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RESEARCH ARTICLE

Determining seed longevity and vigor in finger millet [*Elusine coracana* (L.) Gaertn.] and its genetic implication under storage

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Abstract

The seeds of any crop variety maintain vigor and viability to a certain period, *i.e.,* seed longevity, also known as aging resistance and storability, which is genetically controlled and influenced by the storage environment. The study enabled the categorization of 33 cultivars of finger millet with different potentials of seed storability, seedling growth and vigor traits at different periods of natural seed aging under ambient storage conditions. The genotypic (G) differences among the cultivars for seed viability and vigor traits at eight durations of the aging period (E) were significant for all the traits. The response of genotypes to the aging period differed significantly as revealed by the genotype (G) x aging period (E) interaction. The seed germination and emergence were reduced gradually after two years of storage. The majority (88%) of cultivars maintained the standard seed germination (75%) until two years (E4) of aging. After three years (E6), 21 cultivars maintained seed viability above the standard germination (75%). Extending the aging period to three and half years (E7) further reduced to eight cultivars with >75% seed viability. At the end of the four years (E8), only four cultivars could retain seed viability to the standard (75%), *i.e.,* G14 (86%), G26 (81%), G12 (79%) and G22 (75%) indicating seed aging resistance to maintain desired seed longevity for four years. The outcome enables the plant breeders and seed industry personnel to plan the seed storage and production programs of different seed classes in the generation system of finger millet. Further, the information also provides clues for planning the seed conservation and regeneration programs of finger millet in gene banks and active germplasm sites.

Keywords: Finger millet, Seed aging period, Seed longevity, Seedling growth, Seedling vigor, genotype x aging period interaction.

Introduction

Finger millet (*Elusine coracana* (L.) Gaertn.) is an important food and forage cereal crop grown in the semi-arid tropics and subtropics of the world that strives well under drought-prone areas and contributes to food security. Finger millet is rich in contents of fiber, micronutrients and minerals compared to rice and wheat (Karki et al. 2020). Being an annual robust grass, finger millet is cultivated for grain to use as human food, while the crop residues are a good source of dry matter for livestock during dry seasons. In addition to the advantage of finger millet for sustainability and human dietary food, this crop is also a good source of highly nutritious forage for livestock in several African and Asian countries. In India, finger millet is cultivated in an area of 1.1 million ha with a production of 1.58 million tons and average productivity of 1450 kg/ha. Karnataka state occupies the largest area (0.67 million ha) under finger millet, followed by Tamil Nadu (0.079 million ha), Maharashtra (0.086 million ha), Uttarakhand (0.095 million ha) and Odisha (0.041 million ha) (Bhat et al. 2023).

The continuing efforts on finger millet crop improvement programs resulted in the release of several improved varieties for the benefit of a farming community. The seed carries the genetic potential of these improved varieties, and every farmer should be able to access quality seeds possessing the properties of high vigor and viability potential. The complex traits of seed vigor include aging tolerance, seed dormancy, viability, rapid germination, uniform emergence and establishment of seedlings, particularly under suboptimal field conditions (Reed et al. 2022). Seeds lose their vigor

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progressively during the aging process and, when sowing is done, become susceptible to field stress during the process of imbibition (Bewley et al. 2013). Physiological quality plays a crucial role in crop performance, and therefore, seed vigor and viability are important traits during a breeding program for the development of improved varieties with better quality of seeds (Nerling et al. 2019). Seed longevity is the time period /life span of a variety/genotype that maintains the viability of seeds to the desired level which is critical for planning seed storage and seed production programs of different seed classes in the seed industry. Also seed longevity is more pertinent for planning the conservation and regeneration programs of germplasm in gene banks. A variety with longer storability potential is economically valuable as it requires less frequency of seed production cycles compared to the varieties with poor storability potential. Similarly, in gene banks, the genotypes with longer seed life can reduce the seed regeneration cycles. The inevitable seed aging process during seed storage results in poor quality of seed that leads to the loss of annual crop value to the tune of 25% approximately (McDonald 1999; Rajjou et al. 2008; Schwember and Bradford 2010). The success of improved cultivars on the farm front can be realized only when the quality seeds equipped with potential vigor and viability properties are available to seed growers or farmers. Various studies in cereal crops suggest that the genotypes vary for seed vigor and longevity. The genetic analysis of seed longevity was first reported in rice (Miura et al. 2002), subsequently in soybean (Singh et al. 2008), barley (Nagel et al. 2009) and maize (Revilla et al. 2006). The genetic variability for seed aging and longevity traits were reported among the forage sorghum cultivars (Kannababu et al. 2015), sweet sorghum cultivars (Kannababu et al. 2016), grain sorghum parental lines and crosses (Kannababu et al. 2017) and sorghum landraces (Kannababu et al. 2020).

Recent scenario of global climate change with rising temperatures and humidity, seed longevity and vigor are becoming important traits for crop improvement programs to ensure uniform seedling establishment even under stress conditions that lead to a robust crop production system. Even though farmers adopt high seed rates, the poor vigor and viability status of seeds reduce the uniformity of seedling growth and crop establishment. Scientific literature/information on seed longevity of finger millet is scanty. Both the seed industry and gene bank curators express the need for tools to better understand, improve and predict seed longevity. Systematic studies are required essentially to assess the seed viability and deterioration pattern of finger millet under varying environmental conditions of ambient storage, which can provide some clues for genetic improvement and prediction of seed longevity. With this background in view, the present study was planned to evaluate a set of finger millet cultivars to understand the genetic variability for seed longevity and seedling vigor traits and to identify the potential genotypes.

