

**EFFECT OF AGEING AND PRIMING ON SEED  
STORAGE PROTEINS OF BARLEY.**

*(Hordeum vulgare L.)*

**BY**

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of the requirements for the degree of*

*Master of Science*

*IN*

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*Dedicated*  
*To My*  
*Beloved Parents*

## **CERTIFICATE-I**

This is to certify that this thesis entitled, "**Effect of ageing and priming on seed storage proteins of barley. (*Hordeum vulgare* L.)**" submitted for the degree of **Master of Science** in the subject of **Seed Science and Technology** of the **Chaudhary Charan Singh Haryana Agricultural University, Hisar** is a bonafide research work carried out by **Mr. DAVENDER SINGH CHAUHAN** under my supervision and that no part of the thesis has been submitted for any other degree.

The assistance and help received during the course of investigation has been fully acknowledged.

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## **CERTIFICATE-II**

This is to certify that this thesis entitled, "**Effect of ageing and priming on seed storage proteins of barley. (*Hordeum vulgare* L.)**" submitted by **Mr. DAVENDER SINGH CHAUHAN** to the **Chaudhary Charan Singh Haryana Agricultural University, Hisar** in partial fulfillment of the requirement for the degree of **Master of Science** in the subject of **Seed Science and Technology**, has been approved by the student's advisory committee after an oral examination.

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## **LIST OF ABBREVIATIONS**

APS	AMMONIUM PERSULPHATE
SDS	SODIUM DO DECYL SULPHATE
TCA	TRICHLOROACETIC ACID
TEMED	N,N,N,N-TETRAMETHYLENE DIAMINE
TRIS	TRIS( HYDROXYMETHYL)AMINOMETHANE
EDTA	ETHYLENE DI AMINE TERA ACETIC ACID
NaCl	SODIUM CHLORIDE
CTAB	CETYL TRIMETHYL AMMONIUM BROMIDE
TAE	TRIS ACETIC ACID EDTA BUFFER
OD	OPTICAL DENSITY
DHA	DEHYDROGENASE ACTIVITY
TE	TRIS EDTA BUFFER
RH	RELATIVE HUMIDITY
PEG	POLYETHYLENE GLYCOL
RM	RELATIVE MOBILITY
PAGE	POLY ACRYLAMIDE GEL ELECTROPHORESIS
DNA	DEOXYRIBONUCLEIC ACID
RNA	RIBO NUCLEIC ACID
ATP	ADENOSINE TRINUCLEOTIDE PHOSPHATE
TTC	2,3,5- TRIPHENYL TETRAZOLIUM CHLORIDE
HCl	HYDROCHLORIC ACID
UV	ULTRA VIOLET

## CHAPTER - I

# INTRODUCTION

Seed has been important for agriculture ever since the plants were domesticated. Plant breeders have developed a number of high yielding varieties and to harvest their potential, quality seed has been recognized as an important and cheapest input. The quality of seed is mainly measured by its genetic purity and capacity to develop into a healthy plant. International Seed Testing Association (ISTA, 1999) has proposed a number of standardized vigor and viability tests to check the potential level of activity and performance of the seed during germination and seedling emergence. The quality of the seed and storage capacity are also correlated to each other.

Barley (*Hordeum Vulgare* L.) was one of the earliest domesticated crop, and being cultivated since the beginning of civilization. Besides, a better food value in terms of protein quality and total carbohydrates, barley has industrial use as well and it can play an important role in promoting export of agricultural

commodities (Breweries). It is an important winter season crop of rain fed areas of India, where cultivation of wheat is a problem due to salinity and scarcity of irrigation facilities.

Deterioration of stored seed is a serious problem in tropical and subtropical countries like India where high temperature and humidity accelerate the seed ageing phenomena. Therefore, after knowing the quality of seed lot the important aspect that comes into picture is to define the storage or shelf life of seed. This can be achieved by accelerated ageing of seed at high temperature and high relative humidity (>90%). This helps in predicting the natural deterioration of seed lot due to stored protein degradation during storage (Ram *et al.* 1989).

One of the objectives of ISTA vigor committee is to encourage an increase in the species database for the accelerated ageing test (Hampton, 1998;Tekrony.2001). Wang (1985) suggested that temperature and time combination of 45°C for 48 hours provided reasonable separation of four Pea seed lots, while Hampton *et al.*(2004) used 42°C and 45°C for 48,72 and 96 hours and concluded that 45°C was too severe irrespective of ageing time.

The vigour of seed lots can often be improved by technique generally known as priming (Parera and Contliffe, 1994.), which improves the speed and uniformity (synchrony) of germination.

Seed uptakes water during germination which follows triphasic patterns with an initial imbibition followed by lag phase and finally by second uptake phase and associated with start of seedling growth (Come, 1980; Come & Thevenot, 1982). Barley is a dry land crop and water is always a limiting factor. Thus there are good chances that initial hydration followed by desiccation may lead to the loss of germination in the fields. Beneficial effects of priming of seed on the germination and emergence of many species have been reported earlier. Studies have also shown that seed priming or restricted hydration is associated with increase in protein synthesis, rapid resumption of RNA, nucleic acid synthesis and repair (Bray *et al.*, 1989; Dell'Aquila & Bewley (1989); Davision & Bray, 1991). Therefore, it becomes necessary to characterize the changes in seed stored food, which may get affected and would result in either restoration or loss of viability under restricted hydration conditions or priming.

The use of aqueous solution of PEG-6000 (Polyethylene glycol) for imbibitions appears to hold the maximum potential for inducing early and synchronous germination, (Heydecker, 1973-1974; Burlyn and Merrill.R, 1973). Priming with PEG solution enables the seeds to germinate at lower temperature than, those at which untreated seeds will germinate (Akaleniywot and Bewley, 1977).

Present study was planned to determine the effect of ageing and priming on seed storage proteins of barley under natural and artificial aged conditions. An effort will also be made to define the stability of improvement in seed vigor due to priming, with the following objectives:-

1. To define variables for accelerated ageing test in barley.
2. To study the variation of total and specific seed storage protein after ageing and priming.
3. To access the stability of improvement of seed vigor after priming.

## CHAPTER - II

# REVIEW OF LITERATURE

Viability and vigor are the most important attributes of the seed quality which are controlled by both genetical and environmental factors. Seed possess highest vigor at the time of physiological maturity. Thereafter the seed gradually deteriorates (Meena, 1994). Seed deterioration leads to reduction in seed quality, performance and stand establishment which is a major problem in agricultural production (Christiansen and Rwoland, 1981). Seed viability and vigor tests have been used extensively in a number of crops to predict the planting value of the seed in the field. Also, the climatic conditions of India greatly affect the storage capacity of the seed lot because of high temperature and high relative humidity which in turn deteriorate the quality of seed under ambient storage conditions (Basu, 1976). Therefore, to assess the storage capacity of the seed, accelerated ageing test was proposed by ISTA which takes into account these two factors (Indira *et al.*, 2000).

Seed priming (Pre sowing hydration treatments of seeds) enhance seed performance, notably with respect to rate and uniformity of germination thereby enabling better crop establishment (Heydecker *et al.*,1973; Heydecker, 1978; Bradford, 1986; Taylor *et al.*,1988; Parera & Cantliffe, 1994) and also increase the protein synthesis, nucleic acid synthesis and repair (Bray *et al.*,1989; Dell'Aquila & Bewley, 1989; Davison & Bray, 1991; Clarke & James, 1991; Dell'Aquila & Spada, 1992). The information available on different viability and vigor tests on field crops particularly barley have been reviewed as follows:

### **Standard Germination Test**

Germination is the resumption of growth by the embryo and development of a young plant from the seed. Germination in the laboratory tests, is the emergence and development from the seed embryo of those essential structures which for the kind of the seed being tested, indicate the ability to develop into a normal plant under favorable conditions in the soil (ISTA, 1999).

Germination process occurs in 3 phases. Phase-I involves uptake of water by the seeds followed by Phase-II in which active metabolisms occur (ATP production, initiation of RNA and protein synthesis, DNA repair) and in Phase-III visible germination occurs which starts with cell expansion then DNA replication which leads to mobilization of reserved food sufficient

for radical protrusion (Come & Thevenot, 1982 and Bewley & Black, 1985).

To measure the germination ability of the seed lot, ISTA has proposed an excellent tool called as standard germination test. This test measures the viability of the seed lot as well and also referred as seed quality test. The methodology of this test has been standardized so that results are reproducible, within and among laboratories. Results from the germination tests, that established the maximum plant producing potential of seed lot were found correlated with field emergence under favorable conditions (ISTA, 1999). It has been concluded in various plant species that field emergence was most strongly positively correlated with the standard germination tests (Verma *et al.*,1998 in barley; Andrade *et al.*,2001 in maize, Freitas *et al.*,2002 in cotton; Fessel *et al.*,2001 in lettuce; Kasraie *et al.*,2001 in sorghum.) Similar observations in various crop species were made by various other workers.

### **Rate of germination**

Rate of germination is a test to calculate the rate of emergence. Priming treatment to sunflower significantly reduced the time of 50% germination and time to 50%emergence (Mwale *et al.*, 2003). Haris *et al.*2001, reported that priming of wheat seed overnight resulted in faster, more complete emergence, more vigorous early

growth, better tillering, early flowering, larger ears, early maturity, and higher yield.

### **Electrical conductivity test**

The conductivity test measures the amount of electrolytes which leach out from seed as they deteriorate (ISTA, 1999). This test has been used to measure the seed viability (Presley, 1958) and later developed into a vigour test for prediction of field emergence. However, the electrical conductivity test cannot be taken as a sole indicator of seed deterioration as Abdul- Baki & Anderson (1970) showed that leaching of sugar from mechanically injured seed was higher than from whole seed with equal viability.

An increase in leakage of electrolytes from membrane causes secondary effects also such as during germination it stimulates micro-organism activity and infection which ultimately leads to low vigour of seed lot (AOSA, 1983). Narwal (1995) indicated that all varieties of okra loses their membranes permeability after 180 days of storage in ambient conditions which increased the electrical conductivity values. Similarly, Verma *et al.*, (2001) showed that increase in conductivity values of the seed leachates at different soaking periods were related to the degree of deterioration of seed lots of Brassica species. Also, vigor of seed lot has been estimated by various workers using electrical conductivity test (Vanzolini (1998) in groundnut; Barbedo *et al.*

(1998) in inga seeds; Kasarie *et al.* (2001) in forage sorghum; Kharab (1992) in pigeonpea).

### **Dehydrogenase activity test**

The measurement of the activity of specific enzyme was one of the earliest biochemical techniques used to assess deterioration and predict seed viability (AOSA, 1983). Earlier selenium and tellurium salts were used for estimation of planting value. (Hasegawa, 1933; Eidmann, 1938 & Lakon, 1942). Kittock & law, (1968) gave an indirect method for enzyme activity estimation with reduction of 2,3,5- Triphenyl tetrazolium chloride in the seed by the action of a group of enzymes known as dehydrogenases. These enzymes are involved in hydrogen transfer during respiratory activity of biological systems. This hydrogen reduces the TTC to formazan ( a non-diffusible red coloured compound). The red colour indicates the extent of living parts in the seed. A viable part is stained in red while completely unstained parts are non- viable.

Later on, it was also used to measure the vigour levels of seed lot and developed into a reliable index for evaluation of seed viability ( Abdul- baki & Anderson, 1972). Steiner *et al.*, (1989) reported that dehydrogenase activity test is the best predictor of seedling emergence in wheat. Similar correlations were also observed by Agrawal *et al.* (1974) in paddy, Verma *et al.*

(1999) in triticale, Krishnappa *et al.* (1999) in ground nut and Dahiya *et al.* (1999) in cotton.

### **Vigour Index**

A combination of standard germination test value with seedling length and dry matter production provides a broad evaluation of seed vigor. Vigor index offers the possibility of categorizing seed lots into various classes of seed quality. The vigor index values are more significantly correlated with the emergence rate (Verma *et al.*, 1999). Similarly, Rao *et al.* (1999) showed that seed lot with lowest germination percentage in field had low vigor index

The length of seedling after a specified period is the product of time taken to germination i.e. initiation of growth and subsequent rate of growth. Measuring plumule growth as a vigour indicator for cereals was first suggested by Germ (1960). Later on Singh and Ram (1988) found the root and shoot length to be significantly correlated with field emergence. Similar conclusions were also forwarded by other workers (Verma *et al.* (1999) in barley, Trigo *et al.* (2000) in barley, rye, wheat and triticale).

Woodstock (1976) forwarded the seedling dry weight as a parameter of vigour and approved by AOSA. The earlier work in this concern exhibited the correlation between dry matter of the soybean seedling and vigour (Edge & Burris, 1970). The dry weight of the seedling showed a positive response to water and

nutritional uptake. ( Choi *et al.*, 1997 and Sonmez, 2000). Briggs & Dunn (2000) came to the conclusion that dry matter production in barley was significantly consistent to temperature but not to the germination resistance. Similar observations were also made by Richards & Lukacs (2002) in wheat.

### **Accelerated ageing test**

This test was first developed to measure the relative storability of seeds. In this test samples are placed under stress conditions of high temperature (40-45<sup>0</sup>C) and high relative humidity (>90%) for a certain period of time. A standard germination test is conducted after the seeds have been stressed. The differences observed in standard germination percentages and germination percentages after accelerated ageing reveal the actual physiological condition of seeds. The deteriorated seed lots give higher differences than good seed lots. (Anderson & Baker, 1983)

The use of accelerated ageing test to predict the stand establishment in field of peanuts was proposed by Baskin (1971). Later on, Dubey *et al.* (1988) in pigeonpea, Kumar *et al.* (1989) in Mungbean, Ram *et al.* (1989) in Chickpea also proposed similar observations.

Similarly storability of seed, seedling establishment and other quality parameters can also be predicted by accelerated ageing test (Dahiya *et al.*, 1994, Delouche & Baskin, 1978).

The viability in *Atriplex carbodensis* was found to be sensitive to accelerated ageing and reduced germination according to the time of exposure to ageing conditions (Aiazzi *et al.*, 1996). Similar results were observed in soybean by Stewart & Bewley (1980) and Senaratna *et al.* (1988) in peanut by Sung & Jeng (1994) and Perez & Arguello (1995) in beans by Lin & Pearce (1990) in sunflower by Bailey *et al.* (1996) in brassica by Verma *et al.* (2001), Verma & Singh (2001). The polyphenol content increased by equivalent percentages in both peas and barley when seeds exposed to accelerated ageing (Lachman *et al.*, 1997).

### **Seed storage protein profile**

The polyacrylamide gel electrophoresis (PAGE) technique since its introduction has been improved and adopted to various applications in a variety of scientific fields. The uniformity of seed protein profile, its additive nature and negligible effect by environmental conditions or seasonal fluctuations makes it a unique and powerful tool for genotype characterization. Seed proteins are mainly storage proteins and are not likely to be changed in dry mature seed. Also intrinsic changes in the plant such as chromosomal rearrangements or even doubling of the chromosomes have no or very small effect on the seed protein profile (Draper, 1987).

As proteins are the product of structural genes and thus, can be regarded as markers for structural genes that

encode them. so, analysis of protein composition can be considered to be an analysis of gene expression and can be used as an ideal mean of varietal discrimination (Cooke *et al.*, 1983)

Wagner, (1978) identified barley varieties by means of PAGE of storage proteins and concluded that varieties which could not be separated on the basis of morphological grain characters could be distinguished by electrophoresis. Similar kind of conclusions were also forwarded by various other workers on barley (Shewry *et al.*,1979; Rensburg, 1980; Montembault *et al.*, 1981; Gabre *et al.*, 1986; Smith & Simpson, 1983; Marchylo, 1987; Ellegaard, 1987; Weiss *et al.*, 1991; Perovic *et al.*,1998; Arabi *et al.*, 2001; Bradova *et al.*,2001; Herrmann & Zanetti, 2002; Curtis & Chadwick, 1984).

Cermy *et al.* (1993) concluded the prolamine starch gel electrophoresis as the better technique for determination of varietal trueness in common wheat and common barley. Kristoffersen *et al.* (2000) separated and characterized the basic barley proteins from starchy endosperm through the pH gel electrophoresis that resulted in a new protocol for obtaining internal sequence from proteins.

### **Hordein protein profile**

Hordein protein is a specific storage protein of barley. Popereya and Mozhareno (2001) concluded that PAGE technique developed as a replacement for less accurate starch gel electrophoresis. Binneck *et al.*(2002)observed variation in hordein banding pattern in different cultivars. A high resolution system of acidic PAGE was developed to analyze the hordein polypeptide in barley. After the analysis it was observed that a characteristic hordein pattern, account of high reproducibility, speed, simplicity, and the low cost of analysis, this method can be easily incorporated in barley (Fasoula *et al.*,2003) .

### **Seed priming**

Seed priming (Pre-sowing hydration treatments of seeds) is a widely used technique to enhance seed performance, notably with respect to rate and uniformity of germination thereby enabling better crop establishment (Heydecker *et al.*, 1973; Heydecker, 1978; Bradford, 1986; Parera & Cantliffe, 1994; Taylor *et al.*, 1988). The basis of these technique is that seed water uptake during germination follows a triphasic pattern with an initial rapid imbibitions phase (phase-I) followed by lag phase (phase-II) also referred to as germination *sensu sincto* and finally by a second uptake phase associated with an start of seedling growth (phase-III) (Come, 1980; Come & Thevenot, 1982). Since all the preliminary process for germination are presumed to take place during priming (Heydecker, 1973). The objective of seed

priming is to perform a controlled water uptake by the seeds up to the end of phase-II before the radicals protrude from the seed coats. Furthermore, since most seeds are desiccation tolerant up to these developmental stages, the germination process can be arrested by drying. Numerous studies have demonstrated that priming is associated with an increase in protein synthesis (Bray *et al.*, 1989; Dell'Aquila & Bewley, 1989; Davison & Bray, 1991; Dell'Aquila & Spada, 1992) as well as with nucleic acid synthesis and repair (Bray *et al.*, 1989; Clarke & James, 1991; Bray, 1995).

