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Exploratory studies on components of variability for seed longevity and quality traits in bread (*Triticum aestivum*) and durum (*Triticum durum)* wheat

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ABSTRACT

A set of 53 wheat genotypes were evaluated for different quality and longevity related traits at NBPGR, New Delhi, India. The selected genotypes included both durum and bread wheat varieties released in India between 1970 and 2014, and bread wheat germplasm lines for comparison. Bread wheat varieties were good repositories for sedimentation value (SV), whereas, durum varieties for kernel hardness (KH) and germplasm lines for grain iron (Fe), zinc (Zn), and thousand kernel weight (TKW). Both vigor index-1 and 2 after controlled deterioration (VIACD-1 and VIACD-2) was highest for durum varieties followed by bread wheat germplasm lines and varieties. Therefore, seed longevity of durum varieties, bread wheat germplasm lines, and bread wheat varieties, respectively, was good, intermediate and poor. Broad-sense heritability was high for all the studied traits except germination percentage before controlled deterioration (GPBCD). Genetic advance as per cent mean (GAM) was high for all the studied traits, with the exception of moisture content (MC) and GPBCD. Six genotypes (IC 542394, IC 542391, IC 542416, IC 542431, IC 542426, and IC 542387) were good storers and also contained high Fe and Zn, which can be used in breeding programs to improve seed storability, and Fe and Zn content. Both intra species and inter species variability were observed for all the studied traits. The close association observed between Fe and Zn may help in improving both the traits simultaneously. Electrical conductivity (EC) of the seed leachates can be used as a surrogate trait for indirect selection for seed longevity of genotypes due to its significant negative association.

Key words: Association, Gene bank, Genetic variability, Iron, Zinc

Gene banks are vital for storage of genetic material, which is pre-requisite for a crop improvement. Barton (1961) defined seed longevity as the ability of seeds to remain viable. Seed deterioration in gene banks is the common limitation to long-term storage of valuable germplasm. Pre-storage and storage conditions, their genetic and physiological storage potential are the key determinants for the length of viability of stored seeds (Roberts 1961). Species specific defense mechanisms like structural and chemical features inherent to the seed help in delaying the seed decay, thereby increasing seed longevity (Bartosz 1981). Seed longevity has been related to various seed properties, such as color, weight and membrane composition and the correlation between seed longevity and these traits are often species and in some cases variety/genotype specific (McDonald 1999). Kranner *et al*. (2010) identified different mechanisms of seed aging.

Genetic variability for traits is a pre-requisite for any successful breeding program, as the degree of response to selection depends on the quantum of variability and heritability. Species variation for seed longevity for wheat and other species was studied by Walters *et al.* (2005). Studies of seed longevity under optimal storage conditions would take years to complete, so experimental aging procedures such as accelerated aging (AA) or controlled deterioration (CD) conditions are utilized to accelerate the loss of viability. The CD test has been used to assess the vigor of seed lots and to predict their relative longevity by aging the seeds rapidly at elevated temperature and relative humidity (Powell and Matthews 2005). Nagel *et al*. (2009) studied 55 accessions of barley and reported intra specific variability in seed longevity and they attributed the variability to the genetic component since they had handled all the accessions in a similar way. In a similar study, Nagel *et al*. (2011) tested 42 accessions of *Brassica napus* for viability after

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26 years of storage in ambient conditions. Despite having been grown simultaneously and subjected to the same post-harvest and storage conditions, the accessions displayed variation with respect to seed viability which led them to conclude that there is a genotypic component involved in the determination of seed viability. Intraspecific variability of seed longevity has been observed in sorghum, rye, and linseed (Nagel *et al*. 2010). Quantitative trait loci (QTLs) for seed longevity for both natural and artificial aging have been found in wheat (Landjeva *et al*. 2009). Using the linkage mapping approach, QTLs for seed longevity have been identified in barley (Nagel *et al*. 2009).

Though wheat is the most important staple crop and it makes up the largest share of the seeds stored in the gene banks across the globe, currently there is no sufficient information about the nature of seed longevity. The present study aimed to evaluate bread and durum wheat varieties and bread wheat germplasm lines for interspecies and intraspecies variability for seed longevity and other seed quality traits, and to identify promising genotypes for better longevity and quality.

MATERIALS AND METHODS

The experimental set up consisted of 19 Indian varieties of bread wheat and 9 varieties of durum wheat along with 25 germplasm lines of bread wheat (Table 1). The seeds were collected from the crop grown during 2013-14 at Delhi (Indian Agricultural Research Institute, Research Farm, New Delhi, 28º 38'N, 77º 9'E, 228.6m AMSL), and exposed to a similar set of conditions for seed processing.

