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Original Research Article

Changes in Enzymes Activities during Storage of Pearl Millet Hybrids and their Parental Lines (*Pennisetum glaucum* (L.) R. Br.)

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Keywords

Pearl millet, Amylase, catalase, glutathione reductase, lipoxygenase, peroxidase, protease

A B S T R A C T

Seeds of four hybrids of pearl millet *viz*., GHB 719, GHB 905, GHB 744 and GHB 732 along with their parental lines were stored and their physiological characters were evaluated periodically. It was observed that, during storage, the activities of enzymes, catalase, glutathione reductase and amylase increased with the storage time. In addition there was loss of seed vigour and viability. However, peroxidase and protease activities declined with increase in storage period. The seed lots stored till germination were found to be below the Indian Minimum Seed Certification Standard.

Introduction

As pearl millet (*Pennisetum glaucum* (L.) R. Br.) is grown under hostile environment in marginal soils, its seed quality is an important aspect with seed storage (AOSA, 1983). Most often the stored seeds show decreased vigour and produce weak seedlings which are unable to survive (Atici *et al.*, 2007). Present investigation was undertaken to evaluate physiological quality of pearl millet, during storage on the basis of enzyme activities.

Materials and Methods

The investigation was carried out during the year 2014, at the Department of Seed Science and Technology and Department of Biochemistry, College of Agriculture, Junagadh Agricultural University, Junagadh. The seeds of parents (95222A, J 2454, 04999 A, 98444 A, J 2340 and 96222) of pearl millet hybrids GHB 719, GHB 905, GHB 744 and GHB 732 were procured from Pearl Millet Research Station, Junagadh Agricultural University, Jamnagar. The hybrids were produced in the *kharif* season of 2013 at Sagadividi farm, Department of Seed Science and Technology, J.A.U., Junagadh. The harvesting was done in the month of November 2013 and ear heads were kept for air-drying. Threshing was done in the month of January 2014 and the seeds were stored in the month of February 2014 in plastic containers under ambient conditions. The seed samples were drawn at two months interval till the germination. Activities of enzymes peroxidase (Malik and Singh, 1980), catalase (Aebi, 1984),

glutathione reductase, lipoxygenase (Babitha *et al.*, 2006), amylase (Bernfeld, 1955) and protease (Nayak *et al.*, 1979) were evaluated in the dry stored seeds. The experiment was planned in CRD with four replications for each seed lot. The data were analyzed as described by Gomez and Gomez (1984).

Results and Discussion

Peroxidase activity differed significantly among the entries as well as the storage time (Table 1). There was reduction in peroxidase activity with the advancement of storage. Sundareswaran *et al.*, (2009) also reported the similar results. Scialabba *et al.*, (2002) reported decreased peroxidase activity in aged seeds radish, in comparison to that in fresh seeds. Pallavi *et al.*, (2003) also reported a sharp decline in peroxidase activity during ageing in sunflower seeds. Peroxidase and catalase activities were found to be higher in younger seeds of *Chenopodium rubrum* (Mitrovic *et al.*, 2005). Chauhan *et al.*, (2011) reported gradual decline in peroxidase activity in natural and accelerated aged seeds of wheat. Moreover, many studies have demonstrated that seed ageing is associated with the loss of antioxidant enzymes, including peroxidase (Bailly *et al.*, 1996, Balesevic *et al.*, 2005, Begum *et al.*, 2014). So far as the cultivars were concerned, hybrids recorded lesser activities of peroxasidase enzyme, as compared to their parents. Hence, at all storage periods the heterosis was either negative or non-significant (Table 3). Joshi *et al.*, (1986), however, reported significant positive heterosis for peroxidase and IAA oxidase activities in root and shoot of pearl millet seedling.

The entries, at an individual storage period and their interaction differed significantly with respect to the catalase activity (Table 1), though pooled data over entries did not differed significantly. An overall trend revealed that catalase activity increased with the advancement of storage period. Moisture loss and desiccation continued with the storage time. The desiccation of developing sunflower (Bailly *et al.*, 2003, Bailly, 2004) and wheat seeds (De Gara *et al.*, 2003) has been found to be associated with H_2O_2 detoxification in terms of catalase activity.