Several methods are used to control seed water uptake during treatments; priming with an osmotically active agents, usually a salt or polyethylene glycol (PEG) (Bradford, 1986) with a water absorbing carrier (Solid matrix) (Taylor *et al.*, 1988) or with pure water (prehydration in water) (Tarquis & Bradford, 1992; Job *et al.*, 1997) or with only water vapour (drum priming) (Rowse, 1996). The major problem encountered in seed priming is to control seed imbibition to a level permitting pre-germinative processes to proceed but that block radical emergence. Otherwise the consequence of drying back the seeds for storage purposes can be a total loss of the treated batch. The use of aqueous solution of polyethylene glycol (PEG) for imbibition appears to hold the most potential for inducing early and synchronous germination on a population of seeds. (Heydecker, 1973 ; Burlyn & Merrill, 1973). Priming with PEG solution enables seeds to

germinate at temperature lower than those at which untreated seeds will germinate. (Akalehiywot & Bewley, 1977). There is, therefore, strong interest in the characterization of molecular markers for use by the seed industry in the design of priming protocols because optimization of these treatments rests solely on carrying out germination assays, which can only yield a posterior indications on the priming conditions (e.g. duration, water potential and availability, temperature, oxygen availability).

### **Seed Storage proteins**

Storage proteins in seeds provide the amino acids for the growing seedlings in the early period of their life. Seed protein represents the major source of proteins for both human and animal nutrition. The bulk of the proteins in seeds is formed by the storage proteins, therefore, the nutritional quality of seed proteins largely depends on the amino acid composition of the storage proteins. In addition to their amino acids composition, the physiochemical properties of storage proteins are important because they play a part in food processing. Other proteins existing in more or less discrete amounts in the seeds are important too like enzymes involved in food industries.

Storage proteins are synthesized on rough endoplasmic reticulum (Cameron Mills *et al.*, 1978) and stored in protein bodies (Pernollet, 1976). These stored proteins are usually of four types:

- I Albumins:** soluble in water.
- II Globulins:** Soluble in salt solutions but insoluble in water
- III Prolamins:** Soluble in aqueous alcohols
- IV Glutelins:** Soluble in dilute acids and alkalis

The storage proteins of dicotyledonous seeds are invariably globulin whereas in cereals it is usually prolamines (Maize, barley), glutelins (rice) or both (wheat) (Whitehouse, 1973). All these proteins are rich in either glutamine or asparagines and this is consistent with them acting as nitrogen stores for the rapidly growing embryo during early germination. Barley prolamines for example has a glutamine content of 33.1 moles % (Laurie *et al.*, 1976) and so comprises one in every three amino acid residues. Storage proteins are usually insoluble in cellsap for they have appreciable number of the hydrophobic amino acids proline, leucine, isoleucine and phenylalanine. The high levels of amidated and hydrophobic amino acids results in an imbalance of amino acids which is not ideal for human diet.

#### **Mobilization of seed proteins during germination/priming**

In germinating seeds, we can, at least conceptually separate three stages of proteolysis. First there is an initial hydrolysis of free amino acids for the synthesis of the enzymatic machinery required for the conversion of the various, mainly insoluble reserve substances to form suitable for transport. Next there is a bulk hydrolysis of the main reserve proteins to provide

amino acids to the growing seedling. Finally during the senescence of the reserve depleted storage tissue, the cellular proteins including the various parts of the aforementioned mobilization machinery broken down to provide the last ration of amino acids to the seedling before the onset of auto-tropic growth. It seems that in most seed different proteolytic machinery, at least in the quantitative sense functions at each of those stages.

The proteolytic enzymes and inhibitors detected in germinating seeds can be separated into seven groups. These seem to be two groups of proteinase one already present in the resting seeds and other appearing during germination. Three groups of peptidases (acid carboxypeptidases, naphthylamidases and alkaline peptidases) are apparently present in all the seeds but their roles are quantitatively different in different seeds and at different stages of germination than there are inhibitors of both exogenous and endogenous proteinase. According to current views the former act only as substrates in the proteolysis during germination while the latter group is an essential part of the proteolytic machinery at least in some seeds (Mikola, 1981).

The sequence of events in the mobilization of seed proteins in some respect is easier to study in cereal grains than in most other seeds. Because the various process normally taking place in different organelles of single cells are partly divided between three different tissues; enzyme synthesis and secretion

in the aleuronic layer (and partly in the scultellum), storage and hydrolysis in the starting endosperm, uptake and metabolism of the hydrolysis product in the scultellum (Mikola, 1981).

## **CHAPTER - III**

# *MATERIALS AND METHODS*

The present investigation entitled "Effect of ageing and priming on seed storage protein of barley. (*Hordeum vulgare* L.)" was conducted at the Department of Seed Science and Technology, CCS Haryana Agricultural University. The study was planned to determine the effect of aging and priming on seed storage proteins of barley under natural and artificial aged conditions. An effort was also made to define the stability of improvement in seed vigor due to priming, with following objectives:-

1. To define variables for accelerated aging test in barley.
2. To study the variation of total and specific seed storage protein after aging and priming.
3. To access the stability of improvement in seed vigor after priming.

### **Materials**

The seed material for the present investigation consisted of six genotypes of barley which were obtained from

Department of Plant Breeding, CCS Haryana Agricultural University, Hisar. as detailed in table below.

<b>Genotype</b>	<b>Parentage</b>	<b>Source</b>
ALFA-93	Aurora/Queen/Beka	Karnal
BH-393	California mariout x Ratna	Hisar
BH-75	RD-150 x Ahor 131/68	Hisar
BCU-73	WUM-143 (Yagen)	Karnal
RD-2552	RD-2035/DL-472	Jaipur
K-551	P-464/Jyoti	U.P.(Kanpur)

### **Conditioning of seeds**

The freshly harvested seeds of all the genotypes were subdivided into two lots. The first lot was kept as such and designated as fresh seed lot for further tests and observations. While other lot of all the genotypes was exposed to accelerated ageing conditions and designated as aged seed lot.

### **Accelerated ageing**

To observe the optimum time for accelerated ageing test, i.e., period to 50% reduction of seed germination, sufficient number of seed in a single layer from each seed lot of the six genotypes was taken on wire mesh tray fitted in plastic boxes having 40 ml of distilled water. The boxes were placed in ageing chamber after closing their lids. The seeds were aged at  $40 \pm 1^\circ\text{C}$  at different time intervals and tested for standard germination in

three replications of 100 seeds each. The number of normal seedlings was counted on 7th day and expressed in percentage.

Seed from both seed lot was used to conduct following vigor and viability tests as detailed below. Each test was conducted in a completely randomized design with three replications.

### **Standard Germination Test (%)**

One hundred seeds of each genotype replicated thrice were placed on moistened paper (B.P.) and placed in seed germinator at  $20 \pm 1^\circ\text{C}$  temperature with 90-95 per cent relative humidity. The final count of germinated seeds was taken on 7th day and normal seedlings were expressed as percent germination of total seed (ISTA, 1999).

### **Rate of germination (%)**

The number of seedlings emerged were counted on each day up to final count of seedling and rate of germination was calculated as described by Maguire (1962)

$$\frac{\text{No. of seedlings emerged} + \text{----- No of seedlings emerged}}{\text{first day of count} \quad \text{final day of count}}$$

### **Electrical conductivity test (EC) ( $\mu\text{s/cm/seed}$ )**

Twenty normal and uninjured seeds were soaked in separate beakers each containing 50 ml of distilled water. Seeds

were immersed completely in water and beakers were covered with foil. Thereafter, these samples were placed in germinator at 20°C for 24 hours in dark. The electrical conductivity of seed leachates was measured by conductivity bridge meter (ISTA, 1999).

### **Dehydrogenase activity (DHA) test**

The method was suggested by Kittock and Law (1968). Reduction of 2,3,5-triphenyl tetrazolium chloride to red formazan by dehydrogenase enzymes in seed embryos is the basic principle for topographical tetrazolium test for seed viability but the method described here is a quantitative method, which may be used to determine varying dehydrogenase activity between seed lots of similar viability and therefore, it is a measure of seed Vigor. To conduct DHA test, the representative seed samples of each lot, replicated thrice were grounded to pass through 20-mesh screen. The 200 mg flour was soaked in 5 ml of freshly prepared 0.5% TTC solution having pH 7.0. After shaking, the mixture was incubated at a temperature of 35°C for 2 hours. Then it was centrifuged at 10,000 rpm for 3 minutes and the supernatant was poured off. The formazan was extracted with 10 ml acetone for 16 hours at room temperature. It was then centrifuged for 3 minutes at 10,000 rpm and acetone solution containing formazan was transferred to the cuvette. The absorbance reading of the solution

was taken at 520 nm wavelength using Systronics spectrophotometer 169.

**Seedling length (cm)**

The measurement of linear growth of five randomly selected normal seedlings taken from each seed lot of individual genotypes was measured in centimeters in all three replications at the termination of standard germination test period.

**Seedling dry weight (mg)**

Normal seedlings selected for measuring their length were kept further for taking dry weight. These were dried in hot air oven at 80°C for 24 hours and then seedling dry weight was recorded in milligrams. The average weight of five seedlings was taken for further calculations.

**1000 seed weight (g)**

Three replications of 1000-seeds each were weighed individually and the mean of these observations was recorded as 1000-seed weight.

**Moisture content (%)**

Precent moisture of seeds was calculated according to ISTA (1999) by hot air oven method Ten gram seeds from each categories were kept in moisture box in oven at 130± 1 °c for 2 hrs. the samples were taken out and cooled in dessicater before weighing. Moisture content was calculated as given below: -

$$\text{Moisture percentage} = \frac{M_2 - M_1}{\text{—————}} \times 100$$

$$M_2 - M_1$$

Where

$M_1$  – is the weight of empty bottle and its lid .

$M_2$  – is the weight of bottle and its lid and its content before drying.

$M_3$  – is the weight of bottle, its lid and its content after drying.

### **Vigor index**

Seedling vigor indices were calculated according to methods suggested by Anderson and Abdul-baki (1973).

Vigor index-I = Standard germination (%) x seedling length (cm)

Vigor index-II = Standard germination (%) x seedling dry weight (mg)

### **ELECTROPHORESIS PROTOCOL**

#### **Chemicals required**

1. Acrylmide 'electrophoresis grade'
2. Bisacrylmide (N,N<sup>1</sup>-Methylene-bis-acrylmide)
3. Glycine
4. Glycerol
5. Acetone
6. Tri-hydroxymethyl aminomethane(Tris)
7. Ammonium persulphate
8. Chloroform
9. TEMED(N,N,N,N<sup>1</sup>-tetra methylethylene diamine)
10. Bromophenol blue

11. Coomassie brilliant blue R-250(CBBR)
12. Glacial acetic acid
13. Methanol
14. Sodium dodecyl sulphate(SDS)
15. Hydrochloric acid(HCl)
16. 2-mercapta ethanol

## **B. PREPARATION OF REAGENTS**

### **i. Separating (resolving) gel buffer (1.5M Tris-HCl), pH 8.8**

18.15g of Tris was dissolved in 50 ml of distilled water and pH was adjusted to 8.8 by adding concentrated 4N HCl drop by drop. Final volume was made to 100 ml by adding distilled water and stored in refrigerator.

### **ii. Stacking gel buffer (0.5 M Tris-HCl) pH 6.8**

6.05g of Tris was dissolved in about 50 ml of distilled water and pH was adjusted to 6.8 by adding concentrated 4N HCl drop by drop. Final volume was made up to 100 ml and stored.

### **iii. 30% acrylamide for resolving gel (30%T, 2.6%C)**

73.05g of acrylamide and 1.95g of bisacrylamide were dissolved in distilled water and volume was made up to 250 ml.

### **iv. 30% acrylamide for stacking gel (30%T, 1.3%C):**

29.61g of acrylamide and 0.39g of bisacrylamide were dissolved in distilled water and volume was made upto 100 ml.

**v. SDS stock solution (10% w/v)**

10g SDS was dissolved in distilled water with constant stirring and gentle heating. Volume was made upto 100ml with distilled water and stored at room temperature.

**vi. Ammonium persulphate (10% w/v)**

0.1g ammonium persulphate was dissolved in 1ml distilled water (prepare fresh before use )

**vii. Running (Tank or Electrode) buffer (SDS-Tris glycine, pH 8.3)**

9.075g of Tris, 43.2g of Glycine and 3g SDS were dissolved in distilled water and the volume was made upto 3 liter. Electrode buffer can normally be used for 2-3 subsequent runs.

**viii. Defatting solvent mixture (2:1:1 Chloroform: Methanol Acetone v/v/v respectively)**

To 200ml chloroform, 100ml methanol and 100ml acetone were added to get defatting solvent mixture.

**ix. 0.6M Tris-HCl buffer pH (6.6) :**

Dissolve 1.8g of Tris in 15ml of distilled water and adjust pH to 6.6 by adding 4N HCl by drop by drop. Final volume made to 25ml by adding distilled water.

**x. Stock protein extraction solution**

Dissolve 4g SDS in 10.4 ml of 0.6M Tris-HCl buffer (pH 6.6), 7.9 ml distilled water, 10 ml glycerol and 0.05g of Bromophenol blue. The mixture was warmed gently and mixed well.

**xi. Stock Hordein extraction solution**

Dissolve pyronine G (or methyl green) 0.05% in 2 chloroethanol (20%) containing 18 % urea and monothioglycerol (or 2-mercaptoethanol) 1% (keep cold or prepare fresh).

**xii. Fixing solution (15% TCA)**

150g of Trichloro acetic acid was dissolved in distilled water and the volume was made upto one liter.

**xiii. Staining solution**

To 100 ml of 15% TCA solution, 15 ml of 1% CBBR (dissolve 1.0g of Coomassie Brilliant Blue dye in 100 ml methanol).

**xiv. Extraction buffer (Tris-Glycine buffer, pH 8.3)**

1.5 g of Tris was dissolved in 25 ml of distilled water and pH was adjusted to 8.3 by adding Glycine. The final volume was made upto 50 ml.

**xv. Working solution**

4.25 ml of stock protein extraction solution (i.e. solution no. x) was taken, 0.75 ml 2-mercaptoethanol was added and the final volume was made upto 10 ml with distilled water (Note: always working solution should be freshly prepared i.e., just at the time of sample loading).

**xvi. Destaining solution**

Mix methanol, acetic acid and water in ratio of 50:70:880 v/v/v, respectively.

### **C. PREPARATION OF GEL**

Gel plates were cleaned, dried and assembled in the gel cassette. A gel thickness of 1.0 mm was used.

#### **i. Separating gel (15%)**

The following solutions were mixed to prepare one slab of gel.

Tris buffer pH 8.8	-	6.0 ml
Water	-	3.7 ml
30% resolving acrylamide	-	10.0 ml
10% APS	-	0.1 ml
10% SDS	-	0.2 ml

0.02 ml of TEMED was added just before pouring the gel mixture.

All the reagents were mixed well and poured between the plates of the cassette. The cassette was filled unto the mark with out trapping any bubbles. Distilled water is over layered the gel solution and allowed to polymerize for 15-30 min.

#### **ii. Stacking gel (6%)**

After polymerization of the main gel stacking gel mixture was added over it carefully.

Tris buffer pH 6.8	-	1.50 ml
Water	-	6.00 ml
30% stacking gel acrylamide	-	2.00 ml

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10% APS - 0.20 ml

10% SDS - 0.10 ml

(for one slab of gel)

0.04 ml of TEMED was added just before pouring the solution. After pouring the stacking gel solution a comb was be inserted, (having required number of wells) and gel was left for polymerization for about 10- 15 minutes.

**D. PREPARATION OF PROTEIN SAMPLES**

From each variety five seeds were randomly selected. After removing their seed coat with the help of a razor blade and forceps they were crushed into fine powder with the help of pestle and mortar. The powder was put in test tubes and about 10 ml of defatting solvent mixture was added. The test tubes were covered with aluminium foil and allowed for defatting. After 3 hours the solvent mixture was decanted and the process was repeated three to four times, until the oil was removed totally. The seed powder was then dried by leaving the test tubes open at room temperature.

**E. PROTEIN EXTRACTION (TOTAL TRIS SOLUBLE PROTEIN)**

0.1 gm of defatted seed powder was taken in effendorf tube to 0.5 ml of extraction buffer (Tris-Glycine buffer, pH 8.3) was added. The contents were thoroughly mixed and kept for overnight in refrigerator. The effendorf tubes were taken out,

mixed the contents well and subjected to centrifugation at 10,000 rpm for 10 minutes. The supernatant was decanted and from this supernatant 0.1 ml was taken into a separate eppendorf tube to which 0.3 ml of working solution was added. The contents were boiled for five minutes, cooled and finally it was used as protein source for electrophoresis.

#### **F. ELECTROPHORESIS**

The comb and tygon tube was carefully removed after polymerization. The gel cassette was fixed into the electrophoresis apparatus. Then the wells were properly washed with running buffer. In each well 10-15 $\mu$ l sample was loaded. Electrophoresis was carried out at 1.5 mA per well till the sample migrated to the resolving gel. Later 2 mA per well was given until the tracking dye reached the bottom of the gel.

#### **G. FIXING AND STAINING**

Cassette was removed from the unit and the gel was taken out gently. It was placed in a staining tray and incubated for 10-15 minutes in 15% TCA solution. To this staining solution was added and incubated for 8 hours. The stain was then decanted and gel was rinsed with water. Destaining was done for 1-2 days with destaining solution to clear the gel background. After proper destaining, gels were stored in 7% acetic acid solution.

The banding pattern of proteins was considered as "Finger prints" of the cultivars. The cultivars were separated into

different groups according to number of bands and presence or absence and intensities of some specific bands. The relative migration ( $R_m$ ) value of each band was calculated as follows:

$$R_m = \frac{\text{Distance migrated by the protein band from the origin (cm)}}{\text{Distance migrated by the tracking dye (cm)}}$$

### **3.3.5.2 Gradient gel**

It is also called pore limit PAGE. One of the main advantages of gradient gel electrophoresis is that the migrating proteins are continually entering area of the gel with decreasing pore size. Such that the advancing edge of the migrating protein zone is retarded more than the trailing edge, resulted in marked sharpening of protein bands. In addition, the gradient in pore size increases the range of molecular masses that can be fractionated simultaneously on one gel.

#### **A. APPARATUS FOR GRADIENT GELS**

Polyacrylamide gradient gels are prepared using a linear gradient former( Atto, Japan) and peristaltic pump. The gradient gels are cast usually from top to bottom in which high concentration solution is in the down stream compartment. In this constant mixing of solution in mixing tube is required to form linear gradient.

#### **B. CASTING OF SDS-PAGE GRADIENT GELS**

**i. Reagents**

All reagents that are prepared for single percentage SDS-PAGE are used except 30% acrylamide (resolving gel).

**Preparation of 50% acrylamide for resolving gel (50%T, 2.6% C)**

Dissolve 48.7g of acrylamide and 1.3g of bisacrylamide in distilled water and make the final volume to 100ml.

**ii. Procedure**

Gel casting mold was arranged and decide upon most appropriate acrylamide gradient range of concentration for the protein sample to be analyzed. Now gel mixtures are prepared corresponding to the minimum and maximum concentrations in desired gradient as given below in the table. But TEMED is added just before pouring the solution.