Materials and methods

Thirty-three cultivars of finger millet released for cultivation in India were collected from the project coordinator unit, ICAR- All India Coordinated Improvement Programme (AICRP) on Small millets, Bengaluru. The pedigree details of these cultivars are presented in Table 1. These cultivars were multiplied uniformly during the rainy season and the fresh seeds of all the entries were dried to safe seed moisture content (8%), and packed in moisture-proof rigid gasketed plastic tins. These tins were placed in metal boxes and stored at an ambient storage environment (average 25 ± 2°C and 65 ± 5% RH) for four years at the seed science laboratory, ICAR-Indian Institute of Millets Research (IIMR), Hyderabad, India.

Seed trait evaluations

The seed samples were drawn after every six-month interval of the natural aging (storage) period (E), to evaluate the seed quality traits. The seed vigor and longevity traits were evaluated at eight intervals of the natural aging period (E) *viz.*, 6 months (E1), 12 months (E2), 18 months (E3), 24 months (E4), 30 months (E5), 36 months (E6), 42 months (E7) and 48 months (E8). Accordingly, at every interval of the aging period (E), the seed longevity component traits viz., seed germination (SG), root length (RL), shoot length (SL), seedling dry weight (SDW), and seedling vigor index (SVI-1 and SVI-2) were evaluated. The seed germination test was conducted on top of the paper method in petri plates in four replications as per the rules of the International Seed Testing Association (ISTA 2015). Seeds were germinated in a seed germinator and they were maintained at 25 ± 5°C and 90 ± 3% relative humidity. Germination counts were made on the 8th day and the seedlings were evaluated for growth and vigor traits as well. The seed germination (SG) expressed in percentage was calculated based on the number of normal seedlings produced per 100 seeds. Ten normal seedlings were selected at random for recording seedling characters in all four replications. Root length (RL) was measured from the collar region to the tip of the primary root and shoot length (SL) from the collar region to the tip of the first leaf. Seedlings with abnormal growth were separated. Measurement of the length of root and shoot was carried out on each of the 10 randomly selected normal seedlings. Seedling dry weight (SDW) was measured after drying the 10 normal seedlings in a hot air oven maintained at 80°C for 24 hours. Immediately after completion of drying, seedlings were transferred to desiccators for half an hour for cooling and then the weight was recorded. The mean dry weight of normal seedlings was reported. The seedling vigor index (SVI) was calculated by two ways, *viz.,* multiplying mean germination percentage by seedling length (root + shoot) (SVI-1); and multiplying mean

Table 1. Pedigree of finger millet cultivars released in India

S.No.	Variety	Pedigree	Year of release	Maturity (Days)	Average Yield (q)	Area of Adaption	Special features
1	PRM2	Pure line selection	2010	95-100	20-22	Hills of Uttarakhand	--
2	VL347	VR70 x VL149	2010	95-100	20-22	All finger millet growing areas of India	--
3	KOPN235	Selection from local germplasm	2011	115-120	25-26	Sub mountain and ghat zone of Maharashtra	Resistant to blast
4	OEB526	SDFM30 x PE244	2011	110-115	25-26	Odisha, Bihar, Chattisgarh, Karnataka, Tamilnadu	Moderately resistant to leaf, neck and finger blast diseases.
5	OEB532	GPU26 x L5	2012	110-115	22-25	Odisha, Bihar, Chattisgarh, Karnataka, Tamilnadu	Moderately resistant to blast diseases. Non-lodging and non-shattering.
6	KMR204	GPU26 x GE1409	2012	100-105	30-35	Karnataka	Early duration variety
7	VR936	IE2695 x PR202	2012	115-120	28-30	Andhra Pradesh	Suitable for late conditions. Responsive to nitrogenous fertilizers.
8	Vakula	KM55 x U22/B	2012	105-110	25-30	Andhra Pradesh	Resistant to leaf blast and tolerant to drought.
9	IndiraRagi1	HR911 x GE669	2012	120-125	25-26	Chattisgarh	Non-shattering, nonlodging, responsive to fertilizers.
10	Vl352	VR708 x VL149	2012	95-100	33-35	All Ragi growing areas of country	Moderately resistant to blast.
11	GPU67	Selection from germplasm accession GE5331	2009	114-118	30-35	National	Non lodging (Semi dwarf)
12	KMR301	MR1 x GE1409	2009	120-125	35-40	Southern Dry zone of Karnataka	High grain and straw yield, tolerant to blast
13	GPU66	PR202 x GPU28	2009	112-115	35-40	Karnataka	Green plant parts with narrow leaves, medium compact ears with tip incurved fingers
14	Srichaitanya	GPU26 x L5	2009	110-115	26-28	Andhra Pradesh	Moderately resistant to blast
15	GN5	Selection from local germplasm WWN20	2016	120-130	25-27	Gujarat	Late maturing, White colour seed, Moderately resistant to leaf and finger blast.
16	ML365	IE1012 x Indaf5	2008	110-115	50-55	Zone 5 of Karnataka	--
17	Paiyur2	VL145 x Selection10	2008	110-115	30-35	Tamilnadu	--
18	GN4	Pureline selection	2006	105-115	28-30	Gujarat	--

Contd. ....

