



Effect of accelerated ageing on viability and longevity of wheat (*Triticum aestivum*) seed

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ABSTRACT

The accelerated ageing test (AAT) has been successfully used to rank and determine germinability of seed lots in various kind and varieties. An experiment was conducted to determine the influence of artificial ageing on seed viability and longevity of wheat seed of 2 varieties, viz. GW 496 and Lok 1. Progressive ageing period resulted in lowered seed quality in both wheat varieties. Combined effect of varieties and ageing duration showed the significant effect on all the observed seed quality parameters and recorded significant variation in these characters during (2015-16 and 2016-17) and in pooled analysis. The fresh seeds (without ageing) of wheat variety Lok 1 recorded highest average pooled germination (97.50%), seedling vigour index I (2166), seedling vigour index II (20.32) as well as observed biochemical parameters at the end of 5 days of accelerated ageing. Progressive ageing duration showed negative effect on biochemical parameters of the 5 days aged seeds of wheat variety GW 496 recorded lowest average pooled protein content (9.78%), carbohydrate content (60.36%), α amylase assay (6.19 micromoles/mg/min) and higher electrical conductivity (28.60 μ S/cm/g) due to fast deterioration at the end of artificial ageing.

Key words: Accelerated ageing, α Amylase assay, Carbohydrate, Protein, Seed quality

Wheat (*Triticum aestivum* L.) is the most important stable food crop for more than one third of the world population and contributes more calories and proteins to the world diet than any other cereal crops. It is considered a good source of protein, minerals, B-group vitamins and dietary fibre although the environmental conditions can affect nutritional composition of wheat grains with its essential coating of bran, vitamins and minerals; it is an excellent health-building food.

The deterioration of the stored seed is a natural phenomenon and the seeds tend to lose viability even under ideal storage conditions. Degradation and inactivation of enzymes due to changes in their macromolecular structures is one of the most important hypotheses proposed regarding causes of ageing in seeds. Lipid auto-oxidation and increase in free fatty acid content during storage are the most often mentioned reason for accelerated damage of seed. Controlled atmosphere storage offers a pesticide residue free alternative to conventional storage and protection techniques. Seed in commercial sealed storage is not readily accessible for routine quality evaluation and thus methods of predicting the safe storage period from the outset of storage are desirable.

A model for accelerated ageing at 30–60°C, moderate relative humidity levels was developed which indicated that the condition of the seeds was crucial in determining seed life-period and the accelerated ageing model can be used to accurate prediction of seed life.

Artificial ageing treatments take advantage of the fact that seed ageing process is determined by the seed moisture level and temperature. Manipulation of these factors, therefore, hastens movement of seed through the pattern of deterioration. The controlled deterioration and accelerated ageing (AA) tests have been successfully used to rank and predict relative field emergence potential of seed lots in various crops. The objective of artificial ageing is to study the sequence and relationship of process of deterioration over short period and its effect on seed viability, seedling vigour and biochemical process. Biochemical and physiological deterioration during seed ageing had been studied mostly under accelerated ageing conditions using high temperature and high seed water content (Jatoi *et al.* 2001, Hsu *et al.* 2003). For increasing awareness among farmers about the use of quality seeds, there is also a need to have some reliable parameters that evaluate the seed quality before it is sown in the field. Accelerated ageing has been recognized as a good predictor of the storability of seed lots by investigating the seed quality status of the naturally aged seed vis-a-vis the freshly harvested seeds. The study on post-harvest losses in food grains at different stages of their handling would help to assess the extent and magnitude of losses and identify

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the factors responsible for such losses which are important for scientists, technologists, policymakers, administrators and industrialists. Keeping these above problems in view the present investigation was conducted for evaluation of detrimental effect of artificial ageing on seed viability and longevity.