High concentration should be taken in mixing tube and low concentration in reservoir tube in equal volume. Mixing tube need constant stirring with magnetic stirrer is needed to form linear gradient. Open the connection between the two tubes and at the same time turn the peristaltic pump on. Fill the gel sandwich with gel mixture within 15-20 minutes, depending on concentrations range of gradient gel (more the range of concentrations, more the time of pouring).

Reagents (in ml)	Final concentration (%) of gel					
	5%	10%	12%	15%	18%	20%

<b>50% Acrylamide (50%T, 2.6%C)</b>	1.8	3.6	4.3	5.4	6.1	7.2
<b>Distilled water</b>	11.4	9.6	8.9	5.1	4.4	3.3
<b>Tris buffer, pH 8.8</b>	4.5	4.5	4.5	4.5	4.5	4.5
<b>Glycerol*</b>	-	-	-	2.7	2.7	2.7
<b>SDS (10%)</b>	0.2	0.2	0.2	0.2	0.2	0.2
<b>APS (10%)</b>	0.1	0.1	0.1	0.1	0.1	0.1
<b>TEMED*</b>	0.01	0.01	0.01	0.01	0.01	0.01
<b>Total volume</b>	18.0	18.0	18.0	18.0	18.0	18.0

\* Glycerol used in high concentration mixture only (used to minimize mixing by convective disturbances caused by the heat evolved during polymerization for improved gradient formation).

Stacking gel preparation, loading of protein samples and conducting of electrophoresis are same as described in single percentage SDS-PAGE.

### **Priming of seeds**

Fresh and accelerated aged seeds of genotype BH-75 and BH-393 were primed by soaking in water and in an aqueous solution (27.3 gm/ litre) of polyethylene glycol 6000 (PEG 6000) providing an osmotic potential of -1Mpa (Michel & Kaufmann, 1973) for variable time period, and following seed lots were prepared.

1. Fresh seed non-primed
2. Fresh seed water primed (12,18, 24 & 30 hrs)
3. Fresh seed PEG primed (12,18, 24 & 30 hrs)
4. Accelerated aged seed non primed.

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5. Aged seed water primed (12,18, 24 & 30 hrs)
6. Aged seed PEG primed (12,18, 24 & 30 hrs)

The following observations were recorded on all the seed lots as per ISTA, 1999

1. Standard germination test (%)
2. Electrical conductivity ( $\mu\text{s/cm/seed}$  )
3. Rate of germination.
4. Dehydrogenase activity test (optical Density )
5. Seed vigor index- 1.
6. Seed vigor index-11.
7. Protein profile analysis (SDS-PAGE )

**Statistical analysis**

Experiment was conducted as completely randomized design and analysis of variance was done as per standard method by panse and Sukhatme (1967)

## CHAPTER - IV

# EXPERIMENTAL RESULTS

The result of the experiment entitled, “Effect of ageing and priming on seed storage proteins of Barley (*Hordeum vulgare* L.)” conducted in the laboratory of Seed Science and Technology of Chaudhary Charan Singh Haryana Agricultural University Hisar during the year 2003-04 are presented in this chapter with the help of tables, under following sub heads:

- 4.0 1000 seed weight of fresh seed lot of barley
- 4.1 To define variables for accelerated ageing at 42°C in barley
- 4.2 To define variables for accelerated ageing at 45°C in barley
- 4.3 To study variation of total and specific seed storage protein after ageing and priming.
- 4.4 To access the stability of improvement of seed vigor after priming.

### **4.0 1000 SEED WEIGHT OF FRESH SEED LOT OF BARLEY**

Table (1) shows the data of 1000 seed weight of fresh seed lot of barley. The 1000 seed weight was observed significantly different ranging from 32.805 to 58.543g. Maximum seed weight (58.54) was observed in variety BCU-73 and minimum (32.80) in variety BH 393.

#### **4.1 EFFECT OF ACCELERATED AGEING AT 42°C IN BARLEY**

##### **4.1.1 Seed moisture content**

The seed moisture content was recorded in percentage. The data pertaining to moisture content are shown in Table 3. The moisture content of barley among different accelerated ageing time was significantly different. Seed moisture content increases with time of ageing and it was recorded maximum (28.897) in the treatment of 72hrs on 42°C.

Among different varieties seed moisture content was recorded significantly different. Maximum moisture content was observed in variety K-551 (24.944) and minimum in variety BCU-73 (19.90).

##### **4.1.2 Standard germination**

The standard germination was recorded in percentage. The data pertaining to standard germination are shown in Table 2. The standard germination of barley among different accelerated ageing time was significantly different. The standard germination was significantly higher (37.3) in the treatment of 48 hrs ageing

than the 72 hrs ageing. Whereas, among different varieties the maximum standard germination was observed in BH-393 (86.8) followed by BCU-73 (81.3).

#### 4.1.3 Vigor index-I

The data pertaining to vigor index-I are depicted in Table 2. In barley vigor index-1 decreases with the increase of ageing time. Maximum vigor index -1 was observed at 48 hrs of ageing (1666.68). However, among different barley varieties Alfa-93, BH-393 and BCU-73 were at par with each other and they were significantly superior to other varieties. Whereas, minimum vigor index was recorded in RD-2552 (1445.33)

#### 4.1.4 Vigor index-II

Periodical data recorded on vigor index-II are shown in Table 2. Among different ageing time vigor index-11 was recorded significantly higher (713.94) at 48 hrs.

Among different variety, Alfa-93 and BH-393 showed higher vigor index-11.

#### 4.1.5 Electrical conductivity

The data related to electrical conductivity are shown in Table 3. It was recorded in  $\mu\text{s}/\text{cm}/\text{seed}$ . Electrical conductivity values showed non-significant differences in different ageing time however numerically lower value (49.737) was observed at 48 hrs.

**Table 1 : 1000 Seed Weight Of barley**

Varieties	1000 seed weight
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Alfa-93	43.919
BH-393	32.805
BH-75	43.010
BCU-73	58.543
RD-2552	45.690
K-551	51.979
S.E. (m)	0.315
C.D. at 5%	0.980

**Table 2 : Effect of accelerated ageing (42°C) on germination, vigor index-I and vigor index-II in barley**

Ageing time (hrs)	Germination (%)	Vigour index-1	Vigour index-11
0 hrs	98.00	2762.278	1263.889
48 hrs	37.00	1666.689	713.944
72 hrs	35.00	528.944	376.611
S.E.	0.6566	29.057	5.506
C.D. at 5 %	1.8836	83.363	15.798
Alfa-93	77.00	1805.822	849.444
BH-393	86.00	1712.889	837.556
BH-75	69.00	1642.000	789.778
BCU-73	81.00	1792.111	822.889
RD-2552	64.00	1445.333	668.222
K-551	63.00	1517.667	732.000
S.E.	0.985	41.093	7.787
C.D. 5%	2.6638	117.894	22.342

**Table 3 : Effect of accelerated ageing (42°C) on DHA, seed moisture and electrical conductivity in barley**

Ageing time (hrs)	Dehydrogenase activity	Seed moisture (%)	Electrical conductivity (µs/ cm/seed)
0 hrs	0.176	14.264	47.008
48 hrs	0.107	26.200	49.737
72 hrs	0.085	28.897	50.792
S.E.	0.0019	0.0612	N S
C.D. at 5 %	0.0054	0.1755	
Alfa-93	0.114	22.529	45.126
BH-393	0.118	23.532	26.008
BH-75	0.098	23.692	46.626
BCU-73	0.109	19.906	59.070
RD-2552	0.119	24.119	56.230
K-551	0.117	24.944	60.846
S.E.	0.0027	0.0865	2.3601

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C.D. 5%	0.0077	0.2483	6.7710
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The electrical conductivity among different varieties were significantly different. Among all the varieties electrical conductivity of BH-393 was recorded significantly lower (26.008).

#### **4.1.6 Dehydrogenase activity test**

Table 3 shows the data of dehydrogenase activity test. Among different ageing time, the dehydrogenase activity was decreasing with increase in ageing time. It was significantly higher (0.107) at 48hrs than 72 hrs.

However, in different varieties of barley Alfa-93, BH-393, RD-2552 and K-551 were found significantly higher than other varieties and they were at par with each other.

## **4.2 EFFECT OF ACCELERATED AGEING AT 45°C IN BARLEY**

### **4.2.1 Seed moisture content**

The seed moisture content was recorded in percentage. The data pertaining to moisture content are shown in Table 5. The moisture content of barley among different accelerated ageing time was significantly different. Seed moisture content increases with time of ageing and it was recorded maximum (26.701) in the treatment of 48hrs at 45°C. Among barley varieties seed moisture content was significantly different and maximum moisture content was observed in variety K-551 (21.533).

### **4.2.2 Standard germination**

The standard germination was recorded in percentage. The data pertaining to standard germination are shown in Table

4. The standard germination of barley decreases to 59.88 per cent at 48 hrs ageing. Among different varieties the maximum standard germination was observed in BH-393 (92.0) and RD-2552 followed it.

#### **4.2.3 Vigor index-I**

The data pertaining to vigor index-I are depicted in Table 4. Vigor index-I decreases during ageing.

However, among different barley varieties BH-393 and RD-2552 were at par with each other and they were significantly superior (1592.4) to other varieties. Whereas, minimum Vigor index-I was recorded in BCU-73.

#### **4.2.4 Vigor index-II**

Periodical data recorded on vigor index-II are shown in Table 4. Vigour index II also decrease during aging. Maximum vigor index – II (1022.5) was observed in BCU-73 and minimum (845.16) was recorded in RD-2552.

#### **4.1.5 Electrical conductivity**

The data related to electrical conductivity are shown in Table 5. It was recorded in  $\mu\text{s}/\text{cm}/\text{seed}$ . The electrical conductivity among different treatments was significantly different, electrical conductivity increased with accelerated ageing.

Among different barley varieties minimum electrical conductivity (27.393) was recorded in BH-393.



**Table 4 : Effect of accelerated ageing (45°C) on germination, vigor index-I and vigor index-II in barley**

Ageing time (hrs)	Germination (%)	Vigour index-1	Vigour index-II
0 hrs	98.756	2762.276	1263.889
48 hrs	59.889	715.667	558.500
S.E.	0.5232	28.485	6.642
C.D. at 5 %	1.5274	83.165	19.393
Alfa-93	84.000	1716.333	951.667
BH-393	92.000	1784.166	894.167
BH-75	77.000	1672.166	895.667
BCU-73	67.000	1592.166	1022.500
RD-2552	85.000	1929.000	845.167
K-551	70.000	1740.000	858.000
S.E.	0.906	49.338	11.505
C.D. 5%	2.645	144.046	33.589

**Table 5 : Effect of accelerated ageing (45°C) on DHA, seed moisture and electrical conductivity in barley**

Ageing time (hrs)	Dehydrogenase activity	Seed moisture (%)	Electrical conductivity ( $\mu\text{s/cm/seed}$ )
0 hrs	0.176	14.225	47.008
48 hrs	0.111	26.701	52.320
S.E.	0.002	0.0406	1.481
C.D. at 5 %	0.006	0.118	4.320
Alfa-93	0.134	20.227	42.237
BH-393	0.154	19.555	27.393
BH-75	0.110	21.472	48.820
BCU-73	0.145	18.350	61.237
RD-2552	0.145	21.592	58.393
K-551	0.171	21.533	59.903
S.E.	0.003	0.70	2.566

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C.D. 5%	0.011	0.205	7.493
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#### **4.1.6 Dehydrogenase activity test**

Table 5 shows the data of dehydrogenase activity test. Dehydrogenase activity also decreases with accelerated ageing.

However, out of different varieties of barley dehydrogenase activity was recorded significantly higher (0.171) in variety K-551 than other varieties.

#### **4.3 TO STUDY THE VARIATION OF TOTAL AND SPECIFIC SEED STORAGE PROTEIN AFTER AGEING AND PRIMING**

##### **4.3.1 Protein profile of fresh seed lot of Barley**

The seed protein profile of fresh seed lot of barley genotypes resulted in 19 bands with relative mobility ranging from 0.13 to 0.94 with molecular weight ranging from 165 kilodaltons to 470 kilodaltons (Table 8). Band Number 3, 4, 5, 8, 9, 10, 11, 12, 14, 17, 18 and 19 were common in all the genotypes. However band no. 6 was specific to genotype BH-393. Band number 15 was present only in genotypes ALFA-93, BH-393 and RD-2552. Band number 13 was absent in genotype RD-2552. Band number 7 was absent in genotype K-551.

Similarity index based on the relative mobility of protein band and molecular weight indicated a close association among different genotypes tested. The similarity index values varied from 0.516 to 0.929 (Table 9). The most closely associated genotypes were RD-2552 and K-551(92%), BCU-73 and

K-551(86.7%), BH-393 and BCU-73(87%). Among the 6 genotype RD-2552 and K-551 had maximum similarly index i.e. 92%.

#### **4.3.2 Protein profile of AGED seed lot of barley At 42°C for 48 hrs**

The seed lot was exposed to accelerated ageing at 42°C for 48 hrs and change in seed storage profile was observed as compared to fresh seed lot of different genotypes. The data revealed that in accelerated aged seed lot, the total number of seed protein bands were observed to be 17 instead of 19 as observed in fresh seed lot. The relative mobility of various bands varied from 0.135 to 9.41 and molecular weight from 165 kilodaltons to 470 kilodaltons. Band No, 3, 4, 9, 10, 11, 12, 14, 18, 19 common in all genotypes as in fresh seed lots. Band number 8 was common in fresh seed lot of the all varieties but it was absent in aged seed lot. It is due the to effect of ageing and it was found absent in variety BH-393, After 48 hrs. ageing at 42°C Band No. 15 was found to be present in fresh seed lot of variety ALFA-93, BH-393 and RD-2552, and observed absent after ageing of fresh seed lot. Band number 16 was recorded specific for genotype-ALFA-93, BH-393 in fresh seed but it disappeared after ageing. It showed that accelerated ageing resulted in variability in seed storage protein profile and could reveal the hidden diversity present in the genotypes for stress tolerance.

The similarity index based on relative mobility of protein bands ranged from 0.296 to .741 (Table 9) which was much wider than the fresh seed lot (0.561 to 0.929). According to the storage seed protein profile in the Aged seed, most diverse genotypes were ALFA-93 and BCU-73, ALFA-93 and RD-2552 while most closely related genotypes were RD-2552 and K-551.

#### **4.3.3 Protein profile of AGED seed lot of Barley at 42°C for 72 hrs**

The seed lot was exposed to Accelerated ageing at 42°C for 72 hrs and change in seed storage protein was observed. The data revealed that in accelerated aged seed lot, the total no. of seed protein bands were observed to be 14 instead of 19 as observed in fresh seed lot (Table 6). The relative mobility of various bands varied from 0.135 to 0.941 and molecular weight from 17.2 kilodaltons to 470 kilodaltons (Table 8). Band No. 3, 9, 10, 11, 12, 14, 17, 18 and 19 were observed common in all genotypes. Band No. 15 was observed only in fresh genotypes of BH-393, ALFA-93 and RD-2552 was recorded totally absent after ageing at 42°C for the 72hrs. Band no. 16 and 18 present in fresh genotypes of BH-393 and ALFA-93 were lost after 72hrs ageing (Table 8). It might be due to effect of ageing. Band No.

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 M

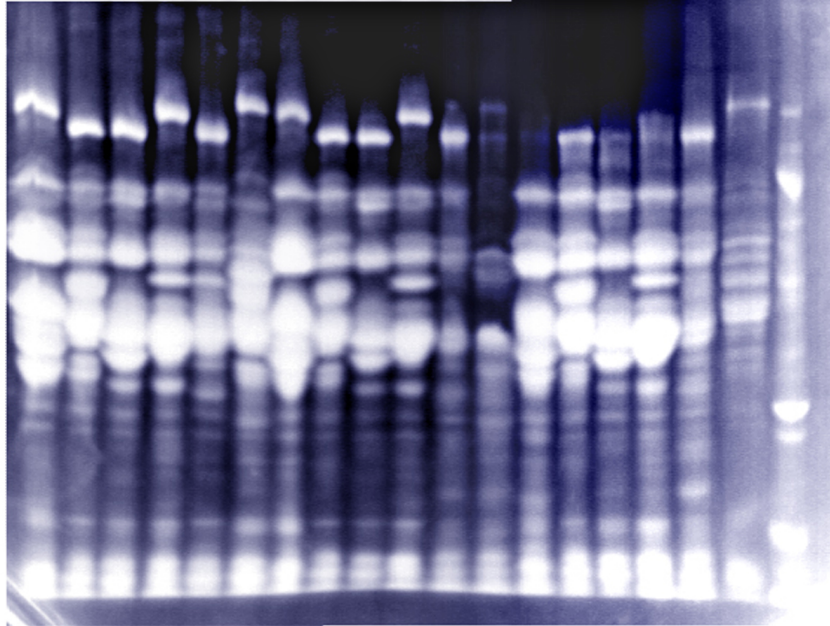


Plate 1 Total seed storage protein profiles of fresh and accelerated aged (42<sup>0</sup>C) seed of Barley genotypes