19	GPU48	GPU26 x L5	2005	95-100	28-30	Karnataka	Early, high yield, blast resistant Suitable for summer also
20	PRM1	Selection from Ekeshwar of Pauri Garhwal Region	2006	110-115	20-25	Hills of Uttarakhand	Resistant to blast
21	VR762	Pureline selection from VMEC134	2006	110-115	26-30	Andhra Pradesh	Moderately resistant to blast
22	L5	Malawi x Indaf 9	1999	120-125	35-40	Karnataka	Late variety, for early sowings
23	GPU26	(Indaf5 x Indaf9) x IE1012	2000	11-105	30-35	Karnataka	Early, blast tolerant, suitable late sowings &summer
24	PR230	Pureline selection	1998	90-100	25-30	Andhra Pradesh	Resistant to blast and blight
25	VL315	SDFM69 x VL231	2004	105-115	26-28	Uttaranchal	Moderately resistant to finger and neck blast
26	TNAU946	Malavi1305 x Co.13	2004	105-110	25-26	Tamilnadu	--
27	VR708	Puerline selection from VMEC36	1998	90-95	20-25	Andhra Pradesh, Uttar Pradesh, Tamil Nadu, Karnataka, Odisha	Photo insensitive& early maturity
28	GPU45	GPU26 x L5	2001	95-100	27-29	Gujarat, Jharkhand, Karnataka, Madhya Pradesh, Maharashtra	Early, blast resistant
29	BM9-1	Mutant from BudhaMandia	1999	103-105	25-30	Karnataka, Andhra Pradesh, Odisha, Madhya Pradesh & Maharashtra	Moderately resistant to blast and brown spot
30	VL324	VL162 x IE3808	2006	110-120	20-22	Lower and mid hills of Uttarakhand	--
31	OEB10	GE68 x GE156	2001	115-120	26-27	Odisha, Madhya Pradesh, Gujarat, Andhra Pradesh & Tamil Nadu	Moderately resistant to blast, resistant to stem borer, late variety
32	GPU28	Indaf5 x (Indaf9 x IE1012	1996	110-115	35-40	Karnataka	Highly blast resistant
33	OUAT2	Mutant of Co.9	1999	120-125	28-30	All finger millet areas in India	Moderately resistant to neck, finger blast, and sheath blight

germination percentage by dry weight of single seedling (SVI-2) and the results (SVI-1 and SVI-2) were expressed to the nearest whole numbers.

Data analysis

After the completion of a four-year storage period (48 months), the data analysis of variance and pair-wise comparisons of means were performed using the statistical software 'Statistics' (version 8.1). Before carrying out the combined ANOVA of the genotypes, an assessment of the homogeneity of variances of the environments (aging periods) was done using Hartley's Fmax test (Hartley 1950). The test revealed that the variances of the population were not significantly different from one another ($p>0.05$). Combined ANOVA was carried out on replicated data obtained from different environments with the package "metan" in R software (Olivoto and Lucio 2020). Considering each aging period as an environment (E), GGE interaction was estimated and biplots were made. Statistical analyses for GGE interaction and biplots were performed using the

Table 2. Pooled ANOVA for seed vigor and longevity traits in finger millet

Source	DF	SG	FE	SL	RL	SDW	SVI-1	SVI-2
Aging period (E)	7	32909.3*	30853.9*	28.9*	234.2*	84.3*	9017577.5*	842973.2*
Replication (R)	24	10.4	10.9	0.3*	0.3	0.1	2998.6	720.8
Genotype (G)	32	2543.7*	2286.3*	1.8*	10.2*	4.9*	404491.8*	39192.5*
G x E	224	242.9*	248.0*	0.3*	1.8*	0.2*	32585.5*	1927.2*
Residuals	768	13	9.9	0.1	0.2	0.1	2734.7	663.6

Note. G = Genotype, E = Natural aging period (Environmental factor), R = Replication, DF = Degrees of freedom, SG = Seed germination percentage, FE = Field emergence percentage, SL = Shoot length (cm), RL = Root length (cm), SDW = Seedling dry weight (mg), SVI-1 = Seedling vigor index-1, SVI-2 = Seedling vigor index-2; * = Level of significance at probability <0.001.

software packages GEA-R (Pacheco et al. 2015). The violin plots were made using '*ggplot2'* package in R software v.4.2.

Results and discussion

Seed vigor is an important index to measure the seed's physiological quality that refers to the comprehensive potential that triggers rapid and uniform emergence and normal growth of seedlings under an extensive range of field conditions. Seed vigor differences were largely determined by the genotype as reported in many crops. The genotypes of the high-vigor-type strains had a greater influence on seed vigor than those of the medium- and low-vigor-type strains (Cheng et al. 2023). Seed longevity is the life span during which the seeds are viable and germinate upon sowing to establish a normal seedling stand. The information on seed longevity in finger millet is not known and it is a major determinant in planning the seed production and conservation programs. The life span of the seeds is a variable trait that depends on genetic and environmental factors.

