MAJOR RESERCH PROJECT

PHYSIOLOGICAL AND MOLECULAR MARKERS FOR ASSESSING VIABILITY OF VEGETABLE SEEDS DURING STORAGE

FINAL REPORT
(July 2015 to July 2018)

SUBMITTED TO

UNIVERSITY GRANTS COMMISSION

Department of Botany
College of Basic Sciences and Humanities
Punjab Agricultural University, Ludhiana-141004
2019

Annexure- IX

University Grants Commission

(Ministry of Human Resource Development, Govt. of India)

Bahadurshah Zafar Marg, New Delhi – 110002

1.	**Title of the Project**	Physiological and Molecular Markers for Assessing Viability of Vegetable Seeds During Storage.
2.	**Name and Address of Principal Investigator**	Dr (Mrs) Seema Bedi Office: Deptt. of Botany, Punjab Agricultural University Ludhiana- 141004 e-mail: sbedipau@yahoo.com Residential: 81 Parkash Colony, Barewal Road Ludhiana- 141012
3.	**Name and Address of the Institution**	Department of Botany Punjab Agricultural University, Ludhiana
4.	**UGC approval letter no. and date**	F. No. 43-130/2014(SR) July 2015
5.	**Date of Implementation**	01.07.2015
6.	**Tenure of the Project**	3 years from 01.07.2015 to 01.07.2018
7.	**Total grant allocated (Rs)**	12,73,000/-
8.	**Total grant received (Rs)**	11,85,700/-
9.	**Final expenditure (Rs)**	11,83,751/-

10.	**Title of the Project**	Physiological and Molecular Markers for Assessing Viability of Vegetable Seeds During Storage.
11.	**Objectives of the project**	The present study was planned with following objectives. i) To study the effects of storage for various durations and accelerated ageing on seed viability and seedling vigour in seeds of hot pepper, bitter gourd and onion. ii) To study the effects of seed storage and accelerated ageing on anti-oxidant defense mechanism, alteration in DNA and proteomics. iii) To determine the efficacy of pre- sowing treatments restoring the viability and vigour of stored and aged seed respectively.
12.	**Whether objectives were achieved (give details)**	yes
13.	**Achievements from the project**	Attached as Annexure -A
14.	**Summary of the findings (500 words)**	• Rapid loss in viability of onion seeds during seed storage is a major problem. Seeds of onion (*Allium cepa* L.) cv. Punjab Naroya harvested in June were subjected to three post harvest storage procedures, viz; refrigerator storage at (5°C; 30% RH) for 4 months (considered as fresh seeds), room temperature storage for 4 months (stored seeds) and accelerated aged seeds. Refrigerator stored seeds were subjected to accelerated ageing (5°C; 90% RH) for 3, 6, 9 and 12 days respectively. The refrigerated stored seeds showed maximum germination percentage (94%) while seeds accelerated aged for twelve days gave least germination percentage (62%). As the duration of ageing increased, there was a marked reduction in seedling length, fresh and dry weight. All the ageing treatments resulted in membrane damage, as is evident from higher solute leakage in aged seeds. Increase in ageing duration led to an increase in total soluble sugars and total free amino acids and an increase in activities of enzymes viz; peroxidase, catalase and α-amylase. The contents of total starch, total soluble proteins, ascorbic acid, α-tocopherol and DNA decreased with accelerated ageing. • Bitter gourd (*Momordica charantia* L.) cv. Punjab 14 seeds harvested in mid-June were subjected to three post-harvest storage procedures, viz., fresh seeds (5°C and 30% RH) for 8 months (stored in

		refrigerator), stored seeds (kept at room temperature) for 8 months and accelerated aged seeds. Fresh seeds stored in refrigerator were subjected to accelerated ageing (45°C; 90% RH) for 3, 6 and 9 days, respectively. Our results showed that increasing ageing duration resulted in reduction in germination percentage and seedling vigour index. All the ageing treatments resulted in membrane damage, which was evident from higher solute leakage in such seeds. Biochemical changes due to ageing included a reduction in content of total soluble proteins whereas, the total soluble sugars and total free amino acids increased with accelerated ageing. There was a decrease in the activities of antioxidant related enzymes, viz., catalase and peroxidase. Exceptionally, α-amylase activity increased with accelerated ageing. The amount of ascorbic acid, α-tocopherol and DNA also reduced with accelerated ageing.. • Freshly harvested seeds of hot pepper var. Punjab Sindhuri and hybrid CH-27 were stored in refrigerator (controlled ageing) and at room temperature (natural ageing) for 12 months. The seeds kept for controlled ageing were drawn at 3 monthly intervals and subjected to accelerated ageing for 3, 6, 9 and 12 days respectively. Ageing significantly affected seed quality in terms of physiological (germination per cent, mean days to germination, seedling vigour index, fresh and dry weight, electrolytic leakage and moisture content), biochemical (content of total soluble sugars, starch, total soluble proteins, total free amino acids, ascorbic acid, α-tocopherol, malondialdehyde, hydrogen peroxide, change in protein profile by SDS PAGE, activity of enzymes, viz; POD,SOD, CAT, α-amylase activity and molecular (alteration in DNA by SSR markers) aspects. To restore the germinability, the seeds were primed in KNO_3, GA_3, PEG and Ascorbic acid. All priming treatments significantly improved germination parameters over control but the treatments differed among themselves in magnitude. The improvement in seed quality and germination parameters due to priming was attributed to increased activity of antioxidant enzymes (POD, SOD, CAT) and reappearance of some protein bands degraded during storage.
15.	**Contribution to the society (give details)**	Physiological, biochemical and molecular aspects of seed deterioration have been studied in three vegetable crops viz., *Allium, Mormordica* and *Capsicum*. These will help to design better storage protocols and may be incorporated into breeding programs for enhancing seed storability. Pre sowing

		treatments to ameliorate seed deterioration occurring during storage have also been suggested.
16.	**Whether any Ph.D. enrolled/produced out of the project**	MSc:2 PhD:1
17.	**No. of publications out of the project**	Research papers: Annexure :B Published/accepted:2 Submitted:4 Abstracts:5

(PRINCIPAL INVESTIGATOR) **(REGISTRAR/ PRINCIPAL)**

(Seal)

(CO-INVESTIGATOR)

Annexure-A

Achievements from the project:

Seed quality undergoes deterioration when subjected to unfavorable storage conditions such as high temperature and high relative humidity. Upon, sowing, the first indication of seed deterioration is loss of seedling vigour followed by decline in viability. Seeds of vegetable crops are particularly susceptible to damage during storage. The duration from harvesting to sowing of seeds may vary from a few months to several years. It is during this time the quality of seeds may decline. Under Indian conditions, due to wide variation in climatic conditions (temperature and relative humidity) and technological shortcomings, safe storage is beyond the reach of many farmers. Consequently, even short term storage of current season's harvest until next sowing poses serious problems to farmers. The present studies were conducted on seeds of vegetable crops.

Momordica charantia :

Methodology:

Freshly harvested seeds of bitter gourd (*Momordica charantia* L. cv. Punjab 14) were separated into three lots and subjected to three post-harvested storage procedures. One lot was stored under optimim conditions in a refrigerator at 5 C at 30 % RH in moisture proof plastic bags for upto 8 months. These seeds were not exposed to any accelerated ageing treatment and were taken as fresh seeds (control). The second lot was kept at room temperature in plastic containers for upto 8 months (stored seeds) whereas the third lot was accelerated aged at 45 C at 90% RH for 3, 6 and 9 days respectively just before subjecting them to germination studies. The high RH conditions were created in desiccators by placing saturated solution of NaCl at the base of desiccators. Estimations were made on seed germination and its speed, seedling growth in terms of length of seedling and its part viz., root and shoot and seedling fresh and dry weight. Biochemical tests of seed vigour such as electrical conductance were also conducted. Total soluble sugars, total soluble proteins, total free amino acids, ascorbic acid and α-tocopherol were estimated at various stages of ageing. Antioxidant enzymes such as catalase, peroxidase and hydrolytic enzymes such as α-amylase activities were determined in the present studies. DNA alteration and SDS-PAGE protein profile were also estimated under different storage conditions.

