-Abundant (LEA) proteins, which accumulate during seed development and desiccation, contributes to the total protein in pea and soybean, since these seeds are desiccation tolerant. However, these proteins are not directly related to seed viability during storage or aging (Grelet *et al.*, 2005). In the present study, despite the temperature treatment of seeds at 60 and 70^{0}C, the total protein content is not decreased significantly (Table 9). It seems that pea and soybean seeds are well adapted to fluctuations in temperature and treatments given to the seeds are not stressful to protein metabolism as well as viability.

Riley (1981) opined that the poor germination of maize seeds at high temperatures (>37^{0}C) was related to the low rate of protein synthesis by the embryo. According to the author, analysis of polysome profiles from the embryos, imbibing at high temperatures, indicated that the low rate of protein synthesis was due to the non availability of active mRNA. The apparatus of translation was not heat labile as was evident when embryos were incubated for 2 hours at 41^{0}C. Cell free extracts from such seeds imbibed for 16 hours were able to translate exogenous mRNA, indicating that ribosome and other sub cellular components were present and functional.

According to Hsiao (1973) and Bewley (1980), drought stress commonly causes a loss of protein from plant tissues as a result of disruption in the normal protein synthesis. But in pea and soybean, such a degradative

process of protein was not evident in temperature stressed seeds because both the control and temperature treated seeds showed similar pattern of degradation of protein in cotyledons, which is a typical characteristic nature of legume seeds. This is an indirect indication that protein metabolism of temperature treated seeds also is almost similar to the control seeds. The histochemical localization of proteins also supports this view (Fig. 23 to 26).

SDS PAGE protein profile showed almost similar bands of proteins in dry control and temperature treated seeds of pea and soybean (Fig. 12 A and 13 A). Since both the seeds are orthodox and get sufficiently desiccated on the mother plant, the chance of new protein synthesis is meager. It was reported that the dry seeds are inactive and unable to respond to the synthesis of new stress proteins though the seeds are subjected to high temperature (Gumilevskaya *et al*., 1996). But in the present study, temperature treatment resulted in the synthesis of 2 new bands on the 3rd day of germination in pea seeds treated at 60 and 70^{0}C and these were not observed either in the control or seeds treated at 50^{0}C. This is in agreement with the views of Gumilevskaya *et al.* (1996) who suggested that heat shock proteins were synthesized in pea seedlings when seeds were subjected to high temperature (38-40^{0}C) for 2 to 4 hours. Many authors supported this view of synthesis of new heat shock proteins as a result of high temperature treatments in the seeds/seedlings of cucumber (Lafuente *et al*., 1991), wheat (Dell' Aquila and Di- Turi, 1996) and barley (Dell' Aquila *et al*., 1998). The two bands

observed in temperature treated seeds of pea are presumed to be heat shock proteins since they are absent in the control.

The soybean seedlings during germination showed the depletion of one protein band each, in seeds treated at 60 and 70^{0}C (Fig. 13 B). This observation is in agreement with the views of Key *et al*. (1981) in soybean seeds treated at 40^{0}C and Riley (1981) in maize seeds treated at 41^{0}C, since in both the plants, protein reduction was noticed during germination. The heat shock proteins (HSPs) are believed to play a role in the acquisition of thermo tolerance (Vierling, 1991; Howarth and Ougham, 1993). Synthesis of HSPs at normal temperatures has also been reported in Salix (Valhala *et al*., 1990), and is suggested to be an ecological adaptation to climates that exhibit large air- temperature fluctuations (50-60^{0}C).

Even though HSPs are not detected in temperature-treated seeds and seedlings of soybean, their role in the thermo tolerance cannot be ruled out because synthesis of HSPs are well known as protectants towards high temperature (Vierling, 1991), but the synthesis and destruction of HSPs are reported to occur during earlier hours of temperature treatment (Iba, 2002). However, the disappearance of some bands during germination of seeds treated at 60^{0}C and 70^{0}C may be due to temperature stress that inhibits germination specific proteins in these samples.

In both pea and soybean seeds, the high temperature stress induced the

formation of selective synthesis of characteristic set of HSPs having low molecular masses (Fig. 12 and 13) as reported by Vierling (1991). The author opined that, with the exposure to high temperature, there is a decline in normal protein synthesis together with the formation of HSPs.

The SDS-PAGE, a potential tool for biochemical characterization of seed protein is relatively simple and the electrophoretic profile of proteins is species specific. The temperature treatment made the seeds less viable and the protein profile showed significant differences in pea seeds in dry state and seedlings of temperature treated seeds (Fig. 12 A and B). The reserve proteins present in dry state of seeds were replaced by new bands at the time of germination. Certain bands of high molecular weight proteins in temperature-treated seeds of both pea and soybean were found to disappear (Fig. 13 A and B). This disappearance of bands is attributed to the degradation of proteins during germination.

One of the important effects of high temperature on plant tissue is production of highly reactive oxygen species. Similarly peroxidase enzyme is widely distributed in all higher plants and protects cells against the destructive influence of H_2O_2 by catalyzing its decomposition through oxidation into O_2 and H_2O (Dionisio-Sese and Tobita, 1998; Sudhakar *et al*., 2001). The seeds of pea and soybean subjected to temperature treatments showed an increased peroxidase activity throughout the period of study (Table 10, Fig. 14 A and

B). The highest peroxidase activity was observed in 70^0C treated seeds and seedlings of both the plants. The increased peroxidase activity, proportional to the increase in temperature is indicative of the tolerance of both pea and soybean seeds towards temperature treatments up to 70^0C.

Peroxidase, catalase and superoxide dismutase play major roles in protecting cells from oxidative damage (Scandalios *et al*., 1980). According to Bhattacharjee and Mukherjee (2006), abiotic stresses like heat and salinity during early germination of *Amaranthus* seeds may result in the induction of oxidative stress in germinating tissues, which increases the vulnerability of newly assembled membrane systems to oxidative damage. Resistance to environmentally induced oxidative stress has been shown to be associated with high levels of peroxidases (Tsang *et al*., 1991; Zheng and van Huystee, 1992; Scandalios, 1993). According to Wise and Naylor (1987), temperature stress is an environmental factor that may cause "oxidative injury". Peroxidases probably play a key defense role against peroxidative stress (Zheng and van Huystee, 1992). Plants possess mechanisms to sense stress and transduce these into a change in activity of different classes of enzymes (Perl-Treves and Galun, 1991). The coordinated induction of antioxidant enzymes in response to environmental stress has been reported in plants (Scandalios, 1990; Perl-Treves and Galun, 1991; Tsang *et al*., 1991). The defensive role of peroxidase activity against temperature stress is evidently shown by both pea and soybean because even the seeds treated at 70^0C

showed high peroxidase activity and a germination of 10% and 17% in pea and soybean respectively, indicating the role of peroxidases in ameliorating the temperature stress.

Histochemical localization of insoluble polysaccharides showed more thickening of cell walls (Fig. 27 to 30) in temperature-treated seeds. This observation can also be correlated to enhanced activity of peroxidases in these tissues (Table 10, Fig. 14 A to D). This is in consonance with the views of van Huystee (1987) who suggested that peroxidases are involved in the lignification of cell walls. According to the author, apart from their role in the free radical scavenging system, peroxidases are involved in a number of processes such as lignifications, inactivation of catalytic proteins, anthocyanin degradation and catabolism of plant growth regulators.

A gradual increase of the total free amino acids was observed during germination of control and temperature treated seeds of both pea and soybean. However, when a comparison is made between control and temperature-treated seeds in the distribution of total free amino acids, only insignificant increase, proportional to increase in temperature treatment was observed. Maximum accumulation was shown by seeds treated at 70^0C in both pea and soybean (Table 11, Fig. 15 A and B). It is evident that, in addition to direct utilization of free amino acids, amino acids formed by proteolytic activities on the storage proteins is also occurring during seed germination (Bewley and

Black, 1994; Copeland and McDonald, 1995; Baskin and Baskin, 2001). During the germination of cottonseed, the total amino acid pool of cotyledons showed a five-fold increase on the third day (Capdevila and Dure, 1977). Collins and Wilson (1975) suggested that amino acids rapidly provide keto acids to allow establishment of respiratory cycles in early stages of germination and even to sugar synthesis in gluconeogenesis.

In seeds treated at high temperature, the total free amino acids are found to accumulate. This may be due to the retarded rate of translocation to the growing seedlings, which are characterized by low seedling vigour as reported earlier (Table 3, Fig. 4). The synthesis of γ amino butyric acid has been reported in temperature stressed seeds and tissues (Taiz and Zeiger, 2002; Shereena, 2005). So the contribution of γ amino butyric acid towards the increased total free amino acid content in the temperature-treated pea and soybean seeds cannot to be ruled out.

A significant increase in proline content was found to occur in temperature treated seeds (Table 12, Fig. A to D). During germination of these seeds, maximum quantity of proline was shown by temperature treated seeds especially in the seeds treated at 70^0C. Proline is considered as an antioxidant and biochemical indicator of water and temperature stress. Plants containing high concentration of proline show considerable resistance to abiotic stresses (Andarwulan *et al.*, 1999). Proline can also act as detoxifying

agent of free radicals (van Rensburg *et al*., 1993). Free proline has been suggested as a metabolic measure of drought and act as a general adaptation to water stress (Hare *et al*., 1998). According to those authors, physiological mechanisms to maintain cellular moisture may be through osmotic adjustment and proline has been advocated to play a significant role in such processes.

As explained earlier, thickening of cell wall is an important character of temperature-treated seeds of pea and soybean (Fig. 23 to 30). Another reason for the thickening of cell wall in seed tissue may be the involvement of phenolics, which serve as precursor of lignin which acts as physical protectants against temperature stress, as reported in *Brassica napus* (Fletcher and Kott, 1999). Both pea and soybean seeds are rich in phenolics (van Staden *et al*., 1989) and the metabolism is reported to be linked with proline content (Buchanan *et al*., 2000). Hence the proline accumulation of temperature-stressed seeds is found to be indirectly related to enhancement of cell wall thickening.

Mobilization of the stored reserve in seeds has been studied in many cereal grains and legume seeds (Khan, 1978; Bewley and Black, 1983; 1985; 1994; Mayer and Poljakoff- Mayber, 1989; Copeland and Mc Donald, 1995). In the present study, the dry control seeds of pea and soybean showed very high amount of starch content, which was found decreased in temperature treated seeds. During germination, in both the seeds, the starch content in the

axis increased gradually and significantly up to 4th day of seedling development and very steeply thereafter. The cotyledons showed maximum starch content in the early days of germination which was found decreased during further growth of the seedlings. Starch reserves in legume seeds are normally degraded during germination and this process has been documented for a range of species (Bewley and Black, 1985). But Smith (1974) and Briarty and Pearce (1982) observed the appearance of starch granules in the cotyledon of *Phaseolus vulgaris* during germination.

Accumulation of starch content in the embryonic axes and concomitant reduction in the cotyledons of both the control and temperature-treated pea and soybean seeds indicate mobilization of soluble carbohydrates and accumulation of starch content due to the lack of utilization in the axes. It seems that the seedlings are in etiolated condition, so that growth retardation is imposed, resulting in a transient accumulation of starch which is entitled to be used for the seedling growth under favourable conditions.

Significant reduction of starch content, particularly in cotyledons of temperature-treated seeds may be due to the mechanical loss of starch grains from the cotyledons, as a result of the cell wall breakage as shown in the histochemical localization of starch (Fig. 27 to 30).

When a comparison is made between the two seeds, the starch content of soybean seeds was found to be only about 50% of that of pea seeds. In all

treatments and control of pea and soybean seeds, the same trend of mobilization was followed during germination (Table 13, Fig. 17 A to D). Bewley and Black (1994) stated that in pea seeds, starch degradation during germination occurs slowly for 5-6 days and the initial starch hydrolysis is by phosphorolysis since starch phosphorylase is the first enzyme to increase the activity in the cotyledonary tissue. In the present study the reduced rate of starch depletion and absence of maltose in pea seeds are readily agreeable with the views of Bewley and Black (1994). If at all starch depletion occurs, it takes place by phosphorolysis, as amylase activity is assumed to be nil in the absence of maltose accumulation. The same trend of starch degradation is shown by the seeds treated at high temperature also (Table 13). Shereena (2005) reported only very feeble amylase activity in pea seeds during germination and seeds treated at 45 and 50^0C showed a further reduction in amylase activity compared to the control seeds.

In histochemical study, no well developed starch grains were seen in soybean, either in control or germinating seeds. However, PAS positive masses and granules were present invariably in dry/control and treatments. However, PAS positive masses occurring as patches over the cells (Fig. 26 to 30) other than cell walls is plausibly due to the interference of lipid content which gets stained with PAS (Pearse, 1972), since soybean seeds are lipid rich. However, depletion of starch content in the cotyledons of temperature-treated soybean seeds is not as significant as that of pea due to the lack of

granular starch content, which is presumed to be lost mechanically due to the breakage during temperature treatment.

Carbohydrate metabolism during seed germination is critical for seedling growth. The starch content is decreased during germination of pea seeds and the amount of soluble sugars is increased continuously (Table 14, Fig. 18 A and B). Soluble sugars are used for energy and as the building blocks for necessary cellular structures. During the initial 4 days of germination/seedling growth of soybean seeds, the quantity of starch was increased but on 5^{th} day it was shown declined. Corresponding to the starch synthesis, decline in oil and protein was occurred. Soluble sugars are utilized initially during germination, possibly as an immediate energy source (Abrahamsen and Sudia, 1966; Adams *et al*., 1980).

Temperature treatments made a slight decrease in the total sugar content at higher temperatures like 60 and 70^0C than in control seeds of pea and soybean. Rapid decrease in total soluble sugars was found to occur in the early stages of germination with an increase in the amount thereafter (Table 14, Fig. 16 A and B). The exorbitant reduction of soluble sugars from imbibition stage onwards in pea seeds compared to the dry seeds is due to the utilization of soluble sugars during imbibition, germination and seedling growth (Bewley and Black, 1983; 1985; Mayer-Poljakoff-Mayber, 1989). The dominant factor responsible for the significant reduction of sugars may be

the expression of the metabolite on a dry weight basis on one hand and the high moisture content due to the exponential rate of imbibition during initial hours, on the other. The same trend was shown by all the temperature-treated seeds also. Further increase of sugar content during seedling growth is due to the impaired metabolism of the etiolated seedlings.

The HPLC analysis of soluble carbohydrates revealed that glucose, fructose, rhamnose, maltose, sucrose, raffinose, stachyose and verbascose are present in the control seeds of pea (Table 15). In the seeds treated at 60 and 70^0C, glucose and fructose are found to disappear. It seems that in dry seeds the rate of respiration is very low and during temperature treatment only these reducing sugars are utilized. On the third day of germination in the control, all the sugars are significantly increased, except maltose, which disappeared during germination. During germination of seeds treated at 60^0C, maltose reappeared but the amount of all other sugars was found reduced. In the seeds treated at 70^0C, only sucrose, rhamnose and raffinose were present in considerable quantities. During germination, starch depletion occurs by starch phosphorolysis in the cotyledons resulting in an increase of all sugars in general and sucrose in particular, for the translocation to the growing seedling. Absence of maltose in the cotyledons of the germinating seeds indicates that starch hydrolysis is mainly by phosphorolysis and not through amylolytic degradation. Since starch catabolism in pea seeds is found to be by phosphorolysis, the product is glucose-1-phosphate, which can be directly

used as a substrate for sucrose synthesis for translocation. The abundant accumulation of sucrose (113.2 mg g^{-1}) in the cotyledons of 3 day old seedlings (Table 16) reveals sucrose synthesis, which is reduced significantly in temperature-treated seeds presumably due to the retardation of sucrose synthase activity.

In addition to the absence of glucose and fructose, pea seeds treated at high temperature showed a significant reduction of raffinose, stachyose and verbascose (raffinose family of oligosaccharides), where as in control seeds these sugars were greater in quantity and the total sugar content also was high (Table 15). Pea seeds come under orthodox category and hence highly tolerant to desiccation. Desiccation tolerance and accumulation of sugars in general and raffinose family of oligosaccharides in particular are related to each other (Blackman *et al.,* 1991; 1992; Sun *et al*., 1994; Brenac *et al.,* 1997).

The amount of raffinose is found decreased rapidly in 70^0C treated dry seeds and the germination percentage also seemed to be very low - only 10% in *P. sativum* and 17% in *G. max.* Disappearance of oligosaccharides and resultant reduction in seed longevity was reported in *Impatiens walleriana* and *Capsicum annum* (Buitink *et al*., 2000). The reduction of raffinose family of oligosaccharides in the temperature treated seeds indicates gradual loss of viability, plausibly due to over desiccation effect. In soybean also, the

association between desiccation tolerance and distribution of sugars, particularly of raffinose family of oligosaccharides, is evident (Table 17). In seeds treated at 60^0C, those sugars are more in amount and is proportional to the viability percentage, while in seeds treated at 70^0C these sugars occur in very low quantities. However, abundant occurrence of sucrose, which gets reduced gradually during temperature treatment, is a characteristic feature of soybean seeds. The distribution of sucrose also is directly related to desiccation tolerance and is in accordance with the views of Bernal-Lugo and Leopold (1992; 1993).

Sugars and sugar derivatives may play a role in both desiccation tolerance and the longevity of dry seeds (Horbowicz and Obendorf, 1994; Brenac *et al*., 1997; Obendorf, 1997; Obendorf *et al.*, 1998). Distribution of soluble carbohydrates, particularly sugars, is related to desiccation tolerance in maturing seeds (Hoekstra *et al*., 1994; Popp and Smirnoff, 1995; Nkang, 2002). The role of sucrose and raffinose has been interpreted as agents of stabilization of membrane lipids during desiccation (Koster, 1991; Koster *et al.*, 1994; Wolkers *et al*., 1998; Buitink *et al*., 2000). Sucrose vitrification around fluid membrane phospholipids stabilizes the membrane during desiccation (Koster *et al*., 1994). According to Buchanan *et al.* (2000) increase in the soluble non-reducing sugars occurs during desiccation. The accumulation of raffinose is associated with desiccation tolerance and it is important for the longevity of mature dry seeds (Bewley and Black, 1994). In

the cotyledons of seeds treated at 60^{0}C, all the sugars occur in reduced quantities but maltose reappears, revealing slight amylolytic activities as reported by Bewley and Black (1994). In the cotyledons of seeds treated at 70^{0}C, the amount of almost all the sugars, except rhamnose and sucrose are reduced because of the non-vigourous seedlings and translocation of sugars seemed to be impaired. Presence of rhamnose is a characteristic feature of desiccated seeds during temperature treatment followed by germination. Abundant occurrence of rhamnose may be due to cell wall degradation since rhamnose is an important deoxy sugar present in the cell wall formed by dehydration of hexoses (Buchanan *et al*., 2000).

In soybean, fluctuations in the distribution of rhamnose sugar are insignificant, presumably due to lack of cell wall degradation as observed in histochemical study (Fig. 23 to 30). In dry seeds of soybean, composed of greater quantity of sugars than that of pea, the temperature treatment resulted in a significant reduction of important sugars in general and sucrose and raffinose in particular. Since raffinose plays a significant role in desiccation tolerance (Blackman *et al*., 1992), significant reduction of this sugar indicates loss of desiccation tolerance resulting in loss of viability in both pea and soybean seeds particularly in samples treated at 70^{0}C (Table 15 to 18, Fig. 19 A and B). Significant reduction of total sugars occurs in temperature-treated seeds. Absence of maltose in dry seeds and cotyledons of germinating seeds on the third day is a characteristic feature of soybean in spite of the presence

of considerable quantity of starch, which remains almost unchanged in the cotyledons of germinating seeds up to 5th day (Table 13). In germinating seeds, the total sugar distribution of cotyledons do not change significantly due to temperature treatment, showing the recovery of desiccation stress, which is confirmed by the HPLC sugar profile of the seeds treated at 60 and 70^{0}C (Table 17 and 18, Fig. 20 and 21).

Lipid content in pea seeds is very low (4%) and remain almost unchanged during germination. Significant reduction of lipid in the seeds treated at 60 and 70^{0}C may be due to the direct volatilization under high temperature. In spite of significant reduction of lipid content in temperature-treated seeds, its quantity did not show any significant variation during germination. So it seems that slight amount of lipid is getting volatilized during temperature treatment.

Soybean seeds are lipid rich and are characterized by gluconeogenesis i.e., starch synthesis occurs during germination as reported by Adams *et al*. (1980) and Bewley and Black (1994). In the present study also, soybean showed significant reduction of lipids, particularly on the 3rd day of germination (Table 19) and the starch content of these samples remains unhydrolyzed and hence not mobilized to the growing axis (Table 13). However, the seeds treated at 60 and 70^{0}C show significant reduction of lipid content during germination. High temperature treatment made the soybean

seeds less viable. Wang *et al*. (2001) stated that soybean seeds contain high saturated fatty acids and hence perform better at a relatively high temperature because their membranes would be more fluid. In the present study, soybean seeds showed more germination percentage in seeds treated at 70^0C compared to pea seeds. The significant reduction of lipids in dry and temperature-treated seeds at 60 and 70^0C may be due to direct volatilization of lipids under high temperature with out further changes during germination (Table 19, Fig. 22 A and B).

The sugar content in the cotyledons of soybean may be gluconeogenetic in origin but their reduced quantity is possibly due to their synthesis for glycerol-1-phosphate of the immediate product of lipase activity. Degradation of free fatty acids may take more days since its catabolism involves the sequential steps of β oxidation and three distinct organelles are involved to complete the process, which actually takes place during later phase of seedling growth. According to Murray (1984), lipid mobilization/utilization is a post-germination process in lipid rich seeds. In soybean seeds it is evident that lipid content was found to decrease from 3rd day of germination in both control and temperature treated seeds (Table 19).

Even though starch accumulation by gluconeogenesis in the lipid rich seeds of soybean is not observed in the present study, the catabolism of lipids is evidently seen in the distribution of sugars. Degradation of lipids by lipase

results in the formation of glycerol and fatty acids. During early days of seedling growth (3rd day of germination in the present study) the glycerol may enter the gycolate pathway and undergo reversal of glycolysis to yield hexoses, or the glycerol after phosphorylation and oxidation may form triose-phosphate, which is converted to pyruvate and then oxidized through citric acid cycle. However, the sugar content of the soybean cotyledons during the 3rd day of germination is comparatively lower than that of pea seeds.

Given the reported occurrence of gluconeogenesis, starch synthesis is not shown by soybean seeds either biochemically or histochemically. Histochemical localization of insoluble polysaccharides is interfered by lipids (Pearse, 1972). So starch grains, if at all formed, cannot be localized by PAS (periodic acid Schiff's) reaction. Considerable reduction of lipids on one hand (Table 18) and lack of starch synthesis (Table 19, Fig. 22 B) and accumulation of sucrose or any other mono or oligosaccharides on the other hand, indicate substantial mobilization of seed reserves (lipid) from the cotyledons to the growing axis and their oxidation as the respiratory substrate during seedling growth retaining significant quantities of lipids in the cotyledons.

Slinkard *et al.* (1994) stated that legumes like *Pisum sativum* and *Glycine max* can live in wide ecological niches having varying climatic conditions, though commonly these legumes prefer temperature below 30^0C.

In the present study, the *P. sativum* and *G. max* seeds showed resistance to temperature treatment up to 70^0C. Given the significant reduction of germination percentage, seed vigour and seedling vigour by temperature treatments up to 70^0C, pea and soybean seeds are tolerant to high temperature to considerable extent, revealing the adaptation of these species to survive under a wide range of temperature regime and/or other environmental conditions.

SUMMARY AND CONCLUSIONS

The present study is an attempt to compare the physiological, biochemical and histochemical aspects of the effect of temperature on pea and soybean seeds which belong to desiccation tolerant orthodox group, during early stages of germination. The seeds were subjected to temperature treatments at 50, 60 and 70^{0}C for ten continuous days as separate lots, using a hot-air oven. The treated seeds were cooled to room temperature, packed and stored in polyethylene bags for further study. The temperature treated seeds were used to study germination percentage, moisture content percentage, seed vigour index, seedling vigour, imbibition and leachate analyses which include, imbibition percentage, hard seed percentage mineral ion concentration in the leachate, electrical conductivity of leachate and leachate sugar content. Biochemical studies were begun with appropriate samplings and included dry weight determination, estimation of total protein, total amino acid, proline, starch, total sugar and total lipid contents, assay of peroxidase activity, SDS-PAGE profiling of proteins and HPLC analysis of sugars. Histochemical studies were also conducted to localize the total proteins and insoluble polysaccharides in the cotyledons of temperature treated and control seeds/ seedlings.

By the temperature treatment, seeds underwent extreme desiccation and adapted and modified themselves to withstand the temperature stress. Though the temperature treatment given was 50, 60 and 70^0C for ten continuous days, the seeds could withstand the effect of the treatment and maintained germination percentage of 10 in pea and 17 in soybean seeds.

Though the mode of germination is different i.e., hypogeal in pea and epigeal in soybean, performance of the seeds in physiological, biochemical and histochemical aspects were of the same pattern. Both the seeds were readily germinable, and after imbibition within 24 hours the radicle protrusion was very clear, completing the germination process rapidly.

