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Effect of priming on physiological seed quality in aged seeds of hot pepper (*Capsicum annuum* L.) var. Punjab Sindhuri and hybrid CH-27

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Abstract
Hot pepper (*Capsicum annuum* L.) is an important spice crop of India and the quality of its seed is an important commercial trait. However, when stored for intervening months between harvesting and next sowing the seeds undergo a loss of quality. Freshly harvested seeds of hot pepper var. Punjab Sindhuri and hybrid CH-27 were subjected to natural ageing (stored at room temperature for 12 months), controlled ageing (stored in refrigerator for 12 months) and accelerated ageing (3,6,9 and 12 days). As the storage duration increases, germination, seedling length, seedling fresh and dry weight decreases while MDG, moisture content and electrolytic leakage increases. Aged seeds were primed in the solutions of Ascorbic acid (2% and 2.5%), PEG (25% and 30%), GA_3 (30ppm and 50ppm) and KNO_3 (1% and 2%) for 12 and 24 hours. All priming treatments significantly improved seed performance in terms of germination percentage, MDG, seedling growth, moisture content and electrolytic leakage. Among the various priming treatments KNO_3 primed seeds excelled over all other treatments.

Keywords: Priming, germination, electrolytic leakage, moisture content

Introduction
Seed deterioration is inevitable and irreversible which causes loss of seed quality with time. It is a natural process which involves cytological, physiological, biochemical and physical changes in seeds during storage. These changes reduce vigour followed by viability and ultimately death of the seed. Storability of seeds is of much concern as seeds undergo deterioration if the storage conditions are not appropriate. High seed moisture content at higher storage temperature hastens the process of seed deterioration resulting in the loss of seed viability (Mosavinik *et al*., 2011) [12]. The older seeds produce less vigorous seedlings which subsequently affect the yield (Rasmussen *et al*., 2015) [16].
The quality of hot pepper seed is a very important commercial trait. In north India, hot pepper is generally sown in the months of October-November (autumn-winter crop) and in February-March (spring-summer crop). The most favourable time for harvesting for seed production is when the fruit attain the deep red color (Tian *et al*., 2014) [21]. For seeds production fruits are harvested in mid-August till October.
In order to quickly obtain information on physiological changes that may occur in a seed during long term storage the technique of accelerated ageing is employed. Through this technique, the alteration that may occur in a seed at the cellular level during long-term storage can be simulated within a comparatively short period of time by exposing seeds to high temperatures (40-45°C) and a high relative humidity (Delouche and Baskin 1973) [6].
The present study was undertaken on hot pepper (*Capsicum annum* L.) hybrid CH-27 and cultivar Punjab Sindhuri seeds to investigate the effect of ageing and priming on viability and vigour. Seed priming treatments are used to reduce the damage of aging and for repair process thereby leading to seed invigoration. The positive effects of priming on the seed performance of many species are attributed to biochemical mechanisms of cell repair *viz*; the resumption of metabolic activity, restoration of cellular integrity, through the synthesis of DNA and RNA,

proteins and the improvement of the antioxidant defense system (Vaktabhai and Kumar, 2017) [22].

The objective of the present study was to study effect of ageing on germination percentage and other related parameters in hot pepper seeds during storage and the effect on moisture content and electrolytic leakage that co-relate with loss of seed viability during seed ageing. Some seed priming treatments were employed to determine the efficacy of these treatments in restoring the germination percentage and hence seed viability and vigour.

Materials and Methods

The experiments were conducted for two seasons, 2016-17 and 2017-18.

Plant Material: Seeds of hot pepper (*Capsicum annum* L.) hybrid CH-27 and cultivar Punjab Sindhuri were obtained from the department of Vegetable Science, PAU, Ludhiana. Seeds were harvested and extracted in September 2016 and 2017 respectively and experiment on two genotypes of hot pepper for storage and ageing studies was started from first week of October for both the years.

Seed Storage: The harvested seeds were subjected to three post-harvest storage protocols.

a) **Controlled ageing:** The seed were stored in refrigerator (5°C; 30% RH) for up to 12 months.
b) **Natural ageing:** Seeds were stored in glass vials which were placed in plastic containers at room temperature for up to 12 months.
c) **Accelerated aged seeds:** The seeds kept for controlled ageing, drawn after 12 months were subjected to accelerated ageing for 3,6,9 and 12 days by placing them in a desiccator with at 90% relative humidity (RH) kept in an incubator at50˚C (Winston and Bates 1960) [24].

Controlled, natural and accelerated aged seeds were subjected to standard germination tests. Germination tests were performed by rolled paper method ISTA (1999) [8] at 25ºC in an incubator. Three replications of 100 seeds each were taken. The coefficient of rate of germination (CRG) was calculated by using the formula: CRG = (ΣN/ ΣDn) ×100

(Where N is the number of seeds germinated on day D, and D is the number of days counted from the beginning of the germination).