MATERIALS AND METHODS

The laboratory experiment was conducted at Anand Agricultural University, Gujarat with factorial CRD design

with 3 repetitions. Seeds of 2 varieties, viz. V_1 : GW 496 and V_2 : Lok 1 were subjected to accelerated ageing for D_1 : 0 day (fresh seed), D_2 : 1 day, D_3 : 2 day, D_4 : 3 day, D_5 : 4 day and D_6 : 5 day. The seed quality parameters, viz. germination (%), seedling shoot length (cm), seedling root length (cm), seedling length (cm), seedling fresh weight (g), seedling dry weight (g), seedling vigour index I, seedling vigour index II and electrical conductivity ($\mu\text{S}/\text{cm}/\text{g}$) as well as biochemical changes, viz. protein (%), carbohydrate (%) and α -amylase assay (micromoles/mg/min) were recorded

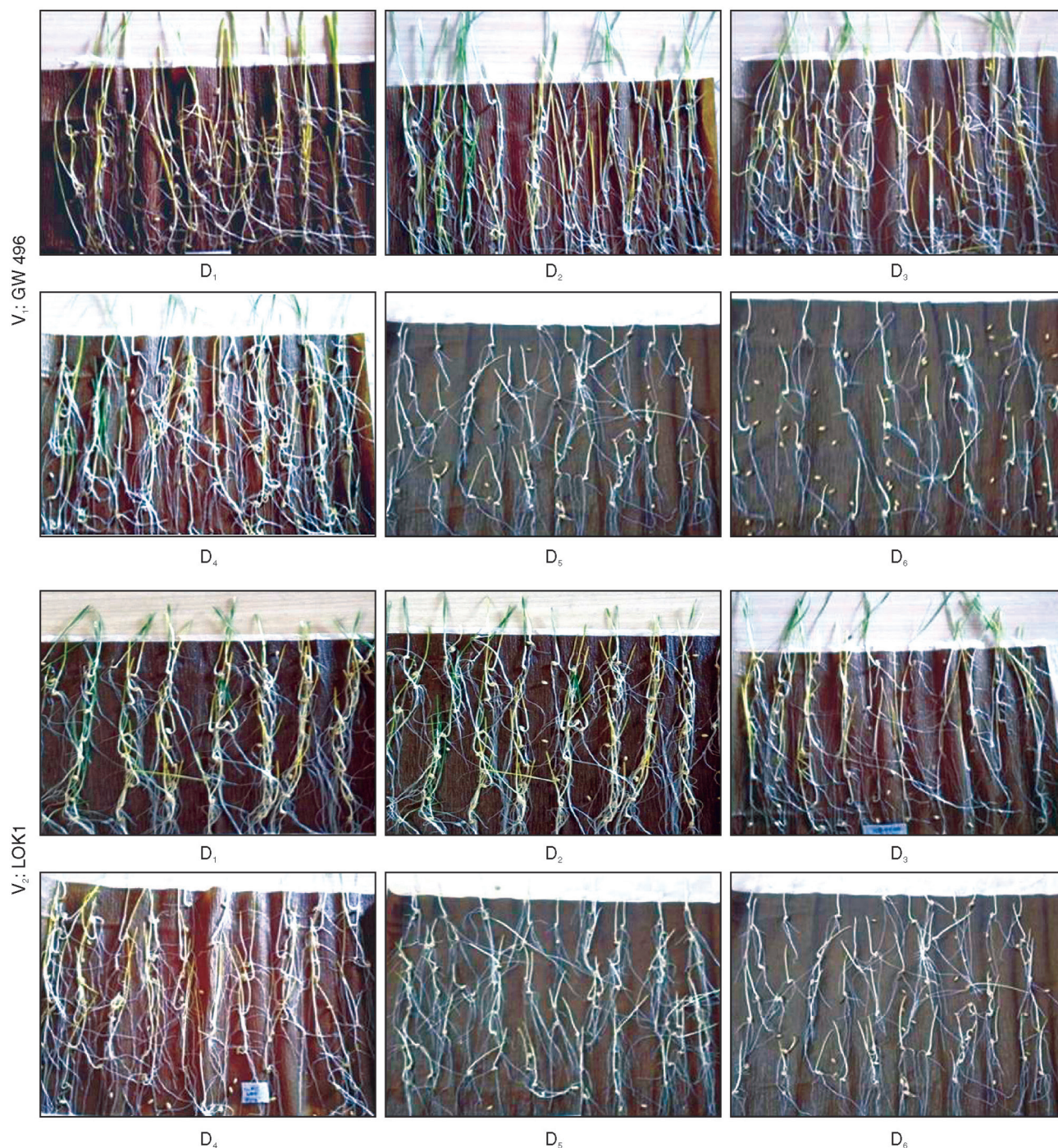


Fig 1 Effect of varieties and ageing period on germination (%) of wheat seed. V_1 : GW 496; V_2 : Lok 1; D_1 : 0 day; D_2 : 1 day; D_3 : 2 day; D_4 : 3 day; D_5 : 4 day; D_6 : 5 day.

for evaluation of seed viability during the year 2015-16 and 2016-17.

Accelerated ageing: The fresh seeds of wheat varieties, viz. GW 496 and Lok 1 were tied in a fine muslin cloth bag