The control seeds of pea showed 12.8% moisture content and cent percent germination. Temperature treatment at 70^0C reduced moisture content to 6.4% and germination to 10%. Soybean seeds subjected to temperature treatment also showed reduction in germination percentage as well as in MC percentage from the maximum rate recorded in control seeds. SVI also found decreased obviously with temperature. Temperature treatment enhanced leakage of solutes including K^+, Ca^{++} ions and sugar and showed very high electrical conductivity. This observation is also related to the loss of viability and germination percentage. The dry matter of seeds was gradually increased with temperature treatment and attained maximum in the 70^0C treated seeds.

On germination, the amount of dry matter in the axes and the

cotyledons showed a gradual decrease. Biochemical analyses of seeds and seedlings showed an increase in quantity of metabolites like protein and starch in the axis along with a corresponding decrease in the amount in cotyledons during germination of control and temperature treated seeds. Due to the tolerant nature of pea and soybean seeds, the heat stress did not induce much difference in the protein profile of temperature treated seeds. The cotyledons of the seedlings of 3rd day growth showed disappearance of protein bands, which was very prominent in control seeds and this lack of protein bands was very clear in temperature treated seeds, especially at 60 and 70^{0}C. Conversely, synthesis of two new protein bands was observed in pea. Histochemical localization of proteins was in agreement with the biochemical estimation.

The guaiacol peroxidase activity also increased with the increase of temperature in the treatments in both pea and soybean seeds and it induced more lignification on the cell walls. Proline content which acts as a biochemical indicator of stress is found to be increased in seeds treated at high temperatures and indirectly related to enhance cell wall thickening also.

Mobilization pattern of seed reserves such as starch, protein, lipid and amino acids during germination is almost uniform in control and temperature-treated seeds, except at very high temperature treatments, at which the solute leakage was very high, affecting the viability and germination of seeds.

Temperature treatments made a slight decrease in the total sugar content at higher temperatures of 60 and 70^0C than the control seeds of pea and soybean. During the initial stage of germination, there was a rapid decrease in the total sugar content and then the amount was found to increase along with the growth of the seedlings in both pea and soybean. The HPLC profile of sugars revealed that glucose, fructose, rhamnose, maltose, sucrose, raffinose, stachyose and verbascose are present in the control seeds of pea and soybean and the amount showed variations in the temperature treated seeds.

Lipid content was found to decease in pea and soybean seeds treated with temperature but during germination the distribution of lipid was almost in the same pattern in control as well as in the experimentals.

The experimental data obtained are interpreted and compared to current views and the following conclusions are drawn.

1. High temperature treatment resulted in a significant reduction of moisture content to 6.4% in pea and 6.8% in soybean seeds while retaining their viability and hence both the seeds are tolerant to temperature up to 70^0C.

2. Hard-seededness is imposed in pea seeds due to temperature treatment and this quality is linked to the phenolic content of the testa, which get oxidized and browned and become hard and impermeable to water where as soybean seeds do not show seed-hardedness.

3. Imbibition is delayed in temperature-treated seeds resulting in reduced rate of germination and concomitant reduction of seed vigour index.

4. Both seed vigour and seedling vigour are reduced in temperature treated seeds of pea and soybean but relatively soybean seeds are more resistant, probably owing to their oil-rich seed reserves.

5. Effect of higher temperature treatment on both pea and soybean results in loss of cell wall integrity resulting in profuse leakage of minerals and metabolites with accompanying higher electrical conductivity and these are directly related to increased imbibitional injury and loss of seed viability.

6. Leakage of calcium is more pronounced in soybean seeds compared to pea in which Ca^{++} ions are involved in the synthesis of non-protein amino acid -γ amino butyric acid (GABA), which is an important effect of temperature treatment.

7. Reserve mobilization during germination of pea and soybean seeds do not vary significantly as the effect of temperature treatment compared to the control seeds. The disorganized physiological aspects such as reduced germination percentage, seed and seedling vigour and increased leakage of minerals and metabolites are found to be reorganized during imbibition and germination and hence changes in

its metabolites are negligible compared to that of control seeds.

8. Biochemical estimation and histochemical localization revealed that protein content of pea and soybean remained almost similar in the temperature-treated and control seeds. However, SDS-PAGE profile of pea showed two new bands in the seeds treated at 60^0C and 70^0C and these proteins are presumed to be heat shock proteins which are absent in the control seeds. The protein profile did not show much change in seeds of soybean treated at different temperatures. However, in the germination specific protein profile, one band each in 60^0C and 70^0C treatments was found to disappear.

9. Enhanced activity of peroxidases is a characteristic feature of both pea and soybean seeds subjected to temperature treatment. This served as a mechanism to protect the tissue from the destructive effect of H_2O_2 generated as a consequence of high temperature treatment.

10. The occurrence of more thickened cell walls in the temperature-treated seeds is correlated to the enhanced peroxidase activity, which is known to involve in the lignifications of cell walls.

11. Accumulation of total free amino acids in the cotyledons indicates the retarded translocation to the seedlings causing reduced seedling vigour, a corollary of the temperature treatment.

12. Accumulation of proline is found to be one of the antioxidative mechanisms against high temperature stress in both pea and soybean seeds.

13. Thickening of cell walls due to increased lignifications in the temperature-treated seeds is also correlated to the proline accumulation because the synthesis of the former takes place under the influence of the latter.

14. Starch content of pea seeds is greater than that of soybean and during germination a general depletion of starch is shown by temperature-treated seeds as a result of cell wall breakage leading to more imbibitional injury resulting in the mechanical leakage of starch grain.

15. Starch hydrolysis of pea seeds during germination is plausibly by phosphorolysis because resultant sugars of starch hydrolysis include no maltose while sucrose is abundant revealing absence of amylolytic degradation of starch.

16. Histochemical localization of starch in soybean seeds shows that granular starch is absent in soybean in which lipid content is stained by PAS reaction.

17. Increased quantity of raffinose family of oligosaccharides in the temperature-treated seeds is seemed to be related to desiccation

tolerance of these seeds.

18. Presence of rhamnose in the temperature treated seeds reveals cell wall disintegration during temperature treatment since rhamnose is an important deoxy sugar of cell wall components.

19. Soybean seeds are lipid rich and gluconeogenesis, synthesis and accumulation of sugars are seen in control and experimental seeds. Lipid depletion, observed in the dry seeds of both in pea and soybean seeds, during temperature treatment may be due to direct volatilization of lipids at high temperature.

20. Given the relatively significant germination percentage, even by temperature treatments up to 70^0C, pea and soybean seeds are tolerant to high temperature to considerable extent, revealing the adaptation of these species to survive under a wide range of temperature regime and/or other environmental conditions.

REFERENCES

Abdul - Baki, A.A. 1969. Relationship of glucose metabolism to germinability and vigour in barely and wheat seeds. ***Crop Sci.*** **9**: 732-737.

Abdul - Baki, A.A. and Anderson, J.D. 1970. Viability and leaching of sugar from germinating barely. ***Crop Sci***. **10**: 31-34.

Abeles, F.B. and Biles, C.L. 1991. Characterization of peroxidases in lignifying Peach fruit endocarp. ***Plant Physiol. 95***: 269-273.

Abrahamsen, M. and Sudia, T.W. 1966. Studies on the soluble carbohydrates and carbohydrate precursors in germinating soybean seeds. ***Am. J. Bot.*** **53**: 108-114.

Adams, C.A., Rinne, R.W. and Fjerstad, M.C. 1980. Starch deposition and carbohydrase activities in developing and germinating soybean seeds. ***Ann. Bot.*** **45**: 577-582.

Ahmad, S. and Banaras, M. 1981. Effect of pre-sowing high temperature stress on growth and yield of seed cotton. ***J. Agri. Res.*** **1**: 61-65.

Alia, P., Mohanty, P. and Matysik, J. 2001. Effect of proline on the production of singlet oxygen. ***Aminoacids*** **21**: 195-200.

Altschul, A.M., Snowden, J.E., Manchon, J.B. and Dechary, J.M. 1961. Intracellular distribution of seed proteins. ***Archs. Biochim. Biophys.*** **95**: 402-404.

Amuti, K.S. and Pollard, C.J. 1977. Soluble carbohydrates of dry and developing seeds. ***Phytochemistry*** **16**: 529-532.

Andarwulan, N., Fardiaz, D., Wattimena, G.A. and Shetty, K. 1999. Antioxidant activity associated with lipid and phenolic mobilization during seed germination of *Pangium edule.* ***J. Agri. Food Chem.*** **47**: 3158-3163.

Anonymous, 1969. ***The Wealth of India***. Raw materials CSIR, New Delhi. Vol. **VIII.** 124-139.

Anonymous, 1995. ***The Wealth of India***. Raw materials CSIR, New Delhi, Vol. **X.** 210-222.

Ashton, F.M. 1976. Mobilization of storage proteins of seeds. ***Annu. Rev. Plant Physiol.*** **27**: 95-117.

Aspinall, D. and Paleg, L.G. 1981. Proline accumulation: Physiological aspects. *In:* L.G. Paleg and D. Aspinall (Eds.) ***The Physiology and Biochemistry of Drought Resistance in Plants.*** Academic Press-Australia. 205-258.

AOSA. 1983**.** ***Seed Vigour Testing Handbook***. East Lasing: AOSA.

Bagley, B.W., Cherry, T.H., Rollins, M.L. and Attschul, A.M. 1963. A study of protein bodies during germination of peanut (*Arachis hypogea*) seeds. ***Am. J. Bot.*** **50**: 523-531.

Bailly, C. 2004. Active oxygen species and antioxidants in seed biology. ***Seed Sci***. ***Res*****. 14:** 93-107.

Bain, J.M. and Mercer, F.V. 1966. Sub cellular organization of the developing cotyledons of *Pisum sativum* L. ***Aust. J. Biol. Sci.*** **19**: 49-67.

Baker, E.H., Bradford, K.J., Bryant, J.A. and Rost, T.L. 1995. A comparison of desiccation-related proteins (dehydrin and QP47) in peas (*Pisum sativum*). ***Seed Sci. Res.*** **5:** 185-193.

Baker, J., Steele, C. and Dure, L. III. 1988. Sequence and characterisation of 6 LEA proteins and their genes from cotton. ***Plant Mol. Biol.*** **11:** 277-291.

Bartels, D., Singh, M. and Salamini, F. 1988. Onset of desiccation tolerance during development of the barley embryo. ***Planta*** **175**: 485-492.

Basara, S.M.A., Ahmad, N., Rehman, K. and Iqbal, N. 2002. Cotton seed invigoration by pre-sowing seed humidification. ***Int. J. Agri. Biol.*** **4:** 127-130.

Baskin, C.C. and Baskin, J.M. 2001. ***Seed: Ecology, Biogeography and Evolution of Dormancy and Germination*.** Academic Press. San Diego. New York.

Bates, L.S., Waldran, R.P. and Teare, I.D. 1973. Rapid determination of free proline for water-stress studies. ***Plant Soil*** **39**: 205-207.

Becker, W.M., Leaver, C.J., Weir, E.M. and Riezman, H. 1978. Regulation of glyoxisomal enzymes during germination of cucumber: 1. Developmental changes in cotyledonary protein, RNA and enzymic activities during germination. ***Plant Physiol. 62***: 542-549.

Bedell, P.E. 1998. ***Seed Science and Technology: Indian Forestry Species.*** Allied Publishers Ltd. New Delhi.

Beevers, H. 1980. The role of glyoxalate cycle. *In*: P.K. Stumpf (Ed.) ***The Biochemistry of Plants: A Comprehensive Treatise*** **Vol. 4, *Lipids: Structure and Function.*** Academic Press, New York. 117-130.

Berjak, P., **Vertucci, C. W. and Pammenter, N.W.** 1993. Effects of developmental status and dehydration rate on charecteristics of water and desiccation- sensitivity in recalcitrant seeds of *Camellia sinensis*. ***Seed Sci. Res*.** **3**: 155-166.

Berlyn, G.P. and Miksche, J.P. 1976. ***Botanical Microtechnique and Cytochemistry***. The Iowa State University Press, Ames, Iowa.

Bernal-Lugo, L. and Leopold, A.C. 1992. Changes in soluble carbohydrates during seed storage. ***Plant Physiol***. **98**: 1207-1210.

Bernal-Lugo, L., Diaz-de Leon, F., Castillo A. and Leopold, A.C. 1993. Embryo sugar composition and seed storage performance. *In*: D. Come, and F. Corbineau, (Eds.) ***Proceedings of the Fourth International Workshop on seeds: Basic and Applied Aspects of Seed Biology.*** ASFIS, Parish. 789-792

Bewley, J.D. 1980. Protein synthesis. *In*: L.G. Paleg and D. Aspinall (Eds.) ***The Physiology and Biochemistry of Drought Resistance in Plants.*** Academic Press, New York. 261-282.

Bewley, J.D. 1997. Seed germination and dormancy. ***Plant Cell 9:*** 1055-1066.

Bewley, J.D. and Black, M. 1983. ***Physiology and Biochemistry of Seeds in Relation to Germination.*** **Vol. I.** Springer – Verlag, Berlin.

Bewley, J.D. and Black, M. 1985. ***Seeds: Physiology of Development and Germination*****.** Plenum Press, New York.

Bewley, J.D. and Black, M. 1994. ***Seeds: Physiology of Development and Germination.*** 2nd Edn. Plenum Press. New York.

Bewley, J.D. and Marcus, A. 1990. Gene expression in seed development and germination. ***Prog. Nucleic Acid Res. Mol. Biol.*** **38:** 165-193.

Bhandari, N.N. and Chitralekha, P. 1984. Degradation of protein bodies in germinating seeds of *Brassica campestris* L. var. *sarson* Prain. ***Ann. Bot. 53***: 793-801.

Bhatnagar, P.S. and Tiwari, S.P. 1991. Soybean in edible oil economy of India. ***Agri. Situ. India*** **46**: 295-301.

Bhattacharjee, S. and Mukherjee, A.K. 2006. Heat and salinity induced oxidative stress and changes in protein profile in *Amaranthus lividus* L. ***Indian J. Plant Physiol***. **11**: 41-47.

Blackman, S.A., Wattlaufer, S.H., Obendorf, R.L. and Leopold, A.C. 1991. Maturation proteins association with desiccation tolerance in soybean. ***Plant Physiol***. **96**: 868-874.

Blackman, S.A., Obendorf, R.L. and Leopold, A.C. 1992. Maturation proteins and sugars in desiccation tolerance of developing soybean seeds. ***Plant Physiol***. **100:** 225-230.

Bramlage, W.J., Leopold, A.C. and Parrish, D.J. 1978. Chilling stress to soybean during imbibition. ***Plant Physiol.*** **61**: 525-529.

Bramlage, W.J., Leopold, A.C. and Specht, J.E. 1979. Imbibitional chilling sensitivity among soybean cultivators. ***Crop. Sci***. **19:** 811-814.

Brenac, P., Smith, N.E. and Obendorf, R.O. 1997. Raffinose

accumulation in maize embryos in the absence of a fully functional Vpl gene product. ***Planta*** **203**: 222-225.

Briarty, L.G. and Pearce, N.M. 1982. Starch granule production during germination in legumes**.** ***J. Exp. Bot. 33***: 506-510.

Bruggink, H., Kraak, H.L., Dijkerna, M.H.G.E. and Bekendam, J. 1991. Some factors influencing electrolyte leakage from maize (*Zea mays* L.) kernels. ***Seed Sci. Res*****. 1**: 15-20.

Bruni, F. and Leopold, A.C. 1991. Glass transition in soybean seed. I. Relevance to anhydrous biology. ***Plant Physiol***. **96**: 660-663.

Buchanan, B.B., Gruissem, W. and Jones, R.L. 2000. ***Biochemistry and Molecular Biology of Plants*****.** American Society of Plant Physiologists, Rodeville, Maryland.

Buitink, J., Hemminga, M.A. and Hoekstra, F.A. 2000. Is there a role for oligosaccharides in seed longevity? An assessment of intracellular glass stability. ***Plant Physiol***. **122**: 1217-1224.

Burris, J.S. 1973. Effect of seed maturation and plant population on soybean seed quality**.** ***Agro. J.*** **65:** 440-441.

Buttery, B.R. and Buzzell, R.I. 1968. Inheritance of peroxidase activity in soybean seed coats. ***Crop Sci***. **9**: 387-388.

Cabrera, E.R. and Boyd, A.H. 1988. Effect of moisture and temperature on germination of cotton seed. ***Proc. Beltwide Cotton Prod. Res. Conf. Nat. Council.*** Memphis. 88- 89.

Callis, J. 1995. Regulation of protein degradation. ***Plant Cell*** **7**: 845-857.

Cammue, B.P.A., Broekaert, W.F., Kellens, J.T.C., Raikhel, N.V. and Paumans, W.J. 1989. Stress-induced accumulation of wheat germ agglutinin and abscisic acid in roots of wheat seedlings. ***Plant Physiol.*** **91**: 1432-1435.

Capdevila, A.M. and Dure, L. 1977. Developmental biochemistry of cotton seed embryogenesis and germination, VIII. Free amino acid pool composition during cotyledon development. ***Plant Physiol***. **59:** 268-273.

Carpenter, J.F., Crowe, L.M. and Crowe, J.H. 1987. Stabilization of phosphofructokinase with sugars during freeze-drying: Characterization of enhanced protection in the presence of divalent cations. ***Biochim. Biophys. Acta.*** **923**: 109-115.

Chen, Y. and Burris, J.S. 1990. Role of carbohydrates in desiccation tolerance and membrane behaviour in maturing maize seed. ***Crop Sci.*** **30**: 971-975.

Chin, H.F. 1988. ***Recalcitrant Seeds. A Status Report***. IBPGR, Rome.

Chin T.Y., Poulson, R. and Beevers, L. 1972. The influence of axis removal on protein metabolism in cotyledons of *Pisum sativum* L. ***Plant Physiol***. **49**: 482-489.

Chu, T.M., Aspinall, D. and Paleg, L.G. 1974. Stress metabolism. VI. Temperature stress and the accumulation of proline in barley and radish. ***Aust. J. Plant Physiol***. **1**: 87-97.

Clegg, J.S. 1978. Interrelationships between water and cellular metabolism in *Artemia* cysts. ***J. Cell. Physiol.* 94**: 123-138.

Colaco, C., Sen, S., Thangavelu, M., Pinder, S. and Roser, B. 1992. Extraordinary stability of enzymes dried in trehalose: Simplified molecular biology. ***Biotechnology* 10**: 1007-1010.

Collins, D.M. and Wilson, A.T. 1975. Embryo and endosperm metabolism of barley seeds during early germination. ***J. Exp. Bot***. **26**: 737-740.

Copeland, L.O. and McDonald, M.B. 1995. ***Principles of Seed Science and Technology*.** 3rd Edn. Chapman and Hall, New York.

Cortes, P.M. and Spaeth, S.C. 1993. Potassium leakage from artificially aged pea (*Pisum sativum* L.) embryos during imbibition ***J. Seed Technol.* 17**: 30-42.

Crowe, J.H. and Crowe, L.M. 1992. Membrane integrity in anhydrobiotic organism: Toward a mechanism for stabilizing dry seeds. *In*: G.N.

Somero, C.B. Osmond and C.L. Bolis (Eds.) ***Water and Life***. Springer-Verlag, Berlin. 87-103.

Darbyshire, B. 1974. The function of the carbohydrate units of three fungal enzymes in their resistance to dehydration. ***Plant Physiol.* 54**: 717-721.

Davies, D.R., Berry, G.J., Health, M.C. and Dawkins, T.C.K. 1985. Pea (*Pisum sativum* L.). *In*: R.J. Summerfield and E.H. Roberts (Eds.) ***Grain Legume Crops.*** William Collins Sons and Co. Ltd. London. 147-198.

de Villalobos, A.E., Pelaez, D.V., Boo, R.M., Mayer, M.D. and Elia, O.R. 2002. Effect of high temperatures on seed germination of *Prosopis caldenia* Burk. ***J. Arid Envir. 52:*** 371-378.

del Monte, J.P. and Tarquis, A.M. 1997. The role of temperature in the seed germination of two species of the *Solanum nigrum* complex***. J. Exp. Bot***. **48:** 2087-2093.

Dell' Aquila, A. and Di -Turi, M. 1996. The germination response to heat and salt stress in evaluating vigour loss in aged wheat seeds. ***Seed Sci. Technol.* 24:** 313-319.

Dell' Aquila, A., Corona, M.G. and Di -Turi, M. 1998. Heat- shock proteins in monitoring, ageing and heat-induced tolerance in

germinating wheat and barley embryos. ***Seed Sci. Res. 8***: 91- 98.

DeRocher, A.E., Helm, K.W., Lanzon, L.M. and Vierling E. 1991. Expression of a conserved family of cytoplasmic low molecular weight heat shock proteins during heat stress and recovery. ***Plant Physiol.*** **96:** 1038-1047.

Dey, P.M. 1990. Oligosaccharides. *In*: P.M. Dey and J.B. Harborne (Eds.) ***Methods in Plant Biochemistry.*** **Vol. 2.** Academic Press, San Diego. 189-218.

Dias, C.F.S., Marcos – Filho, J. and Carmello, Q.A.C. 1996. Potassium leaching test for the evaluation of vigour in soybean seeds. ***Seed Sci. Technol.*** **25**: 7-28.

Dionisio-Sese, M.L. and Tobita, S. 1998. Antioxidant responses of rice seedlings to salinity stress. ***Plant Sci.*** **135**: 1-9.

Doijode, S.D. 1988. Solute leakage in relation to loss of seed viability in chilli cultivars. ***Indian J. Plant Physiol***. **31**: 285-287.

Douglas, J.E. 1975. Seed storage and packaging. *In:* W.P. Feistritzer (Ed.) ***Cereal Seed Technology.*** Food and Agriculture Organization of the United Nations, Rome. 87-107.

Duke, S.H. and Kakefuda, G. 1981. Role of the testa in preventing cellular rupture during imbibition of legume seeds. ***Plant Physiol***. **67**:

449-456.

Duke, S.H., Kakefuda, G. and Harvey, T.M. 1983. Differential leakage of intracellular substances from imbibing soybean seeds. ***Plant Physiol***. **72**: 919-924.

Dure, L. III., Crouch, M.L., Harada, J.J., Ho, T.H.D., Mundy, J., Quatrano, R.S., Thomas, T. and Sung, Z.R. 1989. Common amino sequence domains among LEA proteins of higher plants. ***Plant Mol. Biol***. **12**: 475-486.

Edje, O.T. and Burris, J.S. 1970. Physiology and biochemical changes in deteriorating soybean seeds. ***Off. Seed Analyst*** **60**: 158-166.

Egley, G.H., Paul, R.N., Vaughn, K.C. and Duke, S.O. 1983. Role of peroxidase in the development of water-impermeable seed coats in *Sida spinosa* L. ***Planta*** **157**: 224-232.

Egli, D.B. and Tekrony, D.M. 1996. Seed bed conditions and prediction of field emergence of soybean seed. ***J. Prod. Agri. 9***: 365-370.

Ellis, R.H. and Roberts, E.H. 1981. The quantification of ageing and survival in orthodox seeds. ***Seed Sci. Technol***. **9**: 373-409.

Ellis, R.H. and Roberts, E.H. 1982. Desiccation, rehydration, germination, imbibition injury and longevity of pea seeds (*Pisum sativum*). ***Seed Sci. Technol.*** **10**: 501-508.

Ellis, R.H., Hong, T.D. and Roberts, E.H. 1988. A low moisture content limit to logarithmic relations between seed moisture content and longevity. ***Ann. Bot.*** **61**: 405-408.

Ellis, R.H., Hong, T.D. and Roberts, E.H. 1989**.** A comparison of the low moisture content limit to the logarithmic relation between seed moisture and longevity in twelve species. ***Ann. Bot.*** **63**: 608-611.

Ellis, R.H., Hong, T.D. and Roberts, E.H. 1990. Effect of moisture content and method of rehydration on the susceptibility of pea seeds to imbibitional damage. ***Seed Sci. Technol.*** **18:** 131-137.

Ferguson, I.B., Lurie, S. and Bowen, J.H. 1994. Protein synthesis and breakdown during heat shock of cultured pear cells. ***Plant Physiol***. **104:** 1429- 1437.

Fletcher, R.S. and Kott, L.S. 1999. ***Phenolics and Cold Tolerance of Brassica napus.*** 10th International Rape Seed Congress. Canberra, Australia.

Foyer, C.H. and Noctor, G. 2000. Oxygen processing in photosynthesis: Regulation and signalling. ***New Phytol.*** **146**: 359-388.

Fridowitch, I. 1989. Superoxide dismutase. An adaptation in paramagnetic gas**.** ***J. Biol***. **126**: 93-101.

Fu, J.R., Jin, J.P., Peng, Y.F. and Xia, Q.H. 1994. Desiccation tolerance in

two species with recalcitrant seeds: *Clausena lansium* (Lour.) and *Litchi chinensis* (Sonn.) ***Seed Sci. Res***. **4**: 257-261.

Garcia-Luis, A. and Guardiola, J.L. 1975. Effects of gibberellic acid on the transport of nitrogen from the cotyledons of young pea seedlings. ***Ann. Bot.*** **39**: 325- 330.