Mean days to germination (MDG) was calculated by using the formula: MDG= (1/CRG) × 100

Seedling length was measured from tip of emerging leaf till tip of the longest root. A centimeter scale was used for measurements. For determination of fresh weight, at the end of fifteen days, seedlings were removed from germination papers, blotted dry and their fresh weight was recorded and expressed in grams. The same seedlings were oven-dried at 60°C for 36 hours (to a constant dry weight) and their dry weight was recorded. Electrolyte leakage from seed membrane was determined as per Liu and Huang (2000) [10]. Moisture content was determined in 3 replications with Halogen Moisture Analyzer (Mettler Toledo). The seeds were placed on plate of instrument and initial weight was recorded. The halogen dryer unit dried the seeds and final (dry) weight was recorded after 3 min. The moisture content (%) was displayed on the instrument.

Seed priming: For priming the seeds were allowed to imbibed in the following solutions for 12h and 24h, *viz*; Ascorbic acid (2% and 2.5%), Polyethylene glycol M.W. 6000 (PEG) (25% and 30%), GA_3 (30ppm and 50ppm) and KNO_3 (1% and 2%).

Statistical analysis

Experimental data will be analyzed as per standards statistical procedure for split plot design prescribed by Cochran and Cox (1957) [4] and adapted by Cheema and Singh (1991) [3] in Statistical package CPCS1, software developed by the Department of Mathematics and Statistics, PAU, Ludhiana.

Results

Germination percentage (%)

Both the genotypes of hot pepper *viz*; CH-27 and Punjab Sindhuri showed a significant difference for germination percentage (Table 1). Minimum germination was recorded in control seeds (T_1). Both natural and accelerated ageing have a negative effect on germination and the least germination % was found in seeds subjected to accelerated ageing for 12 days (30% and 29% respectively in both the genotypes). Significant differences were also observed among priming treatments. When compared to control (T_1), all treatments showed increase in germination % but differed in their magnitudes. Seeds treated with KNO_3 and GA_3 recorded highest germination % followed treatment with PEG and AsA. The maximum germination percentage (90% and 88.3% respectively in both the genotypes) was recorded in controlled aged seeds subjected to priming with KNO_3 (Table 1).

Table 1: Effect of different priming treatments on germination percentage (%) in seeds of two genotypes of *Capsicum annuum* under laboratory conditions

Ageing\Priming treatment	CH-27						Punjab Sindhuri					
	Controlled ageing	Natural ageing	3 Days AA	6 Days AA	9 Days AA	12 Days AA	Controlled ageing	Natural ageing	3 Days AA	6 Days AA	9 Days AA	12 Days AA
T1: Control	79.0	38.0	42.0	39.0	35.0	30.0	78.0	37.0	41.0	37.0	34.0	29.0
T2: ASA(2%) 12h soak	86.0	48.0	51.0	49.0	47.0	38.0	85.0	47.0	50.0	47.0	45.7	37.0
T3: ASA(2%) 24h soak	84.0	44.0	46.0	45.0	40.0	33.0	83.0	42.0	46.7	43.0	39.0	35.0
T4: ASA(2.5%) 12h soak	84.0	45.0	48.0	44.0	42.0	35.0	84.0	43.0	47.0	43.0	41.0	36.0
T5: ASA(2.5%) 24h soak	83.0	42.0	46.0	43.0	39.0	32.0	82.0	40.0	45.0	41.0	38.0	33.0
T6: PEG(25%) 12h soak	84.0	44.0	47.0	44.0	38.0	34.0	83.0	42.0	46.0	43.0	37.0	33.0
T7: PEG(25%) 12h soak	85.3	47.0	51.0	46.0	45.0	38.7	85.0	46.0	50.0	45.0	44.0	37.0
T8: PEG(25%) 24h soak	85.0	45.0	48.0	45.0	42.0	37.0	84.0	44.0	47.0	50.7	43.3	36.0
T9: PEG(30%) 12h soak	87.0	50.0	53.0	51.0	49.0	41.0	86.0	48.0	52.0	49.0	48.0	40.0
T10: GA_3(30ppm) 12h soak	87.0	48.0	52.0	47.0	45.7	40.0	86.0	45.0	48.0	46.0	44.0	39.0
T11: GA_3(30ppm) 24h soak	86.0	44.0	49.0	44.0	39.0	33.0	85.0	41.0	44.7	42.0	36.0	32.0
T12: GA_3(50ppm) 12h soak	89.0	51.0	54.0	52.0	50.0	42.0	88.0	47.0	50.0	47.0	49.0	41.0

T13: GA_3(50ppm) 24h soak	87.0	46.0	50.0	46.0	42.0	38.0	86.0	43.0	48.0	43.0	42.0	36.0
T14: KNO_3(1%) 12h soak	90.0	52.0	54.0	52.0	49.7	42.0	88.3	51.0	53.0	51.0	48.0	41.0
T15: KNO_3(1%) 24h soak	87.0	46.0	50.0	48.0	45.0	42.3	86.0	45.3	48.3	47.0	44.0	38.0
T16: KNO_3(2%) 12h soak	89.0	49.0	52.0	48.0	46.7	41.0	88.0	47.0	51.0	46.7	45.0	40.0
T17: KNO_3(2%) 24h soak	86.0	45.0	48.0	46.0	42.7	38.0	85.0	44.0	47.0	45.0	42.0	37.0