G x E interaction

The genotypic (G) differences among the cultivars of finger millet for seed viability and vigor traits at eight periods of natural aging (E) were assessed using ANOVA (Table 2). Genotypic (G) differences were significant for all the traits at a 5% significance level. The variation due to aging periods (E) was also significant for all the traits studied. The response of genotypes to aging periods differed significantly as revealed by the genotype (G) x aging period (E) interaction. Mean values of genotypes (G) across seed aging periods (E) revealed the variation for seed viability potential and seedling vigor traits as well (Figure 1 and Table 3). Under the conditions of an ambient storage environment (average 25 ± 2°C and 65 ± 5% RH) for four years, finger millet cultivars responded differently at every six-month interval of time span. Thirty-three cultivars (G) were assessed at different seed aging periods (E) to know the variation in seed longevity (germination and field emergence), seedling growth (root length, shoot length and dry weight) and vigor (seedling vigor index-1 and index-2) traits to understand on optimum storage period within which the standard seed germination (75% for seed certification) can be maintained (Figs. 1a, 1b and 1c).

The means of eight aging periods (E) across the 33 genotypes (G) were plotted for all these seven traits. The genotypic variation in each of the aging periods (E) was plotted in violin plots to show the dispersion of genotypes in each treatment for seed longevity traits, i.e., germination and emergence (Figures 2a and 2b). After E1 (6 months aging period), the G15 was an outlier with less than 80% seed germination. The outliers recorded until E4 (24 months aging period) for seed germination were G5, G15 and G30, where a similar pattern was noted for emergence. Later, to E4, the genotypes showed the range of response that expanded the quartiles and the genotypes were distributed evenly. It implies that a minimum storage duration of 3.5 years is required to get considerable variability among the genotypes for longevity in finger millet. The clustering based on the response to aging on germination revealed a clear-cut grouping of genotypes. The red highlighted genotypes in the unrooted tree (Figure 3) showed more than 75% germination (standard) after four years of aging, whereas the green highlighted genotypes showed < 25% germination. The other genotypes in the cluster were intermediate to the two groups in the unrooted tree. All the traits indicated the genetic regulations that impart seed longevity and seedling vigor. However, it is evident from the data that the seed germination and emergence reduced gradually after two years of storage. With the passage of time, the variation in seed longevity among the genotypes depends on the response of individual genotypes to varying conditions of temperature, relative humidity and oxygen pressure under ambient storage. Different species and genotypes vary for seed longevity behavior in different ways as influenced by storage conditions (Kannababu et al. 2020; Zinsmeister et al. 2020) and the maximum time period that seeds maintain germination viability is known as seed longevity (Sano et al. 2016).

After completion of two years of natural aging (E4), 25 cultivars had >80% seed viability and four cultivars >75%. Early loss of viability was found with the cultivar G3, which

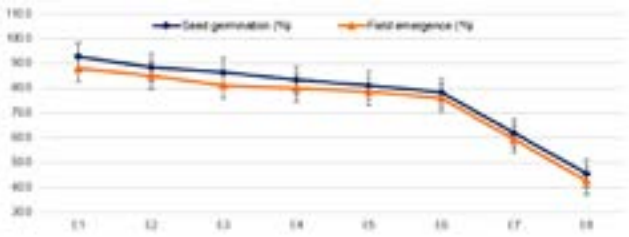
Table 3. Overall mean performance of finger millet genotypes for seed vigor and longevity traits.

Code	Cultivar	SG	FE	SL	RL	SDW	SVI-1	SVI-2
g1	PRM2	78.4	74.3	3.2	6.7	1.22	797.3	104.4
g2	VL347	69.9	67.8	3.3	6.5	1.40	764.8	116.2
g3	KOPN235	59.8	57.0	3.1	6.3	1.13	619.1	83.6
g4	OEB526	74.4	70.3	3.0	6.2	1.26	701.1	103.8
g5	OEB532	69.4	65.8	3.2	6.8	1.09	739.6	91.2
g6	KMR204	83.3	80.6	3.0	6.1	1.19	776.1	106.7
g7	VR936	60.4	57.4	3.2	6.4	0.91	634.2	72.1
g8	Vakula	80.2	77.7	3.3	5.7	0.92	737.4	76.8
g9	IndiraRagi1	80.4	77.6	3.3	6.2	0.92	783.2	80.1
g10	Vl352	83.6	80.6	3.2	6.0	0.95	779.9	85.0
g11	GPU67	71.9	68.1	3.2	6.5	1.10	724.2	89.3
g12	KMR301	88.9	85.0	3.4	6.4	1.11	872.8	103.1
g13	GPU66	81.9	78.9	3.3	6.8	1.10	838.1	98.1
g14	Srichaitanya	91.7	84.9	3.4	7.9	1.11	1038.2	103.4
g15	GN5	61.1	58.6	2.7	5.9	1.24	561.3	85.5
g16	ML365	72.1	69.3	3.3	6.7	1.01	757.1	83.3
g17	Paiyur2	66.9	64.3	3.3	6.7	0.98	690.3	77.6
g18	GN4	83.3	80.0	3.4	6.8	1.53	856.8	132.7
g19	GPU48	73.4	70.4	3.5	7.2	1.60	807.7	125.8
g20	PRM1	84.0	80.8	4.0	7.0	1.73	942.5	151.8
g21	VR762	83.4	79.8	3.6	8.1	1.50	986.1	130.8
g22	L5	88.8	84.3	3.5	6.9	2.05	931.0	185.8
g23	GPU26	83.6	79.8	3.7	7.5	1.94	964.9	170.9
g24	PR230	79.5	76.3	3.5	7.2	1.66	858.5	137.3
g25	VL315	75.2	72.4	3.1	7.4	1.50	816.4	123.0
g26	TNAU946	89.9	85.5	3.2	6.9	1.74	913.1	158.9
g27	VR708	69.7	66.7	3.2	6.5	2.36	764.3	194.9
g28	GPU45	81.6	77.8	3.6	7.4	1.86	915.3	159.2
g29	BM9-1	78.9	75.6	3.4	6.9	1.43	828.0	121.6
g30	VL324	59.4	56.7	2.9	6.4	1.79	612.7	122.4
g31	OEB10	84.3	80.1	3.7	6.6	2.01	882.2	178.3
g32	GPU28	79.9	76.0	3.5	7.5	1.93	895.8	163.1
g33	OUAT2	81.3	78.4	3.5	6.3	1.69	804.5	144.1
CD(5%)		16.1	15.7	0.52	1.4	0.79	253.0	77.7
E1	6 M	92.8	88.1	3.6	8.5	2.66	1120.3	246.9
E2	12 M	88.5	85.2	3.9	7.8	2.29	1033.4	203.3
E3	18 M	86.5	81.2	3.6	7.2	1.91	932.2	166.3
E4	24 M	83.4	80.0	3.8	7.5	1.50	946.6	126.2
E5	30 M	81.2	78.6	3.3	6.7	1.06	809.1	87.2