Garcia-Luis, A. and Guardiola, J.L. 1978. Gibberellic acid and starch breakdown in pea cotyledons. ***Ann. Bot.*** **42**: 337-344.

Gaspar, T., Penel, C., Thorpe, T. and Greppin, H. 1982. ***Peroxidases 1970-1980. A Survey of Their Biochemical and Physiological Roles in Higher Plants.*** University of Geneva press, Geneva, Switzerland.

Gelmond, H., Luria, I., Woodstock, L.W. and Perl. M. 1978. The effect of accelerated ageing of *Sorghum* seeds on seedling vigour. ***J. Exp. Bot.*** **29:** 489-495.

Gidrol. X., Noubhani, A., Mocquot, B., Fournier, A. and Pradet, A. 1988. Effect of accelerated ageing on protein synthesis in two legume seeds. ***Plant Physiol. Biochem.*** **26**: 281-288.

Gillikin, J.W. and Graham, J.S. 1991. Purification and developmental analysis of the major anionic peroxidase from the seed coat of *Glycine max.* ***Plant Physiol***. **96**: 214-220.

Goldberg, R.B., Barker, S.J. and Perez-Grau, I. 1989. Regulation of gene

explosion during plant embryogenesis. ***Cell*** **56:** 149-160.

Gorecki, J.R., Piotrowicz-Cieslak, A. and Obendorf, L.R. 1997. Soluble sugars and flatulence - producing oligosaccharides in maturing yellow lupin (*Lupinus luteus* L.) seeds. ***Seed Sci. Res***. **7**: 185-193.

Granquist, G.A. 1987. Leakage of cations from a carrot seed lot. ***Acta Horti***. **215**: 219-224.

Grelet, J., Benamar, A., Teyssier, E., Avelange-Macherel, M., Grunwald, D. and Macherel, D. 2005. Identification in pea seed mitochondria of a Late-Embryogenesis-Abundant protein, able to protect enzymes from drying. ***Plant Physiol*****. 137:** 157-167.

Greppin, H., Penel, C. and Gaspar, T. 1986. ***Molecular and Physiological Aspects of Plant Peroxidases.*** University of Geneva Press, Geneva, Switzerland.

Guillermond, A. 1941. ***The Cytoplasm of the Plant Cell***. Chronica Botanica Co., Waltham, U.S.A.

Gumilevskaya, N.A., Chumikina, L.V., Arabova, L.I., Zimin, M.V. and Shatilov, V.R. 1996. Effect of high temperature on protein synthesis in the axes of imbibing pea embryos. ***Russ. Rev***. ***Plant Physiol.*** **43**: 215-222.

Gupta. S.C., Bhatia, L.K. and Singh A.K. 1994. Role of proline and

cellular osmotic agents in drought tolerance in cowpea. *In:* K. Singh and S.S. Purohit (Eds.) ***Plant Productivity under Environmental Stress.*** Agro Botanical Publishers, India**.** 237-240.

Hanley, M.E., Frenner, M. and Ne'eman, G. 2001. Pre-germination heat shock and seedling growth of fire-following Fabaceae from four Mediterranean-climate regions. ***Acta Oecol. 22***: 315-320.

Hare, P.D., Cress, W.A. and van Staden, J. 1998. Dissecting the roles of osmolyte accumulation during stress. ***Pant Cell Envir.* 21**: 535-553.

Harrington, J.F. 1972. Seed Storage and longevity. *In*: T.T. Kozlowski (Ed.) ***Seed Biology***, **Vol. 3.** Academic Press. New York. 145-245.

Harris, M., Smith, D.L. and Macender, R.O. 1986. Growth analysis of soybean seedlings during the life span of the cotyledons. ***Ann. Bot***. **57**: 69-79.

Heldt, H.W. 1999. ***Plant Biochemistry and Molecular Biology.*** Oxford University Press. Oxford.

Helm, K.W., Lafayette, P.R., Nagao, R.T., Key, J.T. and Vierling, E. 1993. Localization of small heat shock proteins to the higher plant endomembrane sytem. ***Mol. Cell Biol.* 13**: 238-247.

Hernandez, J.A. and Almansa, M.S. 2002. Short-term effects of salt stress on antioxidant systems and leaf water relations of pea leaves. ***Physiol.***

Plant. **115**: 251-257.

Hernandez, J.A., Ferrer, M.A., Jimenez A., Barcelo, A.R. and Sevilla, F. 2001. Antioxidant systems and O_2/ H_2O_2 production in the apoplast of pea leaves. Its relation with salt induced necrotic lesions in minor veins***.*** ***Plant Physiol.*** **127:** 817-831.

Hershko, A. and Ciechanover, A. 1992. The ubiquitin system for protein degradation. ***Annu. Rev***. ***Biochem***. **61**: 761- 807.

Heydecker, W. 1972. Vigour. *In*: E.H. Roberts (Ed.) ***Viability of Seeds***. Chapman and Hall. Ltd. London. 209-252.

Hildebrand, D.F. 1992. Altering fatty acid metabolism in plants. ***Food Technol***. **1**: 71-74.

Hilhorst, H.W.M. 1995. A critical update on seed dormancy. 1. Primary dormancy. ***Seed Sci. Res***. **5**: 61-73.

Hobbs, P.R. and Obendorf, R.L. 1972. Interaction of initial seed moisture and imbibitional temperature on germination and productivity of soybean. ***Crop Sci.*** **12:** 664-667.

Hoekstra, F.A., Crowe, J.H., Crowe, L.M., van Rockel, T. and Vermeer, E. 1992. Do phospholipids and sucrose determine membrane phase transitions in dehydrating pollen species? ***Plant Cell Envir.*** **15**:

601-606.

Hoekstra, F.A., Haigh, A.M., Tetteroo, F.A.A. and van Roekel, T. 1994. Changes in soluble sugars in relation to desiccation tolerance in cauliflower seeds. ***Seed Sci. Res***. **4:** 143-147.

Horbowicz, M. and Obendorf, R.L. 1994. Seed desiccation tolerance and storability. Dependence on flatulence producing oligosaccharides and cyclitols. Review and Survey. ***Seed Sci. Res*. 4**: 385-405.

Hottiger, T., De Virgilio, C., Hall, M.N., Boller, M.N. and Wiemken, A. 1994. The role of trehalose synthesis for the acquisition of thermo-tolerance in yeast: II. Physiological concentrations of trehalose increase the thermal stabilty of proteins *in vitro.* ***European J. Biochem.*** **219:** 187-193.

Howarth, C.J. and Ougham, H.J. 1993. Gene expression under temperature stress. ***New Phytol.*** **125**: 1-26.

Howarth, C.J. and Skot, K.P. 1994. Detailed characterization of heat shock protein synthesis and induced thermo-tolerance in seedlings of *Sorghum bicolor* L. ***J. Exp. Bot.*** **45**: 1353-1368.

Hsiao, T.C. 1973. Plant responses to water stress**.** ***Annu. Rev. Plant Physiol***. **24**: 519-570.

Iba, K. 2002. Acclimative response to temperature stress in higher plants:

Approaches of gene engineering of temperature treatment. ***Annu. Rev. Plant Biol.*** **53**: 225-245.

ISTA. 1985. International rules for seed testing (ISTA). ***Seed Sci. Technol.*** **13**: 299-355.

ISTA. 1987. Hand book of vigour test methods. 2nd Edn. International seed testing association. Zurich.

Ishida, N., Kano, H, Kobyashi, T., Hamaguchi, H. and Yoshida, T. 1987. Estimation of biological activities by NMR in soybean seeds during maturation. ***Agric. Biol. Chem***. **51:** 301-307.

Ishida, N., Kano, H., Kobayashi, T., Hamaguchi, H. and Yoshida, T. 1988. The relationship between imbibition damage and initial water content of soybean. ***Agric. Biol. Chem***. **52**: 2771-2775.

Issa, M.A., Abdel-Salam, H.S., Hassan, M.S. and Elmalt, E.A. 1994. The effects of germination on carbohydrate contents, trypsin inhibitors and protein digestability (*in vitro*) of some local varieties of cow pea. ***Ann. Agric. Sci***. **32**: 1545-1560.

Johansen, D.A. 1940. ***Plant Microtechnique***. McGraw Hill, New York.

Kalpana. R. and Rao, K.V.M. 1997. Protein metabolism of seeds of pigeon pea (*Cajanus cajan* L.) cultivars during accelerated ageing. ***Seed Sci. Technol. 25***: 271-279.

Karunagaran, D. and Rao, P.R. 1990. Axial control of protease development in the cotyledons of horse gram (*Macrotyloma uniflorum* Lam.) seeds during germination. ***Indian J. Plant Physiol***. ***33***: 232-238.

Kellogg, F.W. and Fridowitch, I. 1975. Superoxide, hydrogen peroxide and singlet oxygen in lipid peroxidation by a xanthine oxidase system. ***J. Biol. Chem.*** **250:** 8812-8817.

Kermode, A.R. 1990. Regulatory mechanisms involved in the transition from seed development to germination. ***Crit. Rev. Plant Sci.*** **9:** 155-195.

Key, J.L., Lin. C.Y. and Chen, Y. M. 1981. Heat shock proteins of higher plants. ***Proc. Nat. Acad. Sci.*** USA. **78:** 3526-3530.

Khan, T.N. 1978. Variation ecology and cultural practices of winged bean. *In*: ***The Winged bean.*** The First International Symposium on Developing the Potentials of Winged bean. Manila, Philippines. 3-11.

Khan, R.A., Ahmad, S. and Hussain, S. 1973. Effects of pre-sowing high temperature stress on seedling emergence and yield of seed cotton. ***Exp. Agri. 9***: 9-14.

Knypl, J.S., Janas, K.M. and Radziwonowska - Jozwiak, A. 1980. Is enhanced vigour in soybean (*Glycine max*) dependent on activation of

protein turnover during controlled hydration of seeds? ***Physiol. Veg.*** **18**: 157-161.

Koller, D. and Hadas, A. 1982. Water relation in the germination of seeds. *In:* O.L. Lange, R.S. Nobel, C.B. Osmond and H. Ziegler (Eds.) ***Encyclopaedia of Plant Physiology: Physiological Plant Ecology III Water Relations and Carbon Assimilation.*** **Vol. 12B.** Springer - Verlag, Berlin. 401-431.

Koster, K.L. 1991. Glass formation and desiccation tolerance in seeds. ***Plant Physiol***. **96**: 302-304.

Koster, K.L. and Leopold, A.C. 1988. Sugars and desiccation tolerance in seeds. ***Plant Physiol***. **88:** 829-832.

Koster,K.L., Webb, M.S., Bryant, G. and Lynch, D.V. 1994. Interactions between soluble sugar and POPC (1-palmityl-2-oleoyl-phosphatidylcholine) during dehydration. Vitrification of sugars alters the phase behaviour of phospholipid***. Biophys. Acta***. **193**: 143-150.

Kumar, J. and Badiyala, D. 2005. Effect of seed rate, row spacing and sowing time on yield attributes of soybean. ***Legume Res***. **28:** 288- 290.

La Beller, D., Picque, E. and Demignot, S. 1986. Peroxidase localization in the seeds of *Brassica juncea*. *In*: H. Greppin, C. Penel and T.

Gaspar (Eds.). ***Molecular and Physiological Aspects of Plant Peroxidases.*** University of Geneva Press, Geneva, Switzerland, 451-454.

Laemmli, U.K. 1970. Cleavage of structural proteins during assembly of the head of bacteriophage T_4. ***Nature*** **227**: 680-685.

Lafuente, M., Teresa, A.B., Guye, M.G. and Saltveit, E.M. 1991. Effect of temperature on conditioning on chilling injury of cucumber cotyledons**.** ***Plant Physiol.*** **95**: 443-449.

Lea, P.J. and Joy, K.W. 1983. Amino acid interconversion in germinating seeds. *In:* C. Nozzolillo, P.J. Lea and F.A. Loewus (Eds.). ***Recent Advances in Phytochemistry.*** **Vol**. **17**, Plenum Press, New York and London 77-109.

Lee, C.K. and Karunanithy, R. 1990. Effects of germination on the chemical composition of *Glycine* and *Phaseolus* beans. ***J. Sci. Food Agri.*** **51**: 437-445.

Lee, S.S., Taylor, A.G., Bere Sniewicz, M.M. and Paine, D.H. 1995. Sugar leakage from aged leek, onion. and cabbage seeds. ***Plant Var. Seeds.*** **8:** 81-86.

Lee, Y.P. and Takahashi, T. 1966. An improved colorimetric determination of amino acids with the use of ninhydrin. ***Anal. Biochem***. **14**: 71-77.

Leopold, A.C. 1980. Temperature effects on soybean imbibition and leakage**.** ***Plant Physiol***. **65**: 1096-1098.

Leopold, A.C., Sun, W.Q. and Bernal-Lugo, I. 1994. The glassy state in seeds: analysis and function. ***Seed Sci. Res*.** **4**: 267-274.

Leprince O., Bronchart, R. and Deltour, R. 1990. Changes in starch and soluble sugars in relation to the acquisition of desiccation tolerance during maturation of *Brassica campestris* seed. ***Plant Cell Envir.*** **13:** 539-546.

Leprince, O., **Hendry G.A.F. and McKersie, B.D.** 1993. The mechanism of desiccation tolerance in developing seeds. ***Seed Sci. Res.*** **3:** 231-246.

Loeffler, T.M., Tekrony, D.M. and Egli, D.B. 1988. The bulk conductivity test as an indicator of soybean seed quality. ***J. Seed Technol***. **12**: 37-53.

Loomis, E.L. and Smith O.E. 1980. The effect of artificial ageing on the concentration of Ca, Mg, Mn, K and Cl in imbibing cabbage seed. ***J. Am. Soc. Horti. Sci.*** **105:** 647-650.

Lott, J.N.A., Cavdek, V. and Carson, J. 1991**.** Leakage of K, Mg, Cl, Ca and Mn from imbibing seeds, grains and isolated seed parts. ***Seed Sci. Res*.** **1**: 229- 233.

Lowell, C.A. and Kuo, T.M. 1989. Oligosaccharide metabolism and

accumulation in developing soybean seeds. ***Crop. Sci.*** **29**: 459-465.

Lowry, O.H., Rosenbrough, N.J., Farr, F.L. and Randall, R.J. 1951. Protein measurement with folin phenol reagent. ***J. Biol. Chem.*** **193**: 265-275.

Maguire, J.D. 1977. Seed quality and germination. *In:* A.A. Khan, (Ed.) ***The Physiology and Biochemistry of Seed Dormancy and Germination,*** Elsevier Biomedical Press. North Holland. 219-235.

Mai-Hong, R., Hong, T.D., Hien, N.T. and Ellis, R.H. 2003. Onset of germinability, desiccation tolerance and hardseededness in the seeds of *Peltophorum pterocarpum* (DC) K. Heyne. (Caesalpinioideae). ***Seed Sci. Res***. **13**: 323-327.

Makasheva, R.K. 1983. ***The Pea.*** Oxonian Press Pvt. Ltd., New Delhi, India.

Mansfield, M.A. and Key, J.L. 1987. Synthesis of the low molecular weight heat shock proteins in plants. ***Plant Physiol***. **84**: 1007-1017.

Marbach, I. and Mayer, A.M. 1976. Respiration and utilization of storage materials in wild and cultivated pea seeds during germination. ***Plant Physiol.*** **38*:*** 126- 130.

Marbach, I. and Mayer, A.M. 1985. The effect of temperature change of leakage from pea seeds. ***J. Exp. Bot***. **36**: 353-358.

Matthews, S. and Bradnock, W.T. 1968. Relationship between seed exudation and field emergence in peas and French beans**.** ***Hortic. Res***. **8**: 89-93.

Matthews, S. and Powell, A.A. 1981. Electrical conductivity test. *In*: D.A. Perry (Ed.) ***Handbook of Vigour Test Methods,*** Zurich: ISTA. 37-42.

Matthews, S. and Rogerson, N.E. 1976. The influence of embryo condition on the leaching of solutes from pea seeds. ***J. Exp. Bot.*** **27**: 961-968.

Mayer, A.M. and Poljakoff – Mayber, A. 1963. ***The Germination of Seeds***. Pergamon Press, Oxford.

Mayer, A.M. and Poljakoff-Mayber, A. 1989. ***The Germination of Seeds.*** 4th Edn. Pergamon Press. New York.

Mazia, D., Brewer, P. A. and Alfert, M. 1953. The cytochemical staining and measurement of protein with mercuric bomophenol blue. ***Biol. Bul***. **104**: 57- 67

McDonald, M.B., Vertucci, C.W. Jr. and Roos, E.E. 1988. Soybean seed imbibition: Water absorption by seed parts. ***Crop Sci.*** **28**: 993-997.

Meng, S.C., Zhang, H.Y. and Kong, X.H. 2003. Effect of dry- heat treatment at 76 ^{0}C in different times and moisture content on seed vigour of Xin-Li- Mci radish. ***Seed Sci. Technol.*** **31**: 193-197.

Misra, N.M. and Kar, R.K. 1990. Regulation of storage protein degradation in cotyledons of germinating cow pea. ***Indian J. Plant Physiol***. **33**: 333- 339.

Moftah, A.E. and Michel, B.E. 1987. The effect of sodium chloride on solute potential and proline accumulation in soybean leaves. ***Plant Physiol. 83***: 238-240.

Monneri, C., Gercia, L. and Guardiola, J.L. 1986. Sugar and Starch changes in pea cotyledons during germination. ***Physiol. Plant.*** **67**: 49-54.

Montgomery, R. 1957. Determination of glycogen ***Arch. Biochem. Biophys***. **67**: 378-386.

Moreau, R.A. and Huang, A.H.C. 1977. Gluconeogenesis from storage wax in the cotyledons of jojoba seedlings. ***Plant Physiol.*** **60**: 329-333.

Mundy, J. and Chua, N.H. 1988. Abscisic acid and water stress induce a novel rice gene. ***Eur. Mol. Biol. Organ. J.*** **7**: 2279-2286.

Munro, K.D., Hodges, D.M., Delong, J.M., Forney, C.F. and Kristie, D.N. 2004. Low temperature effects on ubiquinone content, respiration rates and lipid peroxidation levels of etiolated seedlings of two differentially chilling - sensitive species. ***Physiol. Plant***. **121:** 488-497.

Murphy, J.B. **and Noland, T.L.** 1982. Temperature effects on seed imbibition and leakage mediated by viscosity and membranes. ***Plant Physiol***. **69**: 428-431.

Murray, D.R. 1984. Axis-cotyledon relationships during reserve mobilization. *In:* D.R. Murray (Ed.) ***Seed Physiology*. Vol.2 *Germination and Reserve Mobilization*.** Academic Press. London. 247-280.

Narayan, I.D. and Misra, R.D. 1989. Free proline accumulation and water stress resistance in bread wheat (*Triticum aestivum*) and durum wheat (*Triticum durum*). ***Indian J. Agri. Sci***. **39**: 176-178.

Nichols, M.A. and Heydecker, W. 1968. Two approaches to the study of germination data. ***Proc. Int. Seed Test*. Assoc**. **33***:* 531-540.

Nkang, A. 2002. Carbohydrate composition during seed development and germination in two sub-tropical rainforest tree species (*Erythrina caffra* and *Guilfoylia monostylis*). ***J. Plant Physiol*. 159**: 473-483.

Noggle, G.R. and Fritz, G.J. 1986. ***Introductory Plant Physiology.*** 2nd Edn. Prentice-Hall, Engle Wood Cliffs, N.J., U.S.A.

Obendorf, R.L. 1997. Oligosaccharides and galactosyls in seed desiccation tolerance. ***Seed Sci. Res. 7***: 63-74.

Obendorf, R.L. and Hobbs, P.R. 1970. Effects of seed moisture on

temperature sensitivity during imbibition of soybean. ***Crop. Sci***. **10:** 563-566.

Obendorf, R.L., Dickerman, A.M., Pflum, T.M., Kacalanos, M.A. and Smith, M.E. 1998. Drying rate alters soluble carbohydrates, desiccation tolerance and subsequent seedling growth of soybean [*Glycine max (*L.) Merril] zygotic embryos during *in vitro* maturation. ***Plant Sci.*** **132**: 1-12.

Opik, H. 1966. Changes in cell fine structure in the cotyledons of *Phaseolus vulgaris* L. during germination. ***J. Exp. Bot***. **17:** 427-439.

Pandey, D.K. 1988. Priming induced repair in French bean seeds. ***Seed Sci. Technol*****. 16**: 107-116.

Parrish, D.J. and Leopold, A.C. 1977. Transient changes during soybean imbibition. ***Plant Physiol***. **59:** 1111-1115.

Parrish, D.J. and Leopold, A.C. 1978. On the mechanism of ageing in soybean seeds. ***Plant Physiol***. **61**: 365- 368.

Pearse, A.G.E. 1972. ***Histochemistry: Theory and Applied***, **Vol. 2.** Churchill Livingstone, Edinburgh.

Pelham, H.R.B. 1986. Speculations on the functions of the major heat shock and glucose - regulated proteins**.** ***Cell*** **46**: 959 - 961.

Perl-Treves, R. and Galun. E. 1991. The tomato Cu, Zn Superoxide dismutase genes are developmentally regulated and respond to light and stress, ***Plant Mol. Biol***. **17**: 745-760.

Petruzelli, L. and Taranto, G. 1989. Wheat ageing: the contribution of embryonic and non-embryonic lesion to loss seed viability. ***Physiol. Plant***. **76:** 289-294.

Phillips, J.C. and Youngman, V.E. 1971. Effect of initial seed moisture control on emergence and yield of grain sorghum. ***Crop. Sci***. **11**: 354-356.

Pixton, S.W. 1967. Moisture content - its significance and measurement in stored products. ***J. Stored Prod. Res***. **3**: 35-47.

Pollock, B.M. 1969. Imbibition temperature sensitivity of lima bean seeds controlled by initial seed moisture. ***Plant Physiol***. **44**: 907-911.

Popp, K. and Smirnoff, N. 1995**.** Polyol accumulation and metabolism during water deficit. *In:* N. Smirnoff (Ed.) ***Environment and Plant Metabolism: Flexibility and Acclimation.*** Bios Scientific Publishers. Oxford. 199-215.

Powell, A.A. 1986. Cell membranes and seed leachate conductivity in relation to the quality of seed for sowing**.** ***J. Seed Technol***. **10:** 81-100.

Powell, A.A. and Matthews, S. 1978. The damaging effects of water on dry pea embryos during imbibition. ***J. Exp. Bot. 29***: 1215-1229.

Powell, A.A. and Matthews, S. 1979. The influence of testa conditions on the imbibition and vigour of pea seeds**.** ***J. Exp. Bot***. **30**: 193-197.

Priestly, D.A. and Leopold, A.C. 1983. Lipid changes during natural ageing of soybean seeds. ***Physiol. Plant***. **59**: 467-470.

Pucher, G.W., Leavenwoth, C.S. and Vickery, H.B. 1948. Determination of starch in plant tissues. ***Anal. Chem.*** **20**: 850-853.

Quail, P.H. 1979. Plant cell fractionation**.** ***Annu. Rev. Plant Physiol.*** 30: 425- 484.

Queiroga, V.P. and Parra, N.R. 1989. ***Electrolytes released by sunflower seeds.*** Congresso Brasileiro de Sementes. Brazil.

Reddy, V.V. and Srivastava, G.C. 2003. Superoxide dismutase and peroxidase activities in ripening mango *Mangifera indica* L. fruit. ***Indian J. Plant Physiol***. **8:** 115-119.

Reibach, P.H. and Benedict, C.R. 1982. Biosynthesis of starch in proplastids of germinating *Ricinus communis* endosperm tissue. ***Plant Physiol***. **70**: 252-256.

Reisdorph, N. and Koster, K.L. 1999. Progressive loss of desiccation

tolerance in germinating pea (*Pisum sativum* L.). ***Physiol. Plant.* 105:** 266-271.

Riley, G.J.P. 1981. Effect of high temperature on protein synthesis during germination of maize (*Zea mays* L.). ***Planta.* 151**: 75-80.

Riquelme, A. and Cardemil, L. 1993. Peroxidases in the cell walls of seeds and seedlings of *Araucaria araucana.* ***Phytochemistry* 32**: 15-20.

Roberts, E.H. 1972. Cytological, genetical and metabolic changes associated with loss of viability. *In*: E.H. Roberts, (Eds.) ***Viability of Seeds***. Chapman and Hall Ltd., London. 253-286.

Roberts, E.H. 1973. Predicting the storage life of seeds. ***Seed Sci. Technol***. **1:** 499-514.

Roberts, E.H. and Ellis, R.H. 1989. Water and seed survival. ***Ann. Bot.* 63**: 39-52.

Rolston, M.P. 1978. Water impermeable seed dormancy. ***Bot. Rev***. **44**: 365-396.

Rosenberg, L.A. and Rinnie, R.W. 1988. Protein synthesis during natural and precociously matured soybean seeds [*Glycine max* (L.) Merril]. ***Plant Physiol.* 87**: 474-478.