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

LSD (p=0.05)

Factor A: Genotypes	0.288
Factor B: Ageing	0.498
Factor C: Treatments	0.838
Genotypes x Ageing	NS
Genotypes x Treatments	NS
Ageing x Treatments	2.053
Genotypes x Ageing x Treatments	NS

Mean days to germination

Seed ageing significantly affected the mean days to germination (MDG) with ageing MDG increases i.e; seeds take more time to germinate. Among the different ageing treatments, the highest value of MDG was found in controlled seeds (T_1). The value of MDG increased with both natural and accelerated ageing. Seeds that had been accelerated aged for 12 days showed highest value of MDG (6.71d and 6.73d respectively in both the genotypes). Significant differences were observed among priming treatment. When compared to control (T_1), all treatments showed reduction in MDG but the extent of difference varied. Among various priming treatments, treatment with KNO_3 (T_{14}, T_{15}, T_{16}, T_{17}) and GA_3 (T_{10}, T_{11}, T_{12}, T_{13}) recorded minimum MDG. Among these, the treatment T_{14} showed minimum MDG (Table 2).

Table 2: Effect of different priming treatments on mean days to germination (MDG) in seeds of two genotypes of *Capsicum annuum* under laboratory conditions

Ageing\Priming treatment	CH-27						Punjab Sindhuri					
	Controlled ageing	Natural ageing	3 Days AA	6 Days AA	9 Days AA	12 Days AA	Controlled ageing	Natural ageing	3 Days AA	6 Days AA	9 Days AA	12 Days AA
T1: Control	3.02	5.61	5.16	5.23	5.78	6.71	3.05	5.67	5.18	5.25	5.41	6.73
T2: ASA(2%) 12h soak	2.81	4.13	3.84	4.08	4.27	6.05	2.83	4.15	3.85	4.09	4.29	6.07
T3: ASA(2%) 24h soak	2.92	4.28	4.09	4.23	4.36	6.28	2.93	4.29	4.09	4.23	4.36	6.29
T4: ASA(2.5%) 12h soak	2.89	4.22	3.96	4.17	4.31	6.17	2.90	4.22	3.97	4.19	4.30	6.19
T5: ASA(2.5%) 24h soak	2.96	4.34	4.16	4.29	4.41	6.32	2.98	4.36	4.18	4.30	4.42	6.34
T6: PEG(25%) 12h soak	2.88	4.23	3.97	4.19	4.27	6.16	2.89	4.24	3.98	4.20	4.34	6.16
T7: PEG(25%) 12h soak	2.81	4.14	3.83	4.12	4.25	6.06	2.82	4.16	3.84	4.15	4.25	6.07
T8: PEG(25%) 24h soak	2.84	4.18	3.89	4.15	4.31	6.11	2.86	4.19	3.90	4.17	4.30	6.12
T9: PEG(30%) 12h soak	2.78	4.11	3.74	4.01	4.19	5.96	2.80	4.12	3.75	4.04	4.21	5.98
T10: GA_3(30ppm) 12h soak	2.75	4.07	3.60	3.95	4.12	5.90	2.76	4.08	3.60	3.96	4.13	5.91
T11: GA_3(30ppm) 24h soak	2.83	4.12	3.71	3.98	4.16	5.94	2.84	4.13	3.71	3.99	4.17	5.95
T12: GA_3(50ppm) 12h soak	2.70	4.05	3.60	3.93	4.10	5.88	2.72	4.06	3.60	3.95	4.11	5.89
T13: GA_3(50ppm) 24h soak	2.79	4.10	3.65	3.95	4.13	5.91	2.81	4.12	3.65	3.96	4.15	5.91
T14: KNO_3(1%) 12h soak	2.68	4.03	3.57	3.89	4.06	5.86	2.69	4.04	3.59	3.90	4.07	5.87
T15: KNO_3(1%) 24h soak	2.72	4.07	3.63	3.93	4.11	5.89	2.73	4.08	3.64	3.94	4.13	5.91
T16: KNO_3(2%) 12h soak	2.70	4.07	3.62	3.93	4.09	5.88	2.71	4.08	3.63	3.95	4.11	5.89
T17: KNO_3(2%) 24h soak	2.75	4.09	3.65	3.95	4.13	5.90	2.77	4.10	3.47	3.96	4.15	5.92

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

LSD (p=0.05)

Factor A: Genotypes	NS
Factor B: Ageing 0.023 Factor C: Treatments	0.039
Genotypes x Ageing NS Genotypes x Treatments	NS
Ageing x Treatments	0.096
Genotypes x Ageing x Treatments	NS

Seedling length

Under laboratory conditions, both the genotypes *viz*; CH-27 and Punjab Sindhuri varied significantly for seedling length (Table 3). Upon germination, in 14 day old seedlings, mean minimum seedling length was recorded in controlled seeds (T_1). When compared to control, all the treatments showed increased seedling length but the magnitude of increase varied. Among various priming treatments, treatment with KNO_3 and GA_3 recorded maximum root, shoot and seedling length followed by PEG and AsA. The maximum germination percentage (11.10 cm and 10.80 cm respectively in both the genotypes) was recorded in controlled aged seeds subjected to priming with KNO_3 (Table 3).