However, there are several reports which revealed decrease in antioxidant enzymes in aged soybean, cotton, sunflower, wheat and pearl millet (Bailly *et al.*, 1996, Chauhan *et al.*, 2011, Sundareswaran *et al.*, 2009). Although hybrids showed better performance with regards to field emergence, no distinct classification could be made with respect to storability in relation to the trend of catalase activity. However, Hosamani *et al.*, (2013) classified soybean cultivars for their storability, based on catalase activity during storage.

The activity of glutathione reductase (GR) in different entries as influenced by storage time is presented in (Table 1). The differences due to entries and storage period were significant along with the interaction between them. In general, there was an increase in GR activity with storage period. However, loss of viability has been reported to be associated with reduction in GR activity (Bailly *et al.*, 1996 and Kuchlan, 2006).

Hosamani *et al.*, (2013) reported, increase as well as decrease in the activity of GR with ageing, and they classified genotypes as poor storer and good storer, based on the activity of GR. However, in the present investigation, no such classification was possible. Except for GHB 732 in the later storage period, no significant positive heterosis was observed with respect to the GR activity (Table 3).

Table.1 Peroxidase activity ($\Delta A_{460.}$ mg^{-1}protein. min^{-1}), catalase activity ($\Delta A_{240.}$ mg^{-1} protein. min^{-1}) and glutathione reductase activity ($\Delta A_{340.}$$mg^{-1}$protein. min^{-1}) in seed of pearl millet entries (four hybrids and their respective parents) after different periods of storage

Treatment	Feb-14	Apr-14	June-14	Aug-14	Oct-14	Pooled	Feb-14	Apr-14	June-14	Aug-14	Oct-14	Pooled	Feb-14	Apr-14	June-14	Aug-14	Oct-14	Pooled
	Peroxidase activity ($\Delta A_{460.}$ mg^{-1}protein. min^{-1})						Catalase activity ($\Delta A_{240.}$ mg^{-1} protein. min^{-1})						Glutathione reductase activity ($\Delta A_{340.}$$mg^{-1}$protein. min^{-1})					
GHB 719	1.54	1.83	0.57	0.11	0.12	0.83	0.066	0.079	0.115	0.152	0.138	0.110	0.007	0.025	0.013	0.021	0.023	0.018
95222 A	3.76	1.87	1.04	0.12	0.13	1.38	0.257	0.215	0.115	0.057	0.161	0.161	0.012	0.019	0.018	0.089	0.040	0.036
J 2454	2.49	2.42	1.10	0.10	0.10	1.24	0.079	0.081	0.132	0.180	0.322	0.159	0.032	0.030	0.016	0.012	0.013	0.021
GHB 905	1.70	1.72	0.72	0.08	0.08	0.86	0.083	0.036	0.117	0.514	0.377	0.225	0.009	0.015	0.011	0.024	0.021	0.016
094999 A	2.04	2.05	0.87	0.09	0.11	1.03	0.168	0.051	0.129	0.524	0.133	0.201	0.013	0.027	0.013	0.023	0.052	0.026
GHB 744	2.96	2.84	0.82	0.11	0.12	1.37	0.103	0.050	0.151	0.695	0.158	0.231	0.007	0.017	0.011	0.033	0.031	0.020
98444 A	3.15	2.99	0.96	0.10	0.10	1.46	0.099	0.040	0.125	0.104	0.123	0.098	0.007	0.018	0.012	0.022	0.023	0.016
J 2340	2.26	2.51	1.15	0.12	0.09	1.23	0.143	0.080	0.186	0.161	0.223	0.159	0.008	0.022	0.013	0.019	0.020	0.016
GHB 732	3.67	2.62	1.03	0.10	0.10	1.50	0.164	0.065	0.149	0.154	0.147	0.136	0.016	0.023	0.010	0.124	0.118	0.058
96222 A	4.39	3.33	1.04	0.11	0.12	1.80	0.166	0.087	0.154	0.596	0.167	0.234	0.008	0.024	0.011	0.044	0.094	0.036
Mean	2.80	2.42	0.93	0.10	0.11		0.133	0.078	0.137	0.314	0.195		0.012	0.022	0.013	0.041	0.043	
S.Em.±	0.13	0.10	0.05	0.01	0.01	0.20	0.005	0.009	0.008	0.035	0.035	0.054	0.002	0.002	0.001	0.004	0.004	0.009
C.D. at 5 %	0.37	0.30	0.13	0.02	0.02	0.57	0.014	0.025	0.023	0.100	0.106	NS	0.005	0.006	0.001	0.010	0.022	0.027
C.V. %	9.13	8.47	9.70	11.74	11.33	11.96	7.2500	21.75	11.32	22.1	37.6	27.12	30.580	18.790	8.080	17.370	34.930	30.130
D																		
S.Em.±						0.14						0.038						0.007
C.D. at 5 %						0.40						0.110						0.019
D×E																		
S.Em.±						0.08						0.023						0.004
C.D. at 5 %						0.21						0.065						0.011