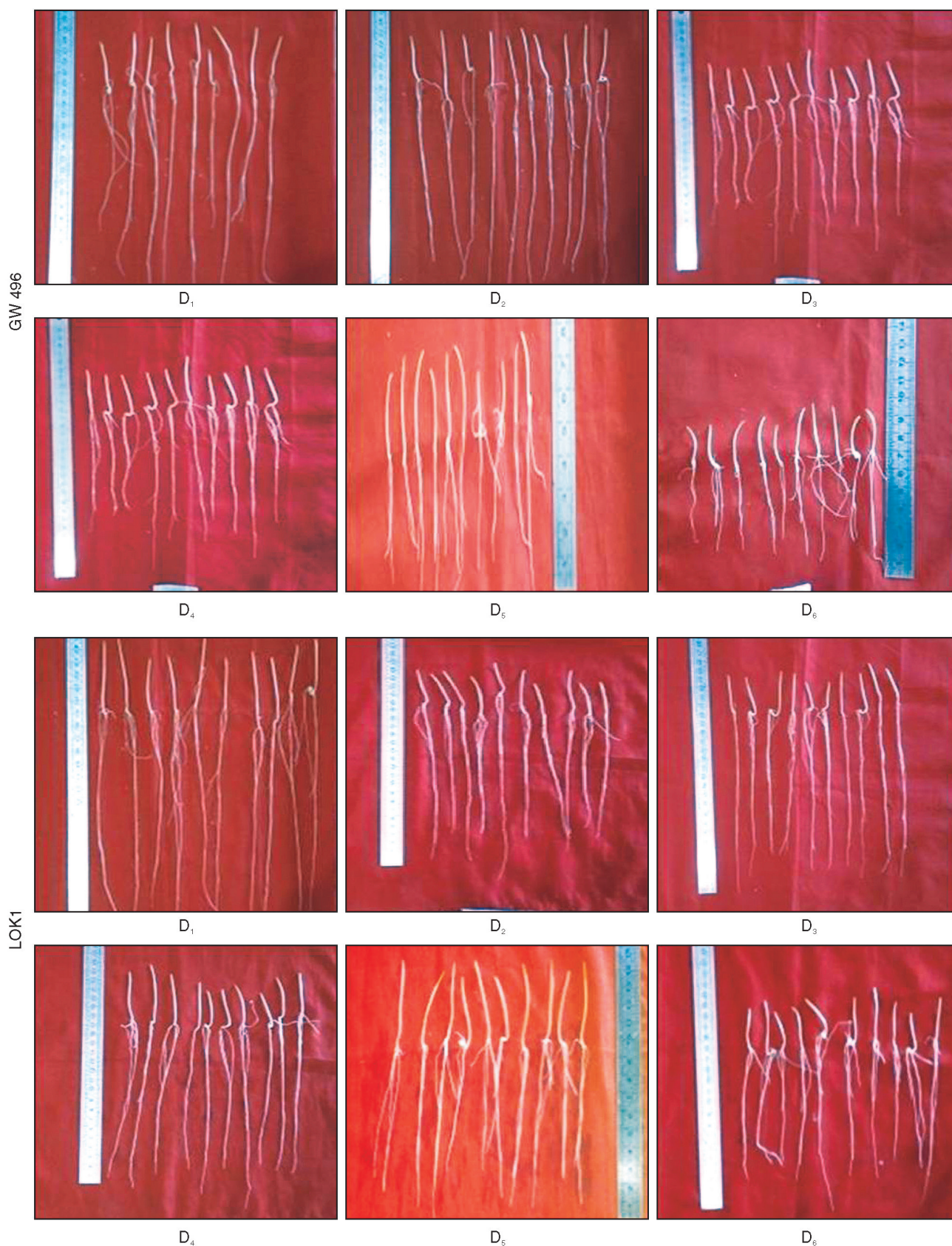


Fig 2 Effect of varieties and ageing period on seedling root length (cm) and shoot length (cm) of wheat seed. V₁, GW 496; V₂, Lok 1; D₁, 0 day; D₂, 1 day; D₃, 2 day; D₄, 3 day; D₅, 4 day; D₆, 5 day.

in 3 repetitions. The tied seeds were placed in desiccators on a wire mesh. The lower part of the desiccators was filled with water. Common salt was added in the water for maintaining high humidity. The desiccators were covered with the lid and sealed with paraffin wax to make it air tight and then placed in hot air oven at 45°C for 0 (control), 1,2,3,4 and 5 days. The desiccators were removed after specified period and the seeds were placed for cooling at room temperature. The aged seeds were then tested for normal germination test and other seed quality parameters.

Electrical conductivity ($\mu\text{S}/\text{cm}/\text{g}$): Electrical conductivity was measured following the standard procedure given by Agrawal and Dadlani (1992). Five gram of seeds in 3 replications were imbibed in 25 ml of deionised water at $20 \pm 1^\circ\text{C}$ for 24 h, the electrical conductance of the leachate was measured at room temperature and expressed as $\mu\text{S}/\text{cm}/\text{g}$.

Estimation of biochemical parameters (%): The protein and carbohydrate content was determined by Micro-Kjeldhal method (AOAC 1990) and phenol-sulphuric acid method (Dubois *et al.* 1956) respectively and α -amylase activity was assayed as per the procedure given by Bernfed (1955).