M 1 2 3 4 5 6 7 8 9 10 11 12

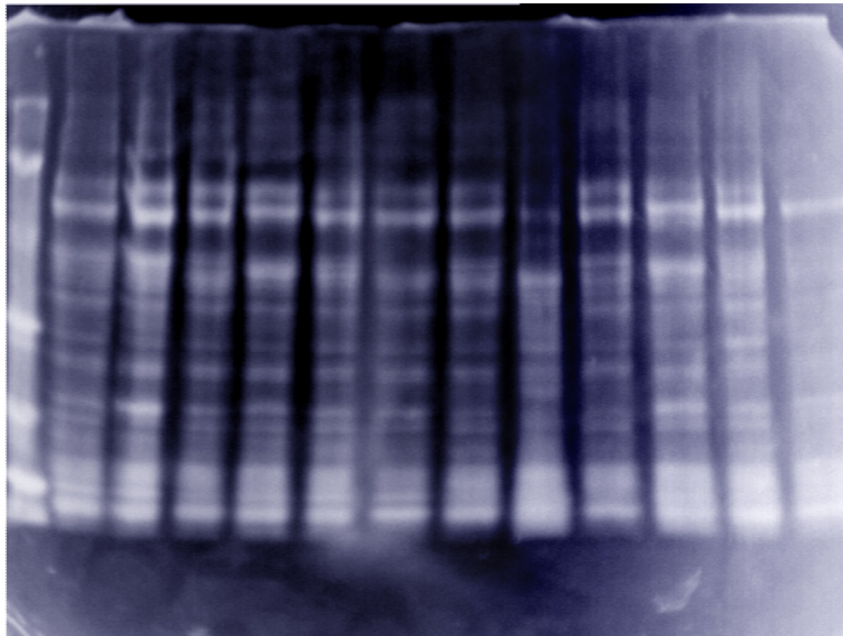


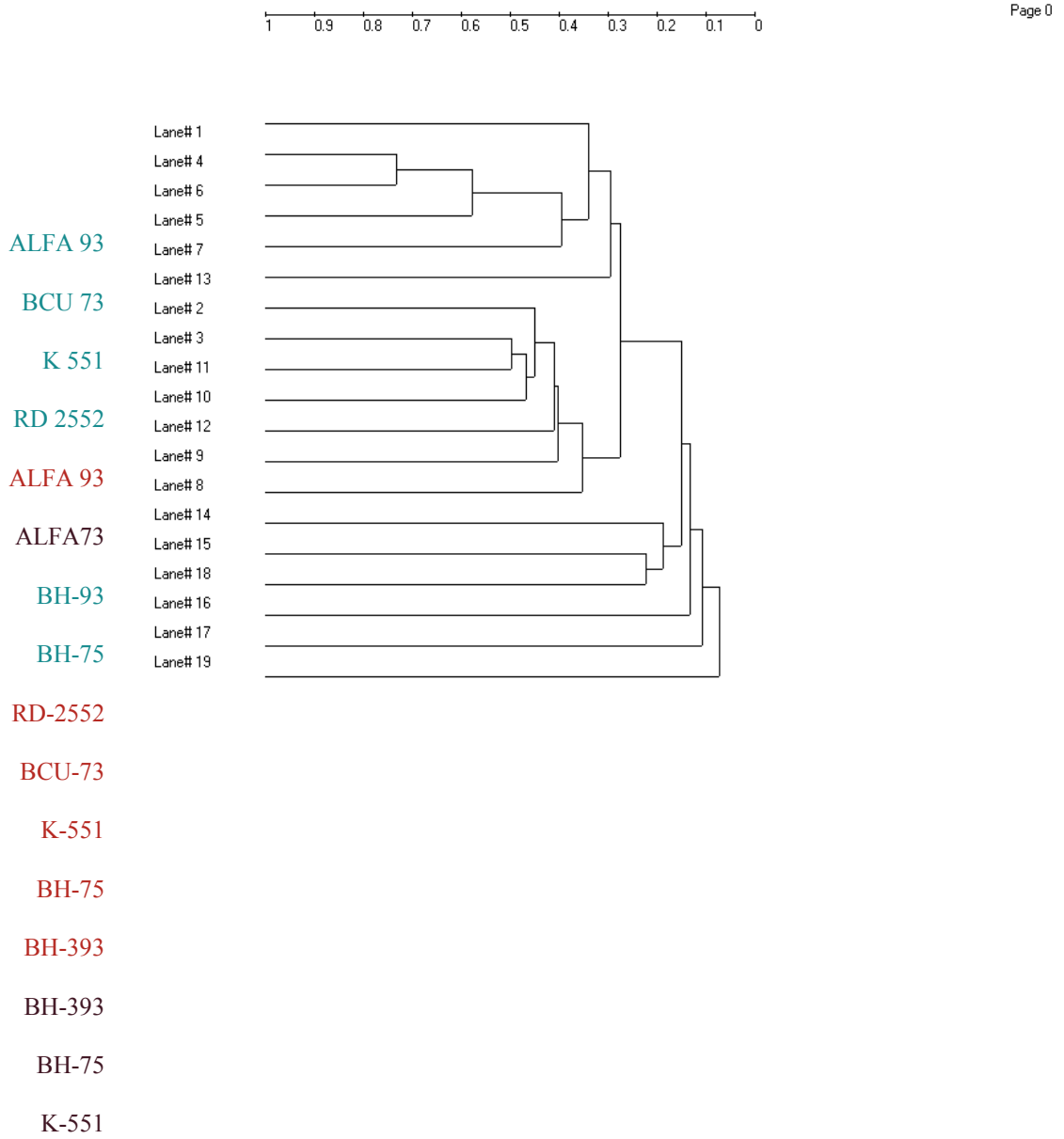
Plate 2 Total seed storage protein profiles of fresh and accelerated aged (45<sup>0</sup>C) seed of Barley genotypes

**Table 6 : Protein profile of total seed soluble Proteins by SDS-PAGE after ageing at different time interval on 42°C in barley**

<b>Variety</b>	<b>Number of bands in fresh lot</b>	<b>Number of bands in aged variety at 48 hrs</b>	<b>Number of bands in aged variety at 72 hrs</b>
Alfa-93	16	14	13
BH-393	19	17	15
BH-75	16	14	14
BCU-73	16	16	14
RD-2552	16	14	13
K-551	14	13	13

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Table 7: Dendrogram of accelerated aged at 42°C (protein)



1 to 6 are fresh varieties

7 to 12 are accelerated aged for 48 hrs

13 to 19 are accelerated aged for 72 hrs

**Table 8: Band map of protein of fresh and aged seed lot (42°C) of barley based on RM values.**

S.No.	RMValues	L1	L2	L3	L4	L5	L6	L7	L8	+L9	L10	L11	L12	L13	L14	L15	L16	L17	L18
1	0.135	-	+	+	+	+	-	-	+	-	+	-	-	-	-	-	-	-	-
2	0.188	-	+	+	+	+	+	-	+	-	+	+	-	-	-	-	+	+	+
3	0.229	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
4	0.300	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+
5	0.340	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+	-	-	-
6	0.370	-	+	-	-	-	-	-	+	-	-	-	+	-	+	-	-	+	+
7	0.441	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	-
8	0.470	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	-
9	0.494	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
10	0.517	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
11	0.594	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
12	0.652	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
13	0.672	+	+	+	+	-	+	+	+	+	+	-	+	+	+	+	+	-	+
14	0.758	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
15	0.788	+	+	-	-	+	-	-	-	-	-	+	-	-	-	-	-	-	-
16	0.823	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
17	0.852	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
18	0.900	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
19	0.941	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

ALFA-93,BH-393,BH-75,BCU-73,RD-2552,K-551(Fresh Genotypes),

ALFA-93,BH-393,BH-75,BCU-73,RD-2552,K-551(Aged for 48 hrs)

ALFA-93,BH-393,BH-75,BCU-73,RD-2552,K-551(Aged for 72 hrs)

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**Table 9: Similarity index of protein 42°C**

		Alfa-93	BH-393	BH-75	BCU-73	RD-2552	K-551	Alfa-93	BH-393	BH-75	BCU-73	RD-2552	K-551	Alfa-93	BH-393	BH-75	BCU-73	RD-2552	K-551	Marker	
	no	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	
Alfa-93	1	1																			
BH-393	2	0.788	1.000																		
BH-75	3	0.581	0.667	1.000																	
BCU-73	4	0.667	0.875	0.667	1.000																
RD-2552	5	0.710	0.533	0.786	0.733	1.000															
K-551	6	0.516	0.733	0.857	0.867	0.929	1.000														
Alfa-93	7	0.600	0.690	0.519	0.759	0.667	0.667	1.000													
BH-393	8	0.400	0.552	0.815	0.621	0.593	0.667	0.615	1.000												
BH-75	9	0.200	0.552	0.593	0.414	0.519	0.444	0.385	0.538	1.000											
BCU-73	10	0.387	0.733	0.643	0.667	0.643	0.643	0.296	0.741	0.667	1.000										
RD-2552	11	0.483	0.643	0.769	0.429	0.692	0.385	0.400	0.560	0.560	0.615	1.000									
K-551	12	0.581	0.533	0.500	0.467	0.500	0.571	0.370	0.519	0.667	0.500	0.692	1.000								
Alfa-93	13	0.483	0.714	0.615	0.571	0.615	0.538	0.400	0.640	0.320	0.615	0.500	0.538	1.000							
BH-393	14	0.667	0.414	0.148	0.552	0.444	0.519	0.615	0.231	0.077	0.222	0.320	0.370	0.480	1.000						
BH-75	15	0.345	0.143	0.077	0.357	0.077	0.308	0.480	0.320	0.080	0.000	0.167	0.308	0.333	0.560	1.000					
BCU-73	16	0.387	0.400	0.286	0.333	0.214	0.214	0.222	0.148	0.222	0.214	0.308	0.143	0.154	0.296	0.462	1.000				
RD-2552	17	0.286	0.074	0.160	0.222	0.240	0.320	0.167	0.250	0.250	0.320	0.174	0.160	0.174	0.167	0.087	0.480	1.000			
K-551	18	0.267	0.276	0.519	0.345	0.222	0.370	0.308	0.385	0.462	0.296	0.400	0.519	0.240	0.154	0.320	0.148	0.417	1.000		
Marker	19	0.261	0.364	0.100	0.182	0.100	0.100	0.105	0.211	0.211	0.200	0.111	0.300	0.222	0.316	0.222	0.200	0.000	0.105	1.000	

1 to 6 fresh varieties

7 to 12 are accelerated aged for 48 hrs

13 to 18 are accelerated aged for 72 hrs

8 was specifically absent in all the genotypes after ageing. It showed that the ageing time also affects the banding pattern of variety. The similarity index based on presence or absence of protein bands ranged from 0.087 to 0.417 (Table 9) which was much diverse than fresh seed lot (0.561 to 0.929). According to the storage seed protein profile in aged seed, the most diverse Genotypes were BH-75 and RD-2552 (8.7%), ALFA-93 and BCU-73 (14.3%), ALFA-93 and RD-2552 (16%), K-551 and BH-393 (15.4%), while most closely related Genotypes were BCU-73 and BH-75 (46.2%), BH-393 and ALFA-93 (48.0%).

#### **4.3.4 Protein profile of aged seed lot of Barley aged at 45°C for 48 hrs**

The seed lot of Aged genotypes was exposed to Accelerated ageing and change in seed storage profile was also observed. The data revealed that in Accelerated Aged seed lot, the total number of seed protein bands were observed to be 12 instead of 19 as observed in fresh seed lot. The relative mobility of various bands varied from 0.135 to 0.941 (Table 12) and molecular weight ranged from 17.2 kilodaltons to 470 kilodaltons. Band No. 3, 18, 19 were common in all genotypes. However, Band No. 5 was present only in BH-393. Band No. 5 was present all the fresh genotype but after ageing was observed consistent in BH-393. It was due to effect of ageing. However Band No. 10 is

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specifically absent in aged seed lot of RD-2552 which was present in fresh seed lot of this variety (Table 12).

The similarity index based on presence or absence of protein bands ranged from (0.435 to 0.87) (Table 13) which was quite different from the fresh seed lot (0.561 to 0.929). According to the storage seed protein profile in Aged seed lot, most diverse genotypes were RD-2552 and BH-75 (43.5%), BH-75 and K-551 (43.5%), BH-75 and BCU-73 (43.5%) while most closely related genotypes were BH-393 and ALFA-93 (87%), BCU-73 and BH-393 (81.8%).

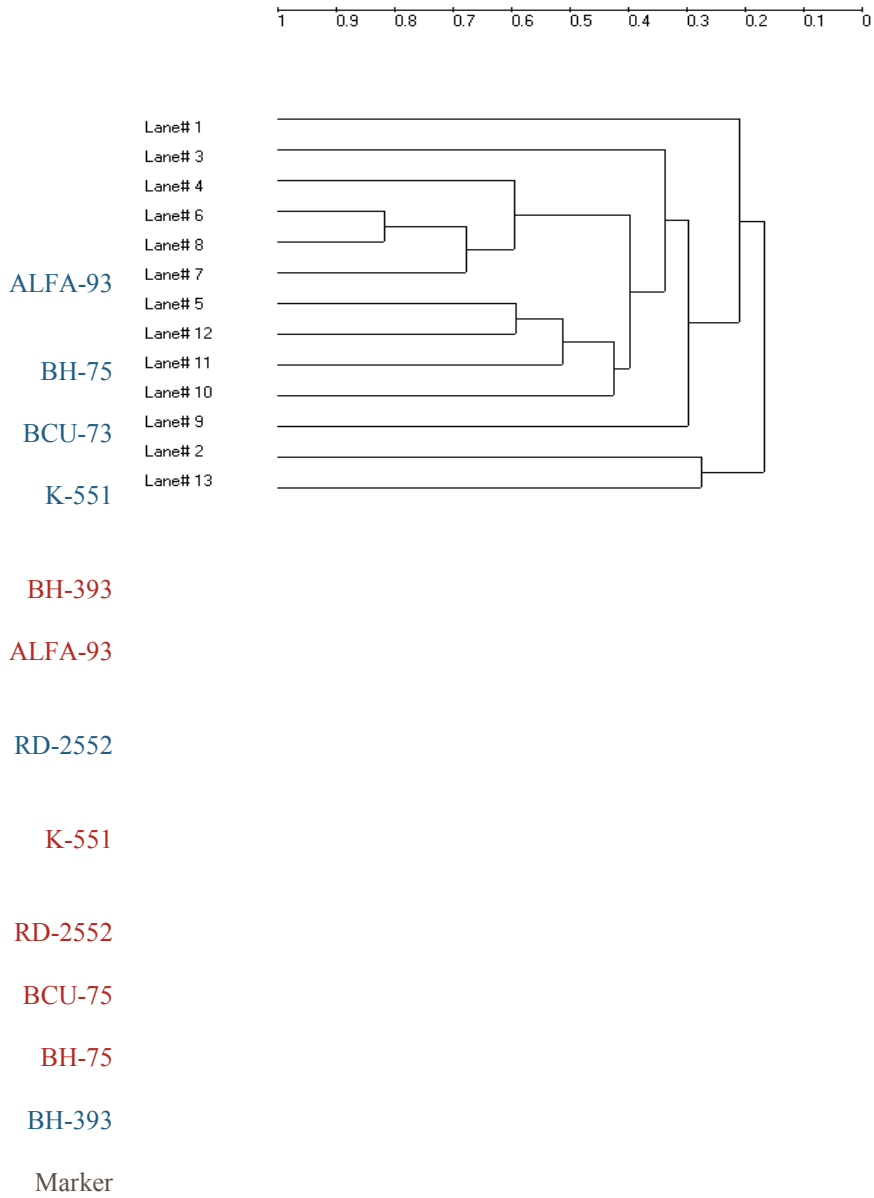
The protein data was converted into binary data as 1 for presence and 0 for absence which was further used to construct a dendrogram. The dendrogram of total seed storage revealed three clusters. Cluster 1 comprised of 6 genotypes Fresh ALFA-93, Fresh BCU-73, Fresh K-551, Fresh RD-2552, Aged ALFA-93 (48hrs), Aged ALFA-93 (72hrs), cluster 2 comprised of 7 genotypes Fresh BH-75, Aged RD-2552 (48 hrs.), Aged BCU-73 (48hrs), Aged K-551 (48hrs), Aged BH-75 (48hrs), Aged BH-393 (48hrs) and cluster 3 comprised of 3 genotypes Aged BH-393 (72hrs), Aged BH-75 (72hrs), Aged K-551 (72hrs), Aged. Two genotypes were diverse not falling under any cluster and these were Aged BCU-73 (72 hrs.) and Aged RD-2552 (72hrs).

Fresh and Aged genotypes of some variety most present in same cluster but in all the varieties change in

**Table 10: Protein profile of total seed soluble Proteins by SDS-PAGE after ageing at different time interval on 45°c in barley**

<b>Variety</b>	<b>Number of bands in fresh lot</b>	<b>Number of bands in aged variety at 48 hrs</b>
Alfa-93	16	10
BH-393	19	10
BH-75	16	10
BCU-73	16	10
RD-2552	16	11
K-551	14	12

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**Table 11: Dendogram of accelerated aged at 45°C (Protein)**

1 to 6 are fresh varieties

7 to 12 are accelerated aged for 48 hrs

**Table 12: Band Map of protein profile of fresh and accelerated aged at 45°C for 48hrs.**

No.	Rm value	Lane1	Lane2	Lane3	Lane4	Lane5	Lane6	Lane7	Lane8	Lane9	Lane10	Lane11	Lane12
1	0.135	-	+	+	+	+	-	-	-	-	-	-	-
2	0.188	-	+	+	+	+	+	-	-	-	+	+	+
3	0.229	+	+	+	+	+	+	+	+	+	+	+	+
4	0.300	+	+	+	+	+	+	-	+	+	-	-	+
5	0.340	+	+	+	+	+	+	-	+	-	-	-	-
6	0.370	-	+	-	-	-	-	+	-	-	-	+	+
7	0.441	+	+	+	+	+	-	+	-	+	+	+	-
8	0.470	+	+	+	+	+	+	-	+	+	+	+	-
9	0.494	+	+	+	+	+	+	-	-	-	+	+	+
10	0.517	+	+	+	+	+	+	+	+	+	+	-	+
11	0.594	+	+	+	+	+	+	+	+	+	-	-	+
12	0.652	+	+	+	+	+	+	-	-	-	-	+	+
13	0.672	+	+	+	+	-	+	+	+	+	+	-	+
14	0.750	+	+	+	+	+	+	+	+	+	+	+	+
15	0.788	+	+	-	-	+	-	-	-	-	-	-	-
16	0.823	+	+	-	-	-	-	-	-	-	-	-	-
17	0.852	+	+	+	+	+	+	+	-	-	-	+	-
18	0.900	+	+	+	+	+	+	+	+	+	+	+	+
19	0.941	+	+	+	+	+	+	+	+	+	+	+	+

ALFA-93,BH-393,BH-75,BCU-73,RD-2552,K-551(Fresh Genotypes)

ALFA-93,BH-393,BH-75,BCU-73,RD-2552,K-551(Aged for 48 hrs)

**Table 13: Similarity index Protein 45°C**

		<b>Alfa-93</b>	<b>BH-393</b>	<b>BH-75</b>	<b>BCU-73</b>	<b>RD-2552</b>	<b>K-551</b>	<b>Alfa-93</b>	<b>BH-393</b>	<b>BH-75</b>	<b>BCU-73</b>	<b>RD-2552</b>	<b>K-551</b>	<b>Marker</b>
		1	2	3	4	5	6	7	8	9	10	11	12	13
<b>Alfa-93</b>	1	1.000												
<b>BH-393</b>	2	0.111	1.000											
<b>BH-75</b>	3	0.211	0.480	1.000										
<b>BCU-73</b>	4	0.333	0.250	0.720	1.000									
<b>RD-2552</b>	5	0.118	0.261	0.500	0.522	1.000								
<b>K-551</b>	6	0.125	0.273	0.609	0.545	0.571	1.000							
<b>Alfa-93</b>	7	0.111	0.333	0.480	0.500	0.348	0.273	1.000						
<b>BH-393</b>	8	0.235	0.348	0.417	0.522	0.636	0.476	0.522	1.000					
<b>BH-75</b>	9	0.111	0.417	0.800	0.500	0.522	0.273	0.417	0.522	1.000				
<b>BCU-73</b>	10	0.222	0.417	0.480	0.750	0.522	0.455	0.333	0.435	0.500	1.000			
<b>RD-2552</b>	11	0.533	0.476	0.364	0.381	0.300	0.316	0.571	0.500	0.381	0.476	1.000		
<b>K-551</b>	12	0.375	0.455	0.522	0.455	0.762	0.600	0.182	0.571	0.455	0.273	0.316	1.000	
<b>Marker</b>	13	0.333	0.417	0.160	0.083	0.261	0.273	0.333	0.174	0.333	0.250	0.381	0.273	1.000

banding pattern was recorded (Table 7). It may be due to effect of Ageing.