Table 3: Effect of different priming treatments on seedling length (cm) in seeds of two genotypes of *Capsicum annuum* under laboratory conditions

Ageing\Priming treatment	CH-27						Punjab Sindhuri					
	Controlled ageing	Natural ageing	3 Days AA	6 Days AA	9 Days AA	12 Days AA	Controlled ageing	Natural ageing	3 Days AA	6 Days AA	9 Days AA	12 Days AA
T1: Control	9.30	4.20	6.50	5.70	4.70	3.00	8.90	4.20	6.20	5.30	4.20	2.70

T2: ASA(2%) 12h soak	10.00	6.50	7.70	6.70	6.00	4.00	9.70	6.20	7.40	6.50	5.70	3.80
T3: ASA(2%) 24h soak	9.80	5.70	7.20	6.50	5.40	3.70	9.30	5.70	7.00	6.20	5.10	3.43
T4: ASA(2.5%) 12h soak	9.80	6.20	7.40	6.50	5.70	3.70	9.40	5.90	7.10	6.20	5.40	3.60
T5: ASA(2.5%) 24h soak	9.60	5.60	7.00	6.20	5.20	3.50	9.20	5.30	6.70	6.00	4.90	3.20
T6: PEG(25%) 12h soak	9.70	6.30	7.50	6.40	5.60	3.70	9.50	6.00	7.20	6.30	5.40	3.50
T7: PEG(25%) 12h soak	10.00	6.70	8.00	6.80	6.10	4.10	9.70	6.50	7.80	6.70	5.90	3.80
T8: PEG(25%) 24h soak	10.00	6.70	7.90	6.80	5.90	3.90	9.80	6.50	7.70	6.60	5.70	3.70
T9: PEG(30%) 12h soak	10.40	7.10	8.30	7.30	6.40	4.40	10.20	6.80	8.10	7.10	6.20	4.20
T10: GA_3(30ppm) 12h soak	10.60	7.10	8.40	7.30	6.50	4.50	10.40	6.80	8.20	7.10	6.20	4.30
T11: GA_3(30ppm) 24h soak	10.00	6.70	7.80	6.80	6.10	3.90	9.80	6.50	7.50	6.60	5.80	3.70
T12: GA_3(50ppm) 12h soak	10.80	7.50	8.60	7.70	6.70	4.80	10.60	7.30	8.10	7.40	6.50	4.60
T13: GA_3(50ppm) 24h soak	10.30	7.00	8.00	7.10	6.40	4.20	10.20	6.80	7.90	6.80	6.20	3.80
T14: KNO_3(1%) 12h soak	11.10	7.80	9.00	8.00	7.10	5.20	10.80	7.60	8.80	7.80	6.90	4.90
T15: KNO_3(1%) 24h soak	10.80	7.30	8.60	7.70	6.70	4.80	10.60	7.20	8.30	7.40	6.50	4.70
T16: KNO_3(2%) 12h soak	10.90	7.40	8.80	7.80	6.80	5.00	10.60	7.20	8.50	7.50	6.50	4.80
T17: KNO_3(2%) 24h soak	10.50	7.00	8.20	7.40	6.50	4.60	10.30	6.80	8.00	7.10	6.00	4.40

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

LSD (p=0.05)

Factor A: Genotypes	0.116
Factor B: Ageing	0.020
Factor C: Treatments	0.339
Genotypes x Ageing	NS
Genotypes x Treatments	NS
Ageing x Treatments	NS
Genotypes x Ageing x Treatments	NS

Seedling fresh and dry weight

Both the genotypes of hot pepper *viz*; CH-27 and Punjab Sindhuri varied significantly for both seedling fresh and dry weight (Table 4, 5). With increase in ageing, seedling fresh and dry weight showed a gradual reduction in both the genotypes. Minimum seedling fresh and dry weight was recorded in controlled seeds (T_{14}). Seeds that had been accelerated aged for 12 days showed least value of seedling fresh weight (0.12g and 0.09g in both the genotypes) and seedling dry weight (0.012g and 0.011g respectively in both the genotypes). When compared to control, all treatments showed increased seedling fresh and dry weight but differed in their magnitudes. Among various priming chemicals, treatment with KNO_3 and GA_3 recorded maximum fresh and dry weight. Among all the priming treatments, T_{14}, T_{15}, T_{16} and T_{17} showed better results and T_{14} recorded maximum seedling fresh weight (Table 4, 5).