Salient Findings:

- There was a significant reduction in percent germination and its speed with progressive storage. Highest percent germination was observed in refrigerator stored fresh seeds (93.34%) and least (46.6%) in seeds that had been accelerated aged for 9 days.

  Seeding length, root and shoot length recorded a reduction with storage period. The maximum average root and shoot length was recorded from fresh seeds (15.76 and 8.67

cm respectively) and the shortest average root and shoot length was recorded from seeds that had been accelerated aged for 9 days (7.76 and 4.68 cm respectively). This reduction might be due to the damaging effect of ageing on enzymes,that are necessary to convert reserve food in the embryo to usable form and ultimately production of normal seedlings.

Seed membrane damage in terms of electrical conductivity of seeds increased significantly in seeds that had been accelerated aged for 9 days as compared to unaged seeds. This indicates a reduction in seed vigour and viability.

Table 1: Effect of different storage conditions and accelerated ageing on germination percentage (G %), mean days to germination (MDG),seedling vigour index (SVI), shoot (SL), root (RL), seedling lengths (SDL), seedling fresh weight (FW), dry weight (DW) and electrolyte leakage (EL) in bitter gourd (*Momordica charantia* L.) cv. Punjab 14 seeds

Treatments	**G %**	**MDG**	**SVI**	**SDL (cm)**	**RL(cm)**	**SL (cm)**	**FW (g)**	**DW (g)**	**EL (%)**
Fresh seeds	93.34	0.359	23.2	24.43	15.76	8.67	2.443	0.249	73.04
Stored seeds	86.67	0.405	18.4	20.88	12.92	7.95	2.198	0.212	75.92
AA 3 days	73.3	0.472	14.6	17.91	10.9	7.00	2.143	0.200	78.70
AA 6 days	60	0.668	10.5	15.3	9.74	5.56	1.964	0.174	82.27
AA 9 days	46.6	1.11	5.46	12.44	7.76	4.68	1.503	0.117	85.51
CD ($p \leq 0.05$)	12.42	0.039	2.25	1.67	1.23	0.625	0.221	0.016	5.44

- The biochemical analysis revealed that accelerated ageing led to significant increase in the level of total soluble sugars with concomitant increase in the activity of α- amylase. Total soluble proteins showed a reduction with increasing accelerated ageing duration while total free amino acid increases. The possible reason for this increase is increased enzymatic degradation of proteins by proteases leading to a sharp increase in total free amino acids. With increase in ageing time period, both peroxidase and catalase enzymes showed sharp decline in their activity. The loss in enzyme activity may result from age induced protein deterioration. There was also reduction in antioxidants such as ascorbic acid and α-tocopherol than control. Maximum ascorbic acid and α-tocopherol content was observed in refrigerator stored fresh seeds (0.799 µg g^{-1} FW and 5.64 µg g^{-1} FW) and minimum ascorbic acid and α-tocopherol content was observed in seeds that had been accelerated aged for 9 days (0.155µg g^{-1} FW and 0.75 µg g^{-1} FW).

- With regard to seed reserves, at the end of storage period, the maximum value of total soluble sugars was observed in seeds that had been accelerated aged for 9 days and minimum value was observed in fresh seeds stored in refrigerator (Table 2). There was a significant decline in the

  soluble protein content with increase in ageing time period.The amount of total soluble proteins was less in seeds that had been accelerated aged for 9 days (0.63 mg g^{-1} FW) while it was maximum in fresh seeds (3.40 mg g^{-1} FW) stored in refrigerator (Table 2). On the other hand, the maximum value of total free amino acids was observed in seeds that had been accelerated aged for 9 days (0.63 mg g^{-1} FW) while minimum value was observed in fresh seeds (0.228 mg g^{-1} FW) stored in refrigerator (Table 2). The activities of various antioxidants and enzymes were adversely affected by ageing. The peroxidases showed sharp decline in its activity under accelerated ageing conditions (Table 2). It's activity in unaged or fresh seeds was found to be maximum (0.0583 ΔA min^{-1} g^{-1} FW) as compared to seeds that had been accelerated aged for 9 days (0.0296 ΔA min^{-1} g^{-1} FW). Likewise, highest catalase enzyme activity was observed in fresh seeds (0.411 ΔA min^{-1} g^{-1} FW) while the lowest value was observed in seeds that had been accelerated aged for 9 days (0.015 ΔA min^{-1} g^{-1} FW) (Table 2). However, α-amylase activity gets increased as the ageing duration increases (Table 2). Maximum activity was observed in seeds that had been accelerated aged for 9 days (0.0667 μg maltose produced/ml/min.) while minimum activity was observed in fresh seeds (0.028 μg maltose produced/ml/min.) Among the antioxidants, the ascorbic acid content decreased significantly as the duration of accelerated ageing increased (Table 2). The maximum value of ascorbic acid was recorded from fresh seeds (0.799μg g^{-1} FW) while minimum value was observed in seeds that had been accelerated aged for 9 days (0.155 μg g^{-1} FW). Similarly, the content of α-tocopherol decreased in seeds subjected to accelerated ageing (Table 2). The maximum value of α-tocopherol was recorded from fresh seeds (5.64 μg g^{-1} FW), while the minimum value was observed in seeds that had been accelerated aged for 9 days (1.75 μg g^{-1} FW).
- Increase in DNA degradation is one of the key physiological events occurring during seed deterioration. In the present study, maximum DNA content was observed in fresh seeds (200 ng/μL) while seeds that had been accelerated aged for 12 days exhibit negligible DNA content.

- A significant fall in the content of DNA was evident in the ageing bitter gourd seeds under different storage conditions (Table 3; Fig 1). Maximum DNA content was observed in refrigerator stored (fresh) seeds (200ng/μL) while seeds that had been accelerated aged for 12 days negligible DNA content was observed.

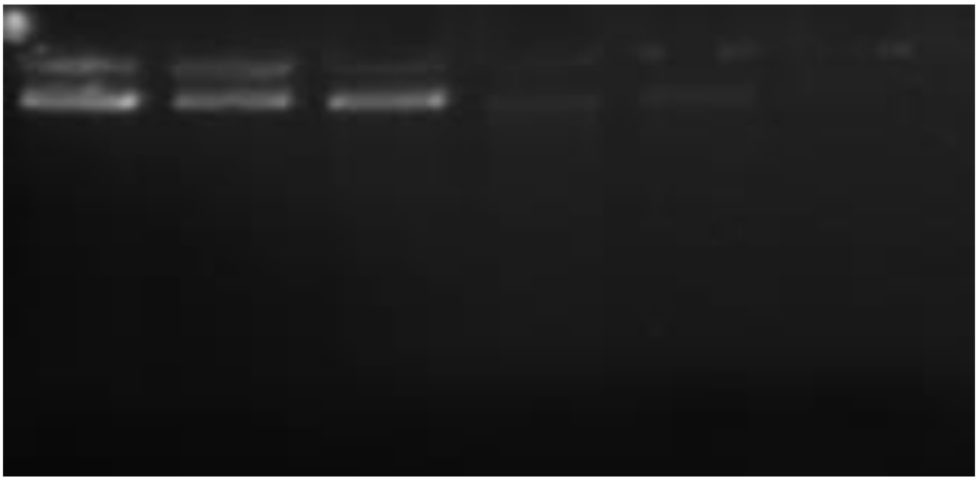
Table 2: Effect of different storage conditions and accelerated ageing on biochemical parameters (total soluble sugars (TSS), total soluble proteins (TSP), total free amino acids (TFAA), peroxidase (POD), catalase (CAT), α-amylase, ascorbic acid (ASA) and α-tocopherol) in bitter gourd (*Momordica charantia* L.) cv. Punjab 14 seeds:

Treatments	TSS (mg g^{-1}) FW	TSP (mgg^{-1}) FW	TFAA (mgg^{-1}) FW	POD (ΔA $min^{-1}g^{-1}$) FW	CAT (ΔA $min^{-1}g^{-1}$) FW	α-amylase (μgmaltose produced/ml/min)	ASA (μgg^{-1}) FW	α-tocopherol (μgg^{-1}) FW
Fresh seeds	4.18	3.40	0.228	0.0583	0.411	0.028	0.799	5.64
Stored seeds	5.13	2.99	0.318	0.0510	0.227	0.0326	0.432	4.92
AA 3 days	5.57	1.84	0.45	0.0460	0.098	0.039	0.368	4.22
AA 6 days	6.89	1.45	0.564	0.0393	0.047	0.0526	0.258	2.81
AA 9 days	8.66	0.63	0.63	0.0296	0.015	0.0667	0.155	1.75
CD ($p\leq0.05$)	1.22	0.418	0.0616	0.00381	0.0828	0.0043	0.135	0.691

Table 3: Effect of different storage conditions and accelerated ageing on DNA content in bitter gourd (*Momordica charantia* L.) cv. Punjab 14 seeds:

Treatments	DNA content (ng/μL)
Fresh seeds	200
Stored seeds	100

AA 3 days	100
AA 6 days	50
AA 9 days	50
AA 12 days	Negligible



A B C D E F

Fig. 1: Effect of accelerated ageing on DNA content in bitter gourd (*Momordica charantia* L.) cv. Punjab 14 seeds under different storage conditions

A = Fresh seeds; B = Stored seeds;

C = Accelerated aged (3 days); D = Accelerated aged (6 days);

E = Accelerated aged (9 days); F = Accelerated aged (12 days)

The SDS-PAGE profile of bitter gourd seeds subjected to accelerated ageing showed a changed banding pattern as indicated by their Rm values. A total of 7 bands were observed with Rm value ranging from 0.416-0.85 (Table 4). The maximum intensity of protein bands was observed in fresh seeds while intensity of protein bands decreases as duration of ageing increases while the number of bands did not differ among the various ageing treatments.

Table 4: Effect of accelerated ageing on relative mobility of total soluble protein profile in bitter gourd (*Momordica charantia* L.) cv. Punjab 14 seeds under different storage conditions:

Band no.	**Rm value**					
	Fresh seeds (Refrigerator stored)	**Room temp. stored seeds (8 months)**	**Accelerated ageing (3 days)**	**Accelerated ageing (6 days)**	**Accelerated ageing (9 days)**	**Accelerated ageing (12 days)**
1	0.43	0.43	0.43	0.416	0.416	0.416

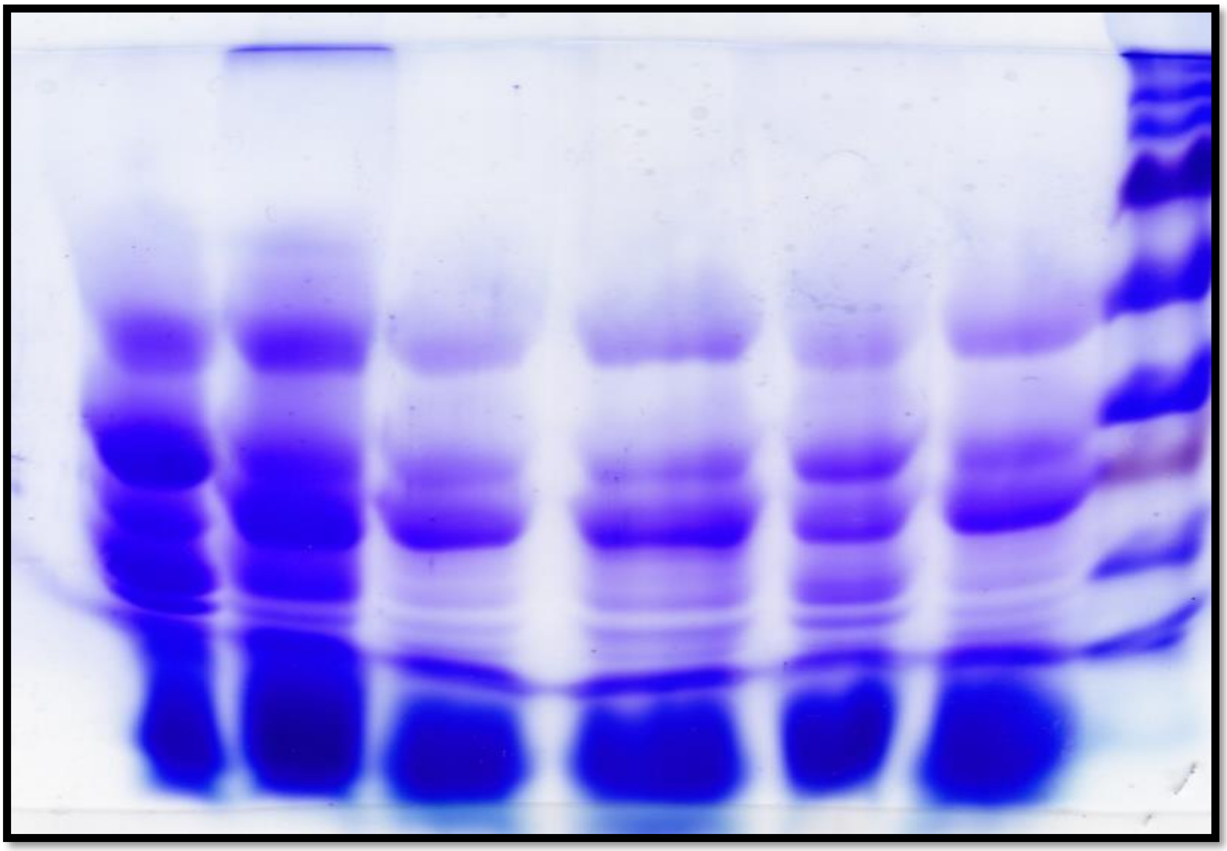
2	0.583	0.583	0.583	0.583	0.55	0.53
3	0.63	0.667	0.667	0.667	0.63	0.616
4	0.73	0.73	0.75	0.75	0.73	0.7
5	0.75	0.76	0.783	0.783	0.75	0.73
6	0.76	0.783	0.80	0.80	0.783	0.76
7	0.783	0.80	0.83	0.85	0.80	0.80

The intensity of bands showed significant difference between aged and control samples. In the present study, soluble proteins of various vigour categories along with control, revealed that the intensity of bands decreased with a reduction in seed vigour (Table 5, Fig 2). The maximum intensity of protein bands was observed in fresh seeds while intensity of protein bands decreases as duration of ageing increases. While the number of bands did not differ among the various categories.

Table 5: Effect of accelerated ageing on intensity of total soluble protein profile in bitter gourd (*Momordica charantia* L.) cv. Punjab 14 seeds under different storage conditions:

Band no.	**Fresh seeds (Refrigerator stored)**	**Room temp. stored seeds (8 months)**	**Accelerated ageing (3 days)**	**Accelerated ageing (6 days)**	**Accelerated ageing (9 days)**	**Accelerated ageing (12 days)**
1	++++	++++	++	++	++	++
2	++++	++++	++	++	+++	+++
3	++++	++++	+++	+++	+++	+++
4	++++	++++	+	+	++	+
5	++++	++++	+	+	++	+
6	++++	++++	+	++	+	+
7	++++	++++	+	+	+	+

Where + very light, ++ light, +++ dark and ++++ very dark



A B C D E F M

Fig. 2: Effect of accelerated ageing on electrophoretic banding patterns of proteins in bitter gourd (*Momordica charantia* L.) cv. Punjab 14 seeds under different storage conditions

A = Fresh seeds; B = Stored seeds;

C = Accelerated aged (3 days); D = Accelerated aged (6 days);

E = Accelerated aged (9 days); F = Accelerated aged (12 days);

M = Protein ladder

From the present study, it is concluded that seed ageing has great impact on seed germination and seedling vigour under unfavourable storage conditions of high temperature and high relative humidity. As the storage duration in seed increases, various quality parameters viz., total soluble proteins, α-tocopherol and ascorbic acid content decreases while total soluble sugars and total free amino acid content increases. Ageing also leads to deterioration of membranes as indicated by an increase in conductivity of seed leachate, which intern affects the activities of various enzymes such as catalase, peroxidase and α-amylase. DNA in low viability seeds has a lower mean molecular weight than in high viability seeds. The electrophoretic banding pattern of seed proteins showed deteriorative changes during ageing of seed at high temperature and high relative humidity. Present study showed that higher molecular weight protein deteriorates at accelerated ageing as well as ambient conditions and with increase in storage period.