Cluster analysis of seed storage protein profile of Fresh and Aged genotype of 6 varieties aged at 45°C for 48hrs were observed and in Aged seed lot (Table 11), the diverse wideness among the genotypes. The cluster were identified from dendrogram which revealed 3 cluster. Cluster I comprised of 5 genotypes; Fresh BH-75, Fresh BCU-73, Fresh K-551, Aged ALFA-93 (48hrs), Aged BH-393(48hrs), cluster 2 comprised of 4 genotypes Fresh-RD-2552, Aged K-551 (48hrs), Aged RD-2552 (48hrs), Aged BCU-73 (48hrs) and Aged BH-75 (48hrs) two genotypes were diverse from all other, these were Fresh BH-393 and Aged BH-75 (48hrs) Fresh and aged genotypes of same variety should present in same cluster but change in banding pattern was observed in all varieties . So it may be due to effect of Ageing.

#### **4.3.5 Hordein Protein profile of fresh seed lot of Barley**

The seed Hordein protein profile of fresh seed lot of various barley genotypes resulted in 19 Bands with relative mobility ranging from 0.169 to 0.974 (Table 16) and molecular weight ranging from 16.5 kilodaltons to 470 kilodaltons. The Band No. 7,9, 10, 11, 12, 13, 17, 18 and 19 were common in all the genotypes. However Band No. 5 was specific to genotype ALFA-93, Band No. 14 was specific to genotypes ALFA-93, BH-75 and K-551; However band No. 2 was absent in genotypes ALFA-93 and

RD-2552. Band No. 6 was absent from genotype BH-75 and RD-2552. Band No. 8 was absent from genotypes ALFA-93 and BH-75 (Table 16).

The similarity index based on the relative mobility of protein band and molecular weight indicated a close association. Among various genotypes tested, the similarity index varied from 0.400 to 0.733 (Table 17). The most closely associated genotypes were BH-75 and RD-2552 (73.3%) and most diverse genotypes were RD-2552 and ALFA-93 (40%).

#### **4.3.6 Hordein protein profile of barley aged at 42°C for 48 hrs**

The seed lot was exposed to accelerated ageing and change in seed storage hordein protein profile was also observed. The data revealed that in accelerated aged seed lot, the total number of hordein protein bands were observed 14 instead of 19 bands in fresh one (Table 15).

Band No. 7, 10, 12, 13, 18 and 19 were common in all genotypes. Band No. 3 was specifically present in Genotype BH-393 this band also was observed in fresh seed lot of BH-75 and K-551 but this band disappeared in these varieties after ageing. It may be due to affect of ageing. Band No. 5 was present in genotype BH-75, band No 6 was specifically present in genotype BH-75 which was also observed in fresh seed lot of genotype ALFA-93 and BH-393



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1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 M

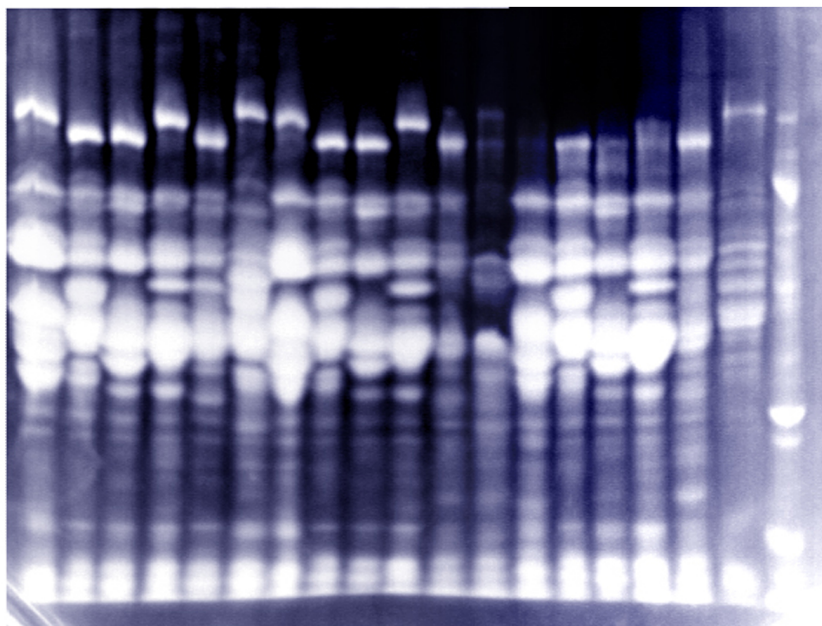


Plate 3 Specific seed storage protein (hordein) profiles of fresh and accelerated aged (42<sup>0</sup>C) seed of Barley genotypes

M 1 2 3 4 5 6 7 8 9 10 11 12

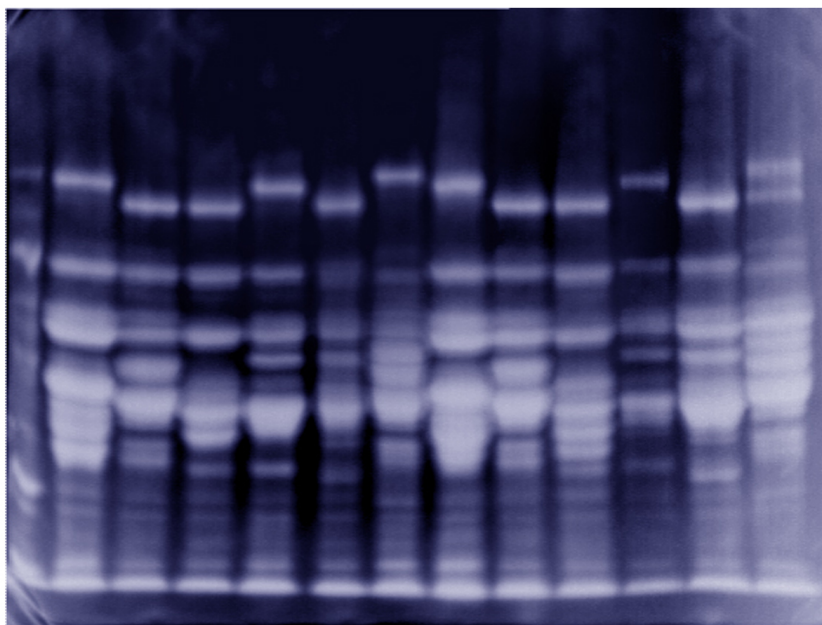


Plate 4 Specific seed storage protein (hordein) profiles of fresh and accelerated aged (45<sup>0</sup>C) seed of Barley genotypes

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before ageing. Bands 15 and 16 were present only in genotype ALFA-93 (Table 16).

The similarity index based on relative mobility of hordein protein bands ranged from 0.376 to 0.667 (Table 17). According to storage protein profile in aged seed lot the most diverse genotypes were RD-2552 and K-551 (37%) while most closely related genotypes were RD-2552 and ALFA-93 (66.7%), BH-393 and BCU-73 (66.7%).

**4.3.7 Hordein protein profile of barley aged at 42°C for 72 hrs.**

Seed lot was exposed to accelerated ageing and storage hordein protein profile was observed. The total number of bands were observed 9 instead of 19 as observed in fresh seed lot (Table 15). The relative mobility varied from 0.169 to 0.974 (Table 17). Only one band at 10 was observed common to all the genotypes. Band No. 3 was specifically present in BH-75 which was also observed in fresh seed lot of genotype K-551. Bands No. 4 and 7 were absent in genotype K-551. Bands 15, 16 were specially absent in all the genotypes while it was observed to be present in fresh and aged seed lot of genotype ALFA-93. It may be due to the effect of ageing (Table 16).

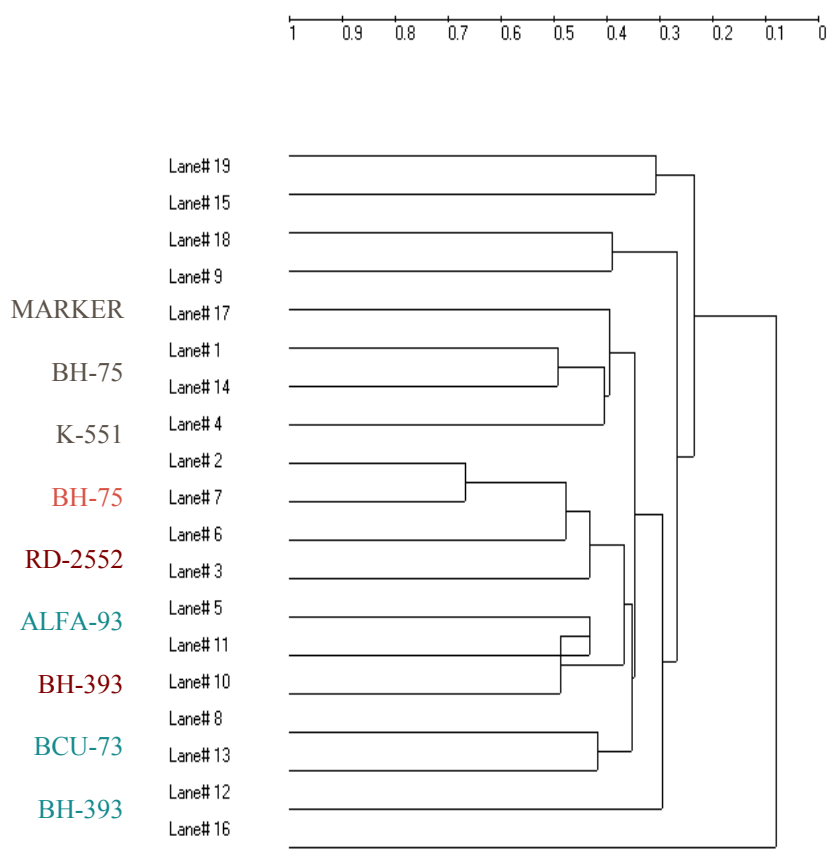
The similarity index based on relative mobility of protein bands ranged from 0.105 to 0.593 (Table 17). According to storage seed hordein protein profile in aged

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**Table 14: Protein profiles of total seed soluble hordein protein by SDS-PAGE after ageing at different time interval on 42°C in barley.**

<b>Variety</b>	<b>Number of bands in fresh lot</b>	<b>Number of bands in aged variety at 48 hrs</b>	<b>Number of bands in aged variety at 72 hrs</b>
ALFA-93	15	14	10
BH-393	14	13	13
BH-75	14	11	11
BCU-73	13	12	12
RD-2552	12	10	9
K-551	13	9	8

Table 15: Dendrogram of accelerated aged at 42°C (Hordein)



1 to 6 are fresh varieties

7 to 12 are accelerated aged for 48 hrs

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13 to 19 are accelerated aged for 72 hrs

**Table 16: Band map of Hordein protein of fresh and aged seed lot (42°C) of barley based on RM values.**

S.No.	RMValues	L1	L2	L3	L4	L5	L6	L7	L8	+L9	L10	L11	L12	L13	L14	L15	L16	L17	L18
1	0.169	+	-	-	+	-	+	+	-	-	+	-	+	-	-	-	+	-	+
2	0.205	-	+	+	-	+	-	-	+	+	-	+	-	-	+	+	-	+	-
3	0.282	-	+	+	-	-	+	-	+	-	-	-	-	-	+	-	-	-	-
4	0.307	+	+	+	+	+	-	+	+	+	+	+	-	+	+	+	+	+	-
5	0.338	-	+	+	-	-	-	-	-	+	-	-	-	-	-	+	-	-	-
6	0.379	+	-	-	+	-	-	-	+	-	-	-	-	+	+	-	-	-	-
7	0.430	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
8	0.461	-	+	-	+	+	+	-	-	-	+	-	+	-	-	-	+	-	+
9	0.492	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	-
10	0.543	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
11	0.574	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+	+	+
12	0.600	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
13	0.651	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+
14	0.692	+	-	+	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-
15	0.717	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
16	0.764	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
17	0.866	+	+	+	+	+	+	+	+	-	+	-	+	+	+	-	+	+	+
18	0.912	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+
19	0.974	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-

ALFA-93,BH-393,BH-75,BCU-73,RD-2552,K-551(Fresh Genotypes),

ALFA-93,BH-393,BH-75,BCU-73,RD-2552,K-551(Aged for 48 hrs)

ALFA-93,BH-393,BH-75,BCU-73,RD-2552,K-551(Aged for 72 hrs)

**Table 17: Similarity index of Hordein at 42°C**

	marker	Rd-2552	K-551	Alfa-93	BH-393	BH-75	BCU-73	RD-2552	K-551	Alfa-93	BH-393	BH-75	BCU-73	RD-2552	K-551	Alfa-93	BH-393	BH-75	BCU-73	
	19	18	17	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	
Marker	19	1.000																		
K-551	18	0.563	1.000																	
RD-2552	17	0.414	0.387	1.000																
Alfa-93	1	0.333	0.563	0.621	1.000															
BH-393	2	0.267	0.625	0.690	0.667	1.000														
BH-75	3	0.400	0.313	0.552	0.400	0.533	1.000													
BCU-73	4	0.276	0.452	0.643	0.621	0.552	0.552	1.000												
RD-2552	5	0.600	0.313	0.414	0.400	0.400	0.733	0.483	1.000											
K-551	6	0.286	0.533	0.667	0.571	0.643	0.500	0.667	0.429	1.000										
Alfa-93	7	0.400	0.563	0.483	0.733	0.733	0.467	0.414	0.533	0.571	1.000									
BH-393	8	0.345	0.452	0.500	0.759	0.621	0.552	0.571	0.690	0.519	0.621	1.000								
BH-75	9	0.286	0.533	0.222	0.500	0.714	0.500	0.519	0.286	0.692	0.571	0.667	1.000							
BCU-73	10	0.500	0.467	0.444	0.643	0.357	0.571	0.519	0.643	0.462	0.571	0.667	0.462	1.000						
RD-2552	11	0.667	0.500	0.483	0.533	0.533	0.733	0.414	0.733	0.357	0.667	0.552	0.286	0.643	1.000					
K-551	12	0.444	0.276	0.615	0.370	0.444	0.519	0.385	0.296	0.640	0.444	0.385	0.400	0.400	0.370	1.000				
Alfa-93	13	0.500	0.467	0.519	0.643	0.571	0.429	0.519	0.571	0.385	0.571	0.593	0.462	0.769	0.571	0.560	1.000			
BH-393	14	0.690	0.645	0.643	0.621	0.621	0.414	0.571	0.483	0.667	0.690	0.500	0.370	0.519	0.414	0.538	0.593	1.000		
BH-75	15	0.643	0.467	0.222	0.357	0.357	0.214	0.222	0.214	0.308	0.286	0.296	0.385	0.462	0.286	0.320	0.462	0.370	1.000	
BCU-73	16	0.000	0.174	0.100	0.095	0.095	0.286	0.300	0.190	0.211	0.095	0.200	0.105	0.211	0.190	0.333	0.105	0.200	0.105	1.000

1 to 6 fresh varieties

7 to 12 are accelerated aged for 48 hrs

13 to 18 are accelerated aged for 72 hrs

seed, the most diverse genotype were BH-75 and BCU-73 (10.5%) while most closely related genotypes were BH-393 and ALFA-93 (59.3%).

#### **4.3.8 Hordein protein profiles of barley aged at 45°C for 48hrs**

Seed lot was exposed to Accelerated ageing at 45°C for 48 hrs and storage hordein protein profile was observed. Total number of bands were observed to be 10 instead of 19 as observed in fresh seed lot (Table 18). The relative mobility varied from 0.169 to 0.974 (Table 20). Band No. 9, 17, 18 were observed common in all genotypes. Band No. 5 was absent in all genotypes while it was present in fresh seed lot of genotype BH-75. Bands number 16, 17, were specifically absent in all genotypes while these were observed in fresh lot, it may be due to the effect of ageing (Table 18).

The similarity index based on relative mobility of hordein protein bands ranged from 0.179 to 0.571. According to storage hordein protein profile in aged seed lot, of the most diverse genotypes are ALFA-93 and K-551 (18%), while most closely related genotypes were ALFA-93 and RD-2552 (47.1%) (Table 21).