Table 4: Effect of different priming treatments on seedling fresh weight (g) in seeds of two genotypes of *Capsicum annuum* under laboratory conditions

Ageing\Priming treatment	CH-27						Punjab Sindhuri					
	Controlled ageing	Natural ageing	3 Days AA	6 Days AA	9 Days AA	12 Days AA	Controlled ageing	Natural ageing	3 Days AA	6 Days AA	9 Days AA	12 Days AA
T1: Control	0.86	0.38	0.41	0.21	0.18	0.12	0.84	0.37	0.38	0.19	0.15	0.09
T2: ASA(2%) 12h soak	0.98	0.56	0.68	0.42	0.26	0.18	0.96	0.55	0.67	0.40	0.25	0.16
T3: ASA(2%) 24h soak	0.94	0.54	0.63	0.38	0.23	0.15	0.93	0.53	0.61	0.38	0.22	0.13
T4: ASA(2.5%) 12h soak	0.96	0.54	0.65	0.39	0.25	0.16	0.94	0.52	0.64	0.38	0.24	0.15
T5: ASA(2.5%) 24h soak	0.92	0.51	0.62	0.36	0.20	0.14	0.90	0.49	0.61	0.34	0.19	0.12
T6: PEG(25%) 12h soak	0.95	0.55	0.66	0.41	0.25	0.17	0.94	0.54	0.65	0.40	0.24	0.16
T7: PEG(25%) 12h soak	1.01	0.57	0.70	0.43	0.28	0.20	1.00	0.55	0.69	0.42	0.27	0.18
T8: PEG(25%) 24h soak	0.99	0.57	0.68	0.43	0.27	0.18	0.98	0.55	0.67	0.41	0.26	0.17
T9: PEG(30%) 12h soak	1.03	0.59	0.72	0.45	0.30	0.21	1.01	0.58	0.70	0.44	0.28	0.20
T10: GA_3(30ppm) 12h soak	1.04	0.61	0.74	0.47	0.33	0.23	1.03	0.60	0.73	0.45	0.32	0.22
T11: GA_3(30ppm) 24h soak	1.01	0.60	0.73	0.46	0.32	0.22	1.00	0.58	0.72	0.44	0.31	0.21
T12: GA_3(50ppm) 12h soak	1.05	0.62	0.75	0.48	0.34	0.25	1.04	0.61	0.73	0.47	0.32	0.23
T13: GA_3(50ppm) 24h soak	1.02	0.61	0.74	0.46	0.33	0.23	1.01	0.59	0.73	0.45	0.32	0.22
T14: KNO_3(1%) 12h soak	1.07	0.63	0.77	0.49	0.36	0.26	1.06	0.61	0.76	0.48	0.35	0.25
T15: KNO_3(1%) 24h soak	1.05	0.62	0.75	0.48	0.33	0.24	1.03	0.61	0.73	0.47	0.32	0.22
T16: KNO_3(2%) 12h soak	1.05	0.62	0.76	0.48	0.35	0.25	1.04	0.60	0.75	0.47	0.33	0.24
T17: KNO_3(2%) 24h soak	1.03	0.61	0.75	0.47	0.33	0.22	1.02	0.59	0.73	0.46	0.31	0.20

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

LSD (p=0.05)

Factor A: Genotypes	0.007
Factor B: Ageing	0.012
Factor C: Treatments	0.024
Genotypes x Ageing	NS
Genotypes x Treatments	NS
Ageing x Treatments	0.048
Genotypes x Ageing x Treatments	NS

Table 5: Effect of different priming treatments on seedling dry weight (g) in seeds of two genotypes of *Capsicum annuum* under laboratory conditions