Capsicum annuum

Methodology:

The study was carried out on two genotypes of hot pepper viz; Punjab Sindhuri and CH-27 during 2016-17 and 2017-18. The freshly harvested seeds of both genotypes of hot pepper were stored in refrigerator at 5ºC at 30% RH in moisture proof plastic bags (controlled ageing) and at room temperature in plastic containers (natural ageing) for 12 months. After every 3 months, the controlled aged seeds were further aged by exposing to high temperature and high humidity (45°C at 90% RH) for 3, 6, 9 and 12 days respectively (accelerated ageing).The high RH conditions were created in desiccators by placing saturated solution of NaCl at the base of desiccators.In the second consecutive experiment, the same seed lot after 12 months of storage in refrigerator and room temperature and further accelerated aged for 3,6,9 and 12 days were subjected to priming treatments viz. polyethylene glycol (25% and 30%), KNO_3 (1% and 2%), gibberellic acid (30ppm and 50ppm) and ascorbic acid (2% and 2.5%) solutions. Observations were recorded on seed germination and its speed, seedling vigour index, seedling growth in terms of length of seedling and its part viz., shoot and root, seedling fresh and dry weight and moisture content. Conductivity of electrolytic leakage from aged and primed seeds were recorded. Total soluble sugars, starch content, total soluble proteins, total free amino acids, ascorbic acid and α-tocopherol were estimated at various stages of ageing. The MDA and H_2O_2 were determined. Activity of antioxidant enzymes such as catalase, peroxidase, superoxide dismutase and hydrolytic enzyme such as α-amylase were determined. Alteration in DNA by SSR markers and change in protein profile by SDS- PAGE were also estimated in seeds subjected to different storage conditions and after priming treatment also.

Salients Findings:

- There was a reduction in germination percentage, among the seeds subjected to different ageing treatments. Maximum germination (86.5% and 85.9% respectively in both the years) was recorded in seeds subjected to controlled ageing and minimum germination (38.3% and 37.5% respectively in both the years) was recorded in seeds subjected to accelerated ageing for 12 days. There was a progressive reduction in the germination of seeds with increase in the storage duration in both the years. The maximum germination (74.0% and 73.9% respectively in both the years) was observed in freshly harvested seed and minimum germination (43.3% and 42.8% respectively in both the years) was recorded in seeds subjected to 12 months of storage (Table 1).
- The effect of ageing and storage on the rate of germination is expressed as Mean days to germination (MDG). MDG increases with increase in ageing as well as duration of

storage. The seedling vigour index also shows a decline with increase in ageing duration (Table 2). Maximum SVI was recorded in seeds subjected to controlled ageing and least SVI was recorded in seeds subjected to accelerated ageing for 12 days. Duration of storage also had significant effect on the SVI of hot pepper seeds. There was a progressive reduction in the seedling vigour with increase in the storage duration in both the years. Root, shoot and seedling length varied significantly (Table 3).

- The biochemical analyses revealed that accelerated ageing led to significant increase in the level of total soluble sugars and decrease in starch content. Total soluble proteins showed a reduction with increasing ageing and duration of storage, while total free amino acid increases (Table 4,5). The possible reason for this increase is increase in enzymatic degradation of proteins by proteases which leads to a sharp increase in total free amino acids.

TABLE 1: Response of ageing and duration of storage on germination (%) in hot pepper seeds under laboratory conditions. (The data are mean values for two genotypes viz; CH-27 and Punjab Sindhuri)

Ageing / Duration of Storage	Controlled ageing		Natural ageing		3 Days AA		6 Days AA		9 Days AA		12 Days AA		Mean	
	2016-17	**2017-18**	**2016-17**	**2017-18**	**2016-17**	**2017-18**	**2016-17**	**2017-18**	**2016-17**	**2017-18**	**2016-17**	**2017-18**	**2016-17**	**2017-18**
0 month	93.5	93.8	91.5	92.0	78.0	77.0	72.5	73.5	60.0	59.0	48.5	48.0	**74.0**	**73.9**
3 month	91.5	90.5	77.5	77.5	73.5	74.0	67.5	67.5	55.0	54.0	42.0	42.0	**67.8**	**67.6**
6 month	88.0	87.5	59.5	59.0	64.0	63.5	58.0	57.5	47.0	46.0	38.0	37.0	**59.1**	**58.4**
9 month	81.0	80.0	49.0	48.0	56.5	57.5	53.0	52.0	42.0	42.0	33.3	32.0	**52.5**	**51.9**
12 month	78.5	77.5	37.5	36.5	41.5	42.5	38.0	38.5	34.5	33.5	29.5	28.5	**43.3**	**42.8**
Mean	**86.5**	**85.9**	**63.0**	**62.6**	**62.7**	**62.9**	**57.8**	**57.8**	**47.7**	**46.9**	**38.3**	**37.5**		

AA= Accelerated ageing. (Controlled aged seeds were subjected to AA)

LSD (p=0.05) **2016-17** **2017-18**

Factor A: Genotypes 0.59 0.52

Factor B: Ageing 1.02

0.09

Factor C: Duration of storage 0.93

0.82

Genotypes x Ageing NS

NS

Genotypes x Duration of storage NS

NS

Ageing x Duration of storage 2.28 2.02

Genotypes x Ageing x Duration of storage NS NS

TABLE 2: Response of ageing and duration of storage on mean days to germination (MDG) in hot pepper seeds under laboratory conditions. (The data are mean values for two genotypes viz; CH-27 and Punjab Sindhuri).

Ageing / Duration of storage	Controlled ageing		Natural ageing		3 Days AA		6 Days AA		9 Days AA		12 Days AA		Mean	
	2016-17	**2017-18**	**2016-17**	**2017-18**	**2016-17**	**2017-18**	**2016-17**	**2017-18**	**2016-17**	**2017-18**	**2016-17**	**2017-18**	**2016-17**	**2017-18**
0 month	2.74	2.72	2.79	2.79	3.05	3.06	3.86	3.87	4.10	4.15	5.27	5.28	**3.64**	**3.65**
3 month	2.78	2.80	2.84	2.85	3.18	3.21	3.94	3.96	4.18	4.21	5.62	5.64	**3.76**	**3.78**
6 month	2.84	2.84	3.57	3.57	3.63	3.64	4.03	4.06	4.26	4.27	5.77	5.78	**4.02**	**4.03**
9 month	2.91	2.92	4.37	4.38	4.02	4.05	4.13	4.14	4.38	4.38	5.94	5.96	**4.30**	**4.31**
12 month	3.03	3.03	5.64	5.65	5.17	5.18	5.24	5.25	5.60	5.59	6.72	6.73	**5.23**	**5.24**
Mean	**2.86**	**2.87**	**3.85**	**3.85**	**3.81**	**3.83**	**4.24**	**4.26**	**4.51**	**4.52**	**5.87**	**5.88**		

AA= Accelerated ageing. (Controlled aged seeds were subjected to AA)

LSD (p=0.05)	**2016-17**	**2017-18**
Factor A: Genotypes	NS	NS
Factor B: Ageing	0.18	0.22
Factor C: Duration of storage	NS	NS
Genotypes x Ageing	NS	NS
Genotypes x Duration of storage	NS	NS
Ageing x Duration of storage	0.43	0.48
Genotypes x Ageing x Duration of storage	NS	NS

TABLE 3: Response of ageing and duration of storage on seedling vigour index (SVI) in hot pepper seeds under laboratory conditions. (The data are mean values for two genotypes viz; CH-27 and Punjab Sindhuri).