Statistical analysis method: The results were illustrated as the means and standard deviation of 3 repetitions with pooled analysis. Statistical analyses were performed by two factor analysis (ANOVA), with Factorial CRD design at the significance level $P < 0.05$. The drysoft software for Windows was used.

RESULTS AND DISCUSSION

Seed quality and biochemical parameters significantly differed in both the varieties due to artificial ageing during the year 2015-16 and 2016-17 (Tables 1–3; Fig 1-2).

Effect of varieties on seed quality and biochemical parameters: Ageing treatment showed significant effect on germination (%) in both wheat varieties GW 496 and Lok 1 irrespective of ageing duration. The appraisal of mean data pertaining to varieties significantly higher germination (79.94%), (84.00%) and (81.97%), seedling vigour index I (1359), (1583) and (1471), seedling vigour index II (13.87), (14.80) and (14.33). The biochemical parameters, significantly higher protein content (11.80%), (11.55%) and (11.68%), carbohydrate content (66.63%), (69.90%) and (68.27%), higher α -amylase assay (8.60 micromoles/mg/min), (8.85 micromoles/mg/min) and (8.73 micromoles/mg/min) and lowest electrical conductivity (23.43 $\mu\text{S}/\text{cm}/\text{g}$), (19.89 $\mu\text{S}/\text{cm}/\text{g}$) and (21.66 $\mu\text{S}/\text{cm}/\text{g}$) was recorded by the variety Lok 1 for the year 2015-16 and 2016-17 and in pooled analysis respectively. The another variety GW 496 recorded lower germination as well as other quality attributes and biochemical parameters and faster deterioration with higher values of electrical conductivity during the same experimental duration.