Table 1 List of genotypes

Genotype	Pedigree	Released	Remarks
A-9-30-1	A 206 / GAZA	1970	Durum wheat variety
NI5439	REMP 80 /3* NP 710	1973	Bread wheat variety
HB208	SPO/MTA//MQ/2*RNW //3/PJ'S7P14/KT54B	1981	Bread wheat variety
HI977	GLL/AUST 61.157/CNO/NO 66/3/Y50E/3/KAL	1984	Bread wheat variety
PBW343	ND/VG9144//KAL/BB/3/YCO"S" /4/VEE#S "S"	1995	Bread wheat variety
PDW233	YAV"S" / TEZ "S"	1995	Durum wheat variety
HI8498	CR "S"-GS"S" /A-9-30-1//RAJ911	1999	Durum wheat variety
GW1139	MACS2340/IWP5070	1999	Durum wheat variety
HD4672	BIJAGA RED /PBW34 //ALTAR 84	1999	Durum wheat variety
HD2781	BOW /C 306 //C591/HW 2004	2002	Bread wheat variety
GW322	GW173 /GW196	2002	Bread wheat variety
HD2864	DL 509-2/ DL 377-8	2004	Bread wheat variety
HI1531	HI 1182/CPAN 1990	2005	Bread wheat variety
AKDW2997-16	CPAN6140 /RAJ 1555	2006	Durum wheat variety
HD2932	KAUZ/STAR//HD2643	2007	Bread wheat variety
HI1544	HD 2402 / HW 3007	2007	Bread wheat variety
WH1021	NYOT95/SONAK	2007	Bread wheat variety
HD4713	SCOT29/TARAI//AJAIA	2008	Durum wheat variety
HD2987	HI 1011/HD 2348//MENDOS/IWP 72/DL 153-2	2009	Bread wheat variety
HD2967	ALD/COC//URES/HD 2160 M/HD 2278	2009	Bread wheat variety
HD2985	PBW 343/PASTOR	2010	Bread wheat variety
PDW314	AJAIA 12/F3 LOCAL (SEL.ETHIO.135.85)//PLATA13/3/SOMAT 3/4/SOOTY9/RASCON 37	2010	Durum wheat variety
HD3043	PJN/BOW//OPATA*2/3/CROC_1/Ae.squarrosa(224)//OPA	2011	Bread wheat variety
DPW-621-50	KAUZ//ALTAR84/AOS/3/MILAN/KAUZ/4/HUITES	2011	Bread wheat variety
HD3086	DBW14/HD2733/HUW468	2013	Bread wheat variety
HD3059	KAUZ//ALTAR84/AOS/3/MILAN/KAUZ/4/HUITES	2013	Bread wheat variety
HI8713	HD 4672 / PDW 233	2013	Durum wheat variety
WH1124	MUNIA/CHTO/AMSEL	2014	Bread wheat variety

Contd.

Table 1 (*Concluded*)

Genotype	Pedigree	Released	Remarks
IC542479	Germplasm	–	Bread wheat germplasm
IC542483	Germplasm	–	Bread wheat germplasm
IC542477	Germplasm	–	Bread wheat germplasm
IC542416	Germplasm	–	Bread wheat germplasm
IC542406	Germplasm	–	Bread wheat germplasm
IC535675	Germplasm	–	Bread wheat germplasm
IC542595	Germplasm	–	Bread wheat germplasm
IC542473	Germplasm	–	Bread wheat germplasm
IC542423	Germplasm	–	Bread wheat germplasm
IC542426	Germplasm	–	Bread wheat germplasm
IC542387	Germplasm	–	Bread wheat germplasm
IC542394	Germplasm	–	Bread wheat germplasm
IC542420	Germplasm	–	Bread wheat germplasm
IC542431	Germplasm	–	Bread wheat germplasm
IC543318	Germplasm	–	Bread wheat germplasm
IC542494	Germplasm	–	Bread wheat germplasm
IC542391	Germplasm	–	Bread wheat germplasm
IC542385	Germplasm	–	Bread wheat germplasm
IC542419	Germplasm	–	Bread wheat germplasm
IC542500	Germplasm	–	Bread wheat germplasm
IC542956	Germplasm	–	Bread wheat germplasm
IC542516	Germplasm	–	Bread wheat germplasm
IC542493	Germplasm	–	Bread wheat germplasm
IC542498	Germplasm	–	Bread wheat germplasm
IC542434	Germplasm	–	Bread wheat germplasm