Rupley, J.A., Gratton, E. and Careri, G. 1983. Water and globular

proteins. ***Trends Biochem. Sci.* 8**: 18-22.

Saio, K., Nikkuni, I., Ando, Y., Otsuru, M., Terauchi, Y. and Kito, M. 1980. Soybean quality changes during model storage studies. ***Cereal Chem***., **57**: 77-82.

Sanhewe, A.J. and Ellis, R.H. 1996. Seed development and maturation in *Phaseolus vulgaris*. II**.** Post-harvest longevity in air-dry storage. ***J. Exp. Bot***. **47**: 959-965.

Saravitz, D.M., Pharr, D.M. and Carta. T.E. 1987. Galactinol synthase activity and soluble sugars in developing seeds of soybean. ***Plant Physiol.* 83:** 185-189.

Saunders, B.C., Holmes–Siedle, A.G. and Stark, B.P. 1964. ***Peroxidase: The Properties and Uses of a Versatile Enzyme and Some Related Catalysts***. Butterworth, London

Saure, M.C. 2005. Calcium translocation to fleshy fruit: its mechanism and endogenous control. ***Sci. Horti.* 105:** 65- 89.

Scandalios, J.G. 1990. Response of plant antioxidant defence genes to environmental stress. ***Adv. Genet***. **28:** 1-41.

Scandalios, J.G. 1993. Oxygen stress and superoxide dismutases. ***Plant Physiol***. **101**: 7-12.

Scandalios, J.G., Tong, W.F. and Roupakias, D.G. 1980. Cat 3, a third gene locus coding for a tissue-specific catalase in maize: Genetics, intracellular location, and some biochemical properties. ***Mol. Gen. Genet.* 179:** 33-41.

Schoettle, A.W. and Leopold, A.C. 1984. Solute leakage from artificially aged soybean seeds after imbibition. ***Crop. Sci.* 24**: 835-838.

Senaratna, T. and McKersie, B.E. 1983. Characterisation of solute efflux from dehydration injured soybean [*Glycine max* (L.) Merr.] seeds. ***Plant Physiol***. **72**: 911-914.

Sharma, S.G. 1988. Regulation of reserve protein metabolism during germination and early seedling establishment in chickpea. ***Indian J. Plant Physiol***. **31**: 193-196.

Shereena, J. 2005. Effect of temperature on physiology of reserve mobilization in *Pisum sativum* L. seeds during germination. Ph.D. Thesis submitted to University of Calicut.

Shereena, J. and Nabeesa- Salim. 2006. Effect of temperature on protein profile of *Pisum sativum* L. seeds during germination. ***J. Biol. Sci***. **6:** 1148-1150.

Shewry, P.R., Napier, J.A. and Tatham, A.S. 1995. Seed storage proteins-structure and biosynthesis. ***Plant Cell 7:*** 945-956.

Shutov, A.P. and Vaintraub, J.A. 1987. Degradation of storage proteins in germinating seeds. ***Phytochemistry*** **26**: 1557-1566.

Simon, E.W. 1974. Phospholipids and plant membrane permeability**.** ***New Phytol***. **73**: 377-420.

Simon, E.W. 1978. Membranes in dry and imbibing seeds. *In*: J.H. Crowe and J.S. Clegg (Eds.) ***Dry Biological Systems***. Academic Press, New York. 205-244.

Simon, E.W. 1984. Early events in germination. *In:* D.R. Murray (Ed.) ***Seed Physiology Vol.2. Germination and Reserve Mobilization***. Academic Press, London. 77-115.

Simon, E.W. and Mathavan, S.O. 1986. The time-course of leakage from imbibing seeds of different species. ***Seed Sci. Technol.*** **14**: 9-13.

Simon, E.W. and Raja-Harun, R.M. 1972. Leakage during seed imbibition. ***J. Exp. Bot***. **23**: 1076-1085.

Simon, E.W. and Wiebe, H.H. 1975. Leakage during imbibition, resistance to damage at low temperature and the water content of peas. ***New Phytol***. **74**: 407-411.

Singh, K. and Afria, B.S. 1985. Evaluation of moisture stress tolerance in castor cultivars. ***Proc. Indian Nat. Sci. Acad***. (**Part B**). **51**: 211-217.

Slinkard, A.E., Basur, G. and Hernandez - Bravo, G. 1994. Biotic and abiotic stresses of cool season food legumes in the w*es*tern hemisphere. *In*: F.J. Muelbauer and W.J. Kaiser (Eds.) ***Expanding the Production and Use of Cool Season Food Legumes***. Kluwer Academic Publishers, Netherlands, 195-203.

Smallwood, M. and Bowles, D.J. 2002. Plants in a cold climate. ***J. Biol. Sci.* 357**: 831-847.

Smith, D.L. 1974. A histological and histochemical study of the cotyledons of *Phaseolus vulgaris*. L during germination. ***Protoplasma* 79:** 41-57.

Smith, D.L. and Flinn, A.M. 1967. Histology and histochemistry of the cotyledons of *Pisum arvense* L. during germination. ***Planta* 74**: 72-85.

Snedden, W.A., Arazi,T., Fromm, H. and Shelp, B.J. 1995. Calcium/ calmodulin activation of soybean glutamate decarboxylase. ***Plant Physiol***. **108**: 543-549.

Sosulski, F.W. and Holt, N.W. 1980. Amino acid composition and nitrogen to protein factors for grain legumes. ***Can. J. Plant Sci.* 60**: 1327- 1331.

Spears, J.F., Tekrony, D.M. and Egli, D.B. 1997. Temperature during seed

filling and soybean seed germination and vigour. ***Seed Sci. Technol***. **25:** 233-244.

Stupnikova, I., Benamer, A., Tolleter, D., Grelet, J. and Borovskii, G. 2006. Pea seed mitochondria are endowed with a remarkable tolerance to extreme physiological tempratures. ***Plant Physiol***. **140**: 326-335.

Sudhakar, C., Lakshmi, A. and Giridarakumar, S. 2001. Changes in the antioxidant enzyme efficacy in two high yielding genotypes of mulberry (*Morus alba* L.) under NaCl salinity. ***Plant Sci.*** **161**: 613-619.

Sun, W.Q., Irving, T.C. and Leopold, A.C. 1994. The role of sugar, vitrification and membrane phase transition in desiccation tolerance. ***Plant Physiol***. **90**: 621-628.

Sung, D.Y., Kaplan, F., Lee, K.J. and Guy, C.L. 2003. Acquired tolerance to temperature extremes. ***Trends Plant Sci.*** **8:** 179-187.

Swain, R.R. and Dekker, E.E. 1966. Seed germination studies. II Pathways for starch degradation in germinating pea seedlings. ***Biochem. Biophys. Acta***. **122:** 87-100.

Taiz, L. and Zeiger, E. 2002. Plant Physiology. 3rd Edn. Sinaur Associates, Inc. Sunderland.

Takayanagi, K. and Murakami, K. 1968. Rapid germinability test with

exudates from seeds. ***Nature*** **218**: 493.

Taylor, A.G. and Prusinski, J. 1990. The influence of seed moisture content and mechanical damage on germination, imbibitional chilling injury and leakage. ***Ann. Rep. Bean Improv. Cooper***. **33**: 143-144.

Taylor, A.G., Paine, D.H. and Paine C.A. 1993. Sinapine leakage from *Brassica* seeds. ***J. Am. Hort. Sci.*** **118-** 546-550.

Teixeira, J. Pereira, S. and Salema, R. 2003. Different GS isogenes play distinct roles in the response to salt stress: Differential mRNA and polypeptid accumulation in potato callus tissue. www.sefv.org/www.sefv.org/web/resumenes_aceptados/s1/125.pdf: 2003.

Tekrony, D.M. and Egli, D.B. 1997. Relationship between standard germination accelerated ageing, germination and field emergence in soybean. *In:* R.H. Ellis, A.J. Black, M. Murdoch and T.D. Hong (Eds.) ***Basic and Applied Aspects of Seed Biology***. Kluwer Academic Publishers, Boston. 593-600.

Thomas, P.B., Morris, E.C. and Auld, T.D. 2003. Interactive effects on heat shock and smoke on germination of nine species forming soil seed banks within the Sydney region. ***Aust. Ecol.*** **28**: 674-683.

Tombs, M.P. 1967. Protein bodies in the soybean. ***Plant Physiol.*** **42**:

797-813.

Torrent, M., Geli, M.I. and Ludevil, M.D. 1989. Storage protein hydrolysis and protein body breakdown in germinated *Zea mays* L. seeds. ***Planta*** 180: 90-95.

Trawatha, S.E., Tekrony, D.M. and Hiderbrand, D.F. 1995. Relationship of soybean seed quality to fatty acid and C_6-aldehyde levels during storage. ***Crop Sci. 35***: 1415-1422.

Tsang, E.W.T., Bowler, C., Herouart, D., van Camp. W., Villarroel, R., Genetello, C., van Montagu, M. and Inze. D. 1991. Differential regulation of superoxide dismutase in plants exposed to environmental stress. ***Plant Cell*** **3**: 783-792.

Tully, R.E. and Beevers, H. 1978. Proteases and peptidases of castor bean endosperm. Enzyme characterization and changes during germination. ***Plant Physiol***. **62**: 746-750.

Tully, R.E., Musgrave, M.E. and Leopold, A.C. 1981. The seed coat as a control of imbibitional chilling injury. ***Crop. Sci***. **21**: 312-317.

Umezurike, G.M. and Numfor, F.A. 1979. Changes in the content of starch and activities of some enzymes of carbohydrate metabolism in cotyledons of germinating seeds of *Voandzeia subterranea*. ***J. Exp. Bot***. **30:** 583-588.

Valhala, T., Eriksson, T. and Engstrom, P. 1990. Heat shock proteins in willow (*Salix viminalis*). ***Physiol. Plant.* 80**: 301-306.

van Huystee, R.B. 1987. Some molecular aspects of plant peroxidase biosynthetic studies. ***Annu. Rev. Plant Physiol***. **38**: 205-219.

van Rensburg, L., Krüger, G.H.J. and Krüger, H. 1993. Proline accumulation as drought-tolerance selection criterion: Its relationship to membrane integrity and chloroplast ultrastructure in *Nicotiana tabacum* L. ***J. Plant Physiol*. 141**: 188-194.

van Staden, J., Manning, J.C. and Kelly, K.M. 1989. Legume seeds- the structure: function equation. *In*: C.H. Stirton and J.L. Zarucchi, (Eds.) ***Advances in Legume Biology***. St. Louis: Missouri Botanical Garden. 417-450

Varner, J.E. and Schidlovsky, G. 1963. Intracellular distribution of proteins in pea cotyledons**.** ***Plant Physiol.* 38**: 139-144.

Vertucci, C.W. and Leopold, A.C. 1984. Bound water in soybean seed and its relation to respiration and imbibitional damage. ***Plant Physiol.* 75**: 114-117.

Vertucci, C.W. and Leopold, A.C. 1986. Physiological activities associated with hydration level in seeds. *In:* A.C. Leopold, (Ed.) ***Membranes, Metabolism and Dry Organisms***. Cornell University Press. Ithaca.

35-49.

Vertucci, C.W. and Leopold, A.C. 1987. Water binding in legume seeds. ***Plant Physiol***. 85: 224-231.

Vertucci, C.W., Ellenson, J.L. and Leopold, A.C. 1985. Chlorophyll fluorescence characteristics associated with hydration level in pea cotyledons. ***Plant Physiol.*** **79:** 248-252.

Vierling, E. 1991. The roles of heat shock proteins in plants**.** ***Annu. Rev. Plant Physiol. Plant Mol. Biol.*** **42**: 579-620.

Vierstra, R.D. 1987. Ubiquitin, a key component in the degradation of plant proteins. ***Physiol. Plant.*** **70**: 103-106.

Vierstra, R.D. 1993. Protein degradation in plants. ***Annu. Rev. Plant Physiol. Plant Mol. Biol*** .**44**: 385- 441.

Villiers, T.A. 1973. Ageing and the longevity of seeds in field conditions. In: W. Heydecker (Ed.) ***Seed Ecology.*** Butterworth and Co. Ltd. London. 265-288.

Waggoner, P. E. and Parlange, J. 1976. Water uptake and water diffusivity of seeds. ***Plant Physiol.*** **57**: 153-156.

Wang, T., Harp, T., Hammond, E.G., Burris, J.S. and Fehr, W.R. 2001. Seed physiological performance of soybeans with altered saturated

fatty acid contents. ***Seed Sci. Res***. **11**: 93-97.

Washitani. I. 1988. Effects of high temperatures on the permeability and germinability of the hard seeds of *Rhus javanica* L. ***Ann. Bot. 62***: 13-16.

Washitani, I. and Sacki, T. 1984. Leafy-canopy inhibition of germination as a mechanism for the disappearance of *Amaranthus patulus* Berttol in the second year of a secondary succession. ***Jpn. J. Ecol.*** **34**: 55-61.

Washitani, I. and Takenaka, T. 1986. "Safe sites" for the seed germination of *Rhus javanica*: a characterization by responses to temperature and light. ***Ecol. Res.***, **1:** 74-82.

Webster, B.D. and Leopold, A.C. 1977. The ultra-structure of dry and imbibed cotyledons of soybean. ***Am. J. Bot.*** **64**: 1268-1293.

Whelan, W.J. 1955. Starch, glycogen, fructosans and similar polysaccharides. *In*: K. Peach, and M.V. Tracey (Eds.) ***Modern Methods of Plant Analysis***. **Vol. 2**. Springer - Verlag. Berlin. 145-196.

Williams, R.J. and Leopold, A.C. 1989. The glassy state in corn embryos. ***Plant Physiol.*** **89**: 977-981.

Wilson, R.F. 1987. Seed metabolism. *In:* J.P. Wilcox and W. Madison (Eds.) ***Soybean: Improvement, Production and Uses.*** 2nd Edn. American

Society of Agronomy. 643-686.

Wise, R.R. and Naylor, A.W. 1987. Chilling-enhanced photo-oxidation. Evidence for the role of singlet oxygen and superoxide in the breakdown of pigments and endogenous antioxidants. ***Plant Physiol.*** **83**: 278-282.

Wisniewski, M., Close, T.J., Artlip, T. and Arora, R. 1996 Seasonal patterns of dehydrins and 70-kilodalton heat-shock proteins in bark tissues of eight species of woody plants. ***Physiol. Plant.*** **96**: 496-505.

Wolkers, W.F., Bochicchio, A., Selvaggi, G. and Hoekstra, E.A. 1998. Fourier transform infrared micro-spectroscopy detects changes in protein secondary structure associated with desiccation tolerance in developing maize embryos. ***Plant Physiol***. **116**: 1169-1177.

Wood, I.M. 1990. Response of seedlings of soybean, sunflower and sorghum to added mineral nutrients. ***Aust. J. Exp. Agri.*** **30:** 833-839.

Woodstock, L.W. and Pollock, B.M. 1965. Physiological pre-determination: Imbibition, respiration, and growth of Lima bean. ***Seed Sci.*** **150:** 1031-1032.

Woodstock, L.W. and Taylorson, R.B. 1981. Soaking injury and its reversal with polyethylene glycol in relation to respiratory metabolism in high and low vigour soybean seeds. ***Physiol. Plant.*** **53**: 263-268.

Woodstock, L.W., Furman, K. and Leffler, H.R. 1985. Relationship between weathering, deterioration and germination, respiratory metabolism and mineral leaching from cotton seeds. ***Crop sci. 25:*** 459-466.

Yaklich, R.W., Kulik, M.M. and Anderson, J.D. 1979. Evaluation of vigour tests in soybean seeds: Relationship of ATP, conductivity, and radioactive tracer multiple criteria tests to field performance. ***Crop Sci***. **19:** 806-810.

Zheng, X. and van Huystee, R.B. 1992. Anionic peroxidase catalysed ascorbic acid and IAA oxidation in the presence of hydrogen peroxide: A defence system against peroxidative stress in peanut plant. ***Phytochemistry*** **31:** 1895-1898.

Figure 1: Effect of Temperature on Germination Percentage in *Pisum sativum* and *Glycine max* Seeds

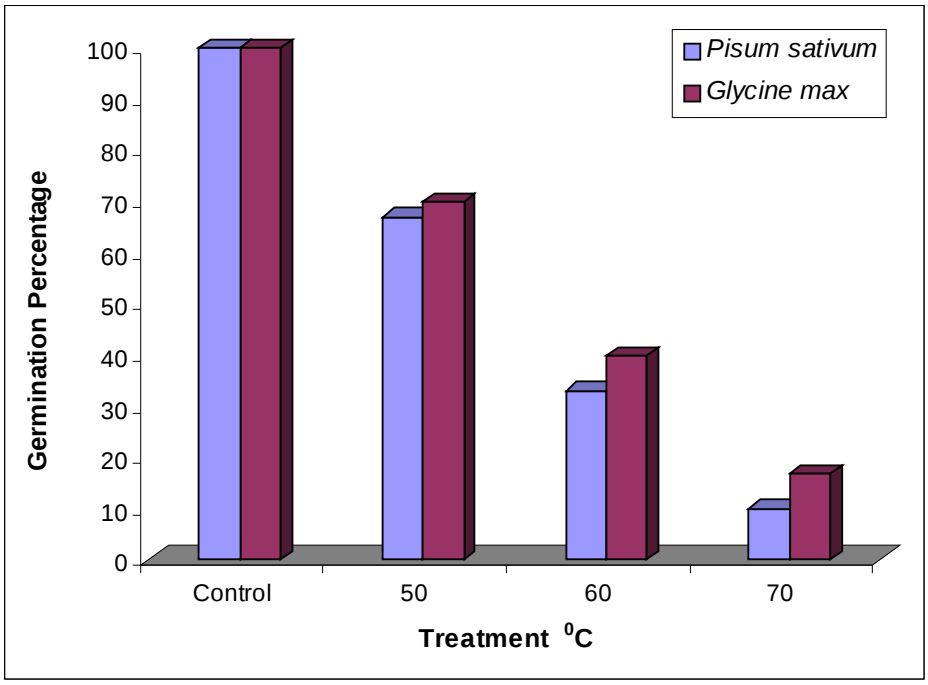


Figure 2: Effect of Temperature on Moisture Content Percentage in *Pisum sativum* and *Glycine max* Seeds

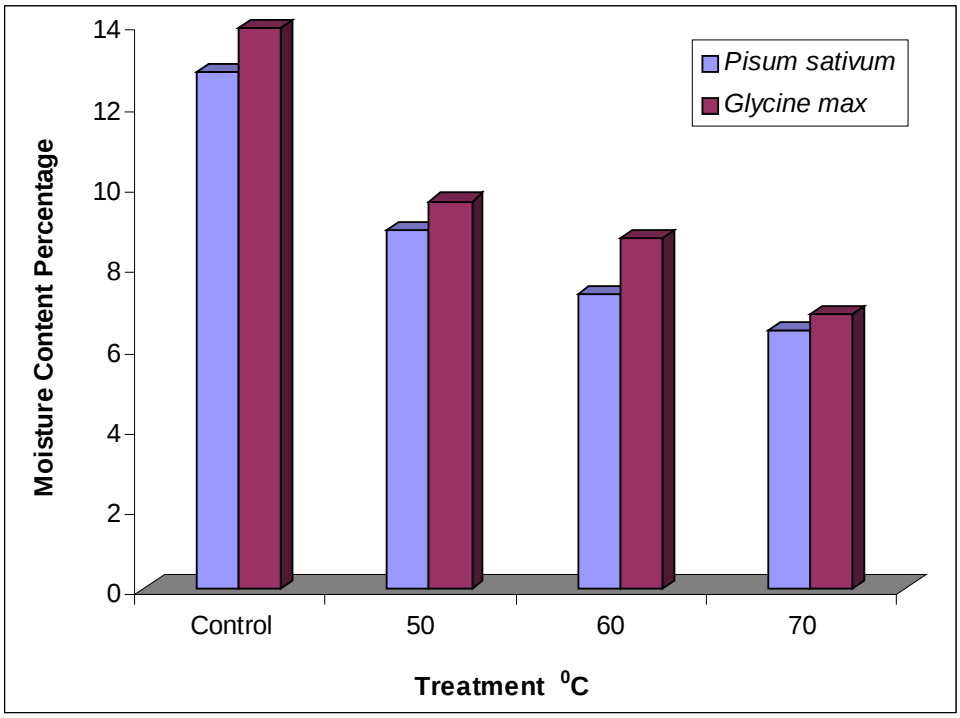


Figure 3: Effect of Temperature on Seed Vigour Index in *Pisum sativum* and *Glycine max* Seeds

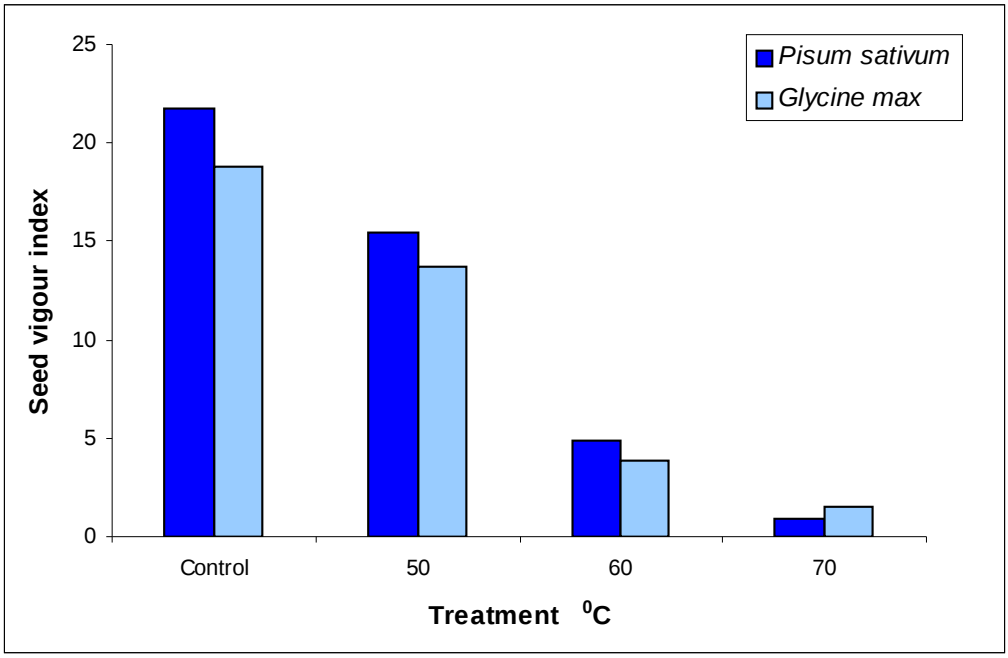


Figure 4: Effect of Temperature on Seedling Vigour in *Pisum sativum* and *Glycine max*

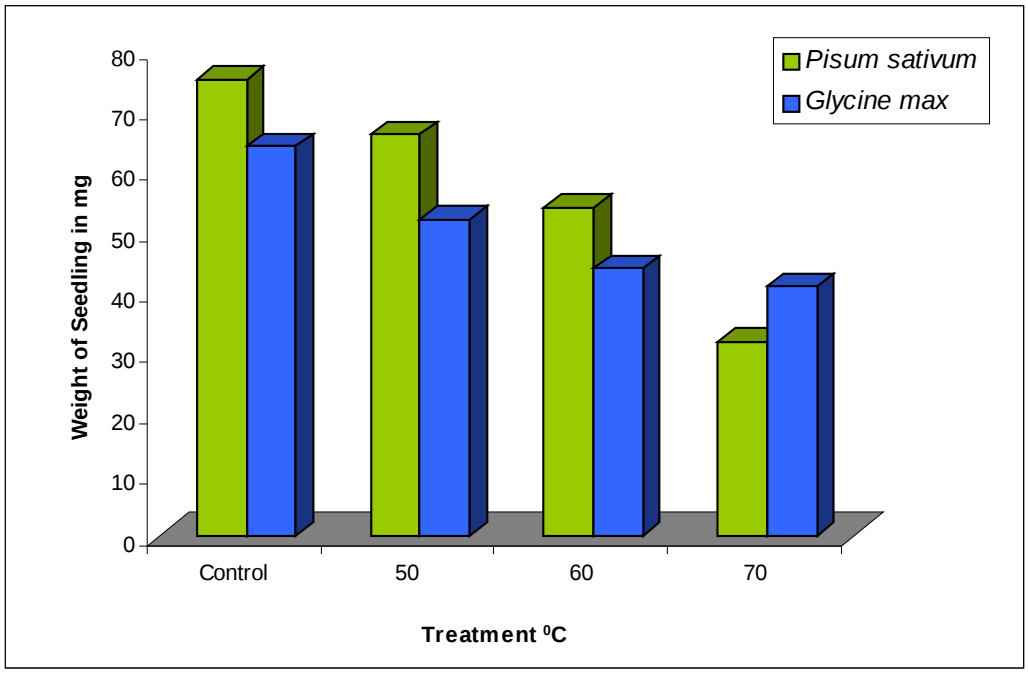


Figure 5: Effect of Temperature on Imbibition and Germination in *Pisum sativum* and *Glycine max* Seeds