Significant variation in seed quality attributes and biochemical parameters due to artificial ageing among varieties might be due to varietal differences and depicting specific genotypic potential to withstand under adverse condition. Both the varieties differ greatly in their response

to accelerated ageing. These differences revealed that the rate of deterioration varied with the varieties and some of the varieties showed faster deterioration as compared to others. The knowledge of differences in rate of seed deterioration in different varieties may be very useful to predict seed longevity during long-term storage. Lucia *et al.* (2004) and Ananthi *et al.* (2015) reported rate of deterioration in the seeds at different temperatures, depicting specific genotypic potentials. These differences revealed that the rate of deterioration varied with the varieties (Kapoor *et al.* 2010). The distinguishing behaviour of these varieties could be attributed to genotypic differences to sustain the ageing treatments. The differences in the seed growth potential have also been observed by Kalpana and Madhav Rao (1995) in pigeon pea. Merritt *et al.* (2003) reported that, the rate of seed deterioration can vary among various plant species. The negative relationship between seed size and germination index, root and shoot length was also observed by Kaya *et al.* (2008). Likewise, differential response of genotypes to withstand the ageing has been reported for naturally aged seeds of cotton by Goel *et al.* (2002). The experimental findings are in accordance with Anderson and Gupta (1986), Gidrol *et al.* (1998), Filho *et al.* (2001) in soybean and Kapoor *et al.* (2011) in rice.

Effect of ageing duration on seed quality and biochemical parameters: The seed quality of both varieties was significantly affected due to ageing duration and significant variation found during both the experimental years. Significantly, higher germination (96%), (97.67%) and (96.83%), seedling vigour index I (1910), (2125) and (2017), seedling vigour index II (18.73), (20.03) and (19.38), higher protein content (12.52%), (12.57%) and (12.55%), carbohydrate content (70.28%), (72.28%) and (71.28%), higher α -amylase assay (10.41 micromoles/mg/min), (11.07 micromoles/mg/min) and (10.74 micromoles/mg/min) and lowest electrical conductivity (20.22 $\mu\text{S}/\text{cm}/\text{g}$), (16.19 $\mu\text{S}/\text{cm}/\text{g}$) and (18.46 $\mu\text{S}/\text{cm}/\text{g}$) was recorded by the seeds without ageing treatment for the year 2015-16, 2016-17 and in pooled analysis respectively. At par values were recorded by 1 day aged seeds in most of the observed characters during both the years and pooled analysis. Increasing duration of ageing drastically decreased the seed quality. Lowest seed quality parameters were recorded by 5 days aged seeds.

Combined effect of variety and ageing duration on seed quality and biochemical parameters: Without aged seeds of Lok 1 variety recorded significantly higher germination (97.00%), (98.00%) and (97.50%), seedling vigour index I (2055) and (2276), seedling vigour index II (19.72), (20.91) and (20.32), higher protein content (12.53%), (12.64%) and (12.59%), carbohydrate content (70.85%) and (72.76%) and lowest electrical conductivity (19.68 $\mu\text{S}/\text{cm}/\text{g}$) and (15.78 $\mu\text{S}/\text{cm}/\text{g}$) for the year 2015-16, 2016-17 and in pooled analysis respectively. Whereas, the 5 days aged seeds of wheat variety GW 496 recorded significantly lower seed quality and biochemical parameters indicating the rapid deterioration of seeds due to artificial ageing. The α -amylase