Ageing\Priming treatment	CH-27						Punjab Sindhuri					
	Controlled ageing	Natural ageing	3 Days AA	6 Days AA	9 Days AA	12 Days AA	Controlled ageing	Natural ageing	3 Days AA	6 Days AA	9 Days AA	12 Days AA
T1: Control	0.052	0.026	0.037	0.026	0.017	0.012	0.050	0.024	0.035	0.024	0.016	0.011
T2: ASA(2%) 12h soak	0.064	0.043	0.047	0.039	0.025	0.016	0.063	0.042	0.046	0.039	0.024	0.015
T3: ASA(2%) 24h soak	0.060	0.039	0.043	0.035	0.023	0.014	0.059	0.038	0.042	0.034	0.021	0.013
T4: ASA(2.5%) 12h soak	0.062	0.041	0.045	0.037	0.023	0.015	0.061	0.041	0.044	0.038	0.022	0.014
T5: ASA(2.5%) 24h soak	0.059	0.037	0.042	0.035	0.021	0.013	0.058	0.036	0.041	0.033	0.020	0.012
T6: PEG(25%) 12h soak	0.063	0.043	0.048	0.039	0.026	0.015	0.062	0.042	0.047	0.038	0.025	0.013
T7: PEG(25%) 12h soak	0.065	0.046	0.051	0.042	0.028	0.018	0.064	0.045	0.049	0.041	0.027	0.017
T8: PEG(25%) 24h soak	0.065	0.044	0.049	0.040	0.027	0.016	0.063	0.043	0.048	0.039	0.026	0.015
T9: PEG(30%) 12h soak	0.067	0.048	0.052	0.043	0.030	0.019	0.066	0.046	0.051	0.042	0.028	0.018
T10: GA_3(30ppm) 12h soak	0.071	0.051	0.057	0.047	0.034	0.019	0.070	0.050	0.056	0.046	0.033	0.018
T11: GA_3(30ppm) 24h soak	0.068	0.048	0.053	0.044	0.031	0.016	0.067	0.047	0.052	0.043	0.031	0.015
T12: GA_3(50ppm) 12h soak	0.072	0.053	0.058	0.049	0.036	0.020	0.071	0.051	0.057	0.048	0.035	0.019
T13: GA_3(50ppm) 24h soak	0.069	0.050	0.056	0.045	0.033	0.017	0.068	0.048	0.054	0.044	0.032	0.016
T14: KNO_3(1%) 12h soak	0.075	0.057	0.061	0.052	0.039	0.021	0.074	0.056	0.059	0.051	0.038	0.020
T15: KNO_3(1%) 24h soak	0.072	0.055	0.059	0.049	0.036	0.018	0.071	0.054	0.058	0.047	0.035	0.017
T16: KNO_3(2%) 12h soak	0.073	0.055	0.059	0.051	0.037	0.019	0.072	0.054	0.059	0.050	0.036	0.018
T17: KNO_3(2%) 24h soak	0.070	0.054	0.055	0.048	0.034	0.017	0.069	0.052	0.056	0.047	0.032	0.016

AA= Accelerated ageing. (Controlled aged seeds were subjected to AA)
LSD (p=0.05)
Factor A: Genotypes 0.001
Factor B: Ageing 0.001
Factor C: Treatments 0.002
Genotypes x Ageing 0.002
Genotypes x Treatments NS
Ageing x Treatments NS
Genotypes x Ageing x Treatments NS

Electrolytic leakage

Both genotypes of hot pepper *viz*; CH-27 and Punjab Sindhuri varied significantly for electrolytic leakage (Table 6). Both natural and accelerated ageing have a significant effect on electrolytic leakage. Among the different ageing treatments, maximum electrolytic leakage (96.12% and 96.97% respectively in both the genotypes) was recorded in seeds subjected to 12 days of accelerated ageing. Priming treatments lead to repair and re-organization of cellular membrane and thereby reduce electrolytic leakage. When compared to control, all treatments result in the decrease of electrolytic leakage but differed in their magnitudes. Among various priming chemicals, treatment with KNO_3 and GA_3 recorded minimum electrolytic leakage followed by PEG and AsA. The minimum electrolytic leakage (48.22% and 48.17% respectively in both the genotypes) was recorded in controlled aged seeds subjected to priming with KNO_3 (Table 6).

Table 6: Effect of different priming treatments on electrolytic leakage (%) in seeds of two genotypes of *Capsicum annuum* under laboratory conditions

Ageing\Priming treatment	CH-27						Punjab Sindhuri					
	Controlled ageing	Natural ageing	3 Days AA	6 Days AA	9 Days AA	12 Days AA	Controlled ageing	Natural ageing	3 Days AA	6 Days AA	9 Days AA	12 Days AA
T1: Control	58.34	91.50	84.92	89.21	92.52	96.12	58.94	91.57	86.04	90.03	93.55	96.97
T2: ASA(2%) 12h soak	53.64	79.96	75.64	84.72	86.94	90.34	54.12	78.88	76.71	85.68	87.72	91.28
T3: ASA(2%) 24h soak	54.99	81.77	76.98	85.91	87.79	91.32	55.34	82.66	77.78	86.83	88.84	91.97
T4: ASA(2.5%) 12h soak	54.24	80.65	76.21	85.11	87.31	90.91	55.04	81.32	77.26	86.07	88.13	91.78
T5: ASA(2.5%) 24h soak	55.64	82.84	77.36	86.31	88.22	90.44	56.07	83.92	78.42	87.42	89.21	91.51
T6: PEG(25%) 12h soak	52.84	78.31	74.64	84.34	86.83	89.14	53.74	78.89	75.60	85.14	87.70	89.83