	Controlled	Natural ageing	3 Days AA	6 Days AA	9 Days AA	12 Days AA	Mean

Ageing / Duration of storage	ageing													
	2016-17	**2017-18**	**2016-17**	**2017-18**	**2016-17**	**2017-18**	**2016-17**	**2017-18**	**2016-17**	**2017-18**	**2016-17**	**2017-18**	**2016-17**	**2017-18**
0 month	8.51	8.46	7.87	7.87	5.85	5.71	4.50	4.57	3.03	2.98	1.26	1.20	**5.17**	**5.13**
3 month	7.96	7.82	5.97	5.96	4.71	4.70	3.75	3.77	2.21	2.16	0.82	0.83	**4.24**	**4.21**
6 month	6.82	6.70	3.16	3.11	3.55	3.48	2.73	2.67	1.65	1.57	0.59	0.56	**3.08**	**3.01**
9 month	5.11	4.99	1.96	1.87	2.32	2.39	1.96	1.90	0.93	0.91	0.45	0.42	**2.12**	**2.08**
12 month	4.01	3.92	0.94	0.88	1.50	1.51	0.95	0.95	0.57	0.57	0.34	0.31	**1.39**	**1.36**
Mean	**6.48**	**6.38**	**3.98**	**3.94**	**3.59**	**3.56**	**2.78**	**2.77**	**1.68**	**1.64**	**0.69**	**0.66**		

AA= Accelerated ageing. (Controlled aged seeds were subjected to AA)

LSD (p=0.05)	**2016-17**	**2017-18**
Factor A: Genotypes	0.08	0.09
Factor B: Ageing	0.14	0.16
Factor C: Duration of storage	0.12	0.15
Genotypes x Ageing	NS	NS
Genotypes x Duration of storage	NS	NS
Ageing x Duration of storage	0.30	0.36
Genotypes x Ageing x Duration of storage	NS	NS

The three major antioxidant enzymes, viz. peroxidase, catalase and superoxide dismutase were studied. Maximum peroxidase activity (0.94 ΔAmin^{-1} g^{-1} FW and 0.93 ΔAmin^{-1} g^{-1} FW respectively in both the years) was recorded in seeds subjected to controlled ageing which was significantly better than the seeds subjected to natural ageing (0.69 ΔAmin^{-1} g^{-1} FW and 0.68 ΔAmin^{-1} g^{-1} FW respectively in both the years). Ageing duration reduced the peroxidase activity thus minimum activity (0.27 ΔAmin^{-1} g^{-1} FW and 0.26 ΔAmin^{-1} g^{-1} FW respectively in both the years) was recorded in seeds subjected to accelerated ageing for 12 days. Duration of storage had significant effect on the peroxidase activity of hot pepper seeds. The maximum peroxidase activity (0.85 ΔAmin^{-1} g^{-1} FW and 0.84 ΔAmin^{-1} g^{-1} FW respectively in both the years) was observed when seed were extracted and minimum (0.34 ΔAmin^{-1} g^{-1} FW and 0.33ΔA min^{-1} g^{-1} FW respectively in both the years) was recorded in seeds stored for 12 months (Table 6).

TABLE 4: Response of ageing and duration of storage on total soluble proteins (mg g^{-1}) FW in hot pepper seeds under laboratory conditions. (The data are mean values for two genotypes viz; CH-27 and Punjab

Sindhuri).

Ageing / Duration of storage	Controlled ageing		Natural ageing		3 Days AA		6 Days AA		9 Days AA		12 Days AA		Mean	
	2016-17	**2017-18**	**2016-17**	**2017-18**	**2016-17**	**2017-18**	**2016-17**	**2017-18**	**2016-17**	**2017-18**	**2016-17**	**2017-18**	**2016-17**	**2017-18**
0 month	0.48	0.47	0.45	0.45	0.36	0.36	0.30	0.29	0.28	0.28	0.19	0.19	**0.34**	**0.34**
3 month	0.42	0.42	0.40	0.40	0.32	0.32	0.28	0.28	0.25	0.25	0.17	0.17	**0.31**	**0.30**
6 month	0.38	0.38	0.22	0.21	0.23	0.23	0.20	0.20	0.18	0.18	0.13	0.13	**0.22**	**0.22**
9 month	0.33	0.33	0.16	0.16	0.19	0.19	0.17	0.17	0.15	0.15	0.11	0.11	**0.19**	**0.18**
12 month	0.30	0.30	0.07	0.06	0.12	0.12	0.09	0.09	0.07	0.07	0.05	0.05	**0.12**	**0.11**
Mean	**0.38**	**0.38**	**0.26**	**0.26**	**0.25**	**0.24**	**0.21**	**0.20**	**0.19**	**0.18**	**0.13**	**0.13**		

AA= Accelerated ageing. (Controlled aged seeds were subjected to AA)

LSD (p=0.05)	**2016-17**	**2017-18**
Factor A: Genotypes	0.003	0.001
Factor B: Ageing	0.005	0.003
Factor C: Duration of storage	0.004	0.002
Genotypes x Ageing	NS	NS
Genotypes x Duration of storage	NS	NS
Ageing x Duration of storage	0.010	0.006
Genotypes x Ageing x Duration of storage	NS	NS

Similar trend was recorded in CAT, SOD, and α-amylase activity (Table 7,8,9). Since enzymes are also proteins, cross linking would seriously disturb their functioning. So, there is a direct correlation between decline in enzyme activity and loss of viability. There was also reduction in non-enzymatic antioxidants such as ascorbic acid and α-tocopherol with increase in ageing. Maximum value of ascorbic acid (1.27 μg g^{-1}FW in both the years) was recorded in controlled ageing which was significantly more than in the seeds subjected to natural ageing (1.04 μg g^{-1}FW and 1.03 μg g^{-1}FW respectively in both the years) (Table 10).

TABLE 5: Response of ageing and duration of storage on total free amino acids (mg g^{-1}) FW in hot pepper seeds under laboratory conditions. (The data are mean values for two genotypes viz; CH-27 and Punjab

Sindhuri).

Ageing / Duration of storage	Controlled ageing		Natural ageing		3 Days AA		6 Days AA		9 Days AA		12 Days AA		Mean	
	2016-17	**2017-18**	**2016-17**	**2017-18**	**2016-17**	**2017-18**	**2016-17**	**2017-18**	**2016-17**	**2017-18**	**2016-17**	**2017-18**	**2016-17**	**2017-18**
0 month	0.11	0.12	0.12	0.12	0.15	0.15	0.17	0.17	0.23	0.24	0.37	0.37	**0.19**	**0.20**
3 month	0.13	0.13	0.14	0.14	0.16	0.16	0.18	0.18	0.26	0.27	0.38	0.38	**0.21**	**0.21**
6 month	0.14	0.14	0.19	0.19	0.17	0.17	0.20	0.20	0.29	0.29	0.38	0.39	**0.23**	**0.23**
9 month	0.16	0.16	0.22	0.23	0.18	0.19	0.22	0.23	0.29	0.30	0.40	0.40	**0.25**	**0.25**
12 month	0.17	0.17	0.35	0.35	0.20	0.20	0.26	0.26	0.37	0.37	0.42	0.43	**0.29**	**0.29**
Mean	**0.14**	**0.14**	**0.20**	**0.20**	**0.17**	**0.17**	**0.20**	**0.21**	**0.29**	**0.29**	**0.39**	**0.39**		

AA= Accelerated ageing. (Controlled aged seeds were subjected to AA)

LSD (p=0.05)	**2016-17**	**2017-18**
Factor A: Genotypes	0.003	0.002
Factor B: Ageing	0.005	0.003
Factor C: Duration of storage	0.005	0.002
Genotypes x Ageing	NS	NS
Genotypes x Duration of storage	NS	NS
Ageing x Duration of storage	0.011	0.006
Genotypes x Ageing x Duration of storage	NS	0.008

Ageing duration reduced the ascorbic acid content and minimum value (0.53 μg g^{-1}FW in both the years) was recorded in seeds that had been accelerated aged for 12 days. Duration of storage had significant effect on ascorbic acid. The maximum value (1.15 μg g^{-1}FW and 1.14 μg g^{-1}FW respectively in both the years) was observed in freshly harvested seeds and minimum value (0.73 μg g^{-1}FW and 0.72 μg g^{-1}FW respectively in both the years) was recorded in seeds subjected to 12 months of storage. α-Tocopherol is an important antioxidant and is an important non-enzymatic inhibitor of lipid peroxidation in vivo. Maximum value of α-Tocopherol (4.57 μgg^{-1}FW and 4.58 μgg^{-1}FW respectively in both the years) was recorded in controlled ageing seeds which was significantly more than seeds subjected to natural ageing (3.56 μgg^{-1}FW and 3.55 μgg^{-1}FW respectively in both the years). Ageing duration reduced the α-tocopherol and minimum value (0.99 $\mu g g^{-1}$FW in both the years) was recorded in seeds that had been accelerated aged for 12 days (Table 11).