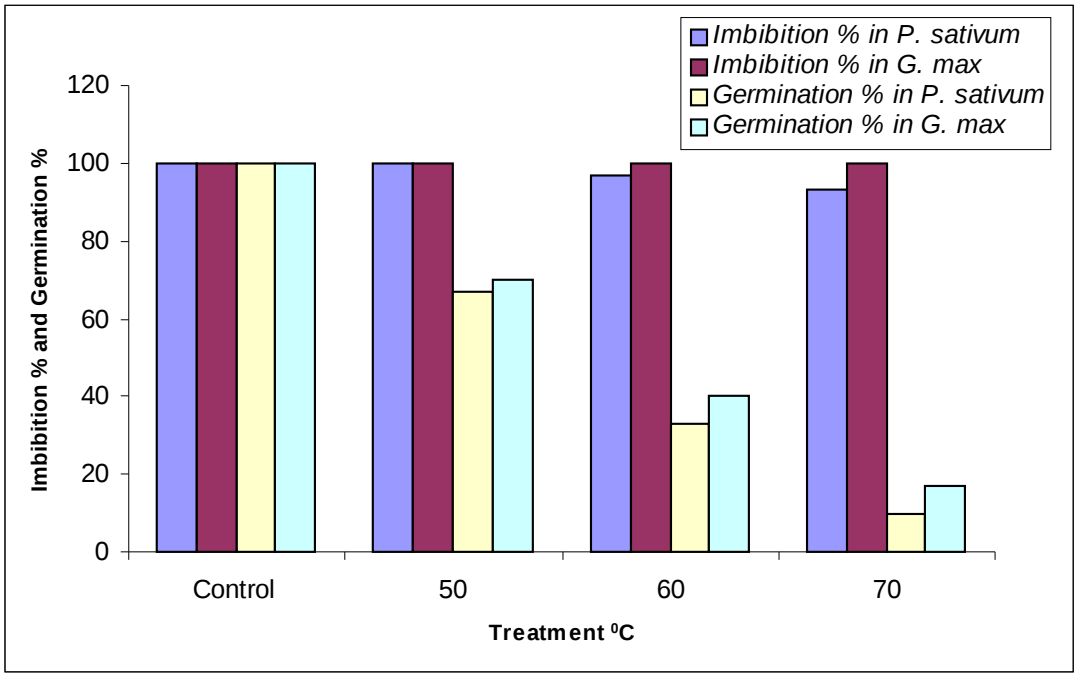


Figure 6: **Effect of Temperature on Potassium Ion (K^+) Concentration in the Leachate of *Pisum sativum* and *Glycine max* Seeds during Imbibition**

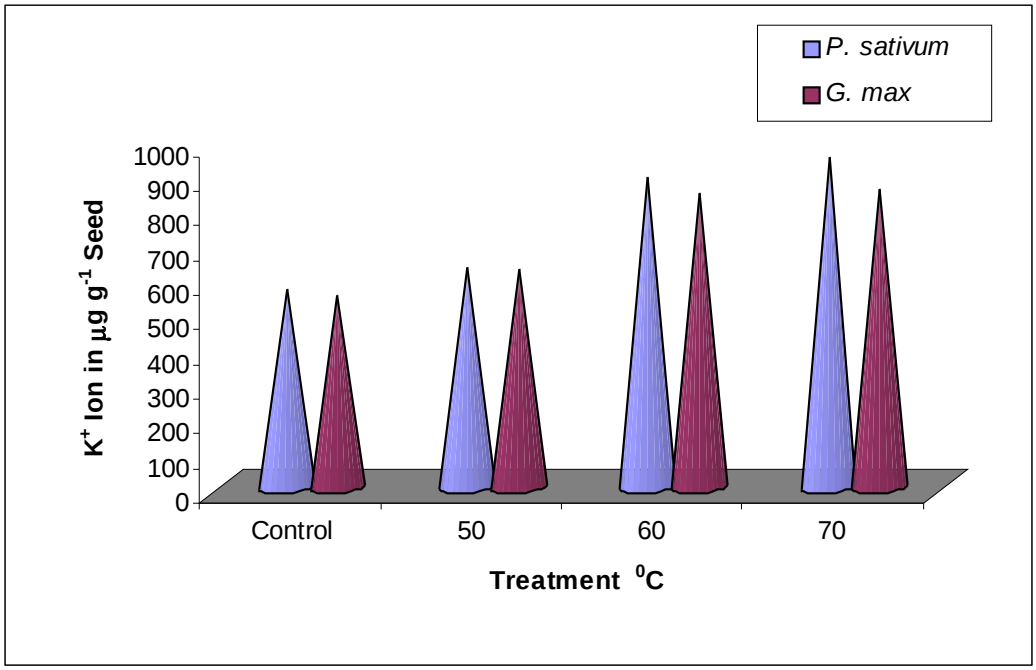


Figure 7: **Effect of Temperature on Calcium Ion (Ca^{++}) Concentration in the Leachate of *Pisum sativum* and *Glycine max* Seeds during Imbibition**

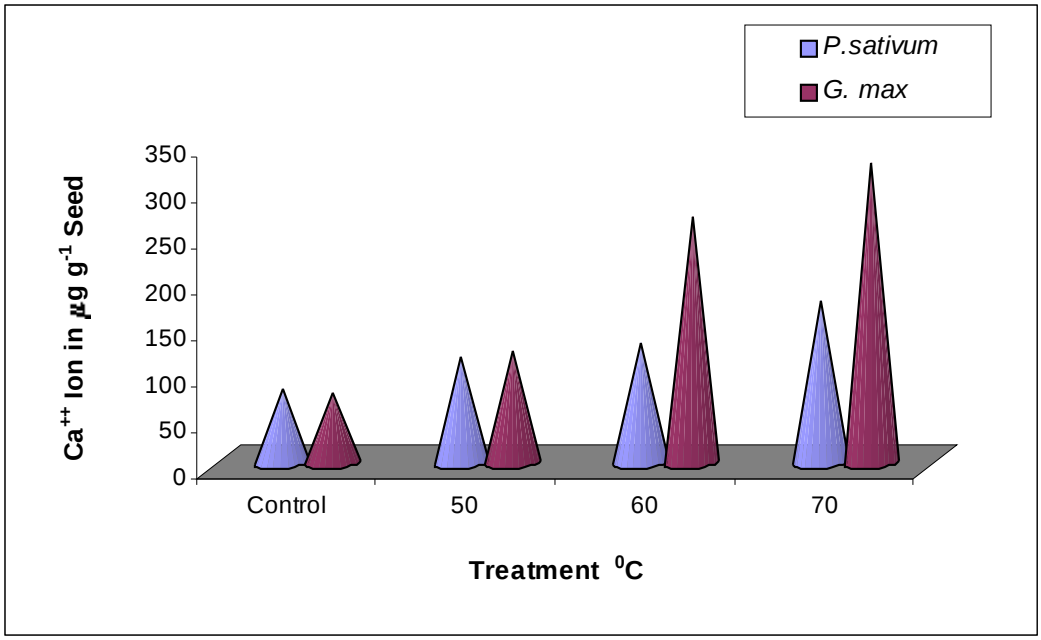


Figure 8: Effect of Temperature on Electrical Conductivity of the Leachate in *Pisum sativum* and *Glycine max* Seeds during Imbibition

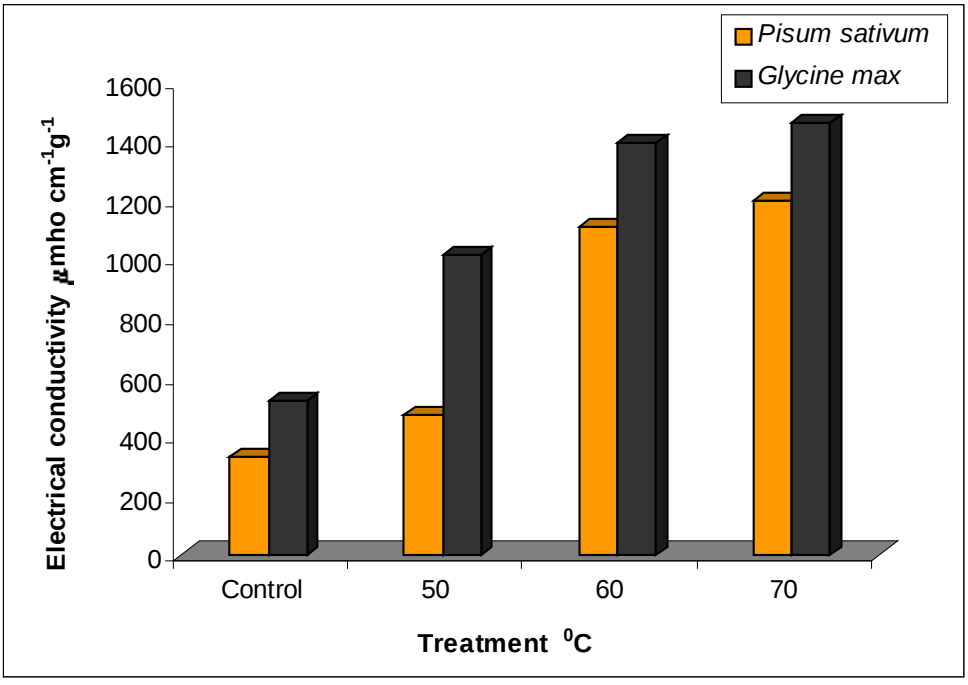


Figure 9: Effect of Temperature on Total Sugar in the Leachate of *Pisum sativum* and *Glycine max* Seeds during Imbibition

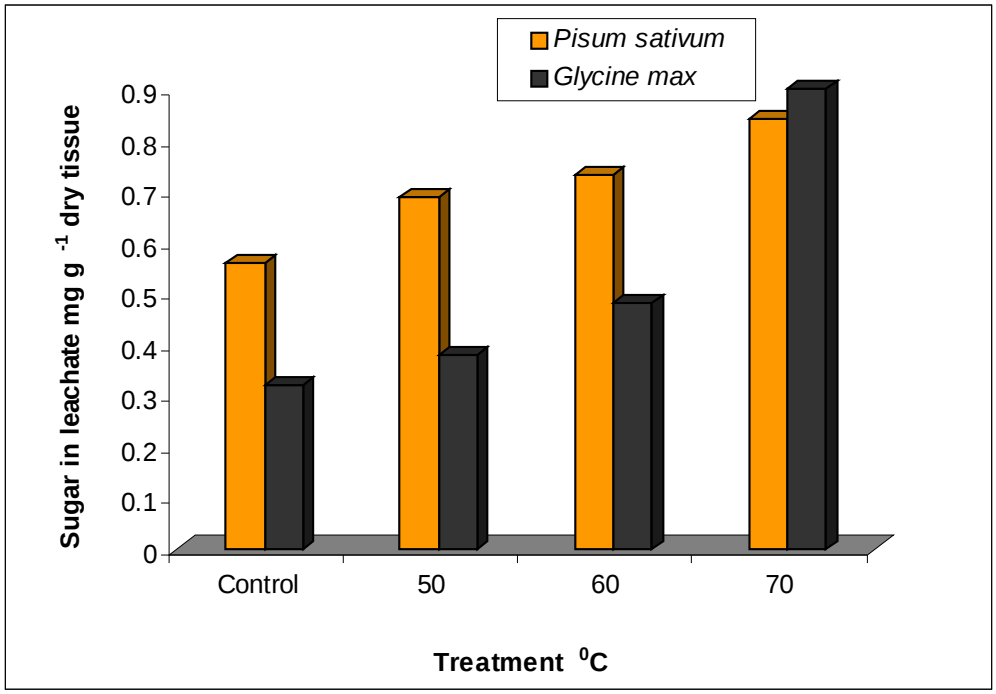
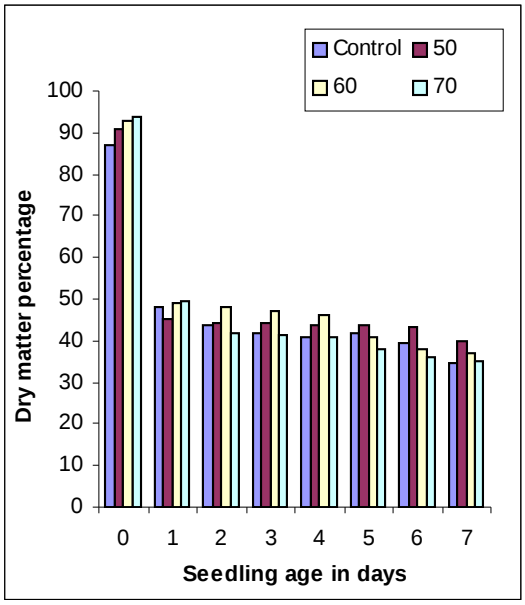
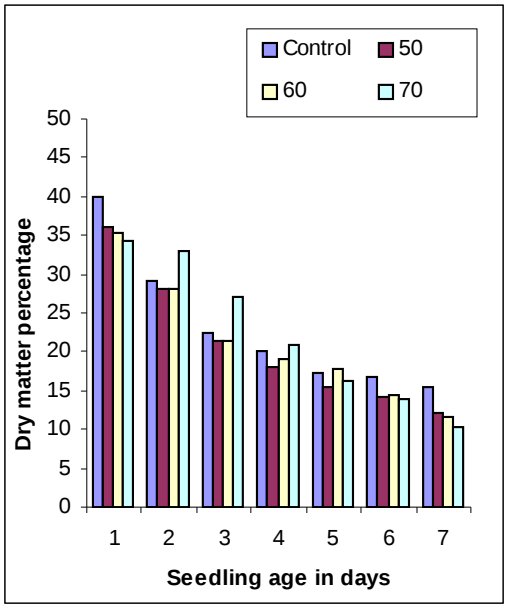


Figure 10: Effect of Temperature on the Dry Matter Distribution in the Seeds/Seedlings of *Pisum sativum* and *Glycine max*

A. *Pisum sativum* cotyledon



B. *Pisum sativum* axis



C. *Glycine max* cotyledon

D. *Glycine max* axis

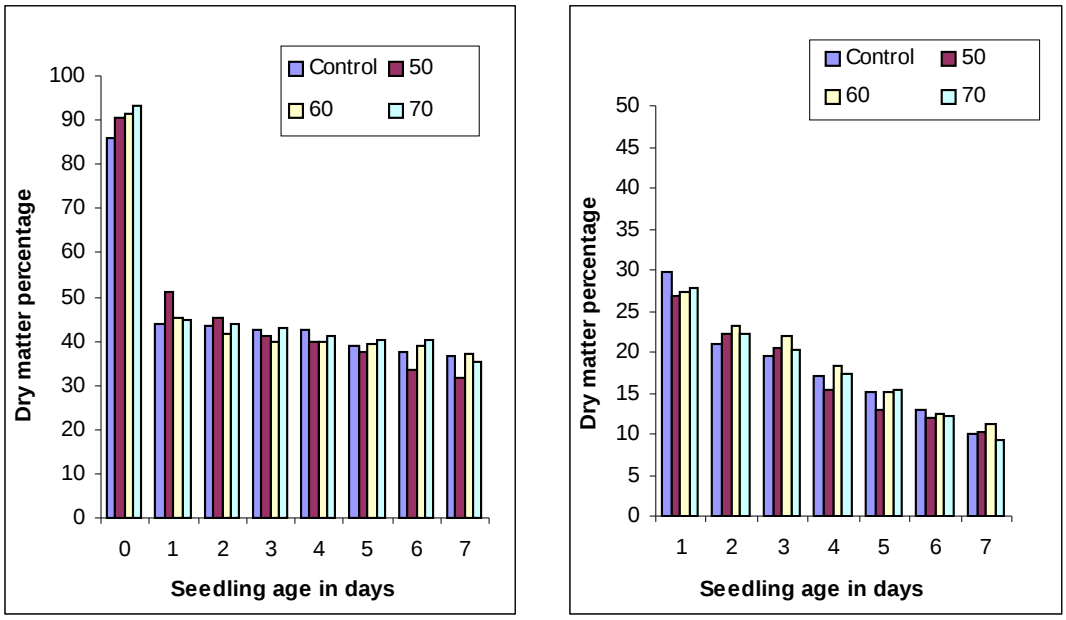
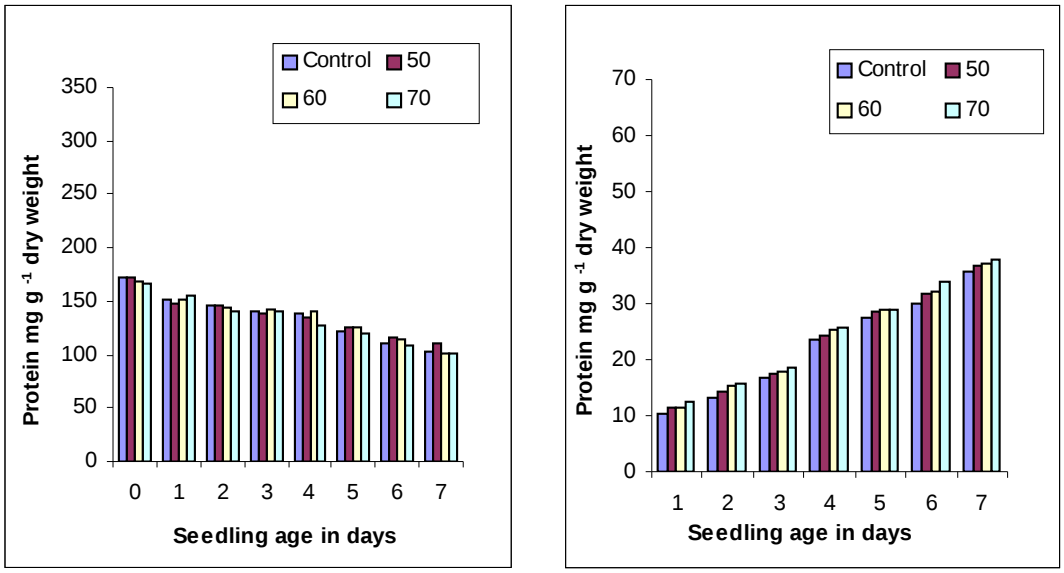


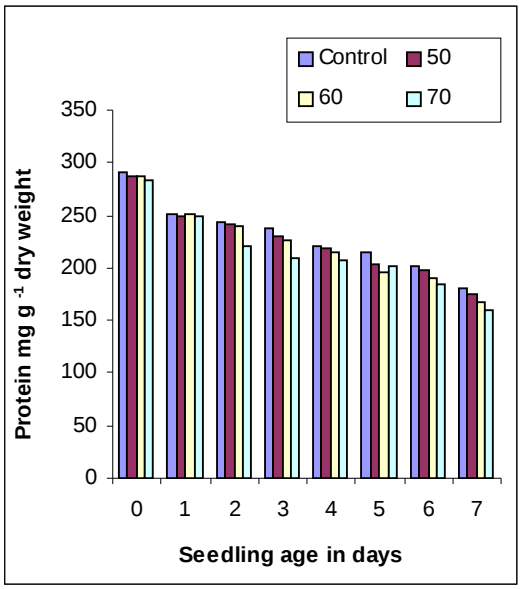
Figure 11: Effect of Temperature on Total Protein in the Seeds/ Seedlings of *Pisum sativum* and *Glycine max*

A. *Pisum sativum* cotyledon

B. *Pisum sativum* axis



C. *Glycine max* cotyledon



D. *Glycine max* axis

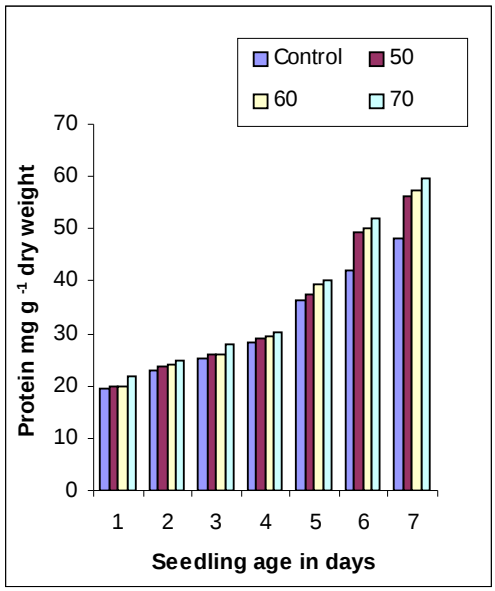


Figure 14A: Effect of Temperature on Guaiacol Peroxidase activity in the Seeds/ Seedlings of *Pisum sativum*

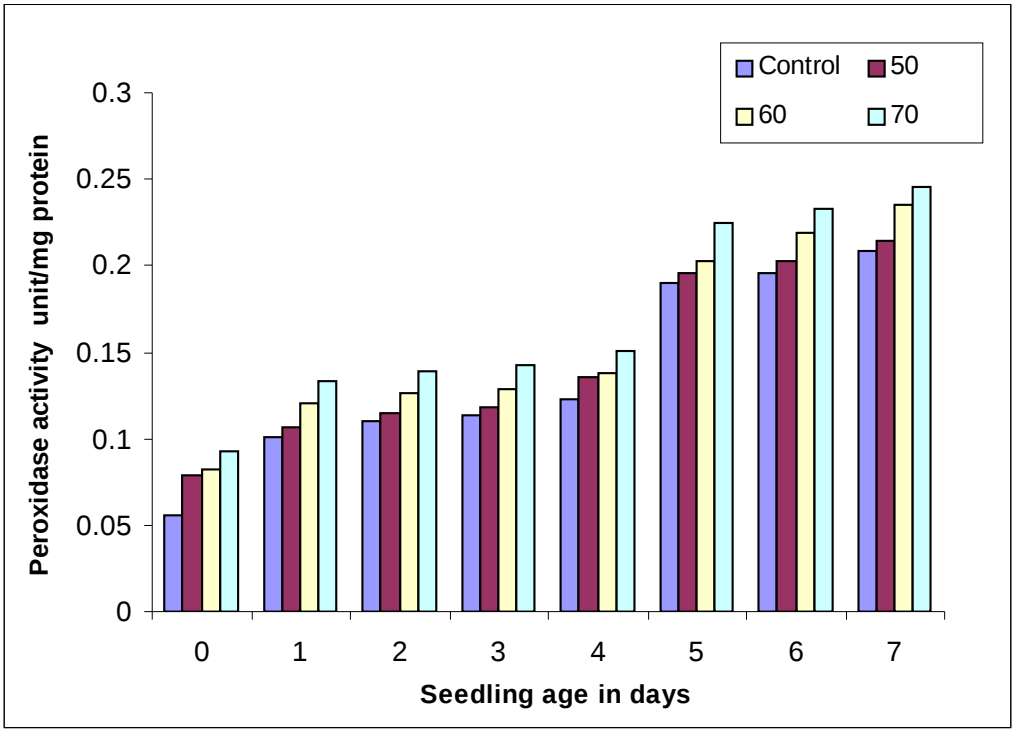


Figure 14B: Effect of Temperature on Guaiacol Peroxidase activity in the Seeds/ Seedlings of *Glycine max*

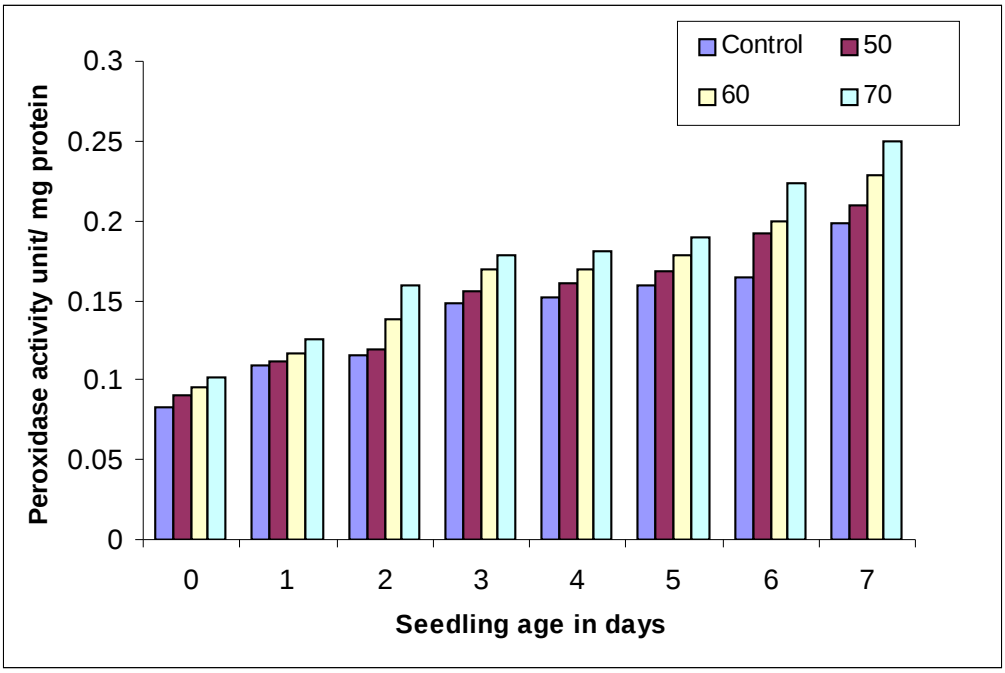


Figure 15A: Effect of Temperature on Total Free Amino Acids in the Seeds/ Seedlings of *Pisum sativum*