Table 1 Effect of accelerated ageing on seed quality parameter

Treat- ment	Germination (%)						Seedling vigour index I						Seedling vigour index II														
	2015-16			2016-17			Pooled			2015-16			2016-17			Pooled			2015-16			2016-17			Pooled		
	Variety	Mean	V ₁	V ₂	Mean	V ₁	V ₂	Mean	V ₁	V ₂	Variety	Mean	V ₁	V ₂	Variety	Mean	V ₁	V ₂	Variety	Mean	V ₁	V ₂	Variety	Mean	V ₁	V ₂	Variety
D ₁	95.00	97.00	96.00	97.33	98.00	97.67	96.17	97.50	96.83	1764	2055	1910	1973	2276	2125	1869	2166	2017	17.74	19.72	18.73	19.15	20.91	20.03	18.44	20.32	19.38
D ₂	91.33	93.33	92.33	96.33	96.67	96.50	93.83	95.00	94.42	1511	1858	1684	1823	2044	1933	1667	1951	1809	16.13	17.72	16.92	17.98	19.01	18.49	17.05	18.36	17.71
D ₃	90.67	92.67	91.67	93.33	95.33	94.33	92.00	94.00	93.00	1407	1532	1469	1695	1925	1810	1551	1728	1640	13.59	16.66	15.13	14.31	17.79	16.05	13.95	17.23	15.59
D ₄	88.00	90.00	89.00	90.00	92.00	91.00	89.00	91.00	90.00	1092	1379	1235	1413	1685	1549	1253	1532	1392	12.91	14.71	13.81	12.91	15.03	13.97	12.91	14.87	13.89
D ₅	72.67	73.00	72.83	82.00	83.33	82.67	77.33	78.17	77.75	788	946	867	1044	1081	1062	916	1013	965	6.79	10.46	8.63	7.92	11.93	9.92	7.36	11.19	9.27
D ₆	24.00	33.67	28.83	27.67	38.67	33.17	25.83	36.17	31.00	229	387	308	313	491	402	271	439	355	2.07	3.93	3.00	2.59	4.13	3.36	2.33	4.03	3.18
Mean	76.94	79.94	78.44	81.11	84.00	82.56	79.03	81.97	80.50	1132	1359	1246	1377	1583	1480	1254	1471	1363	11.54	13.87	12.70	12.48	14.80	13.64	12.01	14.33	13.17
For comparing the means of	<i>S.E.M</i> ±	<i>C.D.</i> @	<i>S.E.M</i> ±	<i>C.D.</i> @	<i>S.E.M</i> ±	<i>C.D.</i> @	<i>S.E.M</i> ±	<i>C.D.</i> @	<i>S.E.M</i> ±	<i>C.D.</i> @	<i>S.E.M</i> ±	<i>C.D.</i> @	<i>S.E.M</i> ±	<i>C.D.</i> @	<i>S.E.M</i> ±	<i>C.D.</i> @	<i>S.E.M</i> ±	<i>C.D.</i> @	<i>S.E.M</i> ±	<i>C.D.</i> @	<i>S.E.M</i> ±	<i>C.D.</i> @	<i>S.E.M</i> ±	<i>C.D.</i> @	<i>S.E.M</i> ±	<i>C.D.</i> @	<i>S.E.M</i> ±
	5%	5%	5%	5%	5%	5%	5%	5%	5%	5%	5%	5%	5%	5%	5%	5%	5%	5%	5%	5%	5%	5%	5%	5%	5%	5%	5%
V	0.57	1.67	0.53	1.56	0.39	1.11	11.21	32.73	10.27	29.98	7.60	21.61	0.15	0.43	0.14	0.42	0.10	0.29									
D	0.99	2.90	0.93	2.70	1.51	5.47	19.42	56.69	17.79	51.92	44.42	161.52	0.25	0.74	0.25	0.28	1.03										
V × D	1.40	4.10	1.31	3.82	0.96	2.73	27.46	80.17	25.16	73.43	31.48	NS	0.36	1.05	0.35	1.02	0.25	1.71									
CV (%)	3.10	2.75	2.92	3.82	2.94	3.35	4.89	4.65																			

V₁, GW 496; V₂, Lok 1; D₁, 0 day; D₂, 1 day; D₃, 2 day; D₄, 3 day; D₅, 4 day; D₆, 5 day.