TABLE 6: Response of ageing and duration of storage on peroxidase activity ($\Delta A\ min^{-1}\ g^{-1}$)FW in hot pepper seeds under laboratory conditions. (The data are mean values for two genotypes viz; CH-27 and Punjab Sindhuri).

Ageing / Duration of storage	Controlled ageing		Natural ageing		3 Days AA		6 Days AA		9 Days AA		12 Days AA		Mean	
	2016-17	**2017-18**	**2016-17**	**2017-18**	**2016-17**	**2017-18**	**2016-17**	**2017-18**	**2016-17**	**2017-18**	**2016-17**	**2017-18**	**2016-17**	**2017-18**
0 month	1.09	1.09	1.08	1.09	0.93	0.93	0.82	0.82	0.65	0.65	0.50	0.49	**0.85**	**0.84**
3 month	1.08	1.05	0.98	0.98	0.86	0.86	0.71	0.70	0.53	0.52	0.38	0.37	**0.76**	**0.75**
6 month	0.97	0.96	0.60	0.59	0.75	0.75	0.63	0.63	0.41	0.41	0.22	0.21	**0.60**	**0.59**
9 month	0.88	0.88	0.48	0.48	0.61	0.60	0.52	0.52	0.36	0.36	0.15	0.15	**0.50**	**0.50**
12 month	0.69	0.68	0.28	0.27	0.45	0.45	0.38	0.36	0.16	0.15	0.08	0.07	**0.34**	**0.33**
Mean	**0.94**	**0.93**	**0.69**	**0.68**	**0.72**	**0.72**	**0.61**	**0.61**	**0.42**	**0.42**	**0.27**	**0.26**		

AA= Accelerated ageing. (Controlled aged seeds were subjected to AA)

LSD (p=0.05)	**2016-17**	**2017-18**
Factor A: Genotypes	NS	NS
Factor B: Ageing	0.008	0.011
Factor C: Duration of storage	0.008	0.010
Genotypes x Ageing	NS	NS
Genotypes x Duration of storage	NS	NS
Ageing x Duration of storage	0.019	0.025
Genotypes x Ageing x Duration of storage	NS	NS

TABLE 7: Response of ageing and duration of storage on catalase activity($\Delta A\ min^{-1}\ g^{-1}$)FW in hot pepper seeds under laboratory conditions. (The data are mean values for two genotypes viz; CH-27 and Punjab Sindhuri).

Ageing / Duration of storage	Controlled ageing		Natural ageing		3 Days AA		6 Days AA		9 Days AA		12 Days AA		Mean	
	2016-17	**2017-18**	**2016-17**	**2017-18**	**2016-17**	**2017-18**	**2016-17**	**2017-18**	**2016-17**	**2017-18**	**2016-17**	**2017-18**	**2016-17**	**2017-18**
0 month	1.72	1.72	1.71	1.71	1.57	1.57	1.32	1.32	1.14	1.14	0.83	0.83	**1.38**	**1.38**
3 month	1.70	1.70	1.60	1.60	1.43	1.43	1.25	1.25	0.96	0.96	0.77	0.76	**1.29**	**1.28**

6 month	1.63	1.63	1.24	1.24	1.32	1.32	1.14	1.14	0.79	0.79	0.64	0.64	**1.13**	**1.13**
9 month	1.54	1.54	0.84	0.82	1.15	1.15	0.94	0.94	0.66	0.66	0.55	0.54	**0.95**	**0.94**
12 month	1.42	1.42	0.55	0.54	1.07	1.07	0.83	0.83	0.54	0.54	0.47	0.46	**0.81**	**0.81**
Mean	**1.60**	**1.60**	**1.19**	**1.18**	**1.31**	**1.31**	**1.10**	**1.09**	**0.82**	**0.82**	**0.65**	**0.65**		

AA= Accelerated ageing. (Controlled aged seeds were subjected to AA)

LSD (p=0.05)	**2016-17**	**2017-18**
Factor A: Genotypes	NS	NS
Factor B: Ageing	0.013	0.013
Factor C: Duration of storage	0.011	0.012
Genotypes x Ageing	NS	NS
Genotypes x Duration of storage	NS	NS
Ageing x Duration of storage	0.028	0.029
Genotypes x Ageing x Duration of storage	NS	NS

TABLE 8: Response of ageing and duration of storage on superoxide dismutase (units^{-1}g^{-1}) FW in hot pepper seeds under laboratory conditions. (The data are mean values for two genotypes viz; CH-27 and Punjab Sindhuri).

Ageing / Duration of storage	Controlled ageing		Natural ageing		3 Days AA		6 Days AA		9 Days AA		12 Days AA		Mean	
	2016-17	**2017-18**	**2016-17**	**2017-18**	**2016-17**	**2017-18**	**2016-17**	**2017-18**	**2016-17**	**2017-18**	**2016-17**	**2017-18**	**2016-17**	**2017-18**
0 month	2.06	2.05	2.06	2.03	1.87	1.87	1.68	1.67	1.51	1.51	1.38	1.37	**1.76**	**1.75**
3 month	2.04	2.04	1.91	1.91	1.76	1.76	1.54	1.53	1.40	1.40	1.25	1.24	**1.65**	**1.64**
6 month	1.93	1.91	1.48	1.46	1.68	1.70	1.48	1.48	1.36	1.34	1.16	1.15	**1.51**	**1.51**
9 month	1.71	1.70	1.19	1.18	1.54	1.52	1.39	1.38	1.21	1.21	1.07	1.06	**1.35**	**1.34**
12 month	1.49	1.49	1.08	1.08	1.38	1.37	1.20	1.19	1.17	1.17	0.98	0.97	**1.21**	**1.21**
Mean	**1.84**	**1.84**	**1.54**	**1.53**	**1.64**	**1.64**	**1.46**	**1.45**	**1.33**	**1.32**	**1.16**	**1.15**		

AA= Accelerated ageing. (Controlled aged seeds were subjected to AA)

LSD (p=0.05)	**2016-17**	**2017-18**
Factor A: Genotypes	NS	NS
Factor B: Ageing	0.024	0.026
Factor C: Duration of storage	0.022	0.024
Genotypes x Ageing	NS	NS
Genotypes x Duration of storage	NS	NS

Ageing x Duration of storage 0.054

0.058

Genotypes x Ageing x Duration of storage NS NS

TABLE 9: Response of ageing and duration of storage on α-amylase activity (μg maltose produced /ml/min)in hot pepper seeds under laboratory conditions. (The data are mean values for two genotypes viz; CH-27 and Punjab Sindhuri).