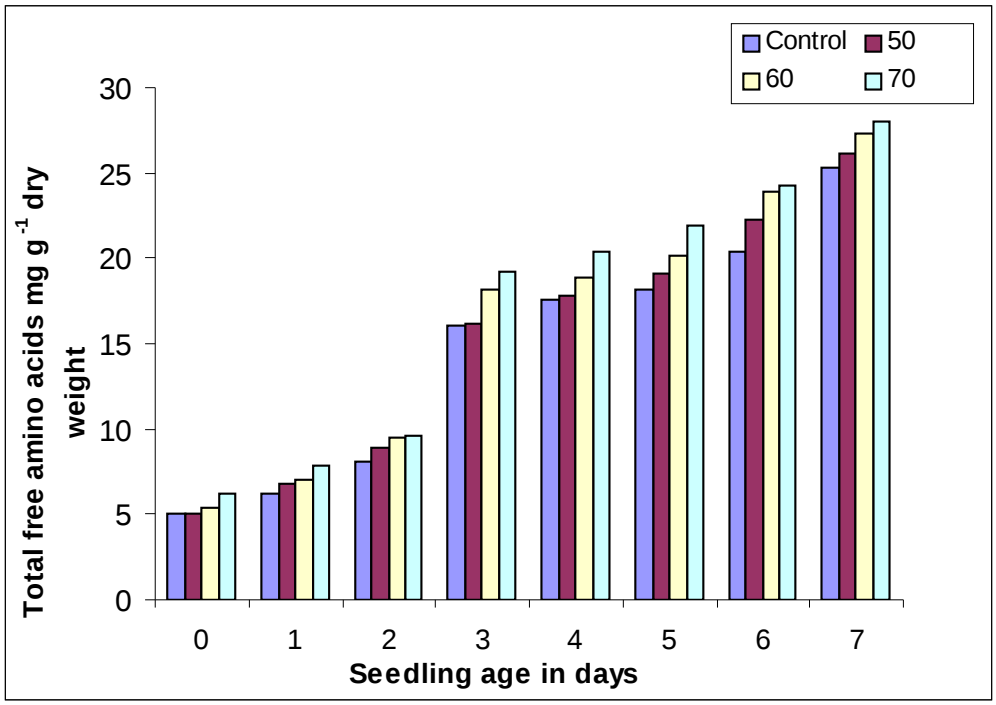


Figure 15B: Effect of Temperature on Total free Amino Acids in the Seeds/ Seedlings of *Glycine max*

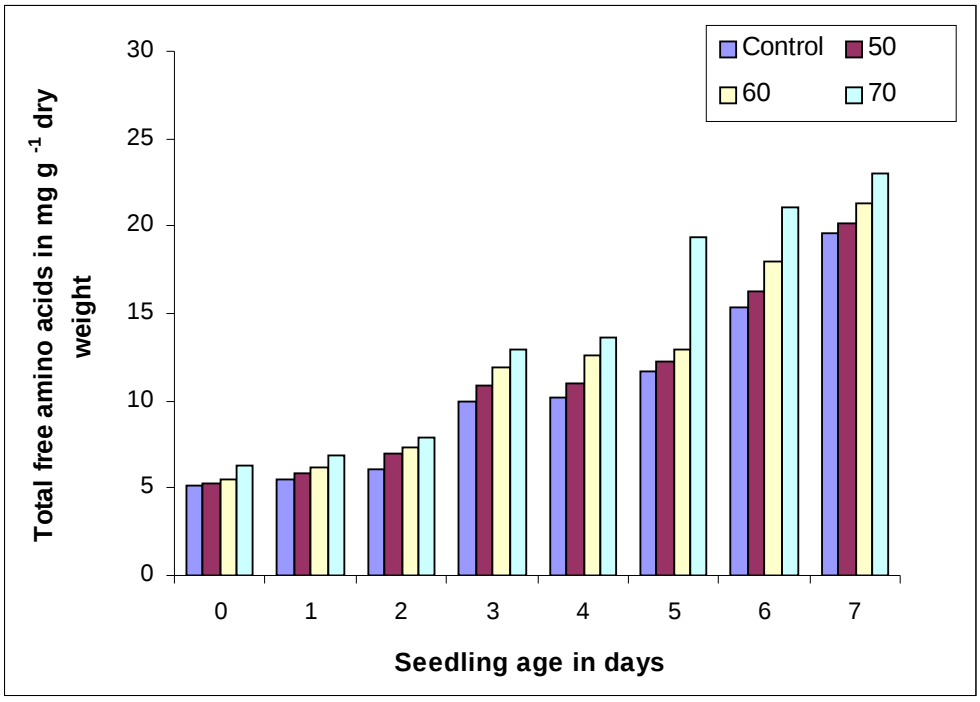
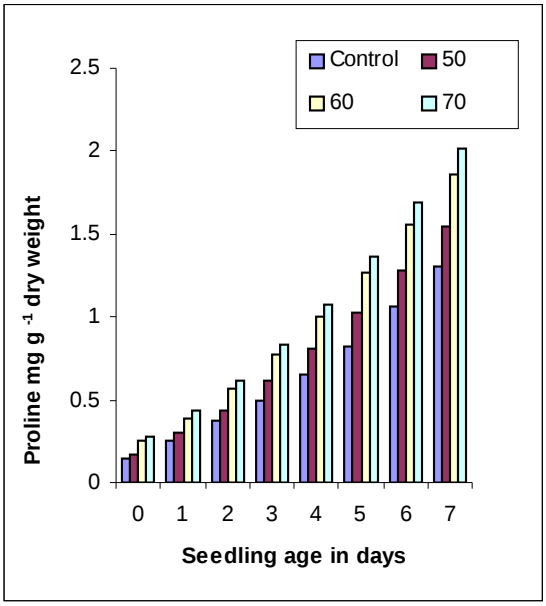
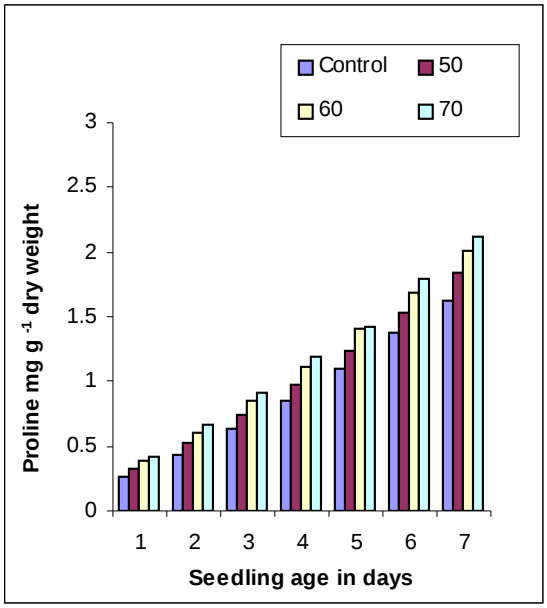


Figure 16: Effect of temperature on the Amount of Proline in the Seeds/ Seedlings of *Pisum sativum* and *Glycine max*

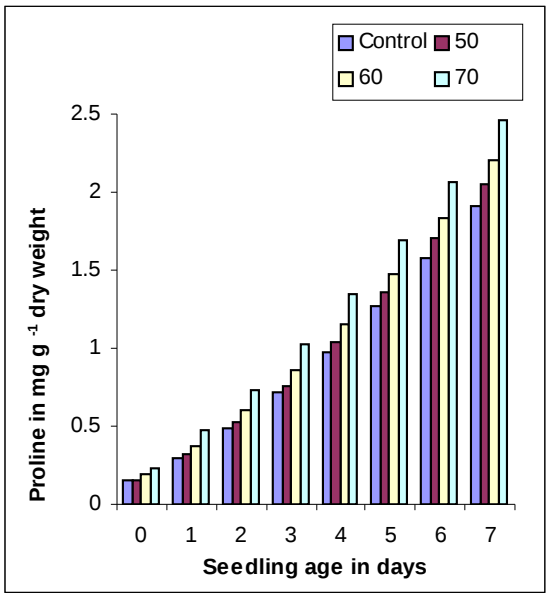
A. *Pisum sativum* cotyledon



B. *Pisum sativum* axis



C. *Glycine max* cotyledon



D. *Glycine max* axis

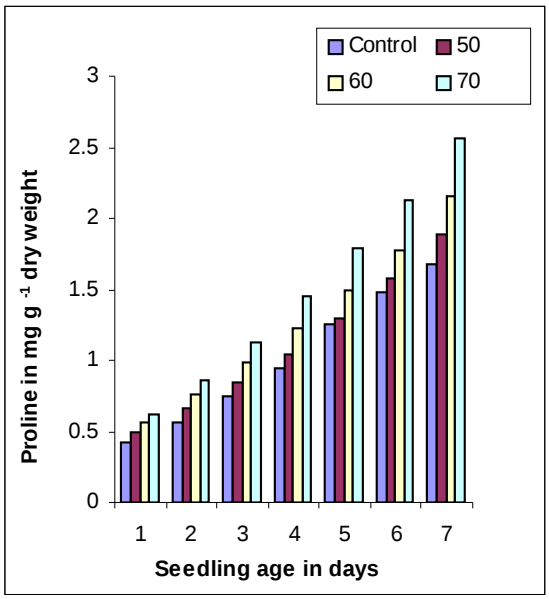
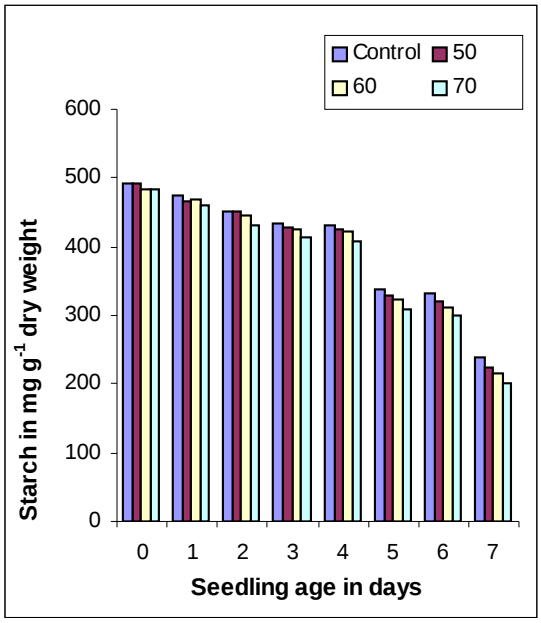
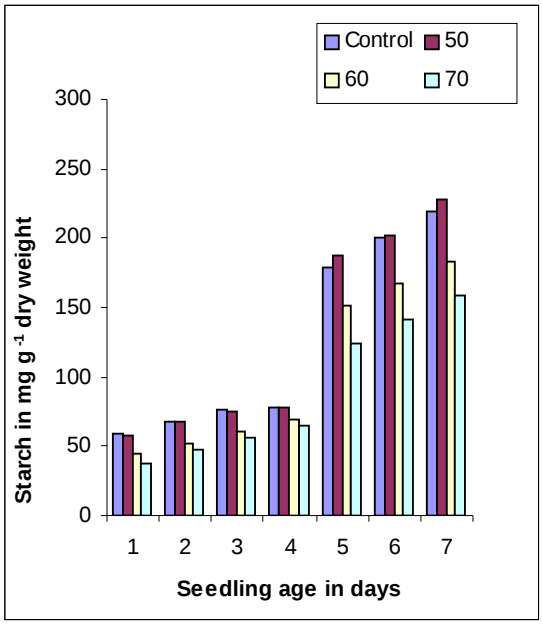


Figure 17 : Effect of Temperature on Starch in the Seeds/ Seedlings of *Pisum sativum* and *Glycine max*

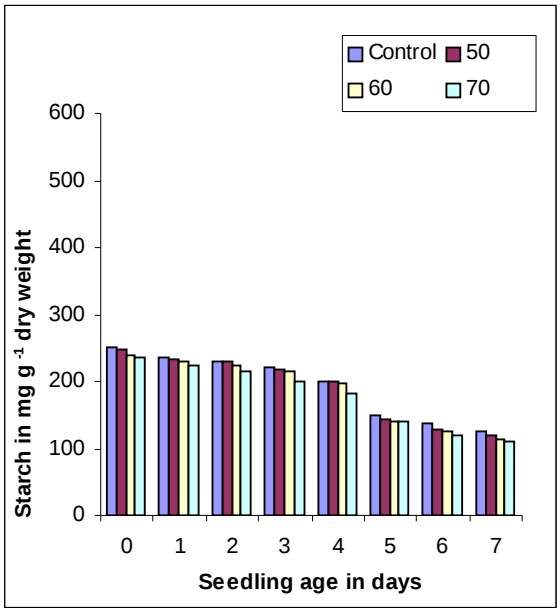
A. *Pisum sativum* cotyledon



B. *Pisum sativum* axis



C. *Glycine max* cotyledon



D. *Glycine max* axis

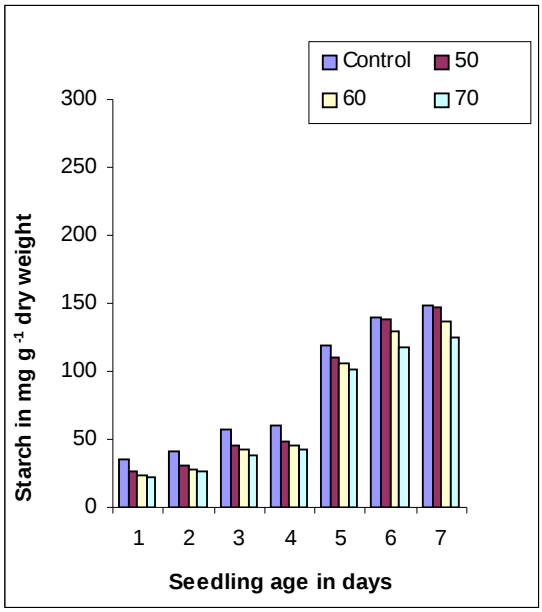


Figure 18A : Effect of Temperature on Total Soluble Sugar in the Seeds/ Seedlings of *Pisum sativum*

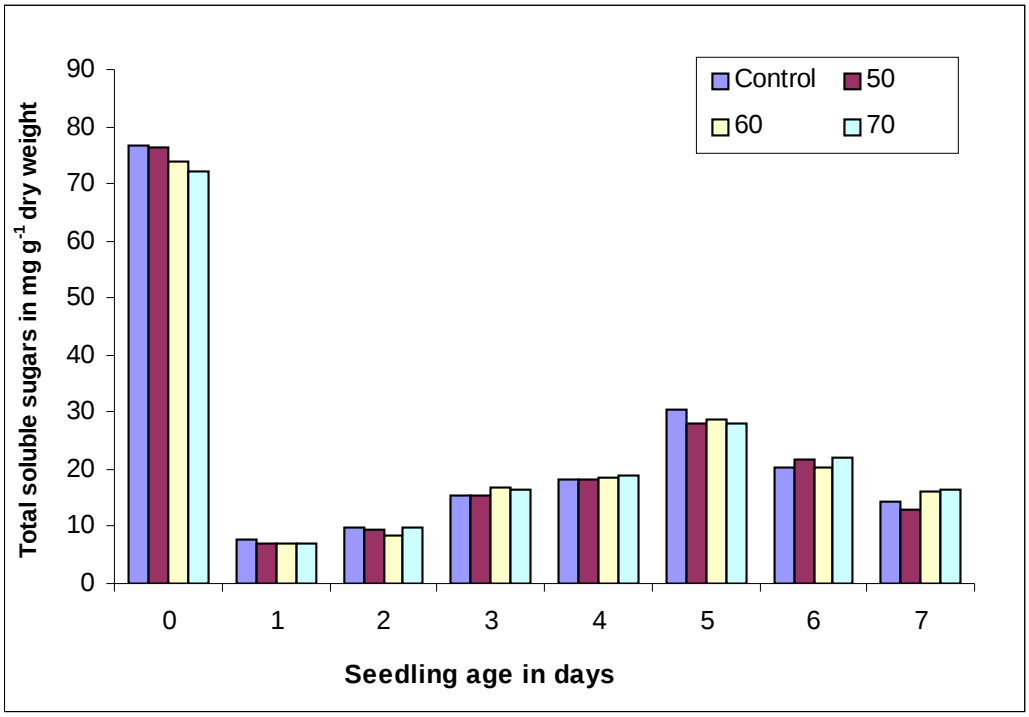


Figure 18B : Effect of Temperature on Total Soluble Sugar in the Seeds/ Seedlings of *Glycine max*

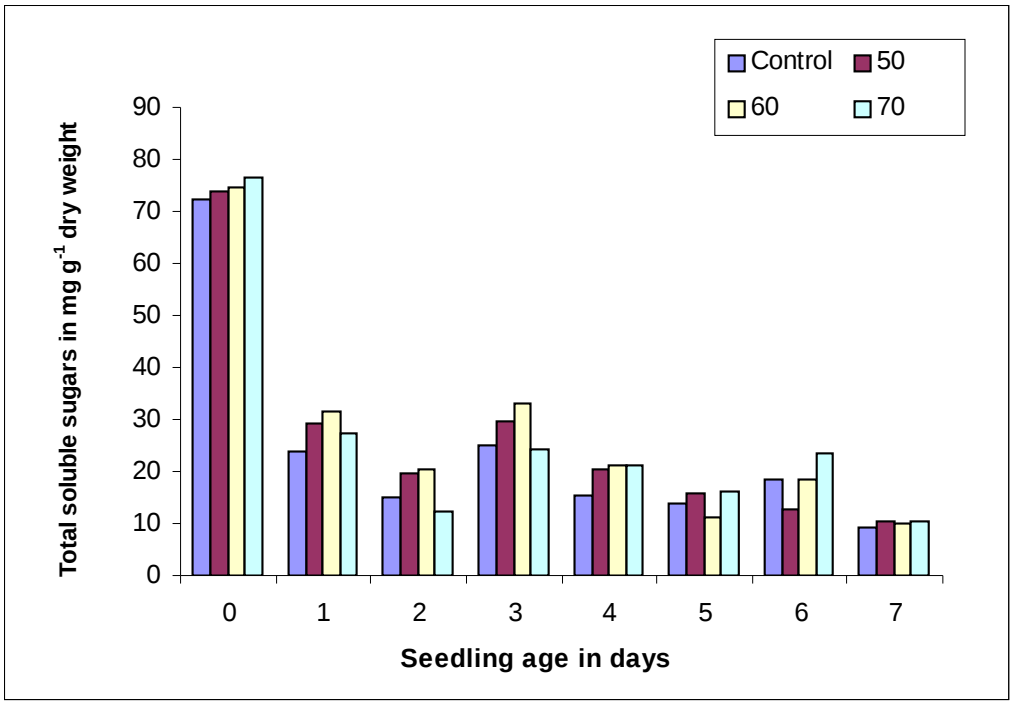


Figure 19A : Effect of Temperature on the Raffinose family of Oligosaccharides in *P. sativum* Seeds/ Seedlings

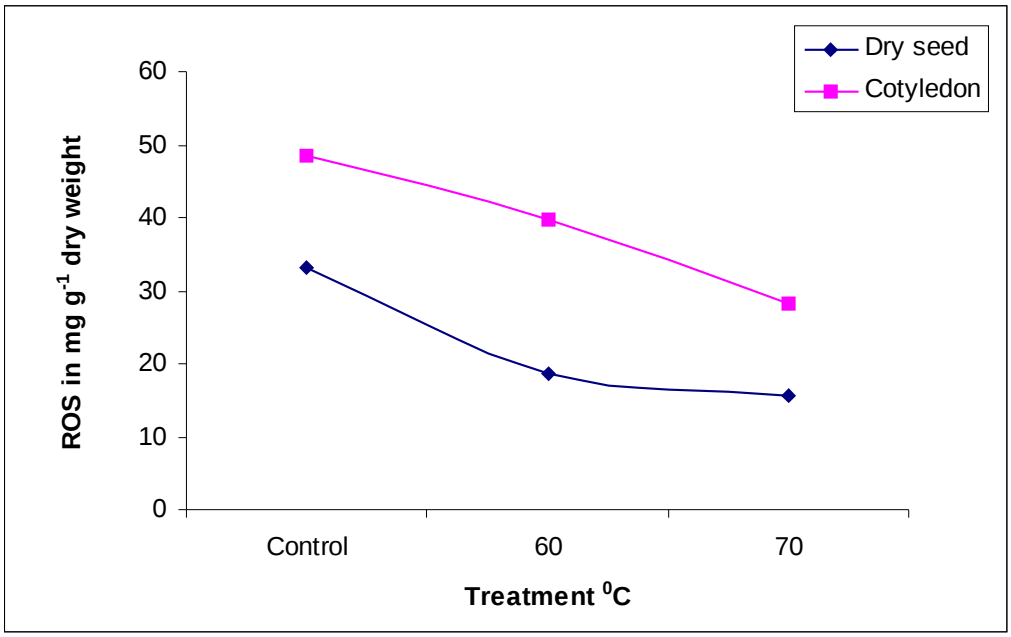


Figure 19B : Effect of Temperature on the Raffinose family of Oligosaccharides in *G. max* Seeds/ Seedlings

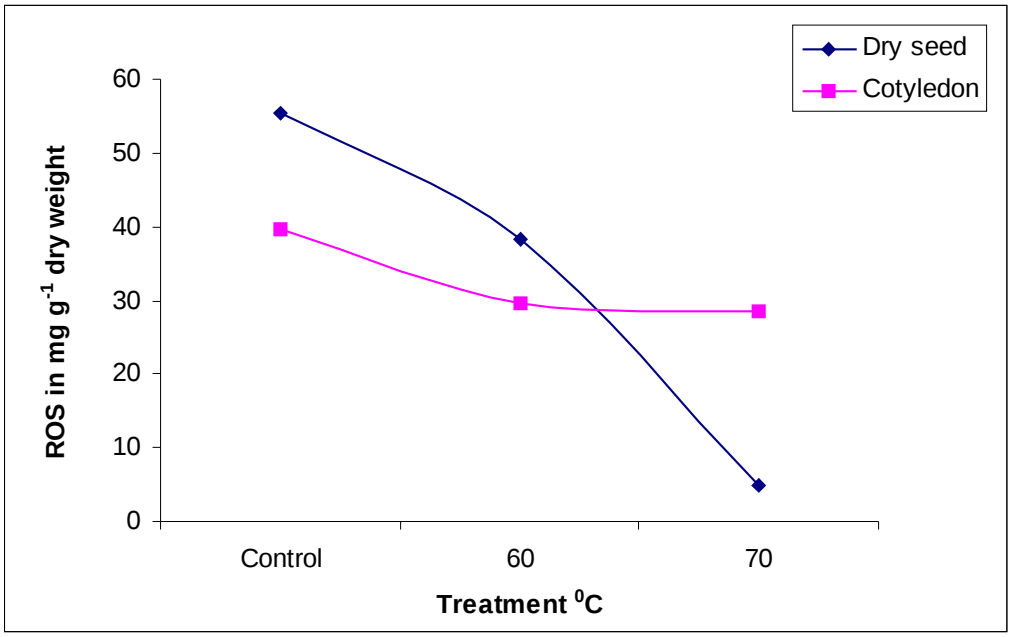


Figure 22A : Effect of Temperature on the Amount of Lipid in the Seeds/Seedlings of *Pisum sativum*

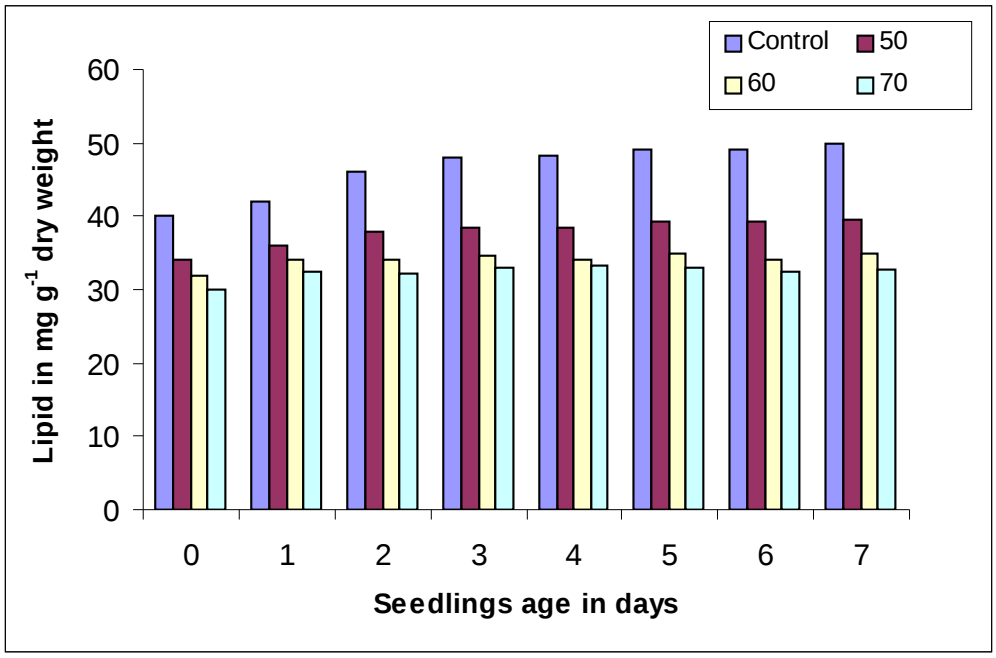


Figure 22B : Effect of Temperature on the Amount of Lipid in the Seeds/Seedlings of *Glycine max*