T7: PEG(25%) 12h soak	51.91	77.14	73.24	83.14	85.31	88.04	52.72	77.78	73.86	83.96	86.79	88.71
T8: PEG(25%) 24h soak	52.09	77.92	73.91	83.78	86.06	88.77	52.83	79.72	74.82	84.44	86.71	89.33
T9: PEG(30%) 12h soak	51.36	76.96	72.88	82.64	84.99	87.63	51.99	77.82	73.64	83.14	85.78	88.89
T10: GA_3(30ppm) 12h soak	49.97	74.91	70.83	79.94	82.76	85.93	50.63	75.03	71.64	80.73	83.99	86.84
T11: GA_3(30ppm) 24h soak	50.94	75.74	71.92	80.42	83.84	86.83	51.84	76.77	72.83	81.66	84.54	87.91
T12: GA_3(50ppm) 12h soak	49.66	74.51	70.13	79.43	82.22	85.32	50.03	75.93	71.01	80.13	83.24	86.04
T13: GA_3(50ppm) 24h soak	50.14	75.17	71.24	80.06	83.21	86.33	51.11	76.21	72.04	80.99	84.01	87.37
T14: KNO_3(1%) 12h soak	48.22	73.36	69.52	78.31	81.10	84.15	48.17	74.44	70.62	79.24	82.13	85.20
T15: KNO_3(1%) 24h soak	49.33	74.10	70.61	79.66	82.14	85.11	49.96	75.17	71.51	80.54	83.31	86.43
T16: KNO_3(2%) 12h soak	48.73	73.84	69.90	78.72	81.63	84.86	49.24	74.97	71.02	79.84	82.71	85.78
T17: KNO_3(2%) 24h soak	50.04	74.85	70.93	79.87	82.80	85.73	50.52	75.82	71.99	81.34	84.02	87.13

AA= Accelerated ageing. (Controlled aged seeds were subjected to AA)
LSD (p=0.05)
Factor A: Genotypes 0.081
Factor B: Ageing 0.141
Factor C: Treatments 0.238
Genotypes x Ageing NS
Genotypes x Treatments NS
Ageing x Treatments 0.582
Genotypes x Ageing x Treatments NS

Moisture content

Both the genotypes of hot pepper *viz*; CH-27 and Punjab Sindhuri varied significantly for moisture content (Table 7) Among the various ageing treatments, maximum moisture content (6.46% and 6.48% respectively in both the genotypes) was recorded in seeds subjected to accelerated ageing for 12 days. Significant differences were also observed among priming chemicals when compared to control. All the treatments result in the decrease of moisture content but differed in their magnitudes. Among various priming chemicals, treatment with KNO_3 and GA_3 recorded minimum moisture content followed by treatments with PEG and AsA. The seed subjected to priming treatment T_{14} had minimum moisture content which was statistically at par with seeds primed with T_{15}, T_{16}, T_{12} and T_{17} (Table 7).

Table 7: Effect of different priming treatments on moisture content (%) in seeds of two genotypes of *Capsicum annuum* under laboratory conditions

Ageing\Priming treatment	CH-27						Punjab Sindhuri					
	Controlled ageing	Natural ageing	3 Days AA	6 Days AA	9 Days AA	12 Days AA	Controlled ageing	Natural ageing	3 Days AA	6 Days AA	9 Days AA	12 Days AA
T1: Control	6.13	6.46	6.34	6.38	6.45	6.46	6.14	6.47	6.36	6.37	6.44	6.48
T2: ASA(2%) 12h soak	6.07	6.35	6.25	6.29	6.35	6.35	6.08	6.36	6.27	6.30	6.36	6.39
T3: ASA(2%) 24h soak	6.09	6.38	6.28	6.31	6.38	6.41	6.11	6.39	6.28	6.32	6.39	6.42
T4: ASA(2.5%) 12h soak	6.09	6.37	6.28	6.30	6.37	6.39	6.10	6.38	6.29	6.32	6.38	6.40
T5: ASA(2.5%) 24h soak	6.10	6.40	6.29	6.32	6.40	6.42	6.12	6.41	6.30	6.33	6.41	6.44
T6: PEG(25%) 12h soak	6.08	6.38	6.27	6.32	6.36	6.40	6.09	6.39	6.28	6.33	6.38	6.41
T7: PEG(25%) 12h soak	6.06	6.35	6.21	6.29	6.34	6.37	6.07	6.37	6.25	6.30	6.35	6.38
T8: PEG(25%) 24h soak	6.07	6.36	6.26	6.31	6.35	6.38	6.08	6.38	6.28	6.32	6.36	6.40
T9: PEG(30%) 12h soak	6.05	6.33	6.22	6.27	6.32	6.35	6.06	6.34	6.24	6.29	6.33	6.37
T10: GA_3(30ppm) 12h soak	6.05	6.33	6.22	6.26	6.32	6.34	6.06	6.32	6.23	6.27	6.31	6.35
T11: GA_3(30ppm) 24h soak	6.07	6.34	6.24	6.29	6.34	6.37	6.09	6.36	6.25	6.30	6.35	6.38
T12: GA_3(50ppm) 12h soak	6.03	6.31	6.20	6.25	6.30	6.32	6.05	6.32	6.22	6.26	6.32	6.34
T13: GA_3(50ppm) 24h soak	6.05	6.34	6.23	6.27	6.33	6.35	6.07	6.35	6.24	6.29	6.34	6.37
T14: KNO_3(1%) 12h soak	6.00	6.28	6.18	6.22	6.28	6.30	6.02	6.30	6.19	6.24	6.29	6.32
T15: KNO_3(1%) 24h soak	6.04	6.31	6.21	6.24	6.31	6.32	6.05	6.32	6.23	6.25	6.32	6.33
T16: KNO_3(2%) 12h soak	6.02	6.30	6.20	6.23	6.29	6.32	6.03	6.31	6.21	6.25	6.31	6.33
T17: KNO_3(2%) 24h soak	6.05	6.32	6.22	6.25	6.32	6.33	6.06	6.33	6.23	6.26	6.34	6.34

