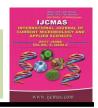


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Evaluation of Seed Quality of Barley Varieties through Controlled Deterioration Test

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ABSTRACT

Keywords

Barley, Controlled Deterioration, Germination, Seedling vigor, Viability

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Five varieties of Barley *viz.*, PL-751, VLB-56, RD-2668, K-713 and DWR-52 were conditioned to 20% moisture content (mc) and subjected to Controlled Deterioration (CD) test at 45°C for 48 and 96 hours, and were analyzed for various physiological parameters to determine the changes after CD. A rapid decline was observed in germination, speed and seedling vigour in all the varieties with increasing duration of CD. Significant differences among the varieties were also observed in all the traits. Seeds aged for 48 and 96 hours deteriorated progressively with 20% moisture content at 45°C which indicates that, physiological changes occur in the seeds under the combination of increasing temperature and moisture content. The extent of deterioration differed in different cultivars indicates the differences among the cultivars and their tolerance or susceptibility to storage conditions.

Introduction

Seed deterioration is a natural phenomenon that occurs in all the seeds and leads to the gradual decline in seed viability. Storage temperature and the seed moisture content are the most important determinants of longevity in storage. The rate at which the seed deterioration process takes place depends on the tolerance of seeds to resist degradation which is specific for different species (Gupta and Aneja, 2004). The processing and storage problems are common in tropical countries like India which has hot and humid tropical and subtropical conditions with fluctuations in relative humidity and temperature throughout the year. Seed deterioration is generally characterized by reduced seedling growth, germination capacity and viability (Mohammadi *et al.*, 2011). The rate of seed deterioration varies greatly among the varieties of the same species (Jatoi *et al.*, 2001).

Barley is cultivated as a rabi crop in India, with sowing being undertaken from October to December and harvesting from March to May. India's annual production of barley has been steadily around 1.2-1.5 million tonnes in the recent years. The area under cultivation has also stabilized at around 0.7-0.8 lakh hectares, with a per hectare yield of around 1944 kg. Although barley seeds are long lived, preliminary studies conducted in this

laboratory revealed that cultivar differences in longevity exist. Establishing the relative longevities of different varieties would facilitate in developing better processing and protocols storage for those which underperform in storage. Seed longevity predictions are made by conducting Accelerated Ageing (AA) tests in many crops seeds. The applications of CD test to grain crops such as wheat and maize were reported, (Modarresi and Van damme, 2003, Tekrony et al., 2005). Hrstkova et al., (2006) applied this test for comparing the performance of spring and winter barley varieties where 45°C and 24% moisture were recommended but their results indicate that this ageing period is insufficient to bring out the differences between the varieties used by them. The present study was thus aimed to determine the relative longevities of five barley varieties by subjecting them to a modified CD test allowing longer period of incubation at 45°C (48 and 96 hours) as well as raising the moisture content to 20% and also determine some of the physiological changes occurring in the deteriorated seeds which can serve as indices of seed quality.

Materials and Methods

The present investigations were undertaken at Germplasm Division of Conservation, National Bureau of Plant Genetic Resources, New Delhi. Five released varieties of barley viz. PL-751, VLB-56, RD-2668, K-713 and DWR-52 procured from National Seeds Corporation, New Delhi were used in present study. The CD test was conducted at 45°C and 20% mc for two durations i.e. 48 and 96 hours. For each variety 50 g seeds of known initial moisture content were placed on moist filter paper and allowed to imbibe to the weight calculated to achieve 20% mc. The achievement of this weight was determined by periodic weighing. Seeds were then kept at 5°C in laminated foil packets for 72 hours to

allow moisture equilibration within and among the seeds. Subsamples of 25g seeds/treatment were sealed in laminated foil packets and incubated at 45°C and removed after 48 and 96 hours (Powell and Matthews, 1981).

The seed lots not subjected to CD served as control. Moisture content of the seeds was determined by high constant temperature oven method (ISTA, 2003).

For germination three replication 50 seeds each were plated in rolled paper and incubated in a seed germinator at 25°C and 95% RH. The seeds were evaluated on the 7th day and normal seedlings were counted for calculation of percent germination (ISTA, 2003).