Table 2 Effect of accelerated ageing on protein content (%) and carbohydrate (%)

Treatment	Protein content (%)						Carbohydrate (%)					
	2015-16			2016-17			Pooled			2015-16		
	V ₁	V ₂	Mean	V ₁	V ₂	Mean	V ₁	V ₂	Mean	V ₁	V ₂	Mean
	Variety	Variety	Variety	Variety	Variety	Variety	Variety	Variety	Variety	Variety	Variety	Variety
D ₁	12.51	12.53	12.52	12.51	12.64	12.57	12.51	12.59	12.55	69.72	70.85	70.28
D ₂	11.98	12.51	12.24	11.84	12.24	12.04	11.91	12.38	12.14	67.45	69.89	68.67
D ₃	11.44	12.38	11.91	11.31	12.24	11.78	11.38	12.31	11.84	65.80	68.06	66.93
D ₄	11.44	11.71	11.58	11.44	11.31	11.38	11.44	11.51	11.48	65.36	67.19	66.28
D ₅	10.91	10.91	10.91	10.51	11.04	10.78	10.71	10.98	10.84	63.80	62.84	63.32
D ₆	9.85	10.78	10.31	9.71	9.85	9.78	9.78	10.31	10.05	59.27	60.93	60.10
Mean	11.36	11.80	11.58	11.22	11.55	11.39	11.29	11.68	11.48	65.23	66.63	65.93
For comparing the means of	<i>S.E.M</i> ±	<i>C.D.@ 5%</i>	<i>S.E.M</i> ±	<i>C.D.@ 5%</i>	<i>S.E.M</i> ±	<i>C.D.@ 5%</i>	<i>S.E.M</i> ±	<i>C.D.@ 5%</i>	<i>S.E.M</i> ±	<i>S.E.M</i> ±	<i>C.D.@ 5%</i>	<i>S.E.M</i> ±
V	0.07	0.21	0.07	0.07	0.19	0.14	0.05	0.14	0.21	0.20	0.62	0.58
D	0.12	0.36	0.12	0.12	0.34	0.24	0.09	0.24	0.37	0.34	1.07	1.00
V × D	0.18	0.51	0.16	0.16	0.48	0.34	0.12	0.34	0.52	0.49	1.52	1.41
CV (%)	2.63		2.48		2.55		1.37		1.22		0.65	NS

V₁, GW 496; V₂, Lok 1; D₁, 0 day; D₂, 1 day; D₃, 2 day; D₄, 3 day; D₅, 4 day; D₆, 5 day.

Table 3 Effect of accelerated ageing on α amylase (micromoles/mg/min) and electrical conductivity (μ S/cm/g)

Treatment	α amylase (micromoles/mg/min)						Electrical conductivity (μ S/cm/g)					
	2015-16			2016-17			2015-16			2016-17		
	V ₁	V ₂	Mean	V ₁	V ₂	Mean	V ₁	V ₂	Mean	V ₁	V ₂	Mean
D ₁	10.28	10.53	10.41	11.02	11.11	11.07	10.65	10.82	10.74	20.76	19.68	20.22
D ₂	9.74	10.02	9.88	9.91	10.17	10.04	9.83	10.10	9.96	22.71	21.94	22.33
D ₃	8.91	9.23	9.07	9.12	9.28	9.20	9.02	9.26	9.14	24.31	23.10	23.71
D ₄	7.31	7.82	7.57	7.88	8.24	8.06	7.60	8.03	7.81	25.78	23.87	24.83
D ₅	6.84	7.39	7.12	7.18	7.61	7.39	7.01	7.50	7.25	27.21	25.62	26.41
D ₆	6.01	6.61	6.31	6.38	6.68	6.53	6.19	6.65	6.42	28.60	26.40	27.50
Mean	8.18	8.60	8.39	8.58	8.85	8.71	8.38	8.73	8.55	24.90	23.43	24.16
For comparing the means of	<i>S.E.M</i> $\pm$	<i>C.D.</i> @ 5%	<i>S.E.M</i> $\pm$	<i>S.E.M</i> $\pm$	<i>C.D.</i> @ 5%	<i>C.D.</i> @ 5%	<i>S.E.M</i> $\pm$	<i>S.E.M</i> $\pm$	<i>C.D.</i> @ 5%	<i>S.E.M</i> $\pm$	<i>S.E.M</i> $\pm$	<i>C.D.</i> @ 5%
V	0.09	0.25	0.08	0.08	0.24	0.17	0.06	0.17	0.10	0.10	0.28	0.06
D	0.15	0.44	0.14	0.14	0.41	0.29	0.10	0.29	0.17	0.17	0.48	0.30
V $\times$ D	0.21	NS	0.20	0.20	NS	NS	0.16	NS	0.23	0.23	0.68	0.28
CV (%)	4.35		3.98		4.16		2.43		2.95		2.68	