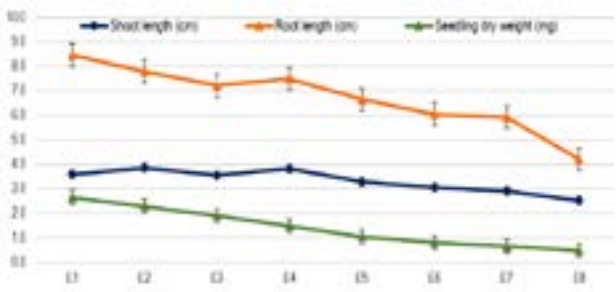
E6	36 M	78.3	76.1	3.1	6.0	0.81	716.1	64.7
E7	42 M	62.0	59.4	2.9	5.9	0.66	551.8	41.5
E8	48 M	45.6	42.6	2.5	4.2	0.50	337.7	24.2
CD(5%)		4.4	4.3	0.15	0.35	0.19	54.6	17.1

Note. g = Genotype, M = Months, E = Natural aging period (Environmental factor), E1 = 6 Months, E2 = 12 Months, E3 = 18 Months, E4 = 24 Months, E5 = 30 Months, E6 = 36 Months, E7 = 42 Months and E8 = 48 Months, SG = Seed germination percentage, FE = Field emergence percentage, SL = Shoot length (cm), RL = Root length (cm), SDW = Seedling dry weight (mg), SVI-1 = Seedling vigor index-1, SVI-2 = Seedling vigor index-2.

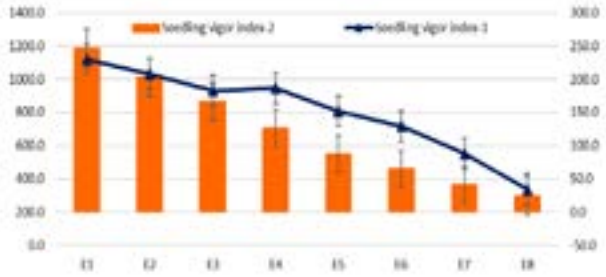
Seed longevity (*SG & FE reduced drastically after E6*)



Seedling growth (*SL, RL & SDW showed gradual decline*)



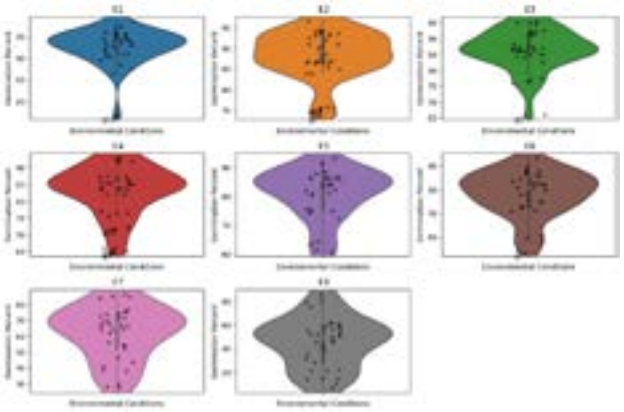
Seedling vigor index (*SVI-1 & SVI-2 showed gradual decline*)



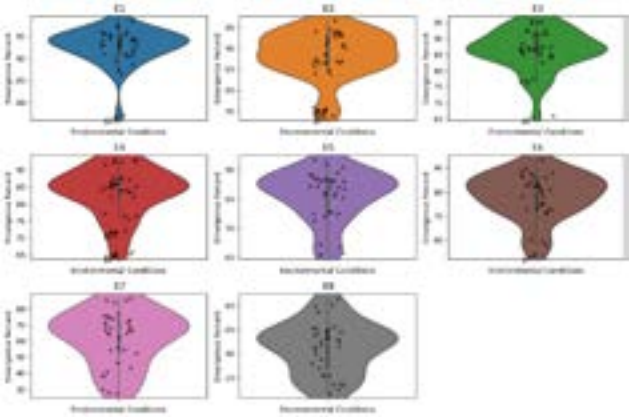
Note. E = Natural aging period (Environmental factor), E1 = 6 Months, E2 = 12 Months, E3 = 18 Months, E4 = 24 Months, E5 = 30 Months, E6 = 36 Months, E7 = 42 Months and E8 = 48 Months, SG = Seed germination percentage, FE = Field emergence percentage, SL = Shoot length (cm), RL = Root length (cm), SDW = Seedling dry weight (mg), SVI-1 = Seedling vigor index-1, SVI-2 = Seedling vigor index-2.