Ageing / Duration of storage	Controlled ageing		Natural ageing		3 Days AA		6 Days AA		9 Days AA		12 Days AA		Mean	
	2016-17	**2017-18**	**2016-17**	**2017-18**	**2016-17**	**2017-18**	**2016-17**	**2017-18**	**2016-17**	**2017-18**	**2016-17**	**2017-18**	**2016-17**	**2017-18**
0 month	1.53	1.53	1.53	1.52	1.41	1.41	1.36	1.36	1.13	1.12	0.84	0.84	**1.30**	**1.30**
3 month	1.52	1.52	1.46	1.46	1.32	1.32	1.22	1.22	1.06	1.05	0.78	0.78	**1.23**	**1.23**
6 month	1.47	1.47	1.21	1.21	1.26	1.25	1.13	1.13	0.92	0.91	0.66	0.66	**1.11**	**1.11**
9 month	1.42	1.42	0.97	0.97	1.18	1.18	0.92	0.92	0.83	0.83	0.54	0.54	**0.98**	**0.97**
12 month	1.40	1.39	0.61	0.61	0.83	0.83	0.63	0.63	0.55	0.54	0.39	0.38	**0.73**	**0.73**
Mean	**1.47**	**1.47**	**1.15**	**1.15**	**1.20**	**1.20**	**1.05**	**1.05**	**0.90**	**0.89**	**0.64**	**0.64**		

AA= Accelerated ageing. (Controlled aged seeds were subjected to AA)

LSD (p=0.05)	**2016-17**	**2017-18**
Factor A: Genotypes	0.003	NS
Factor B: Ageing	0.006	0.010
Factor C: Duration of storage	0.005	0.009
Genotypes x Ageing	NS	NS
Genotypes x Duration of storage	NS	NS
Ageing x Duration of storage	0.012	0.023
Genotypes x Ageing x Duration of storage	NS	NS

With decrease in antioxidants, ageing causes accumulation of ROS. MDA is formed by the peroxidation of membranes that occurs on the onset of stress conditions. Our study showed that MDA content increased with ageing. The lowest MDA content (0.14 μmol g^{-1}FW and 0.15 μmol g^{-1}FW respectively in both the years) was recorded in controlled ageing seeds and highest MDA (0.76 μmol g^{-1}FW and 0.76 μmol g^{-1}FW respectively in both the years) was recorded in seeds that had been accelerated aged for 12 days. Similarly, the lowest H_2O_2 content (157.23μmol g^{-1}FW and 157.62 μmol g^{-1}FW respectively in both the years) was recorded in controlled ageing seeds and highest H_2O_2 (271.69 μmol g^{-1}FW and 272.09 μmol g^{-1}FW respectively in both the years) was observed in seeds that had been accelerated aged for 12 days (Table 12).

TABLE 10: Response of ageing and duration of storage on ascorbic acid (AsA) (µg g^{-1}) FW in hot pepper seeds under laboratory conditions. (The data are mean values for two genotypes viz; CH-27 and Punjab Sindhuri).

Ageing / Duration of storage	Controlled ageing		Natural ageing		3 Days AA		6 Days AA		9 Days AA		12 Days AA		Mean	
	2016-17	**2017-18**	**2016-17**	**2017-18**	**2016-17**	**2017-18**	**2016-17**	**2017-18**	**2016-17**	**2017-18**	**2016-17**	**2017-18**	**2016-17**	**2017-18**
0 month	1.37	1.36	1.33	1.33	1.24	1.24	1.15	1.15	1.05	1.05	0.73	0.73	**1.15**	**1.14**
3 month	1.31	1.31	1.28	1.28	1.20	1.19	1.13	1.13	0.97	0.97	0.62	0.62	**1.09**	**1.08**
6 month	1.29	1.29	1.07	1.07	1.10	1.09	1.05	1.05	0.86	0.86	0.55	0.54	**0.99**	**0.98**
9 month	1.22	1.22	0.84	0.84	1.04	1.04	0.97	0.96	0.77	0.77	0.43	0.43	**0.88**	**0.88**
12 month	1.16	1.16	0.65	0.65	0.86	0.86	0.77	0.77	0.59	0.58	0.33	0.33	**0.73**	**0.72**
Mean	**1.27**	**1.27**	**1.04**	**1.03**	**1.09**	**1.08**	**1.01**	**1.01**	**0.85**	**0.84**	**0.53**	**0.53**		

AA= Accelerated ageing. (Controlled aged seeds were subjected to AA)

LSD (p=0.05)	**2016-17**	**2017-18**
Factor A: Genotypes	0.005	NS
Factor B: Ageing	0.009	0.016
Factor C: Duration of storage	0.008	0.015
Genotypes x Ageing	NS	NS
Genotypes x Duration of storage	NS	NS
Ageing x Duration of storage	0.021	0.036
Genotypes x Ageing x Duration of storage	NS	NS

Increase in DNA degradation is one of the key physiological events occurring during seed deterioration. DNA alterations were analyzed using SSR markers. A total of 32 primers were used to screen seed samples. Out of 32 microsatellite primers variation in three primers (CAMS-647, AVRCD-PP3 and CAMS-194) were detected between freshly harvested and aged seeds. The disappearance of bands from seeds subjected to natural ageing (12 months) coincided with the fact that seed viability was less.

TABLE 11: Response of ageing and duration of storage on α- tocopherol(μg g^{-1}) FW in hot pepper seeds under laboratory conditions. (The data are mean values for two genotypes viz; CH-27 and Punjab Sindhuri).

Ageing / Duration of storage	Controlled ageing		Natural ageing		3 Days AA		6 Days AA		9 Days AA		12 Days AA		Mean	
	2016-17	**2017-18**	**2016-17**	**2017-18**	**2016-17**	**2017-18**	**2016-17**	**2017-18**	**2016-17**	**2017-18**	**2016-17**	**2017-18**	**2016-17**	**2017-18**
0 month	5.62	5.61	5.59	5.59	4.62	4.60	3.75	3.68	2.72	2.70	1.86	1.85	**4.03**	**4.01**
3 month	5.11	5.08	4.98	4.97	3.86	3.81	3.32	3.31	2.13	2.12	1.32	1.32	**3.45**	**3.43**
6 month	4.63	4.62	3.75	3.74	3.08	3.08	2.96	2.96	1.87	1.85	0.98	0.98	**2.88**	**2.87**
9 month	4.06	4.05	2.15	2.13	2.72	2.70	2.54	2.54	1.03	1.03	0.64	0.64	**2.19**	**2.18**
12 month	3.54	3.54	1.34	1.33	1.78	1.78	1.63	1.63	0.72	0.71	0.17	0.17	**1.53**	**1.53**
Mean	**4.59**	**4.58**	**3.56**	**3.55**	**3.21**	**3.19**	**2.84**	**2.82**	**1.69**	**1.68**	**0.99**	**0.99**		

AA= Accelerated ageing. (Controlled aged seeds were subjected to AA)

LSD (p=0.05) **2016-17** **2017-18**

Factor A: Genotypes NS NS

Factor B: Ageing 0.049

0.073

Factor C: Duration of storage 0.045

0.067

Genotypes x Ageing NS NS

Genotypes x Duration of storage NS NS

Ageing x Duration of storage 0.110

0.164

Genotypes x Ageing x Duration of storage NS NS

The SDS-PAGE profile of both genotypes of hot pepper seeds viz; CH-27 and Punjab Sindhuri showed a changed banding pattern in seeds subjected to natural ageing for 12 months as well as seeds accelerated aged for 12 days when compared with freshly harvested seeds. In both the genotypes, a total of 10 bands were observed in freshly harvested seeds. Naturally aged seeds showed absence of some proteins bands (36.71, 19.28, 16.42. 14.43. 13.10 and 12.29 kDa) in both the genotypes of hot pepper but in the seeds which were subjected to accelerated ageing for 12 days, the proteins with 13.10 and 12.39 kDa were absent in CH-27 while in Punjab Sindhuri 21.24, 13.10 and 12.29 kDa were absent. This indicates that seeds subjected to natural ageing showed fewer bands on protein profile as compared to the seeds accelerated ageing for 12 days. Seed ageing leads to changes at morpho-physiological, biochemical and molecular level. All these contribute to loss of seed quality (Table 13, Fig 1).

TABLE 12: Response of ageing and duration of storage on malondialdehyde (MDA) (μmol g^{-1}) FW in hot pepper seeds under laboratory conditions. (The data are mean values for two genotypes viz; CH-27 and Punjab Sindhuri).