Table.2 Lipoxygenase activity ($\Delta A_{234.}$ mg^{-1}protein. min^{-1}), amylase activity (mg maltose released mg^{-1} protein. h^{-1}) and Protease activity (mg peptides released mg^{-1} protein. h^{-1}) in seeds of pearl millet entries (four hybrids and their respective parents) After different periods of storage

Treatment	Feb-14	Apr-14	June-14	Aug-14	Oct-14	Pooled	Feb-14	Apr-14	June-14	Aug-14	Oct-14	Pooled	Feb-14	Apr-14	June-14	Aug-14	Oct-14	Pooled
	Lipoxygenase activity ($\Delta A_{234.}$ mg^{-1}protein. min^{-1})						Amylase activity (mg maltose released mg^{-1} protein. h^{-1})						Protease activity (mg peptides released mg^{-1} protein. h^{-1})					
GHB 719	0.27	2.94	0.70	2.93	0.68	1.50	0.80	0.48	0.98	0.79	0.82	0.77	6.52	5.26	6.09	6.81	6.14	6.16
95222 A	0.46	3.19	0.94	2.41	1.43	1.69	0.61	0.49	1.12	0.76	0.71	0.74	7.05	5.23	5.90	5.81	4.85	5.77
J 2454	0.51	4.34	0.87	2.62	0.33	1.73	0.73	0.26	1.20	0.76	0.69	1.38	6.30	7.84	6.05	5.79	5.13	6.22
GHB 905	0.48	2.77	0.63	1.19	0.55	1.13	0.90	0.51	1.12	0.89	0.82	0.85	7.16	5.17	5.18	5.17	4.26	5.39
094999 A	0.38	3.14	0.76	0.96	0.76	1.20	0.59	0.51	1.37	1.14	1.17	0.96	6.19	5.61	4.67	4.92	3.81	5.04
GHB 744	0.64	3.39	0.80	1.16	0.38	1.28	0.95	0.74	1.64	1.29	0.80	1.08	7.33	7.18	7.17	7.15	6.23	7.01
98444 A	0.69	3.63	0.95	2.09	0.45	1.56	0.97	0.54	1.41	0.78	1.02	0.94	5.13	4.95	5.57	5.39	4.59	5.13
J 2340	0.30	2.54	0.94	2.20	0.47	1.29	0.63	0.35	1.19	0.86	1.00	0.81	6.01	7.21	6.31	6.38	5.46	6.27
GHB 732	0.67	2.70	0.85	2.19	0.47	1.38	1.03	0.59	1.46	1.18	0.70	0.99	8.56	7.45	7.28	7.14	6.12	7.31
96222 A	0.69	4.35	0.91	2.17	0.59	1.74	0.63	0.29	2.23	0.87	1.02	1.01	8.85	6.06	6.58	6.33	6.18	6.80
Mean	0.51	3.30	0.84	1.99	0.61		0.79	0.48	1.37	0.93	1.20		6.91	6.20	6.08	6.09	5.28	
S.Em.±	0.03	0.18	0.03	0.16	0.16	0.19	0.02	0.02	0.07	0.08	0.08	0.09	0.10	0.15	0.16	0.13	0.13	0.28
C.D. at 5 %	0.08	0.53	0.10	0.47	0.19	NS	0.06	0.06	0.20	0.23	0.11	NS	0.30	0.44	0.46	0.38	0.56	0.80
C.V. %	10.46	11.11	8.08	16.27	22.12	15.88	5.05	8.49	10.32	17.41	9.45	11.97	3.01	4.89	5.21	4.27	7.29	4.92
D																		
S.Em.±						0.14						0.06						0.20
C.D. at 5 %						0.39						0.18						0.57
D×E																		
S.Em.±						0.15						0.053						0.15
C.D. at 5 %						0.41						0.148						0.42