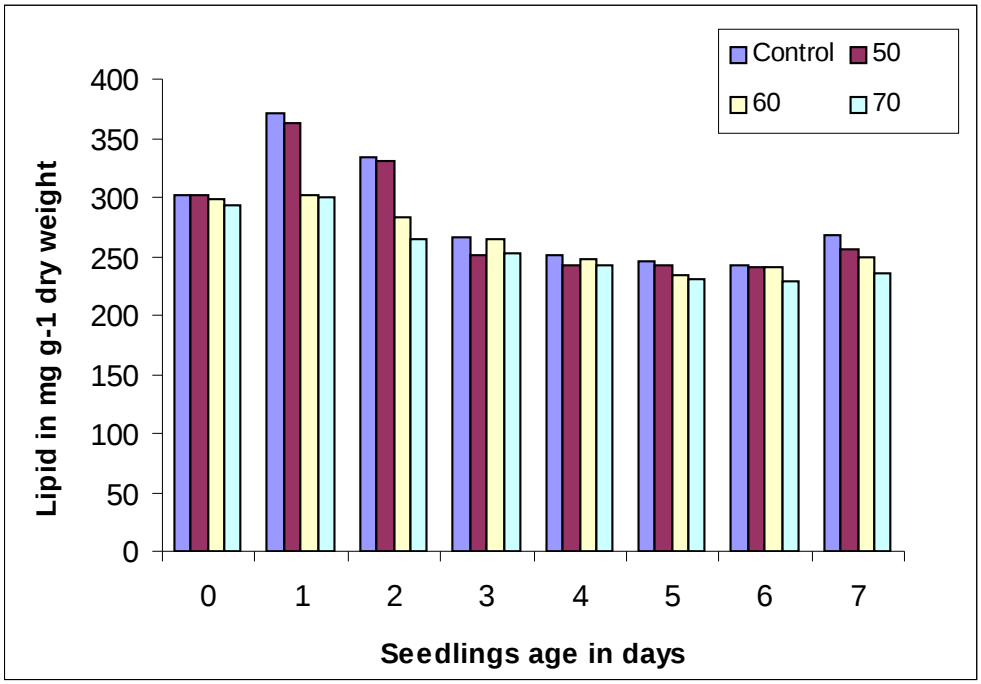


Table 1: Effect of Temperature on Germination and Moisture Content in *Pisum sativum* and *Glycine max* Seeds

Seed Samples	Treatment	Germination Percentage	Moisture Content Percentage
Pisum sativum	Control	100 ± 0	12.8 ± 1.68
	50^0C	67 ± 1.27	8.9 ± 1.64
	60^0C	33 ± 1.51	7.3 ± 1.48
	70^0C	10.0 ± 0.91	6.4 ± 1.12
Glycine max	Control	100 ± 0	13.9 ± 1.16
	50^0C	70 ± 1.24	9.6 ± 1.68
	60^0C	40 ± 1.16	8.7 ± 1.46
	70^0C	17 ± 0.92	6.8 ± 0.95

Table 2: Effect of Temperature on Seed Vigour Index in *Pisum sativum* and *Glycine max* Seeds

Seed Samples	Treatment	Days of Germination					Seed Vigour Index
		1	**2**	**3**	**4**	**5**	
		Daily count of germinated seeds					
Pisum sativum	Control	15 ± 0.90	10 ± 0.96	5 ± 1.80	0	0	21.7 ± 0.99
	50^0C	12 ± 0.78	5 ± 0.89	2 ± 0.32	1 ± 0.62	0	15.4 ± 0.88
	60^0C	2 ± 0.66	3 ± 0.96	2 ± 0.09	2 ± 0.43	1 ± 0.01	4.90 ± 0.92
	70^0C	0	0	2 ± 0.80	0	1 ± 0.01	0.91 ± 0.61
Glycine max	Control	14 ± 1.81	7 ± 0.84	4 ± 0.69	5 ± 0.73	0	20.05 ± 1.52
	50^0C	8 ± 0.89	5 ± 0.68	5 ± 0.56	2 ± 0.68	1 ± 0.01	12.87 ± 1.12
	60^0C	0	3 ± 0.76	4 ± 0.62	3 ± 0.62	2 ± 0.64	3.95 ± 0.94
	70^0C	0	0	3 ± 0.79	2 ± 0.73	0	1.50 ± 0.61

Table 3: Effect of Temperature on Seedling Vigour in *Pisum sativum* and *Glycine max*

Treatment	Dry weight/ seedling in mg	
	Pisum sativum	***Glycine max***
Control	75 ± 5	64 ± 5
50^0C	66 ± 3	52 ± 5
60^0C	54 ± 2	44 ± 3
70^0C	32 ± 4	41 ± 4

Table 4: Effect of Temperature on Imbibition and Germination in *Pisum sativum* and *Glycine max* Seeds

Seed Samples	Treatment	Imbibition %	Germination %	Hard Seed %	Non Viable Seed %
Pisum sativum	Control	100 ± 0	100 ± 0	0	0
	50^0C	100 ± 0	67 ±1.20	0	33 ± 1.12
	60^0C	96.7 ±1.43	33 ± 1.51	3.3 ± 0.16	63.7 ± 1.18
	70^0C	93.3 ± 1.59	10 ± 0.91	6.6 ± 0.17	83.4 ± 1.22
Glycine max	Control	100 ± 0	100 ± 0	0	0
	50^0C	100 ± 0	70 ± 1.24	0	30 ± 1.29
	60^0C	100 ± 0	40 ± 1.16	0	60 ± 1.18
	70^0C	100 ± 0	17 ±1.32	0	83 ± 1.61

Table 5: Effect of Temperature on Mineral Ion Concentration in the Leachate of *Pisum sativum* and *Glycine max* Seeds during Imbibition

Seed Samples	Interval (in hours)	Mineral Ions	Weight of Minerals in mg g^{-1} dry weight			
			Treatment			
			Control	50^0C	60^0C	70^0C
Pisum sativum	12	K^+	570 ± 2.26	630 ± 4.73	890 ± 3.68	950 ± 11.5
		Ca^{++}	80 ± 1.98	115 ± 3.79	130 ± 2.67	175 ± 3.59
Glycine max	12	K^+	550 ± 2.53	625 ± 4.30	845 ± 6.94	855 ± 5.98
		Ca^{++}	75 ± 1.79	120 ± 2.87	265 ± 5.40	325 ±4.82

Table 6: Effect of Temperature on Electrical Conductivity of the Leachate in *Pisum sativum* and *Glycine max* Seeds during Imbibition

Seed Samples	Interval (in hours)	Electrical conductivity [m mhos cm^{-1} g^{-1}]			
		Treatment			
		Control	50^0C	60^0C	70^0C
Pisum sativum	12	331.2 ± 4.20	473.2 ± 6.64	1108.0 ± 12.96	1201.4 ± 12.94
Glycine max	12	526.4 ± 6.61	1017.6 ± 9.30	1393.4 ± 19.79	1463.7 ± 12.71

Table 7: Effect of Temperature on Total Sugar in the Leachate of *Pisum sativum* and *Glycine max* Seeds during Imbibition

Seed Samples	Interval (in hours)	Total Sugar mg g^{-1} dry tissue			
		Treatment			
		Control	50^0C	60^0C	70^0C
Pisum sativum	12	0.56 ± 0.04	0.69 ± 0.01	0.73 ± 0.06	0.84 ± 0.03
Glycine max	12	0.32 ± 0.03	0.38 ± 0.02	0.48 ± 0.03	0.90 ± 0.03

Table 8: Effect of Temperature on the Percentage of Dry Matter Distribution in the Seeds/Seedlings of *Pisum sativum* and *Glycine max*

Seed Samples	Treatment	Tissue	Seedling age (in days)							
			0	1	2	3	4	5	6	7
Pisum sativum	Control	Axis	87.2 ± 2.54	39.89 ± 1.93	29.10± 1.94	22.43 ± 1.46	19.99 ± 1.28	17.38 ± 2.94	16.75 ± 1.32	15.36 ± 1.19
		Cotyledon		48.30 ±1.32	43.85 ± 1.86	41.92 ± 2.65	41.10 ± 2.04	41.69 ± 3.09	39.36 ± 2.02	34.65 ± 2.71
	50^0C	Axis	91.1 ± 2.42	36.19 ± 1.93	28.22 ±1.20	21.36 ± 2.58	17.98 ± 1.89	15.56 ± 2.50	14.19 ± 1.39	12.14 ± 1.42
		Cotyledon		45.42 ± 1.16	44.44 ± 1.30	44.07 ± 2.54	43.85 ± 2.04	43.60 ±2.50	43.03 ±2.83	40.02 ± 3.13
	60^0C	Axis	92.7 ± 2.43	35.31 ± 1.21	28.12 ± 1.56	21.36 ± 1.32	18.99 ± 1.64	17.90 ± 2.49	14.34 ± 1.76	11.53 ± 1.28
		Cotyledon		49.18 ± 1.15	48.08 ± 1.42	47.11 ± 2.56	46.25 ± 2.61	40.92 ± 2.10	38.20 ± 2.83	37.23 ± 2.77
	70^0C	Axis	93.6 ± 2.38	34.16 ± 1.74	32.93 ± 2.38	27.15 ±1.84	20.93 ± 1.46	16.17 ± 1.28	13.88 ± 1.89	10.32 ± 1.96
		Cotyledon		49.32 ± 1.72	41.74 ± 2.07	41.51 ± 2.03	40.94 ± 2.16	37.79 ± 2.74	35.89 ± 2.08	35.21 ± 2.06
Glycine max	Control	Axis	86.1± 2.29	29.73 ± 1 .59	21.00 ± 2.48	19.58 ± 1.73	17.19 ± 1.24	15.12 ± 1.83	13.02 ± 1.97	9.92 ± 1.53
		Cotyledon		44.07 ± 1.56	43.33 ± 2.38	42.65 ± 2.80	42.58 ± 2.07	38.86 ± 1.28	37.65 ± 1.19	36.65 ± 2.47
	50^0C	Axis	90.4 ± 2.37	26.72 ± 1.28	22.18 ± 1.97	20.46 ± 1.91	15.38 ± 1.23	13.01 ± 1.31	11.95 ± 1.24	10.14 ± 1.37
		Cotyledon		50.91 ± 1.83	45.44 ± 1.96	41.26 ± 2.29	39.72 ± 2.34	37.36 ± 1.32	33.4 ± 2.21	31.84 ± 1.27
	60^0C	Axis	91.3 ± 2.21	27.32 ± 1.76	23.18 ± 1.96	21.89 ± 1.82	18.41 ± 1.71	15.08 ± 1.38	12.40 ± 1.50	11.23 ± 1.28

		Cotyledon		45.20 ± 1.97	41.57 ± 1.31	39.93 ± 1.97	39.85 ± 2.17	39.47 ± 1.07	38.74 ± 2.19	37.06 ± 2.49
	70^0C	Axis	93.2 ± 2.33	27.88 ± 1.79	22.17 ± 1.74	20.32 ± 1.39	17.30 ± 1.79	15.26 ± 1.96	12.19 ± 1.57	9.15 ± 1.41
		Cotyledon		44.59 ± 1.97	43.96 ± 1.80	43.09 ± 1.63	41.33 ± 2.52	40.34 ± 2.09	40.27 ±3.09	35.26 ± 2.71

Table 9: Effect of Temperature on Total Protein in the Seeds/Seedlings of *Pisum sativum* and *Glycine max* (mg g^{-1} dry weight)

Seed Samples	Treatment	Tissue	Seedling age (in days)							
			0	1	2	3	4	5	6	7
Pisum sativum	Control	Axis	172.50±3.40	10.21±1.13	13.28±1.11	16.91±1.09	23.54±1.08	27.61±1.03	30.02±1.14	35.63±1.11
		Cotyledon		150.82±4.32	145.90±3.26	140.38±3.22	138.21±4.19	121.50±2.26	110.37±2.18	103.26±2.15
	50^0C	Axis	171.40±3.32	11.46±1.03	14.15±1.01	17.46±1.51	24.41±1.31	28.75±1.28	31.63±1.13	36.94±1.16
		Cotyledon		148.75±3.36	146.82±2.21	138.27±2.15	135.49±2.27	126.32±2.18	115.28±2.29	111.31±2.36
	60^0C	Axis	168.97±3.21	11.58±1.11	15.23±1.57	18.01± 1.49	25.27±1.37	28.96±1.13	32.26±1.09	37.04±1.61
		Cotyledon		152.16±2.24	143.48±2.32	142.15±2.16	140.28±2.19	125.37±2.84	113.36±2.37	101.30±2.91
	70^0C	Axis	166.92±3.34	12.40±1.14	15.56±1.19	18.68±1.21	25.74±1.09	29.10±1.64	33.85±1.43	37.76±1.76
		Cotyledon		156.26±4.23	140.23±4.28	139.76±.3.75	128.17±4.29	120.49±2.85	109.23±2.99	100.50±2.76
Glycine max	Control	Axis	290.71±3.54	19.65±1.77	22.84±1.63	25.40±1.62	28.32±1.15	36.36±1.13	41.90±1.22	48.31±1.36
		Cotyledon		250.46±3.72	243.23±3.18	238.47±3.25	221.00±3.87	215.80±3.65	201.90±3.23	180.40±2.12
	50^0C	Axis	288.15±3.20	19.91±1.23	23.76±1.14	25.96±1.15	29.06±1.13	37.57±1.14	49.19±1.17	56.05±1.11
		Cotyledon		248.30± 3.52	242.26±3.19	230.87±3.39	219.50±3.25	202.73±2.49	198.70±2.74	175.30±2.92

	60^0C	Axis	286.36±3.87	20.04±1.08	24.16±1.12	26.15±1.18	29.45±1.19	39.22±1.14	50.20±1.62	57.48±1.61
		Cotyledon		251.29 ± 3.17	240.16±3.21	225.94±3.28	215.41±3.84	195.68±3.17	190.32±2.14	168.30±3.46
	70^0C	Axis	283.80±3.63	21.65±1.13	24.87±1.17	27.93±1.21	30.16±1.20	40.10±1.11	51.94±1.09	59.49±1.59
		Cotyledon		249.29± 3.16	219.71±3.76	209.34±3.38	206.48±3.66	201.60±3.88	185.20±3.22	160.21±3.24

Table 10: Effect of Temperature on Specific Activity of Guaiacol Peroxidase Enzyme in the Seeds/ Seedlings of *Pisum sativum* and *Glycine max*

Seed samples (Cotyledons)	**Treatment**	**Specific Activity of Peroxidase Enzyme – Units/mg Protein**							
		Seedling age (in days)							
		0	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Pisum sativum	Control	0.056±0.002 (0.49±0.03)	0.101±0.003 (0.93±0.03)	0.110±0.004 (1.12±0.05)	0.113±0.006 (1.20±0.04)	0.123±0.003 (1.31±0.03)	0.190±0.003 (2.14±0.02)	0.196±0.003 (2.70±0.04)	0.208±0.006 (2.96±0.03)
	50	0.079±.003 (0.69±0.04)	0.107±0.004 (1.03±0.06)	0.115±0.002 (1.20±0.04)	0.118±0.008 (1.24±0.03)	0.136±0.002 (1.50±0.03)	0.196±0.002 (2.27±0.08)	0.203±0.002 (2.50±0.03)	0.214±0.009 (2.84±0.02)
	60	0.082±0.003 (0.70±0.04)	0.121±0.002 (1.26±0.01)	0.126±0.001 (1.27±0.01)	0.128±0.003 (1.36±0.03)	0.138±0.001 (1.51±0.04)	0.203±0.003 (2.83±0.03)	0.219±0.003 (2.93±0.08)	0.235±0.002 (3.20±0.02)

	70	0.093±0.003 (0.85±0.04)	0.133±0.002 (1.26±0.03)	0.139±0.004 (1.40±0.04)	0.143±0.003 (1.49±0.06)	0.151±0.006 (1.63±0.02)	0.225±0.005 (2.58±0.02)	0.233±0.002 (2.80±0.06)	0.245±0.003 (3.50±0.02)
Glycine max	Control	0.083±0.003 (0.59±0.03)	0.109±0.002 (0.85±0.02)	0.116±0.005 (0.93±0.03)	0.148±0.006 (1.23±0.04)	0.152±0.003 (1.40±0.03)	0.159±0.003 (1.59±0.02)	0.164±0.005 (1.70±0.05)	0.198±0.006 (2.20±0.02)
	50	0.090±0.003 (0.63±0.04)	0.112±0.005 (0.88±0.05)	0.119±0.004 (0.96±0.06)	0.156±0.003 (1.32±0.03)	0.161±0.002 (1.48±0.02)	0.168±0.005 (1.70±0.06)	0.192±0.003 (2.10±0.09)	0.210±0.002 (2.30±0.10)
	60	0.095±0.002 (0.68±0.05)	0.117±0.003 (0.93±0.06)	0.138±0.003 (1.12±0.03)	0.169±0.004 (1.44±0.02)	0.17±0.003 (1.60±0.04)	0.178±0.002 (1.92±0.03)	0.200±0.001 (2.23±0.02)	0.228±0.003 (2.85±0.07)
	70	0.102±0.004 (0.74±0.03)	0.126±0.003 (1.0±0.05)	0.159±0.002 (1.31±0.02)	0.178±0.003 (1.60±0.04)	0.181±0.001 (1.73±0.02)	0.189±0.003 (2.10±0.05)	0.224±0.002 (2.60±0.03)	0.250±0.003 (3.20±0.02)

(Unit activity in parenthesis)

Table 11: Effect of Temperature on Total Free Amino Acids in the Seeds/Seedlings of *Pisum sativum* and *Glycine max*

Seed Samples (Cotyledon)	**Treatment**	**Amino Acids in mg g^{-1} dry weight**							
		Seedling age (in days)							
		0	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Pisum sativum	Control	5.01 ±0.12	6.20 ± 0.26	8.10 ± 0.38	16.06 ± 1.32	17.52 ± 1.04	18.16 ± 1.94	20.36 ± 1.12	25.29 ± 1.94
	50^0C	5.06 ± 0.18	6.80 ± 0.90	8.96 ±0.73	16.14 ± 1.69	17.82 ± 1.04	19.16 ± 1.99	22.31 ± 1.15	26.16 ± 1.86
	60^0C	5.40 ± 0.62	6.98 ± 0.20	9.52 ± 0.74	18.20 ± 1.28	18.91 ± 1.05	20.16 ± 1.46	23.86 ± 1.25	27.30 ± 1.48

	70^{0}C	6.20 ± 0.24	7.80 ± 0.28	9.60 ± 0.74	19.25 ± 1.34	20.36 ± 1.51	21.93 ± 1.26	24.27 ± 1.39	27.99 ± 1.38
Glycine max	Control	5.12 ± 0.39	5.47 ± 0.30	6.02 ±0.23	9.97 ± 0.95	10.21 ± 1.08	11.63 ± 1.24	15.31 ± 1.32	19.60 ± 1.71
	50^{0}C	5.22 ± 0.19	5.81 ± 0.78	6.95 ± 0.30	10.88 ± 1.23	11.03 ± 1.08	12.22 ± 1.36	16.28 ± 1.92	20.19 ± 1.42
	60^{0}C	5.49 ± 0.13	6.14 ± 0.29	7.28 ± 0.98	11.93 ± 1.06	12.60 ± 1.09	12.98 ± 1.39	17.96 ± 1.41	21.28 ± 1.56
	70^{0}C	6.33 ± 0.14	6.91 ± 0.15	7.91 ± 0.54	12.91 ± 1.75	13.60 ± 1.09	19.31 ± 1.30	21.06 ± 1.81	23.01 ± 1.16

Table 12: Effect of Temperature on Proline content in the Seeds/Seedlings of *Pisum sativum* and *Glycine max*

Seed Samples	Treatment	Tissue	Proline in mg g^{-1} dry weight							
			Seedling age (in days)							
			0	1	2	3	4	5	6	7
Pisum sativum	Control	Axis	0.14 ±0.1 2	0.27 ± 0.03	0.43 ± 0.03	0.63 ± 0.12	0.85 ± 0.13	1.10 ± 0.64	1.38 ± 0.73	1.63 ± 0.95
		Cotyledon		0.25 ± 0.0 3	0.37 ± 0.03	0.50 ± 0.11	0.65 ± 0.12	0.82 ± 0.14	1.06 ± 0.43	1.31 ± 0.61
	50^0C	Axis	0.17 ± 0.18	0.33 ± 0.06	0.53 ± 0.0 4	0.74 ± 0.12	0.97 ± 0.13	1.24 ± 0.44	1.53 ± 0.21	1.84± 0.32
		Cotyledon		0.30 ± 0.0 5	0.44 ± 0.12	0.61 ± 0.24	0.81 ± 0.11	1.03 ± 0.12	1.28 ± 0.11	1.54 ± 0.21
	60^0C	Axis	0.25 ± 0.31	0.38 ± 0.03	0.61 ± 0.12	0.85 ± 0.14	1.11 ± 0.13	1.40 ± 0.15	1.69 ± 0.16	2.01 ± 0.57
		Cotyledon		0.39 ± 0.12	0.57 ± 0.04	0.77 ± 0.11	1.00 ± 0.15	1.27 ± 0.65	1.56 ± 0.16	1.86 ± 0.12
	70^0C	Axis	0.28 ± 0.23	0.41± 0.13	0.66 ± 0.05	0.92 ± 0.13	1.19 ± 0.11	1.43 ± 0.13	1.80 ± 0.16	2.12 ± 0.47
		Cotyledon		0.44 ± 0.12	0.62 ± 0.0 5	0.83 ± 0.12	1.08 ± 0.13	1.37 ± 0.15	1.69 ± 0.19	2.02 ± 0.25
Glycine max	Control	Axis	0.15 ± 0.09	0.42 ± 0.13	0.57 ±0 .15	0.75 ± 0.13	0.94 ± 0.15	1.25 ± 0.11	1.48 ± 0.12	1.68 ± 0.11
		Cotyledon		0.30 ± 0.12	0.49 ± 0.11	0.72 ± 0.13	0.98 ± 0.13	1.27 ± 0.14	1.58 ± 0.19	1.91 ± 0.17
	50^0C	Axis	0.16 ± 0. 07	0.49 ± 0.13	0.66 ± 0.23	0.84 ± 0.13	1.04 ± 0.12	1.29 ± 0.02	1.58 ± 0.13	1.89 ± 0.13
		Cotyledon		0.32 ± 0.16	0.52 ± 0.25	0.76 ± 0.14	1.04 ± 0.13	1.36 ± 0.02	1.70 ± 0.03	2.05 ± 0.22
	60^0C	Axis	0.19 ± 0.08	0.56 ± 0.12	0.76 ± 0.14	0.98 ± 0.14	1.22 ± 0.21	1.49 ± 0.03	1.78 ± 0.31	2.16 ± 0.25
		Cotyledon		0.37 ± 0.12	0.60 ± 0.12	0.86 ± 0.15	1.15 ± 0.21	1.48 ± 0.02	1.83 ± 0.15	2.20 ± 0 .29
	70^0C	Axis	0.23 ± 0.12	0.62 ± 0.13	0.86 ± 0.12	1.13 ± 0.17	1.45 ± 0.23	1.79 ± 0.04	2.13 ± 0.39	2.56 ± 0.15
		Cotyledon		0.47 ± 0.11	0.73 ± 0. 12	1.02 ± 0.13	1.34 ± 0.19	1.69 ± 0.03	2.06 ± 0.22	2.46 ± 0.32

Table 13: Effect of Temperature on Starch content in the Seeds/Seedlings of *Pisum sativum* and *Glycine max*