For the speed of germination, expressed as mean germination time (MGT), 50 seeds in triplicate were plated for germination and counts were taken every day up to 7th day. The MGT was calculated using the following formula:

Mean germination time (MGT) = Σ nd $/\Sigma$ n

Where, n= number of seeds which germinated on day d

d= number of days counted from the beginning of germination test.

Three replications of 25 seeds were plated for seedling vigour expressed as seedling length evaluated by measuring separately the length of the root and shoot on the 7th day, and summing up the two.

Seedling vigour index was calculated as the product of germination per cent and seedling length (Abdul-Baki and Anderson, 1973).

The Tetrazolium Test (TZ) for viability was performed for different varieties of barley

seeds before and after ageing. For this test 10 seeds in three replications were preconditioned by immersing in distilled water at 20°C overnight and sectioned longitudinally through the endosperm and embryo and immersed in one percent solution of TZ at pH 7.0 for 4 hrs in dark at 35°C. They were then evaluated for the staining pattern using the tetrazolium handbook (Moore, 1973) as the guide. The viability was calculated as percentage of properly stained seeds.

The data was subjected to statistical analysis using Statistical Package for Social Sciences (SPSS). Duncan's Multiple Range Test was followed for comparing treatment means.

Results and Discussion

Germination per cent

The changes in seed germination during CD in five barley cultivars are shown in table 1. Initial germination percentage ranged from 86.66 to 98 % in all control non aged seed lots. This trend changed drastically at 48 hours of CD where a significant fall in germination percent was observed in PL-751 (53.33%) and DWR-52 (55.33%) while K-713 (92%) recorded the highest germination followed by RD-2668 (88%) and VLB-56 (84%) which were on par with each other and also with the respective controls (Fig. 1a). The decrease in germination percent was more pronounced in all the varieties after 96 hours of CD (Fig. 1a). The decrease was drastic in PL-751 (6.66%) and DWR-52 (4%), moderate in VLB-56 (53.33%) while K-713(81.33%) maintained highest germination percentage.

Five barley cultivars used in the present study showed initially high quality prior to subjecting to CD. Progressive loss in the seed quality attributes occurred with ageing in all varieties but the intervarietal differences were highly significant. Similar observations have been made by others in several other crops viz., pea (Jatoi et al., 2001), wheat (Siddique et al., 2008, Sterlec et al., 2010), pearl millet (Sundereswaran et al., 2009) and rice (Ramanadane and Ponnuswamy, 2004, Ali et al., 2003). This kind of variability in response to natural and AA can be attributed to genetic constitution and the inherent capacity of a cultivar to withstand stresses. The results imply that K-713 had the highest quality in terms of germination and seedling vigour index. The increase in the germination per cent and vigour index after 48 hours of CD in this variety implies that vigorous metabolic repair leading to invigoration of seeds has taken place due to high moisture content of the seeds as well as the higher temperature to which the seeds were exposed during the CD test.

Mean germination time

MGT for control seed lots ranged from 1.29 to 1.80 (Table 1). The lowest MGT was shown by RD-2668 (1.29) while the highest by K-713(1.80). PL-751(1.54), VLB-56 (1.6) and DWR-52 (1.72) were on par. For all the varieties, CD test for 48 hours increased the length of time to reach the germination except for K-713 (1.19) which registered a significant decrease in MGT as compared to control. The maximum MGT was observed for PL-751(3.08) while VLB-56 (2.28) and DWR-52 (2.4) were on par (Table 1, Fig. 1b). As the ageing duration progressed for 96 hours all the varieties recorded a drastic and significant increase in MGT (Fig. 1b). The highest MGT was recorded for DWR-52 (6.73) which was on par of PL-751 (6.66) whereas the lowest MGT was recorded for K-713(2.48).

When the seed metabolism is disturbed, initial changes occur resulting in slowing down of germination process leading to late emergence of the seedlings.

Table.1 Effect of controlled deterioration test on germination percent and MGT

Varieties	Geri	Germination percent (%)			Mean Germination Time (Speed)		
	Control	CD 48 hrs	CD 96 hrs	Control	CD 48 hrs	CD 96 hrs	
PL 751	86.66b	53.33b	6.66cd	1.54b	3.08a	6.66a	
VLB 56	98.00a	84.00a	53.33b	1.60b	2.28b	4.07b	
RD 2668	94.66a	88.00a	25.33c	1.29c	1.65c	3.66bc	
K 713	86.66b	92.00a	81.33a	1.80a	1.19d	2.48c	
DWR 52	96.00a	55.33b	4.00d	1.72ab	2.40b	6.73a	

Data presented means of three replications.