Cluster analysis of seed storage Hordein protein profile of fresh and aged seed lot were identified from dendrogram (Table

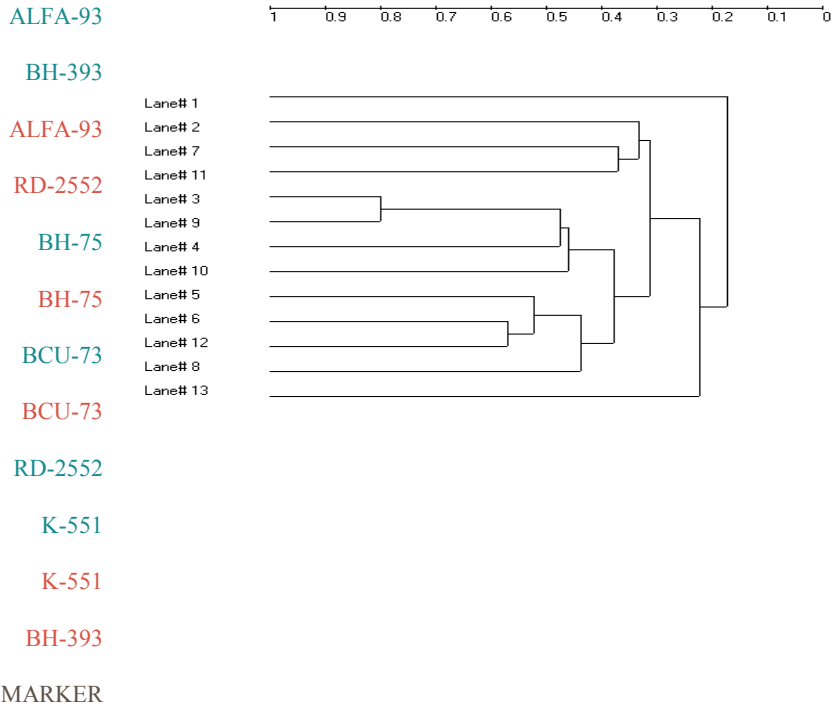
*EXPERIMENTAL RESULTS*

15) which revealed 4 clusters and comprised of 3 genotypes aged BH-75(72hrs) and Aged BH-75

**Table 18: Protein profiles of total seed soluble hordein protein by SDS-PAGE after ageing at different time interval on 45°C in barley.**

Variety	Number of bands in fresh lot	Number of bands in aged variety at 48 hrs
ALFA-93	15	7
BH-393	14	10
BH-75	14	12
BCU-73	13	10
RD-2552	12	9
K-551	13	10

Table 19: Dendrogram of accelerated aged at 45°C (Hordein)



1 to 6 are fresh

7 to 12 are acce

**Table 20: Band Map of Hordein profile of fresh and accelerated aged at 45°C for 48hrs.**

No.	Rm value	Lane1	Lane2	Lane3	Lane4	Lane5	Lane6	Lane7	Lane8	Lane9	Lane10	Lane11	Lane12
1	0.169	+	-	-	+	-	+	-	-	-	+	-	+
2	0.205	-	+	+	-	+	-	-	+	+	-	+	-
3	0.282	-	+	+	-	-	+	-	-	-	-	-	-
4	0.307	+	+	+	+	+	-	+	+	+	+	+	-
5	0.338	-	-	+	-	-	-	-	-	-	-	-	-
6	0.379	+	+	-	+	-	-	+	+	+	-	-	-
7	0.430	+	+	+	+	+	+	-	-	-	+	+	-
8	0.461	-	+	-	+	+	+	-	-	-	+	-	+
9	0.492	+	+	+	+	+	+	+	+	+	+	+	+
10	0.543	+	+	+	+	+	+	-	+	-	+	+	+
11	0.574	+	+	+	+	+	+	-	-	+	-	+	+
12	0.600	+	+	+	+	+	+	+	+	+	-	-	+
13	0.651	+	+	+	+	+	+	+	+	+	+	-	+
14	0.692	+	-	+	-	-	+	-	-	-	-	-	-
15	0.717	+	-	-	-	-	-	-	-	-	-	-	-
16	0.764	+	-	-	-	-	-	-	-	-	-	-	-
17	0.866	+	+	+	+	+	+	-	+	+	+	+	+
18	0.912	+	+	+	+	+	+	+	+	+	+	+	+
19	0.974	+	+	+	+	+	+	+	+	+	+	+	+

ALFA-93,BH-393,BH-75,BCU-73,RD-2552,K-551(Fresh Genotypes)

ALFA-93,BH-393,BH-75,BCU-73,RD-2552,K-551(Aged for 48 hrs)

**Table 21: Similarity index of Hordein 45°C**

		Alfa-93	BH-393	BH-75	BCU-73	RD-2552	K-551	Alfa-93	BH-393	BH-75	BCU-73	RD-2552	K-551	Marker
		1	2	3	4	5	6	7	8	9	10	11	12	13
Alfa-93	1	1.000												
BH-393	2	0.111	1.000											
BH-75	3	0.211	0.480	1.000										
BCU-73	4	0.333	0.250	0.720	1.000									
RD-2552	5	0.118	0.261	0.500	0.522	1.000								
K-551	6	0.125	0.273	0.609	0.545	0.571	1.000							
Alfa-93	7	0.111	0.333	0.480	0.500	0.348	0.273	1.000						
BH-393	8	0.235	0.348	0.417	0.522	0.636	0.476	0.522	1.000					
BH-75	9	0.111	0.417	0.800	0.500	0.522	0.273	0.417	0.522	1.000				
BCU-73	10	0.222	0.417	0.480	0.750	0.522	0.455	0.333	0.435	0.500	1.000			
RD-2552	11	0.533	0.476	0.364	0.381	0.300	0.316	0.571	0.500	0.381	0.476	1.000		
K-551	12	0.375	0.455	0.522	0.455	0.762	0.600	0.182	0.571	0.455	0.273	0.316	1.000	
Marker	13	0.333	0.417	0.160	0.083	0.261	0.273	0.333	0.174	0.333	0.250	0.381	0.273	1.000

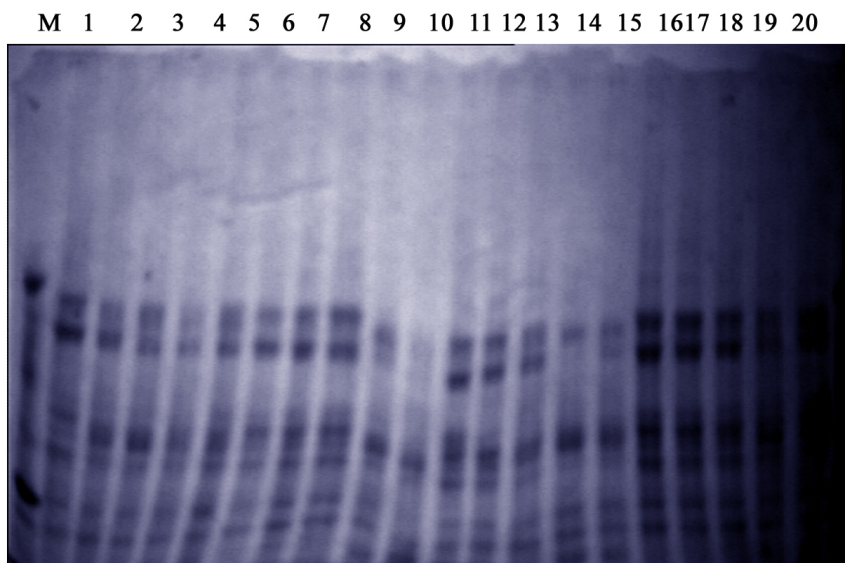


Plate 5 Total seed storage protein profile of variety BH-75 after priming

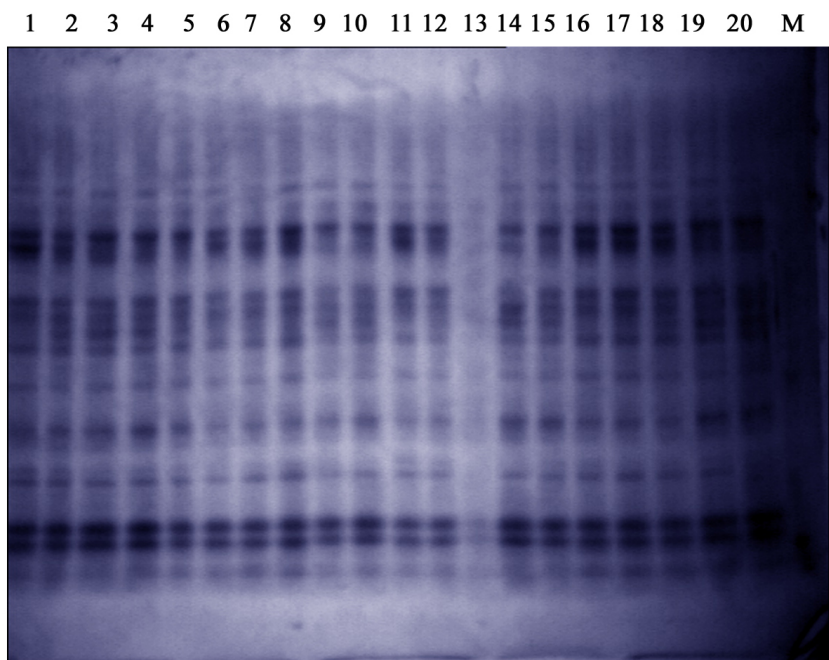


Plate 6 Total seed storage protein profile of variety BH-393 after priming

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(48 hrs), Aged K-551 (72 hrs), cluster 2 comprised of 4 genotypes; Aged RD-2552 (72 hrs) Fresh ALFA-93, aged BH-393 (72hrs), fresh BCU-73, Cluster 3 comprised of 7 genotypes; Fresh BH-393, Aged ALFA-93 (48hrs), Fresh K-551, Fresh BH-75 Fresh RD-2552, Aged-RD-2552 (48hrs) and Aged BCU-73 (48hrs). Cluster-4 comprised of 3 genotypes Aged BH-393 (48hrs), Aged ALFA-93 (72hrs) and Aged K-551 (48hrs) and one genotype- BCU-73 was observed diverse.

Dendrogram of one fresh accelerated aged at 45°C of hordein profile was also observed (Table 19). Clusters were identified from dendrogram which revealed 3 clusters. Cluster No. 1 comprised of 3 genotypes; Fresh BH-393, Aged ALFA-93 (48hrs) and Aged RD-2552 (48hrs), cluster 2 comprised of 4 genotypes; Fresh BH-75, Aged-BH-75 (48hrs), Fresh BCU-73, Aged BCU-73 (48hrs), cluster 3 comprised of 4 genotypes Fresh RD-2552, Fresh K-551, Aged K-551 (48hrs) and Aged BH-393 (48hrs) one genotype was recorded diverse, Fresh ALFA-93.

Fresh and aged genotypes of same variety should be present in same cluster but in all varieties change in banding pattern was recorded. So in same genotype fresh and aged one of same varieties in different clusters observed.

**4.3.9 Variation in protein profiles of BH-75 after priming**

Electrophoresis of seed storage protein extracted from primed seed lot confirms the efficiency of protocol followed. However, protein profile revealed significantly difference in number of protein bands observed in various seed lot. In non-primed seed lot the number of bands observed was 12 with relative mobility varying from 0.375 to 0.966 and molecular weight varied from 89130 daltons to 15850 daltons (Table 23). It was interesting to know that with the increase in priming intervals the protein bands started to disappear (Table 25). This confirms that high molecular weight proteins are first to hydrolyze as soon as the seed is imbibed to disintegrate into lower molecular weight proteins. Rate of protein hydrolysis was more in aged seed lot as compared to fresh seed in both the treatments (water and PEG). It is further to note that the rate of protein hydrolysis is faster in water primed seed as compared to PEG primed seed.

The first biochemical change in the seed after imbibition is hydrolysis of storage proteins. Therefore, electrophoresis of seed storage proteins extracted from various primed seed lot was performed. It was also observed that with a primary treatment aged seeds were fast to loss band as compared to fresh seed (Table 24). The stability of bands revealed that in case of fresh seed band 1, 2 with stand upto 12 hrs in case of water primed, where as upto 24 hrs. in PEG primed. Similarly

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band number 3 was present and withstand upto 24 hrs. in water primed whereas upto 30

**Table 22: Effect of different priming treatments on protein banding pattern in Barley variety BH 75**

Priming interval	Number of bands observed			
	Water primed		Peg primed	
	Fresh	Aged	Fresh	Aged
Fresh non-primed	12	12	12	12
Primed 12 hrs	12	9	12	12
Primed 18 hrs	10	8	12	10
Primed 24 hrs	10	7	12	8
Primed 30 hrs	10	7	8	8
Molecular wt.	6	6	6	6

**Table 23: RM Value of bands and molecular weight of Seed storage protein of BH-75**

Number of Bands	RM values	M.wt.(Kilodalton)
1	0.375	89130
2	0.40	79430
3	0.491	63100
4	0.583	50120

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5	0.641	44670
6	0.666	39810
7	0.751	31260
8	0.791	28180
9	0.825	25120
10	0.833	22390
11	0.925	19950
12	0.966	15850

**Table 24: Banding pattern of total soluble seed protein in barley cultivar BH-75 (various primed and non-primed seed lots) by SDS-PAGE**

Band No.	RM value	FNP	FWP 12 hrs	FWP 18 hrs	FWP 24 hrs	FWP 30 hrs	ANP	AWP 12 hrs	AWP 18 hrs	AWP 24 hrs	AWP 30 hrs	PEG-FP 12 hrs	PEG-FP 18 hrs	PEG-FP 24 hrs	PEG-FP 30 hrs	PEG-AP 12 hrs	PEG-AP 18 hrs	PEG-AP 24 hrs	PEG-AP 30 hrs
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1	0.37	M	L	F	-	-	M	-	-	-	-	M	L	F	-	L	F	-	-
2	0.40	M	L	F	-	-	M	-	-	-	-	M	L	F	-	L	F	-	-
3	0.49	M	M	M	M	F	M	L	F	-	-	M	M	M	-	L	L		
4	0.58	D	D	D	D	M	D	D	D	-	-	D	M	D	-	L	L	F	-
5	0.64	M	M	M	M	M	M	L	L	L	L	L	L	L	L	L	L	L	L
6	0.66	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M
7	0.75	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D
8	0.79	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M
9	0.82	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M
10	0.83	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D
11	0.92	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D
12	0.96	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D

Intensity Bands-D = Dark, L=Light, F=Fant, - = absent

RM= Relative mobility,

FNP = Fresh non-primed, FWP= Fresh water primed

ANP = Aged non-primed, AWP = Aged water primed

PEG-FP = Polyethylene glycol fresh primed

PEG-A.P. = Polyethylene glycol aged primed

hrs. in PEG priming. Band No. 4 to 12 withstand all priming treatment and intervals.

In case of aged seed lot, band number 1, 2 with stand less than 12 hrs. of water priming where as 18 hrs. of PEG priming. Band No. 4 upto 24 hrs. in case of water priming as well as PEG priming.

#### **4.3.10 Protein profile of BH-393 after priming**

Electrophoresis of seed storage protein extracted from primed seed lots confirms the efficiency of the protocol followed. However, protein profile revealed significantly difference in number of protein bands observed in various seed lots. In non primed seed lots, the number of bands observed was 12 with relative mobility varying from 0.277 to 0.966 ( Table 26 ). It was interesting to know that with the increase in priming intervals the protein band started to disappear (Table 25). This confirms that high molecular weight proteins are the first to hydrolyse as soon as the seed is imbibed to disintegrate into lower molecular weight proteins. The rate of protein hydrolysis was more in aged seed as compared to fresh seed in both the priming solutions (water and PEG). It is further to note that the rate of protein hydrolysis is faster in water primed seed as compared to PEG primed seeds. It is due to the negative osmotic regulation by PEG which slower down the rate of imbibition and there after hydrolysis of protein.

**Table 25: Effect of different priming treatments on protein banding pattern in Barley variety BH 393**

*EXPERIMENTAL RESULTS*

Priming interval	Number of bands observed			
	Water primed		Peg primed	
	Fresh	Aged	Fresh	Aged
Fresh non-primed	12	12	12	12
Primed 12 hrs	12	10	12	12
Primed 18 hrs	9	10	10	10
Primed 24 hrs	9	10	9	8
Primed 30 hrs	8	8	8	8
Molecular wt.	6	6	6	6

**Table 26: RM Value of bands and molecular weight of Seed storage protein of BH-393.**

Number of Bands	RM values	M.wt.(Kilodalton)
1	0.277	89130
2	0.298	79430
3	0.367	63100
4	0.426	50120
5	0.489	44670
6	0.546	39810

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7	0.645	31620
8	0.719	28180
9	0.797	25120
10	0.916	22390
11	0.925	19950
12	0.996	15800

**Table 27: Banding pattern of total soluble seed protein in barley cultivar BH-393 (various primed and non-primed seed lots) by SDS-PAGE**

Band No.	RM value	FNP	FWP 12 hrs	FWP 18 hrs	FWP 24 hrs	FWP 30 hrs	ANP	AWP 12 hrs	AWP 18 hrs	AWP 24 hrs	AWP 30 hrs	PEG-FP 12 hrs	PEG-FP 18 hrs	PEG-FP 24 hrs	PEG-FP 30 hrs	PEG-AP 12 hrs	PEG-AP 18 hrs	PEG-AP 24 hrs	PEG-AP 30 hrs
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1	0.277	M	F	-	-	-	M	F	-	-	-	M	L	F	-	L	F	-	-
2	0.298	M	f	F	-	-	M	F	-	-	-	M	L	F	-	L	F	-	-
3	0.367	M	M	M	M	F	M	L	F	-	-	M	M	M	-	L	L		
4	0.426	D	D	D	F	-	D	D	D	-	-	M	L	L	-	L	L	F	-
5	0.489	M	M	M	M	M	M	L	L	L	L	L	L	L	L	L	L	L	L
6	0.546	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M
7	0.645	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D
8	0.719	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M
9	0.797	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M
10	0.916	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D
11	0.925	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D
12	0.996	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D

Intensity Bands-D = Dark, L=Light, F=Fant, - = absent  
 RM= Relative mobility,  
 FNP = Fresh non-primed, FWP= Fresh water primed  
 ANP = Aged non-primed, AWP = Aged water primed  
 PEG-FP = Polyethylene glycol fresh primed  
 PEG-A.P. = Polyethylene glycol aged primed

The protein profile bands observed after various priming treatments in both seeds lots show that during ageing process hydrolysis of various proteins has already been started because of this various protein bands were faster to disappear in aged seeds as compared to fresh seed lot (Table 27). Band numbers 1 and 2 were able to withstand priming upto a period of 8 hrs. in case of fresh seed lots when primed with water. However, the other bands were able to withstand upto 24 hrs when primed with PEG. In case of fresh seed lots band 1 and 2 withstand for less than 12 hrs. in case of water priming, where as upto 18 hrs and 24 hrs in case of PEG priming. Band number 4 was observed on 30 hrs in water primed seed lot while it does not withstand for 30 hrs with PEG primed seed lots.

In case of aged seed band number 1, and 2 withstand 12 hrs of water priming whereas upto 18 hrs. in case of PEG priming, band upto 24 hrs in case of water primed as well as PEG primed quantity of protein was much less in band number 4 after 18 hrs of priming. This confirms that imbibition in case of PEG priming is slower (Table 27).

#### **4.4 To access the stability of improvement of seed vigor after Priming**

In seed priming seed water uptake is the first step as soon as seed is exposed to water and in present experiment the amount water uptake after each priming treatment was measured

in fresh and aged seed lot. The rate of water uptake was faster when priming was done using plain water as compared to aqueous solution of PEG in both seed lots. Within a priming experiment aged seeds were slower to absorb the moisture as compared to fresh seeds. In water primed seeds the water uptake continues till 12 hrs of priming and then slow down due to control hydration in case of PEG.