A random sample of 20 spikes/genotype was harvested after physiological maturity; the spikes were threshed in a clean cloth and the grain was separated from husk in a plastic chaaj. The grain was sampled for Fe and Zn analysis. Care was taken at every step to avoid metal contamination. The grain samples were analyzed at Grain Quality Lab, IARI, New Delhi, using a new cost-effective, high throughput method called EDXRF. The XRF machine model X-Supreme 8000 was calibrated with glass standards in collaboration with James Stangoulis and Georgia Guild. Thousand Kernal weight (TKW) was measured by a random selection of 1000 grains representing the whole sample and the weight was recorded. Kernel hardness (KH) was measured with Single Kernel Characterization System (SKCS) from Perten, Australia. Sodium dodecyl sulphate-sedimentation test was based on the method proposed by Axford *et al*. (1978). Seed moisture content (MC) was estimated following International Seed Testing Association rules (ISTA 1985). Approximately 100 grains from each genotype were ground in a mortar with pestle and transferred into moisture bottles in two replications. Before the transfer of ground material into moisture bottles, the weight of empty bottles was recorded and the final weight of the moisture bottles containing seed sample was recorded. The samples were dried in a forced draft oven at 130ºC for 2 h and weight of moisture bottles containing dried sample was recorded after cooling the samples for 30 min in a desiccator. The moisture content was calculated as follows: MC: (W2-W3/W2-W1)*100. where, W1, weight of empty bottles; W2, weight of the moisture bottle containing seed sample; W3, weight of moisture bottles containing dried sample.

Total 200 seeds in 8 replications of 25 seeds each were uniformly placed in 11 cm petri plates on two layers of moistened filter papers. Distilled water was added at intervals to keep the paper moist. The Petri plates were kept in seed germinators maintaining a constant temperature of 23ºC and relative humidity of 100%. The seedlings were evaluated according to the International Seed Testing Association rules (ISTA 1985). Seedlings which produced normal root and shoots were considered to have germinated (normal germination). The germination was recorded on the seventh day as GPBCD. Seeds which remained un-

germinated but became soft at the end of the testing period were considered dead. The seed samples were subjected to controlled deterioration test, wherein, initial weight and initial seed moisture content of 400 seeds were recorded in four replications. Later, the seed samples were transferred to tri-layered aluminum foil packets and the desired quantity of water was added to bring the moisture content to 20% and sealed air-tightly. Seed samples were shaken at frequent intervals to facilitate uniform absorption of moisture by all the seeds. The seed packets with raised moisture content were incubated in an oven at 45ºC for 72 hrs. Thereafter, the packets were removed from the oven and the samples transferred to Petri plates were dried over laboratory bench for 48 h at room temperature, and used for further studies. The germination test was conducted as mentioned above on the seventh day and recorded as GPACD.

The amount of electrolyte leakage can be assessed by measuring the electrical conductivity of the seed soaked in water, with a conductivity meter. Twenty-five undamaged seeds in three replications from each accession were weighed. The seeds were soaked overnight in 25 ml distilled water at 25 ± 2°C and electrical conductivity was measured using a digital conductivity meter calibrated with 0.01M KCl to a reading of 1.413 µs/cm and expressed as µs/cm/gfw.

Hundred seeds before CD and after CD in four replications of 25 seeds from each genotype were placed in between moist germination paper and kept in a seed germinator maintained at 23ºC and 100% relative humidity for 7 days. On the seventh day, the seedling length of all the normally germinated seedlings was recorded in centimeters. The vigor index-1 was calculated following the method of Abdul-Baki and Anderson (1973) as follows:

VIBCD-1 = Germination percentage × seedling length

VIACD-1 = Germination percentage × seedling length of deteriorated seeds

After measuring seedling length, the fresh weight of the normally germinated seedlings and their dry weight after drying at 80°C for 48 hrs were recorded and vigor index-2 was calculated following the method of Abdul-Baki and Anderson (1973) as follows:

VIBCD-2 = Germination percentage × seedling dry weight

VIACD-2 = Germination percentage × seedling dry (weight of deteriorated seed).

The Standard statistical procedure was used for analysis of variance, and for the genotypic and phenotypic coefficient of variation (Burton and Devane 1953), heritability (Hanson 1956) and genetic advance (Johnnsen 1909). Correlation coefficients were analyzed using the formula suggested by Al-jibouri (1958).