Data followed by the same letter in a column, do not differ at the 5% probability level.

Table.2 Effect of controlled deterioration test on seedling vigour and vigour index of five barley varieties

Varieties	Seedling Vigour				Vigour index		
	Control	CD 48 hrs	CD 96 hrs	Control	CD 48 hrs	CD 96 hrs	
PL 751	30.66d	15.32c	1.72d	2639.90c	821.27c	9.70c	
VLB 56	35.59bc	26.00b	10.53b	3486.70a	b 2181.10b	552.60b	
RD 2668	39.90a	36.09a	5.67c	3777.10a	3181.30a	149.65c	
K 713	37.21ab	37.12a	31.76a	3222.10b	3421.60a	2583.00a	
DWR 52	33.57cd	13.43c	0.44d	3225.10b	751.73c	1.77c	

Data presented means of three replications.

Data followed by the same letter in a column, do not differ at the 5% probability level.

Table.3 Effect of controlled deterioration test on seed viability (TZ) of five barley varieties

Varieties			
	Control	CD 48 hrs	CD 96 hrs
PL 751	100a	63.33b	11.00d
VLB 56	100a	86.66a	43.33b
RD 2668	100a	86.66a	25.33c
K 713	100a	100 a	76.66a
DWR 52	100a	53.33b	6.66d

Data presented means of three replications.

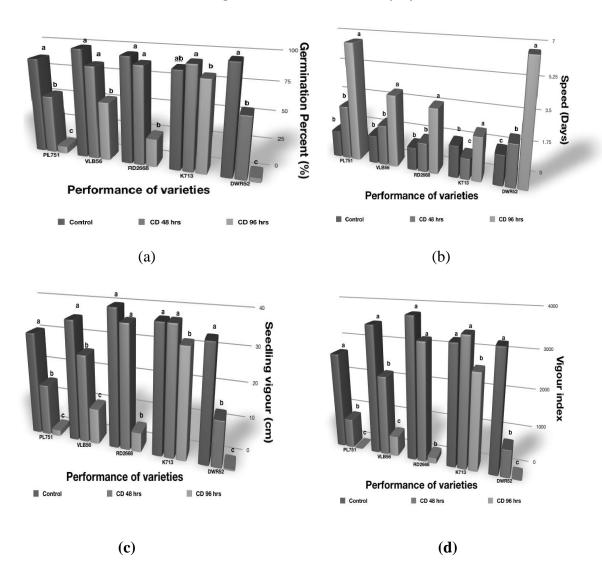
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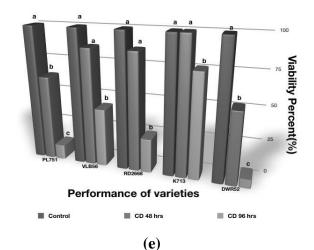
Table.4 Correlation matrix

Parameters	Germination	MGT	Seedling	Seed viability	Vigour
			vigour		index
Germination		823**	.940**		.915**
MGT			817**	849**	778**
Seedling				.949**	.963**
vigour					
Seed viability					.933**
Vigour index					

^{**} Correlation is significant at the 0.01 level (2-tailed).

Fig.1 Effect of controlled deterioration test on five barley varieties exposed to different duration of CD on (a) percent germination, (b) speed (MGT), (c) seedling vigour, (d) vigour index and (e) viability by TZ





Lengthening in the mean germination time is the earliest symptom of ageing (Eski and Demir, 2011). Basak et al., 2006 observed in pepper seed lots that decrease in germination percent was associated with an increase in emergence time of seeds. Seed deterioration expresses itself in a reduction in germination rate and uniformity, reduced tolerance to environmental stresses and consequently inferior seedling emergence and growth (Hampton and Tekrony, 1995). Highly significant negative correlation between germination per cent and MGT (r = -0.823)was obtained indicating that germination rate is an important indicator of seed quality loss (Table 4).