V₁, GW 496; V₂, Lok 1; D₁, 0 day; D₂, 1 day; D₃, 2 day; D₄, 3 day; D₅, 4 day; D₆, 5 day.

assay (micromoles/mg/min) was not found significantly different during both the years and in pooled analysis.

Accelerated ageing resulted in progressive loss of seed viability and vigour. Wheat seeds exhibited an initial germination around 98% which declined progressively with increasing period of artificial ageing. The possible reason of this reduction might be the lowered biochemical activities in seed. Iqbal *et al.* (2002) reported 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. Reduced seed quality mainly attributed to degradation of mitochondrial membrane leading to reduction in energy supply necessary for germination. The ageing treatment caused inability of seeds to maintain the integrity of membranes and this accounts for the reduced germinability of seeds. Important processes during seed germination, such as the establishment of respiration, ATP production and protein synthesis are often perturbed by seed ageing. The chromosomal aberrations and DNA degradation with ageing leads to impaired transcription causing incomplete or faulty enzyme synthesis which is essential for earlier stages of germination.

Increase in stress level, reduced metabolic activities and increased division activity may be the possible reasons for the reduction in the rootlets and shoot growth. The reduction in seed viability is mainly a function of interaction between temperature and seed moisture content. Mohammadi *et al.* (2011) recorded seedling growth reduction due to ageing might be the result of decline in weight of mobilized seed reserve and reduction in growth parameters of artificially aged wheat seeds. Reduced protein content with progressed ageing duration might be attributed to denaturation of protein, elevated superoxide dismutase activity and lack of ATP. Channabasana gowda *et al.* (2008) stated that, the free fatty acid content and free radicals are the main causes of seed ageing and deterioration. Lower carbohydrate content (%) might be associated with denaturation of bio molecules, accumulation of toxic substances and loss of membrane integrity. The increase in thermo-chemiluminescence (TCL) due to higher temperature resulted in activation of carbohydrate hydrolysis in aged seeds. The decrease in carbohydrate content and the increase in moisture content activate the amino carbonyl reaction. Similar findings were also reported by Ravikumar *et al.* (2002) in *Dendrocalamus strictus*, Veselovsky and Veselova (2012) in buck wheat and Lakshmi *et al.* (2014) in edible bamboo seeds. Advanced deterioration due to ageing resulted in accumulation of aldehyde compounds especially methyl jasmonate (MeJA) which is a potential inhibitor of α -amylase. Higher concentration of MeJA reduced the concentration of the enzyme protein and also resulted in inhibition of gibberellin biosynthesis (Norastehnia *et al.* 2007). A gradual decline in amylase activity with progressive ageing duration was also reported by Agarwal and Kharlukhik (1987) in chickpea and Petruzzelli and Taranto (1990) in wheat.

Progressive ageing duration showed negative effect on seed quality attributes and biochemical parameters.

Significantly lower seed germination (%), seedling length (cm), vigour indices and biochemical parameters, viz. protein content (%), carbohydrate content (%), α -amylase assay (micromoles/mg/min) and higher electrical conductivity (μ S/cm/g) was observed at the end of 5 days of accelerated aging.

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