Table.3 Mid-parental heterosis (%) for various enzyme activities in the seeds of four pearl millet hybrids After different periods of storage

Hybrids	Date of sampling														
	Feb-14	Apr-14	June-14	Aug-14	Oct-14	Feb-14	Apr-14	June-14	Aug-14	Oct-14	Feb-14	Apr-14	June-14	Aug-14	Oct-14
	Peroxidase activity ($\Delta A_{460.}$ mg^{-1}protein. min^{-1})					Catalase activity ($\Delta A_{240.}$ mg^{-1} protein. min^{-1})					Glutathione reductase activity ($\Delta A_{340.}$$mg^{-1}$protein. min^{-1})				
GHB 719	-50.6**	-14.77	-46.31*	-2.11	2.21	-60.56*	-46.28	-6.84	27.83	-43.00*	-67.52	3.73	-26.05	-58.86	-13.73
GHB 905	-24.95**	-22.89*	-27.17	-19.02	-21.8	-33.00	-44.95	-10.46	45.86**	65.77**	-61.23	-48.86	-23.57	33.84	-35.19
GHB 744	9.48	3.31	-22.06	1.56	25.25	-15.54	-17.28	-3.22	423.66**	-8.77	-7.49	-14.02	-12.59	65.36	44.61
GHB 732	10.26	-10.2	-5.7	-10.94	-4.51	6.19	-22.66	-12.52	-59.36**	-24.61	94.04	0.72	-15.08	295.61**	107.71*
S.Em. ±	0.22	0.20	0.13	0.05	0.05	0.04	0.06	0.05	0.11	0.11	0.03	0.03	0.01	0.04	0.04
Calculated 't' value															
GHB 719	7.23	1.45	2.26	0.01	0.01	2.39	1.61	0.20	0.78	2.45	0.58	0.03	0.17	1.15	0.14
GHB 905	2.58	2.34	1.22	0.08	0.11	0.96	0.70	0.32	3.81	3.53	0.53	0.54	0.13	0.23	0.44
GHB 744	1.17	0.42	1.07	0.01	0.11	0.44	0.24	0.12	13.25	0.36	0.02	0.11	0.06	0.51	0.37
GHB 732	1.56	1.77	0.09	0.11	0.06	0.23	0.45	0.50	5.30	1.13	0.29	0.01	0.07	3.56	2.36
	Lipoxygenase activity ($\Delta A_{234.}$ mg^{-1}protein. min^{-1})					Amylase activity (mg maltose released mg^{-1} protein. h^{-1})					Protease activity (mg peptides released mg^{-1} protein. h^{-1})				
GHB 719	-44.91*	-21.92**	-22.94*	16.4**	-22.55*	18.42	27.32	-15.2*	4.73	16.14	-2.35	-19.53**	1.91	17.53**	23.15**
GHB 905	8.75	-25.83**	-22.23	-33.8**	0.98	35.44**	32.12	-12.53	-5.91	-12.12	14.72**	-23.09**	-3.4	-3.52	-4.69
GHB 744	29.35	9.94**	-15.05	-45.93**	-17.36	18.87	65.38**	26.15**	56.95**	-21.13*	31.48**	18.19**	20.68**	21.43**	24.15**
GHB 732	34.79	-21.49**	-8.5	0.19	-10.67	63.66**	82.23**	-14.65**	37.26**	-30.87**	15.27**	12.22**	12.86**	12.42**	5.3
S.Em. ±	0.10	0.26	0.11	0.25	0.25	0.09	0.09	0.16	0.17	0.17	0.20	0.24	0.24	0.22	0.22
Calculated 't' value															
GHB 719	2.18	8.25	2.07	4.13	1.98	1.44	1.19	2.04	0.42	1.31	0.79	6.46	0.58	5.15	5.85
GHB 905	0.39	9.65	1.81	6.06	0.05	2.72	1.43	1.87	0.65	1.31	4.66	7.86	0.92	0.95	1.06
GHB 744	1.46	3.06	1.42	9.83	0.80	1.76	3.38	3.94	5.42	2.47	8.88	5.60	6.22	6.39	6.14
GHB 732	1.72	7.40	0.79	0.04	0.56	4.66	3.09	2.91	3.73	3.62	5.75	4.11	4.20	3.99	1.56