Seedling vigour

Seedling vigour was highly positively correlated with germination per cent (r = 0.940) and highly negatively correlated (r = -0.817) with MGT (Table 4). Changes in seedling vigour of different varieties in the control seed lots are given in table 2. Minimum seedling vigour in the control seeds was observed for PL-751(30.66) followed by DWR-52 (33.57) and maximum in RD-2668 (39.9) followed by K-713(37.21). After 48 hours of CD minimum seedling vigour was observed in DWR-52 (13.43) which was on

par with PL-751 (15.32) and maximum in K-713(37.12). After 96 hours of CD all the varieties recorded a significant decrease in seedling vigour as compared to 48 hours aged seed lots (Fig. 1c). Minimum seedling vigour was observed for DWR-52 (0.44) which was on par with PL-751(1.72) while maximum was recorded for K-713(31.76).

Differences in seedling vigour between seed lots are attributed to the rate of germination and seedling growth. There is evidence from comparison of seed lots of onion (Wheeler and Ellis, 1991) and artificially aged seeds of wheat (Guy and Black, 1998) that seeds which germinate earliest have an early start in growth and produce longer seedlings. Iqbal and Smith, 1996 observed a negative impact of seed ageing on root vigour in pea seeds. Seed deterioration during storage resulted in decreased seedling vigour. AA not only affected emergence percentage but also decreased the speed of germination and seedling growth rate resulting in reduced seedling vigour in cucumber seeds which was cultivar dependant (Al-Maskri et al., 2002).

Vigour index

Table 2 indicates that all the varieties maintained a high vigour index before CD

test. Minimum vigour index for control seed lots was observed for PL-751 (2639.90) and maximum for RD-2668 (3777.1) which was on par with VLB-56 (3486.7). K-713 (3222.1) and DWR-52 (3225.1) were on par for this parameter. After CD for 48 hours a significant reduction in vigour index was observed in all the varieties except K-713 (3421.60) in which there was increase in the vigour index followed by RD-2668 (3181.3). Minimum vigour index was exhibited by DWR-52 (751.73) which was on par with PL-751(821.27). As the CD test duration increased to 96 hours, all the five varieties recorded a significant decline in vigour index as compared to 48 hours aged seed lots (Fig. 1d). K-713 (2583) recorded the maximum vigour index while the minimum was observed for DWR-52 (1.77) followed by PL-751 (9.70).

Decrease in seedling vigour index due to AA has been reported by Agarwal and Kharlukhi, 1985 in wheat and chickpea seeds. Similar results were observed by Pallavi et al., 2003 in artificially aged sunflower seeds. Ageing induced loss of vigour is due to delayed and non-synchronous germination which in turn is due to altered sequence of metabolic events eventually leading to seed death. Since seedling vigour index is the product of germination and seedling vigour, increase or decrease in any one of the components or influence its value can Considerably higher vigour index (2583) exhibited by K-713 even after ageing for 96 hours implies its highest stress tolerance capacity despite the fact that its initial germination and vigour index were not the highest (Fig. 1d).

Seed Viability

A good correlation was observed between the viability calculated from TZ test with the germination test (r = 0.962) as well as

seedling vigour index (r = 0.963) indicating that this test can be used as a substitute for germination test in case quick results are desired (Table 4). Before treatment all varieties recorded 100% viability and were hence at par (Table 3). After 48 hours of CD a significant difference was observed between varieties in response to TZ staining. VLB-56 (86.66%),RD-2668 (86.66%) and 713(100%) were on par and differed significantly with PL-751 (63.33%) and DWR-52 (53.33%) (Table 3). After 96 hours of CD all the varieties recorded a significant decrease in viability as compared to 48 hours aged seed lots (Fig. 1e). The lowest viability was observed for DWR-52 (6.66%) and maximum viability was observed for K-713 (76.66%). Similar observations have been reported in aged corn seeds by Bittencourt et al., 2012 and tomato seeds by Santos et al. (2007).

The differences in between the cultivars of barley measured in the present study were highly significant confirming the impact of genotype as well as initial seed quality, especially seed vigour in the performance of the seed lots. The present study clearly indicates that different varieties of barley deteriorated at different rates when exposed to similar conditions. It is also evident that intial vigour rather than the initial germination per cent is a better indicator of a seed lot's performance when exposed to adverse environmental conditions. varieties which had lower vigour than the others deteriorated at a much faster rate than those with higher vigour. The results also indicate the need for special care for processing and storage of varieties with lower vigour potential due to their tendency for accelerated deterioration.

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