Fig. 1. a - c. Effect of extending aging periods on seed longevity, seedling growth and vigor traits in finger millet cultivars

Seed germination (SG) (*Response of genotypes to aging was more prominent after E4*)



Field emergence (FE) (*Response of genotypes to aging was more prominent after E4*)



Note. E = Natural aging period (Environmental factor), E1 = 6 Months, E2 = 12 Months, E3 = 18 Months, E4 = 24 Months, E5 = 30 Months, E6 = 36 Months, E7 = 42 Months and E8 = 48 Months, SG = Seed germination percentage, FE = Field emergence percentage

Fig. 2. a - b. Response of genotypes to aging periods for seed longevity traits of finger millet

lost to 66% after 18 months (E3) of aging, whereas G30 lost to 66% after two years (E4). These results clearly indicate that the majority (88%) of cultivars maintain the standard seed germination (75%) until two years (E4) of natural aging. After three years (E6), 21 cultivars (63%) out of 33, maintained seed viability above the standard germination (75%). Extending the aging period to three and half years (E7) further reduced to eight cultivars with >75% seed viability. At the end of the four years (E8), only four cultivars maintained the standard

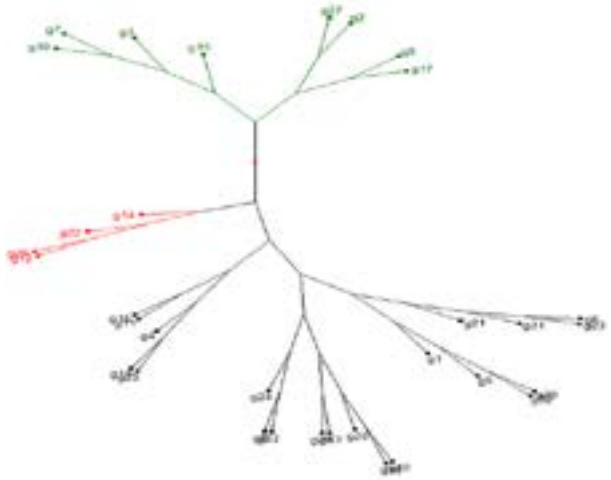
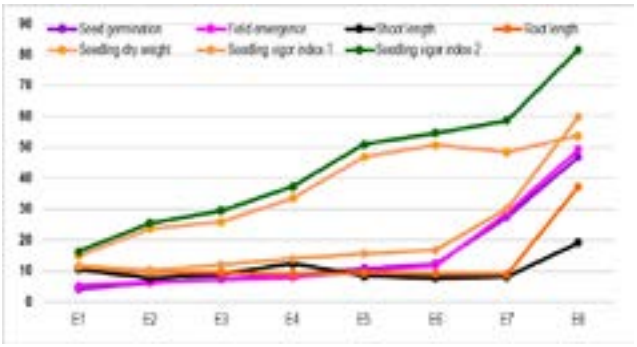


Fig. 3. Clustering of genotypes (g) based on seed germination across aging periods (Red color = High germination, Green color = Low germination)



E = Natural aging period (environmental factor), E1= 6 months, E2= 12 months, E3= 18 months, E4= 24 months, E5= 30 months, E6= 36 months, E7= 42 months and E8= 48 months.

Fig. 4. Coefficient of variation for seed longevity, seedling growth and vigor traits of finger millet due to different aging periods

seed viability (75%), *i.e.,* G14 (86%), G26 (81%), G12 (79%) and G22 (75%), indicating the potential seed longevity. Under specific environmental conditions during seed storage, seed storability, which is also known as longevity and aging resistance, is the crucial factor in maintaining seed viability (Zhou et al. 2024). Seed longevity is a complex trait, and it differs not only at species level (Ewart 1908) but also at genotype/variety level in plant species (McDonald 1999; Mondoni et al. 2018; Kannababu et al. 2020; Guzzon et al. 2021).

The present study with different aging periods has generated variation in the genotypes for all the traits, and the coefficient of variation was estimated to compare the variation for all eight periods across the traits (Fig. 4). Maximum variation in the genotypes was created after E7 (42 months) and E8 (48 months). Among the traits, maximum variation was observed for seedling vigor index-2, followed by seedling dry weight. In general, the initial six months (E1) of aging created considerable genotypic differences for all the traits. Later, the coefficient of variation for aging was almost the same. The consistent reduction in the mean performance with increasing seed age is obvious, which reflects the vulnerability of seeds to aging-induced stress under varying environmental conditions during storage, which is corroborative with earlier findings (Rastegar et al. 2011; Waterworth and West 2023). Due to the prolonged aging period, the seeds eventually lose seed viability (Reed et al. 2022). Seed aging is the result of damage of cellular processes (Zinsmeister et al. 2020) that depends upon seed genetics, maternal environment and storage conditions (Bewley et al. 2013).