AA= Accelerated ageing. (Controlled aged seeds were subjected to AA)
LSD (p=0.05)
Factor A: Genotypes 0.007
Factor B: Ageing 0.013
Factor C: Treatments 0.022
Genotypes x Ageing 0.018
Genotypes x Treatments 0.031
Ageing x Treatments NS
Genotypes x Ageing x Treatments NS

Discussion

Seed priming reduces the damage due to ageing and leads to the repair process thereby leading to seed invigoration. In the present study, priming significantly improved germination percentage which is corroborated by studies in tomato (Nawaz *et al*., 2011) [13] and bitter gourd (Kumar and Singh, 2013) [9]. The beneficial effects of the seed priming technique on the germination of many plant species are attributed to the activity of biochemical mechanisms such as cell cycle related events (De Castro *et al*., 2000) [5], preserving and stability of

cell membrane due to synthesis of nucleic acids (DNA and RNA) and proteins and enhancement in the antioxidant defense system capability (Di Girolamo and Barbanti, 2012) [7].

Priming significantly reduced mean days to germination (MDG) over unprimed seeds. Priming activate and synthesize hydrolytic enzymes e.g. lipases, amylases and proteases which mobilize storage materials in seed (Varier *et al* 2010) [23]. On rehydration quick emergence take place because all pre-germinative processes had already taken place. Priming activates antioxidant enzymes which lower per oxidation in seed thereby maintaining seed vigour which may result in quick germination (Pukacka and Ratajczak 2005) [15].

Our results are corroborated by Basra *et al* 2006 [1] who reported that primed sunflower and wheat seeds could germinate faster and produced longer seedling as compared to untreated seeds. Our study showed highest increase in seedling length when treated with KNO_3 similar increase in seedling length with KNO_3 was reported in cotton seeds (Nazir *et al* 2014) [14]. The difference in the seedling growth may be attributed to physiological and metabolic changes in seeds. The increase in root length with priming treatments might be due to repair of cellular membrane which restore physiological and metabolic processes. Enhancement of growth by soaking in priming chemicals might be the consequence of hydrolysis of complex sugars into simple sugars that are readily utilized in the synthesis of auxins and proteins. Auxins help to soften cell walls and facilitate growth by increased cell division and proteins are utilized in the production of new tissues as seen in tomato (Sabongari and Aliero 2004) [17].

Our results revealed that the seedling fresh and dry weight of primed seeds were significantly higher than control. A similar improvement in seedling fresh and dry weight due to priming treatments were observed in seeds of sunflower (Manjunatha *et al* 2018) [11]. The variation in the seedling fresh and dry weights of different priming treatments in the two genotypes might be due to restricted growth and decrease in seedling length that may occur as a result of loss of change in physiological, biochemical and molecular aspects (Sowmya 2011) [20]. Effective functioning of metabolic pathways leads to development of vigorous seedlings.

The electrolytic leakage was increased with advancement of storage period. This might be because less vigorous or more deteriorated seeds show a lower speed of cell membrane repair during seed water uptake for germination and therefore release greater amounts of solutes to the external environment. Damage to the membrane system can be repaired by priming treatment. The repair is indicated by low electrical conductivity of seed leachate. Protective action of priming chemicals extends the viability and restores vigour of seeds. Reduced leakage of electrolytes by priming has been repaired in seeds of tomato (Nawaz *et al* 2011) [13] and chickpea (Beedi *et al* 2018) [2].

Present study revealed that there is a reduction in the moisture content due to post-priming drying of seeds. Similar results were reported by Schipper *et al* (2001) [18]. Priming treatment allowing a gradual reduction of seed moisture has proved a sound way to restore longevity of seeds of onion (Schwember and Bradford 2011) [19]. This suggested that while seed longevity in storage was reduced, controlled hydration by priming might induce physiological or molecular changes that would render the seeds more resistant to deterioration.

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Conclusion

From the present study, it is concluded that ageing has an impact on seed viability under unfavourable storage conditions of high temperature and high relative humidity. As the storage duration in seed increases, germination, seedling length, seedling fresh and dry weight increases while there is an increase in MDG, electrolytic leakage and moisture. Priming restores the germination percentage and other lost parameters in aged seeds. Overall this leads to enhancement in performance potential of seed. Priming by 1% KNO_3 was best among different treatments.

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