#### **4.4.1 Standard germination**

In table (28, 30, 32 and 34) Effect of various priming treatments was observed on standard germination of fresh and aged seed lot of both cultivars viz., BH-75 and BH-393. It was observed that fresh seed of both cultivars was quite healthy and showed 98% germination, whereas aged seed lot was deteriorated and standard germination was observed as 68% in BH-75 and 69% in BH-393.

Priming treatment to fresh seed lot of both the cultivars did not show any significant improvement in germination whereas, priming of aged seed lot of both the cultivars showed improvement up to significant level. The improvement in aged seed lot of both cultivars was recorded significantly higher in PEG primed seed lots. Among the varieties BH-393 showed 30% improvement as compared to 9% in BH-75 when primed with PEG.

With in the time treatment 12 hours treatment was observed best among all the treatments. In this treatment aged seed lot of BH-393, BH-75 showed 30% and 16% improvement in standard germination respectively.

#### **4.4.2 Vigor index -1**

It was recorded that fresh seed lot of both the varieties (BH-393, Bh-75) did not show any significant improvement in vigor index-1, as depicted by data in table (28, 30, 32 and 34).

But aged seed lot of both varieties showed significant improvement in vigor index-1. Maximum improvement was observed in case of PEG primed seed lot in both cultivars (BH-393, 2081 and BH-75, 1523) .

Within the treatment of time interval, maximum improvement was recorded in aged seed lots of both cultivars of barley at 12 hrs treatments it was also recorded maximum as 2604.00 and 1979.000 in BH-393 andBH-75 respectively.

#### **4.4.3 Vigor index-11**

In table (28, 30, 32 and 34) Periodical data on vigor index -11 showed significant improvement in fresh primed seed lot of BH-393. Maximum vigor index-11 was recorded in PEG primed treatment in cultivars BH-393 (1110.97), whereas BH-75 did not showed any improvement in vigor index-11.

In case of aged primed seed lot it was significantly higher in both the cultivars. It was recorded maximum in PEG primed seed lot 968.760 in BH-393 and 994.807 in BH-75.

In time interval treatment significant improvement in vigor index-11 was observed in fresh seed lot of BH-393 and recorded maximum improvement in 12 hrs treatment, while BH-75 did not exhibited any improvement. Significant improvement was observed in both the varieties of aged seed lot of barley. Maximum improvement was recorded in 12 hrs. Treatment in BH-393(1140) and in BH-75 (1095).

#### **4.4.4 Rate of germination**

Effect of various priming treatments was observed in rate of germination of fresh and aged seed lots of both cultivars BH-75 and BH-393. Quite significant improvement was observed in rate of germination in both the cultivars. In fresh seed lot It was significantly higher in water primed seed lot in both the varieties 23.613 in BH-393 and 20.891 in BH-75. whereas in aged seed lot of both the cultivars showed maximum improvement in PEG primed seed lot of BH-393 (22.493) and BH-75 (19.010) in table (29, 31, 33 and 35).

In case of time interval maximum improvement was observed in both the cultivars in fresh primed seed lots 24.207 and 23.368 in BH-393 and BH-75 respectively. While in case of aged primed seed lots of barley maximum improvement was

observed in 12 hrs treatments and was recorded as 24.177 in BH-393 and 19.880 in BH-75.

#### **4.4.5 Dehydrogenase Activity**

Measurement of activity of specific enzymes was one of the earliest biochemical technique used to assess deterioration and to predict seed viability (AOSA, 1983), and was observed in optical density. Effect of various priming treatment was observed on dehydrogenase activity in fresh and aged seed lots of both primed cultivars in table (29, 31, 33 and 35). It was recorded that dehydrogenase activity in fresh primed seed lot of both the varieties was significantly higher in water primed seed lot and was recorded (0.189) in BH-393 and (0.234) in BH-75.

In aged seed lot of both the varieties it recorded significantly higher in PEG primed seed lot, of BH-393 (0.140) and in BH-75 (0.149).

In case of time interval treatments 12 hour treatment is best among all the treatments. Dehydrogenase activity was observed significantly higher in both fresh primed and aged primed seed lot of both the cultivars. It was recorded 0.2137 and 0.268 in BH-393 and BH-75 in fresh seed lot respectively, while as in aged primed seed lot it was recorded 0.180 and 0.220 in BH-393 and BH-75 respectively.

*EXPERIMENTAL RESULTS*

**Table 28: Effect of priming treatments on germination vigor index-I and vigor-II in fresh barley CV. BH-393**

<b>Priming treatments</b>	<b>Germination (%)</b>	<b>Vigour index I</b>	<b>Vigour index II</b>
Water	96.267	2498.995	1110.993
PEG	95.067	2413.888	997.947
S.E. (m)			7.00740
C.D. at 5%	N.S.	N.S.	20.6767
Control	98.000	2810.640	1176.000
12 hrs	98.000	2737.800	1222.100
18 hrs	95.833	2538.633	1003.383
24 hrs	94.500	2255.466	970.133
30 hrs	92.000	1939.667	900.733
S.E. (m)	0.6791	100.7543	11.0797
C.D. at 5%	2.0037	297.2932	32.6927

**Table 29: Effect of priming treatments on seed germination, dehydrogenase activity and electrical conductivity in fresh barley CV. BH-393**

<b>Priming treatments</b>	<b>Rate of germination (%)</b>	<b>Dehydrogenase activity</b>	<b>Electrical conductivity</b>
Water	23.613	0.189	42.396
PEG	21.909	0.169	44.287
S.E. (m)	0.1292	0.0012	NS
C.D. at 5%	0.37813	0.0034	
Control	21.000	0.105	31.153
12 hrs	24.207	0.213	36.760
18 hrs	23.522	0.210	42.903
24 hrs	23.233	0.176	50.070
30 hrs	21.842	0.191	55.820
S.E. (m)	0.2043	0.0018	1.2021
C.D. at 5%	0.6029	0.0054	3.5470

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*EXPERIMENTAL RESULTS*

**Table 30: Effect of priming treatments on germination vigor index-I and vigor-II in aged barley CV. BH-393**

<b>Priming treatments</b>	<b>Germination (%)</b>	<b>Vigour index I</b>	<b>Vigour index II</b>
Water	86.00	1952.933	896.960
PEG	97.133	2081.493	968.760
S.E. (m)	0.3682	39.9167	24.8095
C.D. at 5%	1.0864	117.7813	8.4081
Control	69.833	1028.733	584.367
12 hrs	99.000	2604.000	1140.500
18 hrs	98.000	2250.800	1046.900
24 hrs	96.333	2242.533	977.700
30 hrs	94.667	1960.000	914833
S.E. (m)	0.5821	63.1139	13.2943
C.D. at 5%	1.7177	186.2286	39.2272

**Table 31: Effect of priming treatments on seed germination, dehydrogenase activity and electrical conductivity in aged barley CV. BH-393**

<b>Priming treatments</b>	<b>Rate of germination (%)</b>	<b>Dehydrogenase activity</b>	<b>Electrical conductivity</b>
Water	21.995	0.127	54.395
PEG	22.499	0.140	49.195
S.E. (m)	0.0916	0.0011	1.3241
C.D. at 5%	0.2703	0.0032	3.9071
Control	17.833	0.091	49.380
12 hrs	24.177	0.180	44.512
18 hrs	23.648	0.149	50.962
24 hrs	23.277	0.137	59.868
30 hrs	22.302	0.110	59.253

*EXPERIMENTAL RESULTS*

S.E. (m)	0.1449	0.0017	2.0936
C.D. at 5%	0.4274	0.0051	6.1777

**Table 32: Effect of priming treatments on germination vigor index-I and vigor-II in fresh barley CV. BH-75**

Priming treatments	Germination (%)	Vigour index I	Vigour index II
Water	91.600	2248.747	1190.629
PEG	90.800	2119.140	1175.312
S.E. (m)	N.S.	N.S.	N.S.
C.D. at 5%			
Control	98.000	2658.667	1101.333
12 hrs	95.667	2403.850	1402.290
18 hrs	94.667	2220.133	1306.677
24 hrs	87.167	1948.000	1122.287
30 hrs	80.500	1689.067	982.267
S.E. (m)	1.5706	72.3140	23.3131
C.D. at 5%	4.6342	213.3750	68.7892

**Table 33: Effect of priming treatments on seed germination, dehydrogenase activity and electrical conductivity in fresh barley CV. BH-75**

Priming treatments	Rate of germination (%)	Dehydrogenase activity	Electrical conductivity
Water	20.891	0.234	25.43
PEG	20.409	0.166	25.66
S.E. (m)	0.1384	0.0020	
C.D. at 5%	0.4083	0.0058	NS
Control	21.137	0.155	34.403
12 hrs	23.368	0.268	21.987
18 hrs	22.742	0.212	23.270

*EXPERIMENTAL RESULTS*

24 hrs	18.205	0.201	23.222
30 hrs	17.797	0.164	24.878
S.E. (m)	0.2188	0.0031	1.5240
C.D. at 5%	0.6455	0.0092	4.4969

**Table 34: Effect of priming treatments on germination vigor index-I and vigor-II in aged barley CV. BH-75**

Priming treatments	Germination (%)	Vigour index I	Vigour index II
Water	74.200	1509.467	849.781
PEG	77.533	1523.760	994.807
S.E. (m)	0.7513		10.1632
C.D. at 5%	2.2168	N.S.	29.9883
Control	68.667	713.667	707.467
12 hrs	84.500	1979.000	1095.557
18 hrs	78.000	1829.500	979.333
24 hrs	74.333	1556.300	895.213
30 hrs	73.833	1504.600	933.900
S.E. (m)	1.1879	63.5037	16.0694
C.D. at 5%	3.5051	187.3787	47.4156

**Table 35: Effect of priming treatments on seed germination, dehydrogenase activity and electrical conductivity in aged barley CV. BH-75**

Priming treatments	Rate of germination (%)	Dehydrogenase activity	Electrical conductivity
Water	17.205	0.138	55.131
PEG	19.010	0.149	54.581
S.E. (m)	0.0875	0.0013	
C.D. at 5%	0.2581	0.0040	NS
Control	15.233	0.088	55.477
12 hrs	19.880	0.220	50.195
18 hrs	19.400	0.175	55.178

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*EXPERIMENTAL RESULTS*

24 hrs	18.572	0.136	56.812
30 hrs	17.453	0.098	58.620
S.E. (m)	0.1383	0.0021	0.7972
C.D. at 5%	0.4081	0.0063	2.3522

#### **4.4.6 Electrical conductivity test**

Electrical conductivity was recorded as  $\mu\text{S}/\text{cm}/\text{seed}$  in table (29, 31, 33 and 35). It was recorded non-significant in fresh seed of both the cultivars, BH-393 and BH-75. It observed significantly different in seed lot of BH-393 in aged primed seed lot while as, it was significantly lower in water primed seed lot of BH-393 (49.195)

Within priming time interval it was observed non-significant in both fresh primed seed lots of both the cultivars, but in aged primed seed lot it recorded significantly lower in BH-393 (44.512) in 12 hours treatment.

## **CHAPTER - V**

# *DISCUSSION*

### **5.1 To define variables for accelerated ageing (at 42°C and 45°C) in barley**

Barley is one of the important crop of rainfed areas of India. Cultivation of barley carries the advantage over wheat because of its low water requirement and fair resistance to salinity to fight with both the above mentioned abiotic stress, seed play an important role. A quality seed will established a strong and healthy plant in the field, which later fight efficiently with various biotic and abiotic stresses. The seed quality is defined by various parameters which have the genetic basis and the variability among genotypes for these parameters is well established (ISTA,1999). The quality of the seed lot not only define its permeability but also define is storability under normal conditions. In present investigation an effort was made to define variables for accelerate ageing at different temperatures in barley.

Deterioration of quality of seed and its longevity during storage are well known. However the extent of losses is governed by a number of internal and external factors. Internal factors include all such variations in seed metabolism which occur due to difference in environmental and adaphic conditions during plant growth and seed development. The external factors include seed storage environment i.e. relative humidity, temperature and oxygen availability in seed store. Harrington (1960) gave a thumb rule to determine the seed longevity as affected by storage environment.

1. For each 1% decrease in seed moisture content, the storage life of seed is doubled.
2. For each 10°.f (5.6°C) decrease in seed storage temperate the storage life of seed is doubled.

The seed longevity affected by internal and external factors carries genetic basis and can be well predicted by conducting accelerated ageing test (ISTA, 1999). In present investigation an effort has been made to determine the variables for accelerated ageing at different temperatures in barley,. This effort will provide an idea of stability of seed viability under various abiotic stresses either during the storage or in the field.

To meet the above discussed objectives the effect of accelerated ageing conditions on various parameters were studied on six genotypes of barley. The results achieved and their significance in seed quality parameters are discussed below.

### **Seed moisture content**

Seed moisture content increases with time of ageing at 42°C and 45°C. Maximum moisture content was recorded when seed lot was exposed to Accelerated ageing at 42°C for 72hrs. It may be due to hygroscopic nature of the seed because as the seed was exposed to high humidity (>95%). Moisture content will remain on increasing till it reached at equilibrium moisture content.

### **Standard Germination**

It determines the plant population of field though, the standard germination is determined in laboratory conditions but it has been well correlated with field establishment in various crops including barley (ISTA, 1999; Andrade *et al.* 2001 and Frietas 2002). All the genotypes tested were having very high level of seed viability as depicted by standard germination. However when such seeds were exposed to accelerated ageing conditions at two temperature 42°C and 45°C for different time intervals 48, 72 hrs; the viability reduced significantly ranging from 40 to 60%. However this reduction in germination was not uniform and genotypes responded variably to the accelerated ageing conditions. Variable responses of genotypes to accelerated ageing conditions have also been reported in barley (Delouche and Baskin, 1978). Similar results were recorded in fenugreek (Indira *et al.* 2000, Ching, 1973). The reduction in viability during

accelerated ageing conditions may be due to several factors. The main factor may be damage to cell wall, resulting in excessive leaching of electrolytes required for germination. Loss of efficiency of germination enzymes may be due to high temperature and chromosome Aberration (Murata *et al.* 1979). In the present study genotypes BH-393, BCU-73 were observed to be tolerant to stress conditions during accelerated ageing at 42°C for 48 hrs. whereas Genotypes BH-393 and RD-2552 were observed most tolerant to stress conditions during accelerated ageing at 45°C for 48 hrs in present study, the genotypes showing high resistance to accelerated ageing test were also showing high standard germination in fresh seed ranging from 94 to 100%. This shows that relative storage potential of these genotypes is better than other genotype. These genotype would also provide better field emergence under average field conditions as compared to other genotypes.

### **Vigor index**

A combination of standard Germination test with seedling length and dry matter provides a broad evaluation of vigor of seed lot. Vigor index offers seed lot to be characterized to the various classes of seed quality. Seed lots which will have more numerical value supported to be high vigorous. It directly affects the planting value of the seed lot. The vigor index define on the basis of seedling length the seedling dry weight have been

designated as vigor index – I and vigor index –II respectively. The results revealed that there is decrease in both the vigor indices when seeds were exposed to accelerated aged conditions at 42°C and 45°C in both the seed lots. Similar results were recorded by Singh *et al.* (2003) in Urd bean and Moong bean when five cultivars of Urd bean and Moong bean were exposed to accelerated ageing. Basra *et al.* (2003) also reported that accelerated ageing not only affected emergence percentage and time but also decreases the seedling vigor as indicated by reduction in root weight, shoot weight and hypocotyl length in cotton. Genotypes were also found to differ significantly for vigor indices. This showed that it was just not the standard germination to determine the planting value of a seed lot but more importantly it was determined that how vigorous the seedling would emerge out of a viable seed.

### **Electrical conductivity**

The estimates of this parameter have been correlated with those of seed viability in same cases. The electrical conductivity of seed leachates have been reported to be higher in aged seeds indicating deterioration of cell permeability and degradation of food reserve in seeds (Ching, 1973 and Delouche and Baskin, 1978). In present experiment, the electrical conductivity of seeds was measured on basis of number of seeds. The increase in electrical conductivity after accelerated ageing

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confirms that accelerated ageing in Barley caused disturbance to the cell wall resulting into permeability and loss of electrolytes to water during imbibition . Similar effect of accelerated ageing have also been reported by Narwal (1995) in Okra. Bailey *et al.* (1996) in sunflower, Kumar *et al.* (2001) in Fanugreek. The present observation on electrical conductivity of exudates indicated that higher humidity coupled with high temperature would have a detrimental effect on seed longevity of barley. It is suggested that to maintain the seed viability and vigor during storage, a combination of high humidity and temperature must be avoided. This also confirms the seed storage principles laid by Harrington (1960).

### **1000 seed weight**

1000 seed weight comprises of weight of 1000 seeds, which determine the volume of food reserves in the seed. The bold seeds have been preferred in barley for malt quality and early vigor of seedlings (ISTA 1999). The 1000 seed weight observed to be ranging from 32.805 to 58.543 g (Table 1). Weight more than 45 g were observed in BCU-73 and RD-2552. A significant variability among genotypes confirms that there is possibility to characterize genotypes on the basis of test weight which may have a bearing on seed quality through better food reserves and better by products like malt.

### **Dehydrogenase Activity (DHA)**

DHA test is an indirect and quick test to ascertain the viability and vigor of the seed lot and have been shown to give positive correlation with field parameter like field emergence in sunflower (Gupta & Agarwal 1980), Okra (Narwal, 1995) and wheat (Steiner *et al.* 1989).

The DHA activities was found to be more in fresh seed lot as compared to aged seed lot which indicates that though in present case DHA did not directly estimate of viability among various seed lot.

## **5.2 To study the variation of total and specific seed storage protein after ageing**

### **5.2.1 Seed storage protein after ageing at 42°C and 45°C for 48 and 72 hrs.**

Presence of genetic diversity in any species is important for plant improvement programme. Barley is a commercially important crop and self pollinated. In present experiment six genotypes were tested fresh and accelerated ageing at different temperature and time and seed protein profile were tested to study variation of total and specific seed storage protein after ageing and priming.

As proteins are the product of structural gene that encode them so analyzing protein profile can be used as an ideal mean of varietal characteristics (Hermann & Zanetti, 2002 and Cooke *et al.* 1983). Analysis of distribution of various protein

bands showed that tested genotypes are quite diverse for the seed storage protein profiles and this strategy can be adopted to define the uniqueness of a genotype. Similar observation has been made by various workers to identified barley variety by mean of PAGE of seed storage protein (Wagner, 1978; Arabi *et al.* 2001 and Bradova *et al.* 2001). However, each genotype was having a specific banding pattern. The band numbers 3,4,5,8,9,10,11,12,14,17,18 and 19 was common in all genotypes and on the other hand same bands were specific to a particular genotypes as band no. 6 was specific to genotypes BH-393. Band no. 15 was specific to genotypes ALFA 93, BH-393, and RD-2552. Band No. 13 is absent in genotypes RD-2552. Band No. 7 is absent in genotype K-551. This indicated that these genotypes could be easily identified from absence/presence of such specific bands.