GGE biplot analysis

Faster and uniform germination and seedling growth are essential for the establishment of seedlings in the field. The seeds of any crop variety maintains the vigor and viability to certain period i.e., seed longevity, which is genetically controlled and influenced by the storage environment. The aging periods were considered environments and genotype genotype-environment (GGE) interaction analysis was done to understand the relationship between the aging period and to visualize genotypes in two dimension biplots *vis-a-vis* aging periods (E) for seed longevity (Figures 5 and 6), seedling growth and vigor traits (Figure 6). Seed vigor determines the potential of seeds for rapid growth, uniform emergence and development under a wide range of field conditions (Rajjou et al. 2012; Ventura et al. 2012). Genetic factors, environmental and storage conditions influence the seed vigor, of which genetic factors determine the seed vigor intensity, and environmental conditions determine the seed vigor expression degree (Sun et al. 2007). During aging process, the seed vigor reduces steadily and becomes sensitive to stress upon germination, leading to poor establishment, which affects the productivity of a crop. However, seed vigor is largely determined by the genotype and the seed vigor variations were reported among different crop species or different strains of the same species, and seed vigor can be inherited (Yu et al. 1999; Tang and Ma 2007; Cheng et al. 2003; Oluwaranti et al. 2020). The genotype, environment, and their interaction had significant effects on seed vigor, and the genetic differences among the strains were the main reason for the variation in seed vigor (Cheng et al. 2023). The GGE model could capture 81.56% of the total variation generated by genotype x environment interaction. Axis-1 accounted for 63.13%, whereas Axis-2 explained 18.43% variation. The aging periods E1 to E3 clustered together in the biplot for seed germination (Fig. 5). The effect of seed age on finger millet was manifesting into reduced germination from E4 and the effect was obvious in E6. The E7 and E8 treatment effects were correlated, where most of the genotypes germinated poorly. The most

stable genotypes with more than 75% germination were G12, G14, G22 and G26 and a similar distribution pattern of aging periods (E) and genotypes (G) was observed on field emergence data (Fig. 6). The genotypic response varied significantly at different periods of aging, and the G x E interaction emphasized that longevity is a genetic trait that can be improved during selection programs of finger millet. The, biplot representation of any two-way data aids in the visualization of the complexity of interaction in the data in a convenient way (Yan 2001). GGE biplot was employed to identify the best combiners for seed quality and storability of parental lines in grain sorghum (Kannababu et al. 2017), and to identify the stable cultivars of maize in different environments (Kumar et al. 2023). The GGE biplot helps to exploit both genotype and environmental variance and the GGE biplots are ideal for which-won-where pattern analysis, genotype, and test environment evaluation (Kumar et al. 2024).

Trait analysis

The correlation between the seed quality traits revealed that seedling dry weight has no correlation with germination and emergence (Fig. 7). The seedling vigor index showed a high correlation with its dependent traits, *viz.* germination, seedling length and seedling dry weight. The rest of the traits are associated significantly with each other. The seed germination indicates whether the embryo packed in the seed is alive or dead. With the progress in aging, most of the biochemical and enzymatic activities are reduced. This confounding effect is recorded as the reduction in germination percentage. For seed aging, oxidative stress is one of the reasons where accumulation and mobility of reactive oxygen species (ROS) through membranes take place along with the decrease in oxidative stress (Kurek et al. 2019); and the aging-induced oxidative stress leads to cellular damage and reduced metabolic activity in seeds (Tongue et al. 2023). The seven traits (SG, FE, RL, SL, SDW, SVI-1 and SVI-2) studied are important indicators of seed longevity and seedling vigor. The most important trait is seed germination, which indicates the longevity potential of a genotype after passing through different periods of aging. Depending on the genotype and environmental factors, seed longevity is a variable trait and it is an important topic of seed science to find out the mechanisms regulating seed aging (Zinsmeister et al. 2020). During the aging process, average performance in all the seven seed quality traits reveals that some traits lose healthy expression gradually, like the seedling dry weight. Whereas the germination percent and the emergence percent is maintained for three years without much loss in the expression; however, the effect is drastic after three years of storage. There are a number of traits at various levels of phenotype (morphological, biochemical and transcription levels) that influence variation in the longevity of seeds (Choudhary et al. 2023). These mechanisms at various levels manifest into a few measurable traits ultimately like

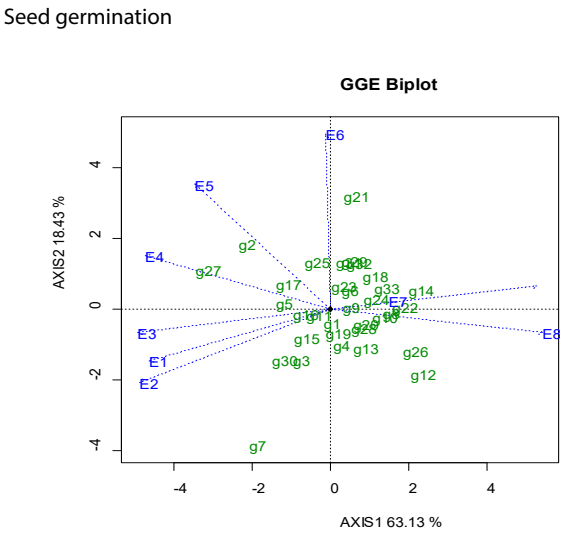


Fig. 5. Distribution of genotypes for seed longevity under varying periods of seed aging (environment factors) – Biplot