Ageing / **Duration of storage**	**Controlled ageing**		**Natural ageing**		**3 Days AA**		**6 Days AA**		**9 Days AA**		**12 Days AA**		**Mean**	
	2016-17	**2017-18**	**2016-17**	**2017-18**	**2016-17**	**2017-18**	**2016-17**	**2017-18**	**2016-17**	**2017-18**	**2016-17**	**2017-18**	**2016-17**	**2017-18**
0 month	0.03	0.04	0.04	0.04	0.27	0.27	0.32	0.33	0.45	0.46	0.52	0.52	**0.27**	**0.27**
3 month	0.04	0.04	0.12	0.13	0.30	0.30	0.45	0.45	0.53	0.53	0.68	0.69	**0.35**	**0.35**
6 month	0.13	0.13	0.53	0.53	0.42	0.42	0.56	0.56	0.63	0.63	0.74	0.74	**0.50**	**0.50**
9 month	0.22	0.22	0.69	0.69	0.59	0.59	0.66	0.66	0.78	0.78	0.84	0.84	**0.63**	**0.63**
12 month	0.34	0.34	0.87	0.88	0.78	0.78	0.83	0.83	0.91	0.92	0.99	1.02	**0.79**	**0.79**
Mean	**0.14**	**0.15**	**0.45**	**0.45**	**0.47**	**0.47**	**0.56**	**0.57**	**0.66**	**0.66**	**0.76**	**0.76**		

AA= Accelerated ageing. (Controlled aged seeds were subjected to AA)

LSD (p=0.05) **2016-17** **2017-18**
Factor A: Genotypes NS
0.002
Factor B: Ageing 0.007
0.003
Factor C: Duration of storage 0.006 0.003
Genotypes x Ageing NS NS
Genotypes x Duration of storage NS NS
Ageing x Duration of storage 0.115
0.007

Genotypes x Ageing x Duration of storage NS NS

Table 13: Protein profiling by SDS page

Controlled (MW)					
CH-27			**Punjab sindhuri**		
Fresh	**stored**	**12 days AA**	**Fresh**	**stored**	**12 days AA**
67.68	67.68	67.68	67.68	67.68	67.68
45.99	45.99	45.99	45.99	45.99	45.99
40.44	40.44	40.44	40.44	40.44	40.44
36.71		36.71	36.71		36.71
21.24	21.24	21.24	21.24	21.24	21.2
19.28		19.28	19.28		19.28
16.42		16.42	16.42		16.42
14.43		14.43	14.43	14.43	14.43
13.10		13.10	13.10		13.10
12.29			12.29		

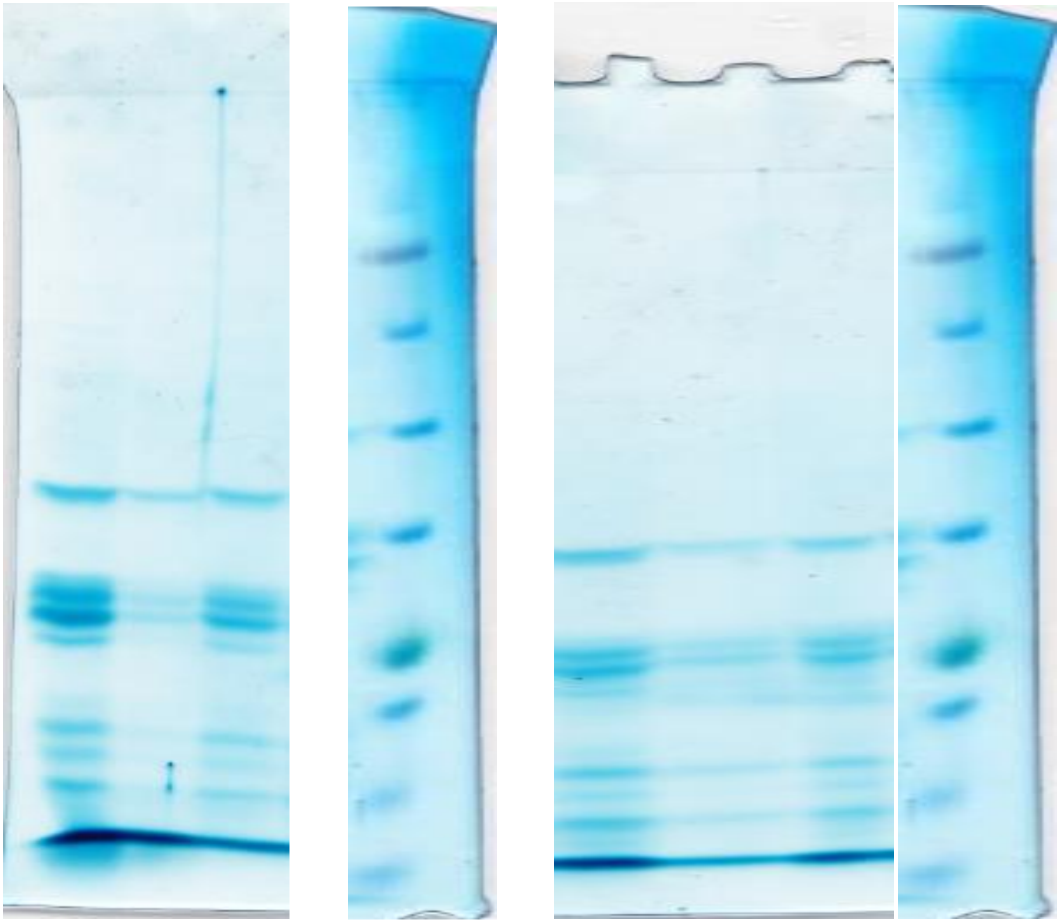


Fig: 1 CH-27 PUNJAB SINDHURI

Annexure-B

Publications

Research papers:

i. Paul A, Bedi S and Singh R (2018) Effect of different storage conditions on physiological and biochemical changes in onion *(Allium cepa* L.) cv. Punjab Naroya seeds. *Indian J. Agric. Biochem* **31**:79-81. (NAAS: 4.6)

ii. Bansal M, Bedi S, Singh R and Hagroo R P (2019) Germination and biochemical changes associated to different storage conditions and accelerated ageing in bitter gourd (*Momordica charantia*) seeds. *Indian J. Agric. Biochem*. (Accepted) (NAAS: 4.6)

iii. R P Hagroo, S Bedi and R Singh (2019) Effect of seed ageing on oxidative damage and priming induced repair process in hot pepper seed. (Submitted)

iv. R. P. Hagroo, S. Bedi and R. Singh (2019) DNA alteration and physiological changes during ageing of hot pepper seeds. (Submitted)

v. R. P. Hagroo, S. Bedi and R. Singh (2019) Protein profiling of aged hot pepper seeds subjected to priming and its relation to viability. (Submitted)

vi. R. P. Hagroo, S. Bedi and R. Singh (2019) Comparison of natural and AA ageing. (Submitted).

Abstracts:

i. Hagroo R P, Kaushal N and Bedi S (2018) Effect of seed priming on seed viability and vigour in hot pepper (*Capsicum annum* L.) seeds. Punjab Science Congress February 7-9, 2018. BP-38.

ii. Kaushal N, Hagroo R P and Bedi S (2018) Studies on seed priming in relation to germination and vigour in aged onion seeds. Punjab Science Congress February 7-9, 2018. BP-42.

iii. Paul A and Bedi S (2017) Effect of different storage conditions on activity of antioxidant enzymes and germination characteristics in onion (*Allium cepa* L.) cv. Punjab Naroya seeds. *Third International Conference on Bioresource and Stress Management,* Jaipur, India during 8-11 Nov. 2017.

iv. Kaushal N and Bedi S (2017) Changes in DNA integrity during storage of onion seeds. *8th Indian youth Science congress,* Mumbai University, Maharashtra. February (16-02-2017).

v. Kaushal N and Bedi S (2016) Effect of seed ageing on DNA content in onion seeds. Proceedings of *National Conference on Basic and Applied Researches in Plants and Microbes* Punjabi University Patiala November (03-05, 2016). p104.