Seed Samples	Treatment	Tissue	Starch in mg g^{-1} dry weight							
			Seedling age (in days)							
			0	1	2	3	4	5	6	7
Pisum sativum	Control	Axis	493.60 ± 8.14	59.79 ± 2.39	67.70 ± 2.55	76.33 ± 3 .15	78.39 ± 3.32	178.53 ± 3.68	200.25 ± 3.34	219.78 ± 3.66
		Cotyledon		474.80 ± 6.80	452.80 ± 5.90	433.80 ± 5.10	430.20 ± 6.60	338.10 ± 5.80	330.60 ± 4.32	239.70 ± 3.49
	50^0C	Axis	491.50 ± 8.49	57.56 ± 2.48	67.44 ± 1.47	75.06 ± 2.61	77.52 ± 1.41	187.85 ± 3.58	202.45 ± 2.29	227.92 ± 4.07
		Cotyledon		467.40 ± 4.20	450.24 ± 4.20	429.02 ± 5.70	425.71 ± 7.01	329.58 ± 4.82	320.41 ± 3.28	225.50 ± 4.45
	60^0C	Axis	484.80 ± 7.62	44.95 ± 1.62	52.57 ± 1.32	60.53 ± 1. 33	69.16 ± 1.28	151.90 ± 3.08	167.45 ± 3.34	183.08 ± 3.46
		Cotyledon		469.05 ± 3.40	444 .30 ± 4.90	426.10 ± 4.80	421.27 ± 4.50	322.70 ± 3.06	310.60 ±3.72	214.20 ± 4.61
	70^0C	Axis	483.70 ± 7.48	37.50 ± 1.38	47.55 ± 1.37	56.77 ± 1.65	65.41 ± 1.68	124.17 ± 2.71	140.97 ± 2.86	158.20 ± 3.64
		Cotyledon		459.80 ± 4.70	431.50 ± 4.40	413.60 ± 4.40	408.64 ± 4.80	309.20 ± 4.36	300.15 ± 4.38	201.50 ± 3.60
Glycine max	Control	Axis	249.90 ± 5.10	34.68 ± 1.49	40.48 ±1.33	57.03 ± 1.72	59.63 ± 1.52	119.63 ± 2.51	140.28 ± 2.28	147.99 ± 3.62
		Cotyledon		237.09 ± 3. 09	230.50 ± 4.20	219.70 ± 5.3	200.38 ± 3.90	147.38 ± 3.78	136.68 ± 3.91	124.90 ± 3.96
	50^0C	Axis	249.20 ± 5.50	26.48 ± 1.56	30.75 ± 1.21	45.37 ± 1.44	48.28 ± 1.38	110.58 ± 2.54	138.20 ± 2.32	146.97 ± 3.58
		Cotyledon		233.69 ± 4. 80	228.70 ± 3.60	218.21 ± 4.80	200.17 ± 4.50	141.90 ± 3.40	128.81 ± 3.64	119.80 ± 3.97
	60^0C	Axis	237.80 ± 4.80	23.16 ± 1.33	27.76 ± 1.57	42.60 ± 1.36	45.49 ± 1.29	105.45 ± 2.46	130.14 ± 2.17	136.90 ± 3.79
		Cotyledon		228.60 ± 5.30	224.90 ± 4.80	216.00 ± 5.30	198.47 ± 5.30	140.90 ±4.40	124.36 ± 3.14	114.90 ± 3.20

	70^0C	Axis	236.10 ± 4.01	22.58 ± 1.39	27.08 ± 1.50	38.97 ± 1.76	42.31 ± 1.28	101.78 ± 2.36	118.36 ± 2.17	125.56 ± 2.50
		Cotyledon		224.10 ± 5.84	215.50 ± 4.04	200.90 ± 4.90	182.56 ±4.90	139.10 ± 3.71	120.72 ± 4.47	110.70 ± 3.59

Table 14: Effect of Temperature on Total Soluble Sugars in the Seeds/Seedlings of *Pisum sativum* and *Glycine max*

Seed Samples	Treatment	Soluble Sugars in mg g^{-1} Dry weight							
		Seedling age (in days)							
		0	1	2	3	4	5	6	7
Pisum sativum	Control	76.71 ± 1.63	7.80 ± 1.92	9.97 ± 1.75	15.53 ± 1.81	18.21 ± 1.72	30.40 ± 1.90	20.17 ± 1.92	14.39 ± 1.79
	50^0C	76.23 ± 1.82	7.16 ± 1.81	9.44 ±1.86	15.42 ± 1.67	18.37 ± 1.61	27.95 ± 1.96	21.74 ± 1.82	13.12 ± 1.64
	60^0C	74.01 ± 1.46	6.10 ± 1.48	8.39 ± 1.75	16.89±1.21	18.46 ± 1.32	28.82±1.06	20.37 ± 1.70	16.22 ± 1.96
	70^0C	72.21 ± 1.86	7.10 ± 1.92	9.89 ± 1.43	16.58 ± 1.38	18.97 ± 1.28	27.92±1.68	22.15 ± 1.76	16.38 ± 1.64
Glycine max	Control	72.46 ± 1.58	23.89 ± 1.48	14.86 ± 1.74	25.06±1.86	15.23 ± 1.64	13.97 ± 1.73	18.36 ± 1.10	9.23 ± 1.83
	50^0C	73.92 ± 1.86	29.31 ± 1.64	19.60 ± 1.74	29.79 ± 1.71	20.36 ± 1.57	15.76± 1.17	12.86 ± 1.97	10.28±1.20
	60^0C	74.51 ± 1.26	31.46 ±1.93	20.57 ± 1.95	33.26 ± 1.85	21.32 ± 1.41	11.07 ± 1.33	18.28 ± 1.28	10.10 ± 1.38
	70^0C	76.51 ± 1.33	27.44 ± 1.20	12.26 ± 1.83	24.09 ± 1.53	21.20 ± 1.43	16.30 ± 1.58	23.57 ± 1.37	10.45 ± 1.53

Table 15: Effect of Temperature on the distribution of Sugars in dry Seeds of *Pisum sativum*

Seed Samples	Sugar	Sugar in mg g^{-1} dry weight		
		Control	**60^{0}C**	**70^{0}C**
Pisum sativum	Glucose	1.3	N.D.	N.D.
	Fructose	1.4	N.D.	N.D.
	Rhamnose	17.7	20.8	20.93
	Maltose	2.8	2.1	2.01
	Sucrose	37.4	34.8	33.5
	Raffinose	13.02	10.4	9.2
	Stachyose	15.4	5.2	4.2
	Verbascose	3.8	3.0	2.3
	Total	**92.82 mg**	**76.3 mg**	**72.14mg**

Table 16: Effect of Temperature on the distribution of Sugars in the Cotyledons of *Pisum sativum* seedlings (3rd day of germination).

Seed Samples (Cotyledons)	Sugar	Sugar in mg g^{-1} dry weight		
		Control	**60^{0}C**	**70^{0}C**
Pisum sativum	Glucose	12.7	9.6	ND
	Fructose	14.12	11.7	ND
	Rhamnose	15.4	26.6	66.5
	Maltose	N.D.	26.3	N.D.
	Sucrose	113.2	29.9	28.01
	Raffinose	28.9	25.3	16.7
	Stachyose	11.2	6.5	4.7
	Verbascose	8.7	7.9	6.7
	Unknown I	ND	13.2	ND
	Total	**204.22 mg**	**157.0 mg**	**122.11 mg**

N.D. – Not detected

Table 17: Effect of Temperature on the distribution of Sugars in dry Seeds of *Glycine max*

Seed Samples	Sugar	Sugar in mg g^{-1} dry weight		
		Control	**60^{0}C**	**70^{0}C**
Glycine max	Glucose	15.8	14.3	13.5
	Fructose	15.9	15.7	7.5
	Rhamnose	27.8	28.6	28.9
	Maltose	N.D.	N.D.	N.D.
	Sucrose	89.2	57.5	17.2
	Raffinose	36.5	25.7	1.12
	Stachyose	9.0	4.9	3.2
	Verbascose	9.8	7.8	0.45
	Total	**204.0mg**	**154.50mg**	**71.87mg**

Table 18: Effect of Temperature on the distribution of Sugar in the Cotyledons of *Glycine max* seedlings (3rd day of germination).

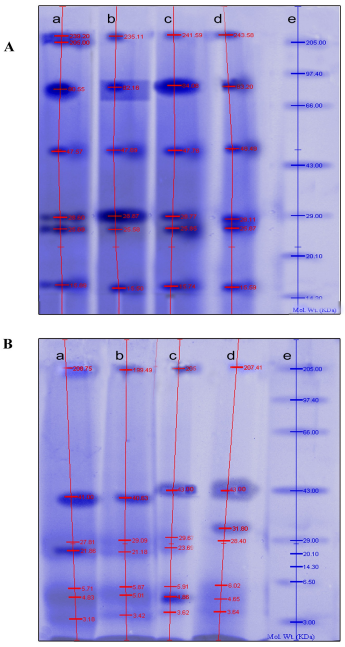
Seed Samples (Cotyledons)	Sugar	Sugar in mg g^{-1} Dry weight		
		Control	**60^{0}C**	**70^{0}C**
Glycine max	Glucose	6.3	7.7	7.7
	Fructose	7.0	8.5	8.5
	Rhamnose	8.93	15.6	15.8
	Maltose	N.D.	N.D.	N.D.
	Sucrose	34.8	34.5	34.7
	Raffinose	26.9	20.1	19.3
	Stachyose	5.4	4.6	4.5
	Verbascose	7.4	4.9	4.6
	Total	**96.73 mg**	**95.90 mg**	**95.10 mg**

N.D. – Not detected

Table 19: Effect of Temperature on Lipid content in the Seeds/Seedlings of *Pisum sativum* and *Glycine max*

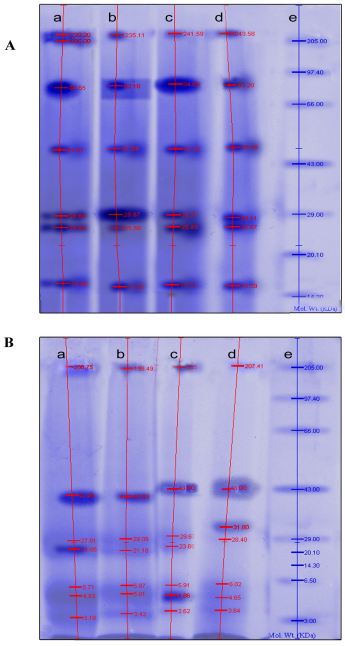
Seed Samples	Treatment	Lipid in mg g^{-1} Dry weight							
		Seedling age (in days)							
		0	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Pisum sativum	Control	40 ± 2.01	42 ± 2.05	46 ± 1.08	48 ± 2.05	48.2 ± 1.04	49 ± 3.08	49.2 ± 2.18	49.9 ± 4.07
	50^0C	34 ± 2.02	36± 1.07	38± 1.09	38.4± 2.04	38.4 ± 1.05	39.2± 1.05	39.2 ± 1.95	39.6 ± 2.03
	60^0C	32± 1.03	34± 1.03	34± 2.05	34.6± 1.03	34.1 ± 2.03	34.9 ± 1.03	34.1 ± 1.63	34.8± 1.05
	70^0C	30± 1.06	32.4± 1.02	32.3± 1.04	33.1± 1.02	33.2± 2.66	33.0 ± 1.05	32.4 ± 1.52	32.7 ± 1.07
Glycine max	Control	301.4 ±3.03	371.4 ± 3.06	334.6 ± 3.07	266 ± 2.07	250.3 ± 2.57	245.4 ± 2.02	242.5 ±2.87	268 ± 2.02
	50^0C	301.1 ± 3.02	363.4 ± 3.03	331 ± 3.03	251 ± 2.08	243.2 ±2.86	242.4 ± 2.06	240.9 ± 2.23	256 ± 3.04
	60^0C	298 ± 2.05	302.2 ± 2.04	282.4 ±2.04	264 ± 2.03	248.2 ± 2.92	234 ± 1.07	240.5 ± 1.84	249.6 ± 2.08
	70^0C	294 ± 2.06	300.2 ± 2.02	264.2 ± 2.02	253 ± 2.04	242.6 ± 2.09	231 ± 1.02	228.3 ± 2.36	236 ± 2.08

Fig. 12. SDS PAGE Protein profile in seeds/seedlings of *Pisum sativum*



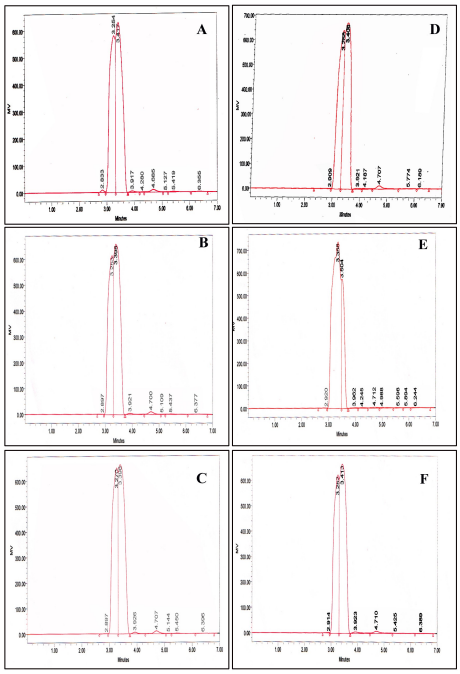
A. Dry seeds ; B. Cotyledons after three days of germination
a. Control; b. 50 ^{0}C; c. 60 ^{0}C; d. 70 ^{0}C; e. Marker proteins

Fig. 12. SDS - PAGE Protein profile in the Seeds/Seedlings of *Pisum sativum*



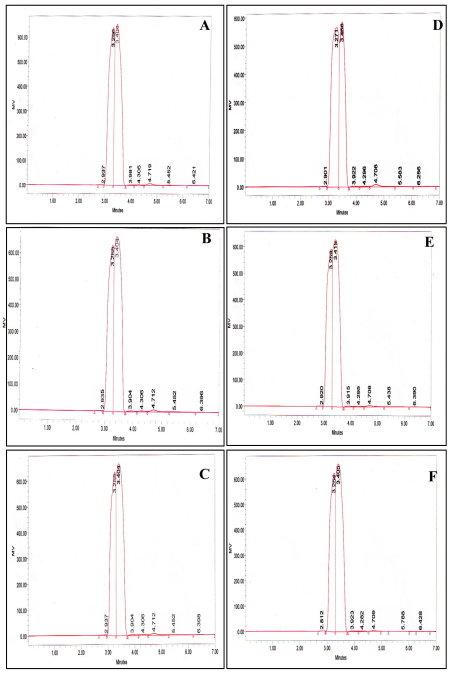
A. Dry seeds ; B. Cotyledons after three days of germination
a. Control; b. 50 ^{0}C; c. 60 ^{0}C; d. 70 ^{0}C; e. Marker proteins

Fig. 20 HPLC Sugar profile of Temperature treated *Pisum sativum* seeds / seedlings



A - C . ***P. sativum*** Dry seeds ; A. Control; B. 60^{0}C; C. 70^{0}C.
D - F . Cotyledon after 3 days germination ; D. Control; E. 60^{0}C; F. 70^{0}C.

Fig. 21 HPLC Sugar profile of Temperature treated *Glycine max* seeds / seedlings



A - C . ***Glycine max*** Dry seeds ; A. Control; B. 60^{0}C; C. 70^{0}C.
D - F . Cotyledon after 3 days germination ; D. Control; E. 60^{0}C; F. 70^{0}C.

Fig. 23 Protein distribution in the cotyledon of dry seeds

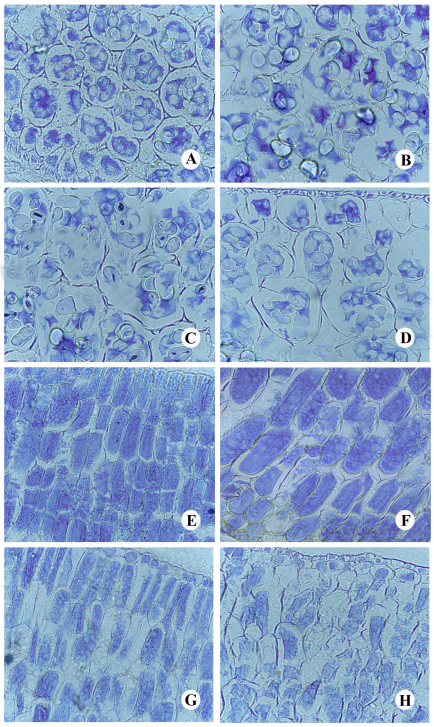


Fig. A -D . ***P. sativum*** ; A. Control; B. 50^{0}C; C. 60^{0}C; D. 70^{0}C.
Fig. E -H . ***G. max*** ; E. Control; F. 50^{0}C; G. 60^{0}C; H. 70^{0}C.

Fig. 24 Protein distribution in the cotyledon on the first day of germination

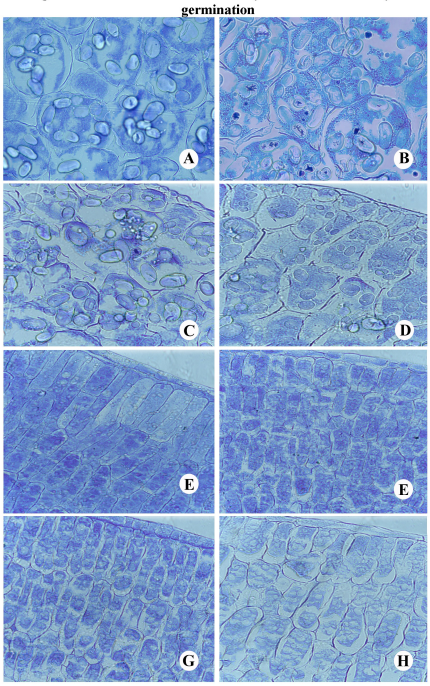


Fig. A -D . ***P. sativum*** ; A. Control; B. 50^{0}C; C. 60^{0}C; D. 70^{0}C.
Fig. E -H . ***G. max*** ; E. Control; F. 50^{0}C; G. 60^{0}C; H. 70^{0}C.

Fig. 27 Starch distribution in the cotyledon of dry seeds

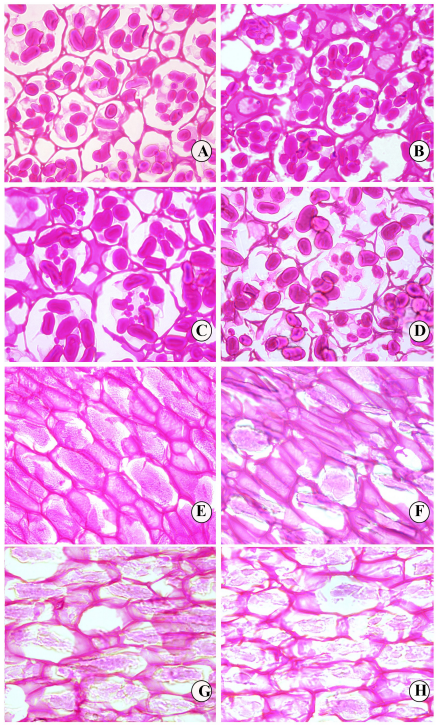


Fig. A -D . ***P. sativum*** ; A. Control; B. 50^{0}C; C. 60^{0}C; D. 70^{0}C.
Fig. E -H . ***G. max*** ; E. Control; F. 50^{0}C; G. 60^{0}C; H. 70^{0}C.

Fig. 28 Starch distribution in the cotyledon on the first day of germination

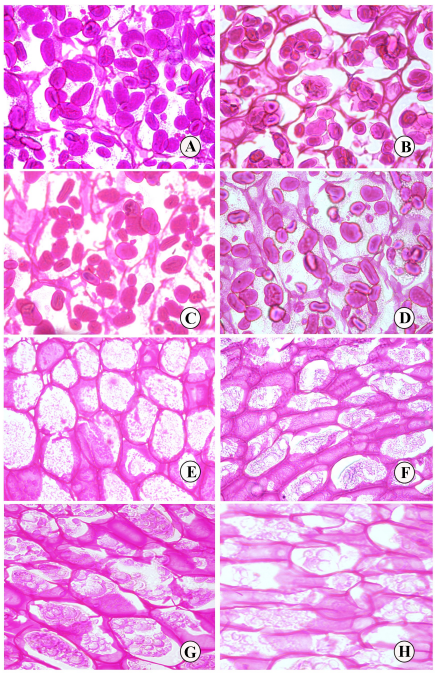


Fig. A -D . ***P. sativum*** ; A. Control; B. 50^{0}C; C. 60^{0}C; D. 70^{0}C.
Fig. E -H . ***G. max*** ; E. Control; F. 50^{0}C; G. 60^{0}C; H. 70^{0}C.

Fig. 29 Starch distribution in the cotyledon on the first day of germination

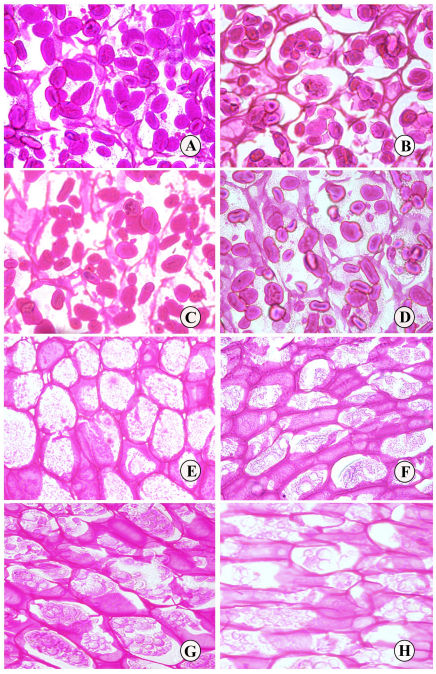


Fig. A -D . ***P. sativum*** ; A. Control; B. 50^{0}C; C. 60^{0}C; D. 70^{0}C.
Fig. E -H . ***G. max*** ; E. Control; F. 50^{0}C; G. 60^{0}C; H. 70^{0}C.

Fig. 30 Starch distribution in the cotyledon on the seventh day of germination

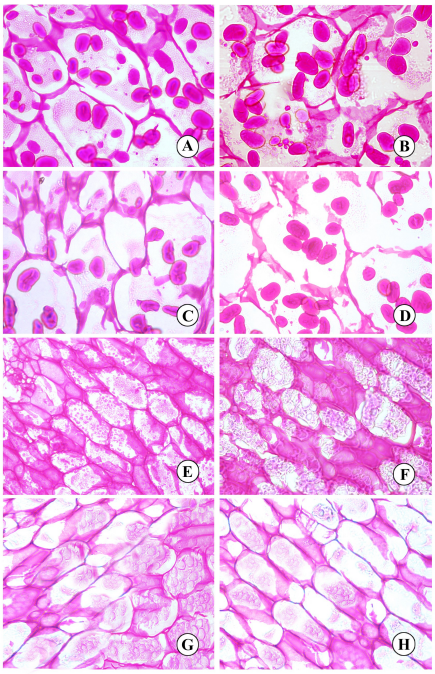
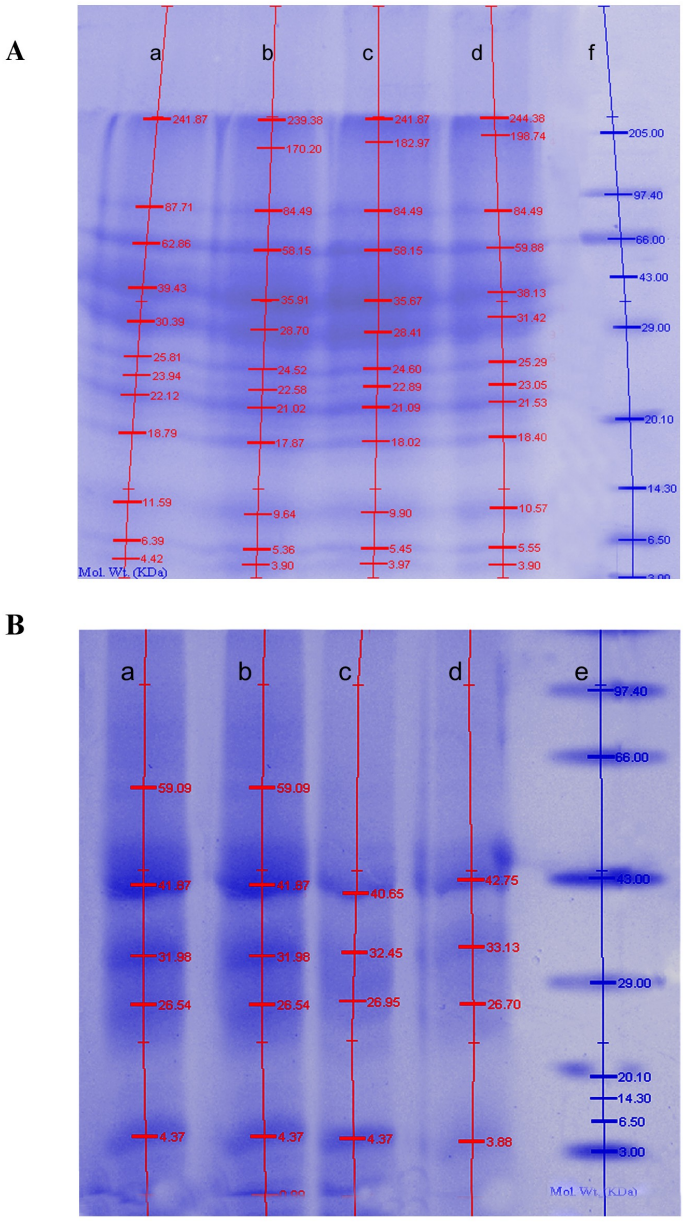


Fig. A -D . ***P. sativum*** ; A. Control; B. 50^{0}C; C. 60^{0}C; D. 70^{0}C.
Fig. E -H . ***G. max*** ; E. Control; F. 50^{0}C; G. 60^{0}C; H. 70^{0}C.

Fig. 13. SDS - PAGE Protein profile in the seeds/ seedlings of *Glycine max*



A. Dry seeds ; B. Cotyledons after three days of germination

a. Control; b. 50 ^{0}C; c. 60 ^{0}C; d. 70 ^{0}C; e. Marker proteins