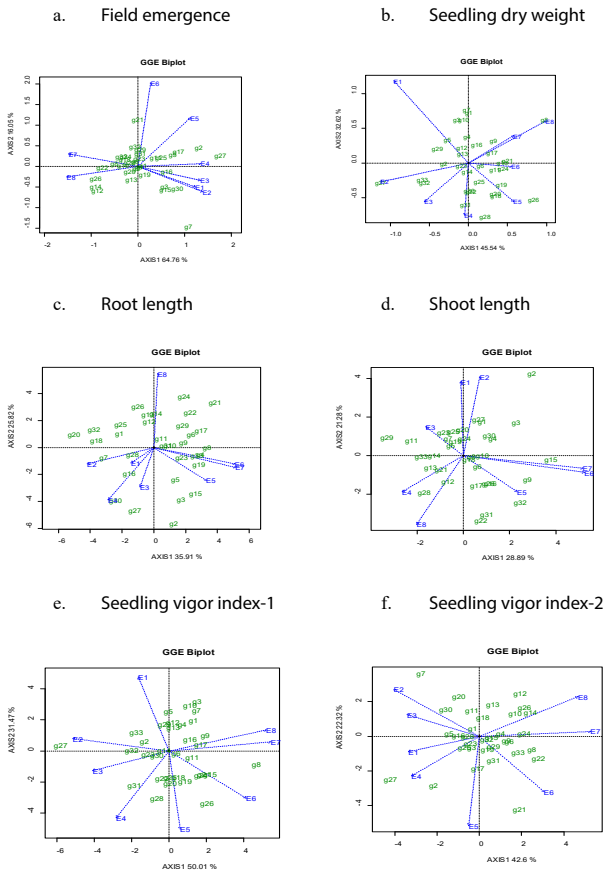


Fig. 6. a - f. Distribution of genotypes (g) for seed longevity, seedling growth and vigor traits under varying aging periods (E: environmental factors) - Biplot

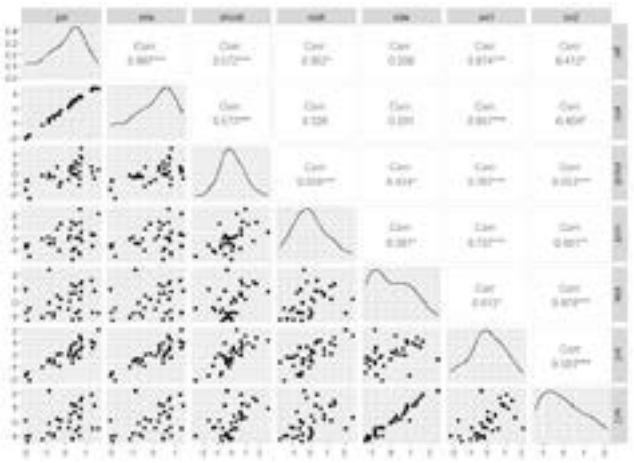


Fig. 7. Correlation among seed longevity, seedling growth and vigor traits of finger millet

test weight, germination percentage, seed vigor and field emergence. During seed aging, the damage to membranes, DNA and, enzymes and proteins could lead to deterioration in viability (Coolbear 1995; McDonald 1999). In addition to DNA and protein damage (Rao et al. 1987; Bailly et al. 2008), lipid peroxidation appears to be the most potent agent causing seed aging (Davies 2005; Wiebach et al. 2020).

In summary, the results brought out scientific information on the varying genetic potential of finger millet cultivars for seed longevity, seedling growth and vigor traits. The differences for these seed quality traits widened after three years of storage under ambient conditions. To study further mechanism of seed longevity in finger millet, the present study recommends a minimum of three and a half years of storage. These findings reveal that seeds of most of the cultivars could be stored at ambient conditions without losing the percentage of germination and emergence until three years, which provides insight into planning/organizing the nucleus seed production of these cultivars at intervals of three years. However, for other commercial seed production, the seedling growth traits and vigor indices also need to be considered along with seed viability status. Genetic influence on seedling vigor and longevity traits appears to be a potential area for breeders to screen cultivars as well as for laboratory applications to predict seedling vigor. Only a few studies are available to explain the variation in seed longevity among accessions of a single plant species (Guzzon et al. 2021). However, more clarification is still needed on the physiological and molecular bases of the complex trait seed longevity regulated by genetic and environmental factors (Liu et al. 2019; Sano et al. 2016; Zinsmeister et al. 2020). Seed longevity is an important trait with respect to the commercialization of cultivars and the economics of seed production and conservation. The present results on different traits help in understanding the pattern of vigor and viability loss in individual cultivars that guides to formulation of the seed storage guidelines and seed multiplication programs of different seed classes in the generation system of finger millet. Further, the information is also pertinent for planning the intervals for regeneration of finger millet seeds in the gene banks and active germplasm sites. It is important for genetic improvement and molecular studies to further deduce the mechanism of loss of seed longevity and vigor traits, as irregular or poor seed viability and vigor affect the uniform stand establishment, which is an important economic constraint that can influence the income of farmers during crop production. Nevertheless, this study is the first of its kind in finger millet to provide information on variability for seed longevity and vigor that can form the basis to unravel the genetic mechanism to enhance seed longevity.

Authors' contribution

Conceptualization of research (NK); Designing of the experiments (NK); Contribution of experimental materials (NK, PGP); Execution of field/lab experiments and data collection (NK); Analysis of data and interpretation (SA, IKD, NK, AVU); Preparation of the manuscript (NK, SA, IKD, PGP, CTS).

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