* Heterotic value significant at 5% levels of significance (Table 't' value = 2.04),

** Heterotic value significant at 1% levels of significance (Table 't' value = 2.75)

The pooled data did not show significant differences among the entries with respect to to the enzyme lipoxygenases activity, but the differences were significant among the entries at individual storage intervals along with significant interaction effect between the storage intervals and the entries (Table 2). The genotypic differences in the lipoxygenase activity have been reported by Hosamani *et al.,* (2013). During present study, the lipoxygenases activity did not revealed any distinct trend with the advancement of storage period, though number of workers have reported a distinct decline with ageing of the soybean seeds (Sung and Chiu, 1995), pigeonpea (Kalpana and Rao, 1993) and pearl miilet (Nagarathna *et al.*, 1992). Contrary to this, Hosamani *et al.,* (2013) reported an increase in the activity of lipoxygenase in the cultivars of soybean. The good storer overall had low level of activity as compared to the poor storer. Thus, the role of seed lipoxygenase in the seeds remained enigmatic (Loiseau *et al.,* 2001). The heterosis for lipoxygenases activity during storage intervals was negative or non-significant during present investigation (Table 3).

The amylase activity did significantly differed at an individual storage interval with respect to the entries, however, it showed increasing trend with storage period (Table 2). The interaction between entries and storage time was significant. However, Sundareswaran *et al.,* (2009) reported significant decrease in amylase activity in imbibed seeds, probaably due to accelerated ageing. Karrer *et al.,* (1993) showed that seedling vigour, amylase activity and accumulation of α-amylase mRNA were positively correlated in the rice cultivars. Similar relationship between α-amylase activity and seed vigour was reported by Krishnasamy and Seshu (1990) in rice, Van der Meulen *et al.,* (2000) in barley and by Sole (1994) and in other cereals. The positive significant heterosis was discernible in the initial storage intervals (Table 3). Joshi *et al.,* (1996b) also reported distinct positive heterosis in the activity of amylase in the endosperm of germinating seeds of pearl millet.

The differences in the entries, storage period and the interaction between them with respect to proteases activity were statistically significant. Storage period registered significant decrease in protease activity with the advancement of ageing (Table 2). So far as mid-parental heterosis in protease activity was concerned, two hybrids GHB 744 and GHB 732 exhibited significant positive heterosis, although. GHB 719 and GHB 905 revealed mixed trend of positive and negative heterosis over storage period (Table 3).

It can thus be concluded that catalase, glutathione reductase and amylase activities increased with the storage of seeds, which was associated with the loss of seed vigour and viability. On the other hand, peroxidase and protease activities declined with increasing storage period, which was also associated with loss in vigour and viability. Lipoxygenase activity however, could not show any distinct trend with respect to storage period.

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