RESULTS AND DISCUSSION

Genetic variability

Dissection of total variability into its components in bread and durum wheat varieties and bread wheat germplasm lines indicated significant differences for all the studied traits (Table 2), suggesting the presence of wider genetic variability to be used for the genetic improvement of wheat. Mean and range for different quality and longevity related traits in bread and durum wheat are presented in Table 3. Highest KH was recorded in durum varieties compared to bread wheat varieties; the reverse was true for Sedimentation value (SV). This interspecies variability for SV was expected because of continuous selection of durum varieties for pasta, and other related products, and of bread wheat varieties for bread and chapathi making. Highest mean value for Fe (41.41±0.96 mg/kg) and Zn (44.08±1.06 mg/kg) was recorded in germplasm lines. Germplasm lines were better repositories of grain Fe and Zn. GPACD mean was highest for durum varieties (64.89±13.03%) followed by germplasm lines (52.50±7.44) and bread wheat varieties (36.89±7.50). Mean of ECBCD (62.36±2.73) and ECACD (71.48±2.77) were lowest in germplasm lines. Vigor index-1 (3090.50±84.28) and 2 (1.32±0.06) before controlled deterioration and vigor index-1 (1421.10±369.78) and 2 (0.63±0.17) after controlled deterioration was recorded highest, respectively, by bread wheat germplasm lines and durum varieties. Though the initial vigor of germplasm lines were better than bread and durum varieties, the vigor after controlled deterioration was better for durum varieties. Durum varieties, bread wheat germplasm lines and varieties were, respectively, good, intermediate and poor gene bank storers. Therefore, seed longevity of durum varieties were better followed by germplasm lines and bread wheat varieties. Intraspecific variability for seed longevity was reported earlier in wheat, oats, barley, and maize (Walters *et al*. 2005) and in barley (Nagel *et al*. 2009).

Estimates of genetic parameters for different quality and longevity related traits are presented in Table 4. PCV and GCV values for the three sets of genetic materials studied were moderate for Fe, Zn, and TKW. Similar results of moderate variability were earlier reported by Yashavanthkumar *et al*. (2014) for Fe and Zn, and for TKW by Mohammed *et al*. (2012). Both GCV and PCV for SV of bread wheat varieties were higher than durum varieties and germplasm lines. Low, moderate and high PCV and GCV values were recorded for KH by durum, bread and germplasm lines, respectively. Our results are in conformity with the earlier study of Mohammed *et al*. (2012) for KH in a set of durum genotypes. PCV and GCV for ECACD were moderate for durum varieties and germplasm lines, whereas, it was high for bread wheat varieties. The magnitude of PCV and GCV was higher for vigor index-1 and 2 both before and after the controlled deterioration in bread wheat varieties followed by germplasm lines and durum varieties. Landjeva *et al*. (2010) reported five QTLs for seed longevity in wheat, which suggests the existence of genetic variability in seed longevity. Broad-sense heritability was higher for all the studied traits except GPBCD, which exhibited moderate broad-sense heritability. GAM was high for all the studied traits, with the exception of MC and GPBCD. Because of the very low variability of these two traits, the GAM was also recorded low, although heritability was high. Similarly,

Table 2 ANOVA for different quality and longevity related traits in bread and durum wheat

Trait	Bread wheat varieties		Durum wheat varieties		Bread wheat germplasm	
	d.f.	MSS	d.f.	MSS	d.f.	MSS
MC	18	0.40***	8	0.12**	24	0.31***
TKW	18	40.07***	8	68.21***	24	84.76***
KH	18	274.51***	8	20.22***	24	410.43***
SV	18	104.87***	8	26.50***	24	53.75***
Fe	18	11.97***	8	16.44***	24	45.90***
Zn	18	48.46***	8	13.94***	24	55.73***
GPBCD	18	6.89*	8	6.00	24	0.59*
GPACD	18	2138.20***	8	3053.72***	24	2770.00***
ECBCD	18	753.30***	8	515.80***	24	371.92***
ECACD	18	1336.76***	8	879.35***	24	382.98***
VIBCD-1	18	655946.85***	8	300670.60**	24	355162.70***
VIACD-1	18	740127.64***	8	2461378.66***	24	1525054.94***
VIBCD-2	18	0.26***	8	0.07*	24	0.21***
VIACD-2	18	0.10***	8	0.49***	24	0.30***

* Significant at P=0.1; ** Significant at P=0.01; *** Significant at P=0.001. MC: Moisture content (%); TKW: Thousand kernel weight (gm); KH: Kernel hardness; SV: Sedimentation value; Fe: Grain iron concentration (mg/kg); Zn: Grain zinc concentration (mg/kg); GPBCD: Germination percentage before controlled deterioration; GPACD: Germination percentage after controlled deterioration; ECBCD: Electrical conductivity before controlled deterioration; ECACD: Electrical conductivity after controlled deterioration; VIBCD-1: Vigour index-1 before controlled deterioration; VIACD-1: Vigour index-1 after controlled deterioration; VIBCD-2: Vigour index-2 before controlled deterioration; VIACD-2: Vigour index-2 after controlled deterioration.