Quantification of the degree of divergence based on protein profile and genetic relationship among genotypes can be exposed as similarity coefficient among various genotypes (Sneath and Sokal, 1973).

The genetic similarity index across all the genotypes was found to be ranging from 0.516 to 0.929 (Table 9). The clusters were identified from dendogram, which revealed five clusters. Cluster 1 comprised of 6 genotypes Fresh ALFA 93, Fresh BCU 73, Fresh K-551, Fresh RD-2552, Aged ALFA 93 (48

hrs.) Aged ALFA 93 (72 hrs.), cluster 2 comprises of 7 genotypes. These genotypes are closely associated with each other. Other cluster also showed the close association between the genotypes.

The present experiment revealed that seed storage protein profiles may not provide a clear cut idea about the uniqueness of a genotypes because of very high degree of similarity among genotypes tested. To explore the hidden variability in seed storage protein profile & the fresh seeds were exposed to accelerated ageing conditions, at different temperature (42°C and 45°C) for different time period (48 hrs., 72 hrs.). The purpose of ageing was to check the stability of available protein bands or appearance of new bands during ageing which may result in exploitation of variability among look like seed storage protein profiles. The protein profile of aged seed lot resulted in 17 bands (Aged at 42° for 48 hrs.) 14 band (Aged at 42°C for 72 hrs.), 12 bands (Aged at 45°C for 48 hrs.) instead of 19 bands observed in fresh seed lot. The number of bands decreased with increase in ageing time and temperature. Protein profile of fresh genotypes were observed quite different from aged profile of same genotypes but a comparative analysis with protein profile of aged seed lot will distinguish all the 6 genotypes from each other.

### **5.2.2 Total specific storage protein (Hordein) After ageing**

The proteins are product of structural gene that encode them. So analysis of protein profile can be used as an ideal mean of varietal characterization (Harmonn & Zanetti 2002 and Cooke *et al.* 1983). Hordein is a fraction of total seed protein of barley. Poperey *et al.* (2002) concluded that PAGE technique developed & replicate for less accurate starch gel electrophoresis.

Analysis of distribution of various protein bands showed that tested genotypes are quite diverse for the seed storage protein profile of hordein protein. Similar observations have been made by various workers to identified barley varieties by mean of SDS-PAGE of seed storage proteins (Binneck *et al.* 2002, Popereya *et al.* 2001, Fasoula *et al.* 2003). However, each genotype has specific banding pattern. The bands are quite different in fresh and aged genotype. There were quite differences in banding pattern and protein profile of fresh and aged genotypes of same variety. It may be due to effect of ageing which leads to the de-naturation of protein and resulted into less number of bands observed in aged genotypes aged at different temperature (42°C and 45°C). This technique could be a powerful tool in genetic purity analysis of aged seed lot where ODVS can easily be identified by exposing samples to accelerated ageing conditions and more after analyzing the seed protein profile.

### **5.2.3 To study the variation of total and specific storage protein after priming**

The seed water uptake in germination followed triphasic pattern with an initial imbibition where seed moisture content increased at a faster rate up to 12 hrs. of priming by both water and aqueous solution of PEG. However, after that period the rate of water uptake decreases in case of water primed seeds whereas it becomes negative in case of PEG primed solution and this reduction in rate continues up to 24 hrs. of priming interval (the period between 12 hrs and 24 hrs. uptake of water). In this case the rate of water uptake increased in all the priming treatments. Above observation is in conformation with the seed water uptake in other species (Come, 1980 and Come & Thevenet, 1982).

The first biochemical changes in seed after imbibition is the hydrolysis of storage protein particularly membrane protein to provide free amino acids for synthesis of enzymatic machinery required for the conservation of the reserved food in the form which is suitable for transport to the other part of seeds. Electrophoresis of seed storage protein extracted from various primed seed lots confirm the efficiency of the protocol followed. However, protein profile revealed significant differences in number of protein bands observed in various seed lots of both the varieties BH-393 and BH-75; It was interesting to know that with

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increase in priming interval the protein bands started to disappear, this conforms that the high molecular weight protein were first to be hydrolyzed as soon as seed is imbibed to disintegrate into lower molecular weight protein. The rate of protein hydrolysis was more in aged seed as compared to fresh seed in both the priming solutions (Water & PEG). It is further to note that the rate of protein hydrolysis is faster in water primed seeds as compared to PEG primed seeds. It is due to the negative osmotic regulation by PEG which slower the rate of imbibition and thereafter hydrolysis of proteins. The protein profile basis observed after various priming treatments both seed lot have showed that during ageing process hydrolysis of various protein has already been started because of this various protein bands were faster to disappear in aged seeds as compared to fresh seeds in both the genotypes, BH-393 and BH-75.

The sequence of immobilization of seed protein in germination or seed hydrolysis starts from enzyme synthesis and it is due to hydrolysis of proteins stored in aleuronic layer and there after hydrolysis of protein and stored food endosperm, which ultimately resulted into metabolism or the hydrolysis product in scutellum and growth of embryo (Mikola, 1981). In case of Barley it was observed that the embryo starts germinating after 2 days (48 hrs.) The present examination of protein profiles showed that initial hydrolysis occurs in higher molecular weight

protein bands upto 34 hrs. of priming and thereafter, it moves to lower molecular weight.

The major effect of both priming treatments was on the improvement in low vigor/aged seeds. This shows that the priming has reversed the seed ageing. Similar observation of beneficial effect of seed priming on low vigor/aged seed have been studied in number of cereals and vegetable seeds. (Basu, 1976 and Goldshorthy *et al.* 1982). The above discussed improvement may be explained in the light of observation made by Wilson & McDonald (1986) in their review. They explained that during imbibition seeds undergo extensive repair and macromolecular turnover. A hydration and dehydration cycle, produce resistance to subsequent damage by accelerated ageing.

In present study we also observed that accelerated ageing and priming changes biochemical effect where in case of fresh seed lot no such positive effects was observed. Therefore it is concluded that seed priming is important to improve the vigor of deteriorated seed lot but the period and method of hydration need to be specified so that biochemical changes occurring in the seeds may not lead to the total loss of seed viability. In case of barley where water stress at the time of germination is normally felt, a suitable priming treatment will certainly add to the uniform germination and field establishment of the seedlings.

### **5.3 To access the stability of improvement of seed vigor after priming**

Rapid and uniform field emergence are two essential prerequisites to increase yield, quality and ultimately profit in annual crops. Uniformity and percentage of seedling emergence of direct seeded crop have a major impact on final yield and quality (Wurr and Fellows, 1983). Rate and uniformity of emergence are inherent to seed quality and environmental conditions during seedling emergence. Slow emergence results in smaller plants (Ellies 1989) and seedling, which are more vulnerable to seed borne diseases (Gubels 1975; Osburn and Schroth 1989). Extended emergence period predispose the planting bed to deterioration and increased soil compaction (Heydecker 1978), particularly under adverse environmental conditions. Various presowing seed treatments have been used to reduce time between seed sowing and seedling emergence and to improve synchronization of field emergence in annual as well as perennial crops. Evenari (1980) reported that ancient Greek farmers soaked cucumber seed in water or milk and honey before sowing to increase germination rate and emergence. In seventeenth century, presowing treatments with salt solution were common practice among Russian farmers (Yapparov and Iskhakov 1974). Heydecker (1973) acknowledges the use of the term 'priming' of seed by Malnassy (1971) to describe the presowing seed treatment

to enhance germination and increase seedlings emergence uniformity under adverse environmental conditions. The focus of present study is to assess the stability of improvement of seed vigor after priming as discussed below:

### **Standard germination**

It determines the plant population in field. Though, the standard germination is determined under laboratory conditions but it has been well correlated with field establishment in various crops including barley (ISTA 1999; Andrede *et al.* 2001 and Frietas, 2002). Effect of various priming treatments was observed on standard germination of fresh and aged seed lot of both cultivars viz., BH-75, and BH-393. It was observed that priming treatment to fresh seed lot of both cultivars did not show any significant improvement in germination. Similar response of fresh seed of Indian tomato to priming has been reported by Pandita and Nagarajan (2000) whereas priming of aged seed lot of both the cultivars showed improvement upto significant level. This type of response has been reported by various workers (Tarquis and Bradford (1992) in lettuce, Goel *et al.* (2003) in cotton, and Benamar *et al.* (2003) in pea seed). Nagarajan and Pandita (2001) reported that osmo priming can delay the onset of deterioration caused by accelerated ageing in tomato. Karssen *et al.* (1989) concluded after studying the effect of priming in tomato, celery, and lettuce seeds that after priming, the seeds contained

increased level of products such as protein (enzymes) Generally, priming of these 3 species permitted rapid and uniform germination by stimulating extensibility of the cell wall in the radicle and by weakening endosperm cell wall in front of the radical tip.

### **Rate of Germination**

Germination rate and uniformity can be improved by priming. The number of different species commercially primed and available to producers is quite small synchronization and rapid seed emergences are the commonly reported benefits of seed priming. Particular advantages of seed priming are augmented under adverse conditions (Knypl and Khan 1981; Wiebe and Muhyaddin 1987). Seed priming promoted early growth of egg plant, pepper, cucumber, and muskmelon plants, but no differences were detected in early and final yield, and fruit size between primed and non primed seeds (Passam *et al.* 1889).

In present experiment, effect of various priming treatments was observed on the of germination of fresh and aged seed lot of both cultivars BH-75 and BH-393. Quite significant improvement was recorded in the rate of germination in both the cultivars. Fresh seed lot of both variety showed maximum improvement in water primed seed lot. Where as aged seed lot of both cultivars showed maximum improvement in PEG primed seed lot. Bailly *et al.* (2002) reported that accelerated ageing

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decreased seed germinability and slowdown the hypocotyl growth, whereas priming with PEG solution resulted in an increase in germination rate and seedling growth in sunflower. Priming of tomato seeds in aerated -0.5 mpa PEG enhanced the germination rate and the extent of percentage of embryo radicals protruding partially or completely through the seed endosperm (Liptay and Zariffa, 1993). PEG and water. Thus priming treatment of average seed lot can improve germination which can provide better field emergence and synchronization of seed germination.

**Vigor index**

A combination of standard germination test with seedling length and dry matter provides a broad evaluation of vigor of seed lot. Vigor index offers a seed lot to be characterized into various classes of seed quality which will have more Numerical value than the specific value or percentage and more specially it directly affects the planting value of the seed lot. The vigor index define on the basis of seedling length and the seedlings dry weight have been designated as vigor index –I and vigor –II respectively. The present experiment revealed that there is non significant improvement in fresh seed lot of both the cultivars and age seed lot of both the cultivars showed significant improvement. Maximum important was observed incase of PEG primed seed lot in both the cultivars. Nagarajan and Pandita (2001), reported that PEG priming reduces the deleterious effect

of ageing and improved germination, speed of germination and vigor in tomato seed which were subjected to accelerated ageing. Nascimento *et al.* (2004) reported that priming increased the germination of seed of low vigor and response was cultivar dependent.

### **Dehydrogenase activity**

Measurement of activity of specific enzymes was one of the earliest biochemical technique used to assess deterioration and to predict seed viability (AOSA, 1983), and was observed as optical density. DHA test is indirect and quick test to ascertain the viability and vigor of seed lot and have been shown to give correlation with field parameters like field emergence in sunflower (Gupta & Agrawal, 1980), Okra (Narwal, 1995), Wheat (Steiner *et al.* 1989).

It was recorded that dehydrogenase activity in fresh primed seed lot in both the variety was significantly higher in water primed seed lot and in aged seed lot of both varieties. It was recorded significantly higher in PEG primed seed lot. DHA activity was found to be more in fresh seed lot. In present study DHA did not directly estimate the viability of seed lot but may indicate the extent of viability among various seed lots.

### **Electrical conductivity**

The estimates of electrical conductivity have been correlated with those of seed viability in same cases. The electrical

*DISCUSSION*

conductivity of seed leachates have been reported to be higher in aged seed indicating deterioration of cell permeability and degradation of food reserves in seed (Chain, 1973 and Delouche and Baskin, 1978). Electrical conductivity of the seed leachates, increased progressively with artificial ageing in cotton (Goel *et al.* 2003). In present experiment electrical conductivity was recorded as non significant in fresh seed lot of both cultivars. The same observation was recorded in sweet corn by Parera and Canttiffe (1994). Basra *et al.* (2003) recorded lower electrical conductivity after 24 hrs. priming in wheat in present experiment electrical conductivity was also recorded significantly lower. In aged primed seed lot of BH-393. Basra *et al.* 2003 recorded lowest electrical conductivity after 24 hrs. hydropriming in case of wheat.

## **CHAPTER - VI**

# *SUMMARY AND CONCLUSION*

Barley is one of the most important cereal crop of rainfed area of India where cultivation of wheat is a problem due to scarcity of irrigation facilities and salinity. Besides a better food value in term of protein quality and total carbohydrate it has industrial use and can play an important role in promoting export of barley products. The quality of the seed is measured by it's genetic purity and storage capacity. International Seed Testing Association has proposed a number of standardized test to check the quality and storability of the seed lot. In tropical and sub tropical countries, deterioration of stored seed is a serious problem and seed priming enhanced the value of deteriorated seed lot (Bray *et al.*, 1989 and Pearman *et al.* 1988). Therefore, present study entitled "Effect of ageing and priming on seed

storage protein of Barley” was carried out at the Department of Seed Science & Technology, CCS Haryana Agricultural University, Hisar with the following objectives:

1. To define variables for accelerated ageing test in barley.
2. To study the variation of total and specific seed storage protein after ageing and priming.
3. To access the stability of improvement of seed vigor after priming.

The experimental material for the present study consisted of six genotypes of barley which were collected from Deptt. of Plant Breeding, CCS HAU, Hisar. All the six genotypes were divided in to two lots. The first lot was kept as such and designated as fresh seed lot while the other seed lot was exposed to accelerated ageing conditions (Temp.  $40\pm 2^{\circ}\text{C}$  and  $45\pm 2^{\circ}\text{C}$  and Relative humidity  $\geq 90\%$ ) designated as accelerated aged seed lot.

Both the seed lots were tested for various vigor and viability parameters in a completely randomized design with three replications. Two genotypes BH-75 and BH-393 were used for evaluation of extent of repair in accelerated aged seed lot by seed priming with water and aqueous solution of PEG 6000 for variable time period. The effect of ageing and priming on protein variation was also studied. The viability and vigor of seeds were tested by using standard procedure of ISTA (1999). Seed storage protein profiles of fresh primed and accelerated aged primed seed lot were

prepared with as per Dalani and Varier (1993). All the gels were analyzed using Biovis Gel Documentation System Supplied by Expert Vision Ltd. Mumbai. The statistical analysis of data for various parameters was carried out according to the standard procedure. The results obtained during the present investigation are summarized as below:

Results revealed that the germination percentage for fresh seed lot was above the minimum seed certification standard whereas in accelerated aged seed lots, the standard germination, vigor index, rate of germination and dehydrogenase activity declined significantly for all the six genotypes. All the genotypes were found significantly different for vigor and viability tests.

After accelerated ageing, the genotypes, BH-393 and BCU-73 were having significantly higher standard germination as compared to other genotypes indicating their superiority over others. A higher electrical conductivity of accelerated aged seed in barley further confirmed the accelerated ageing results in to increase permeability of cell membrane and release of food reserves.

The total seed storage protein profile of fresh seed lot revealed 19 bands with relative mobility value ranging from 0.135 to 0.941 where as aged seed lot revealed 17 bands with relative mobility value ranging from 0.135 to 0.941. The fractional seed storage protein profile (Hordein) of fresh seed lot revealed 19

bands with relative mobility ranged from 0.169 to 0.974 where as aged seed lot revealed 15 band with relative mobility ranged from 0.169 to 0.974.

Priming or restricted hydration of aged seed lot of BH-75 and BH -393 with water and PEG significantly improve all the parameters like standard germination, rate of germination, vigor index, dehydrogenase activity and decrease the permeability of cell membrane by repairing process results in to lower electrical conductivity, clearly forward and confirm that priming boost the repair mechanism of seed as given by Bray *et al.* (1989). Also the protein profile bands observed after various priming treatments of both the seed lot have showed that rate of protein hydrolysis was more in aged seed as compared to fresh seed for both the priming solutions (Water and PEG 6000). The inferences drawn are in brevity:

1. All the six genotypes showed decline in most of seed quality parameters when they were accelerated aged at 42°C and 45°C.
2. Variety BH-393 and BCU-73 were found better tolerant to a biotic stress (accelerated ageing).
3. Priming by water in fresh seed lot and priming by PEG 6000 in aged seed lot showed significantly improvement in seed quality.

*SUMMARY AND CONCLUSION*

4. Among all the priming treatments seed soaking of 12 hrs was found most effective.
5. Accelerated ageing leads to loss of protein contents as compared to fresh seed lot.

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ABSTRACT

<b>Title of Dissertation</b>	:	<b>Effect of ageing and priming on seed storage proteins of barley. (<i>Hordeum vulgare</i> L.)</b>
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<b>Admission No.</b>	:	2003A66M
<b>Title of Degree</b>	:	Master of Science
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**Key words : Barley, Ageing, Priming, Protein, Hordein, Seed quality**

Barley owing to its suitability for salinity and water stress tolerance was selected to define variability available in improved cultivars for seed vigor, viability and storability. An effort was also made to study the effect of ageing and priming on seed storage proteins on the basis of total seed protein profiles. To undertake the above studies six improved genotypes viz. Alfa-93, BH-393, BH-75, BCU-73, RD-2552, K-551 were taken define the changes in storage protein in fresh and accelerated aged seeds. It was observed that all the six genotypes were quite variable for seed quality parameters. The fresh seed lots of all the genotypes were having standard germination above 90%. However, accelerated ageing reduced it to nearly 50%, owing an adverse effect on all the seed quality parameters. On the basis of seed storage protein profiles of fresh and accelerated aged seeds it was revealed that protein profile of fresh seeds was less variable over accelerated aged seeds. An effort was also made to enhance the value of deteriorated seeds (accelerated aged) of BH-75 and BH-393 by giving priming treatments. It was revealed that priming with water and aqueous solution of PEG 6000 significantly increased the standard germination, rate of germination, dehydrogenase activity and vigor index by boosting the repair mechanism of seed. The protein profile of primed seeds revealed that priming results in hydrolysis of seed storage proteins.

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