Table 3 Mean and range for different quality and longevity related traits in bread and durum wheat

Trait	Bread wheat varieties		Durum wheat varieties		Bread wheat germplasm	
	Mean	Range	Mean	Range	Mean	Range
MC	11.42±0.10	10.55-12.24	10.71±0.08	10.28-10.99	10.45±0.08	9.39-11.38
TKW	36.51±1.03	28.53-44.50	40.63±1.95	33.27-50.14	40.66±1.30	27.21-51.58
KH	84.58±2.69	44.53-100.47	96.42±1.06	91.53-101.6	71.41±2.87	31.48-87.50
SV	43.61±1.66	32.00-58.50	31.50±1.21	27.00-37.50	41.8±1.037	30.50-51.00
Fe	38.09±0.56	32.55-42.00	37.81±0.96	32.40-43.10	41.41±0.96	35.35-51.10
Zn	41.66±1.13	34.50-53.20	41.23±0.88	36.20-43.65	44.08±1.06	32.65-56.90
GPBCD	98.00±0.43	94.00-100.00	96.67±0.578	94.00-99.00	99.72±0.11	98.00-100.00
GPACD	36.89±7.50	0.00-90.00	64.89±13.03	1.00-93.00	52.50±7.44	0.00-97.00
ECBCD	107.85±4.45	83.96-164.17	101.10±5.35	83.36-136.15	62.36±2.73	39.87-92.17
ECACD	117.56±5.93	84.96-195.3	110.93±6.99	86.67-145.28	71.48±2.77	50.04-101.66
VIBCD-1	2331.60±131.38	1066.9-3371.5	2027.10±129.24	1312.6-2517.14	3090.50±84.28	2401.00-4004.50
VIACD-1	409.12±139.56	0.00-1982.73	1421.10±369.78	6.46-2716.26	831.27±174.64	0.00-2713.71
VIBCD-2	0.81±0.08	0.07-1.80	0.96±0.06	0.52-1.21	1.32±0.06	0.82-2.14
VIACD-2	0.18±0.06	0.00-0.74	0.63±0.17	0.00-1.20	0.36±0.08	0.00-1.09

Table 4 Estimates of genetic parameters for different quality and longevity related traits in bread and durum wheat

Trait	Bread wheat varieties				Durum wheat varieties				Bread wheat germplasm			
	PCV	GCV	$h^2_{b.s}$	GAM	PCV	GCV	$h^2_{b.s}$	GAM	PCV	GCV	$h^2_{b.s}$	GAM
MC	3.93	3.88	97.4	7.88	2.27	1.92	71.7	3.35	3.76	3.69	96.3	7.45
TKW	12.26	12.19	98.8	24.95	14.37	14.36	99.8	29.54	16.01	15.86	98.1	32.34
KH	13.95	13.79	99.1	28.29	3.30	3.15	91.2	26.19	20.06	19.95	98.8	40.85
SV	16.61	16.41	97.6	33.38	11.56	11.04	91.3	21.73	12.40	12.13	95.7	24.44
Fe	10.42	9.67	77.8	10.29	11.59	10.96	84.2	13.15	15.57	15.23	94.3	22.47
Zn	15.82	15.62	96.7	23.54	10.40	10.03	88.9	21.72	15.98	15.79	96.9	23.9
GPBCD	1.89	1.18	39.0	1.52	1.79	1.38	59.3	2.19	0.54	0.30	29.5	0.33
GPACD	88.62	87.93	98.4	179.71	60.22	59.84	98.8	122.53	70.89	70.61	99.2	144.9
ECBCD	17.99	17.25	91.9	34.07	15.88	14.71	85.8	28.09	18.87	19.21	94.3	42.48
ECACD	21.99	21.31	93.9	42.52	17.90	16.84	89.1	34.70	18.36	17.36	89.9	35.86
VIBCD-1	24.56	23.02	87.8	44.42	19.13	16.33	74.7	29.43	13.64	12.74	87.2	24.51
VIACD-1	148.69	146.29	96.8	296.5	78.10	76.9	97.0	156.04	105.05	102.23	94.7	204.96
VIBCD-2	44.54	41.25	85.8	78.72	19.84	13.31	45.0	18.40	24.30	21.25	76.5	38.28
VIACD-2	128.07	125.38	95.8	252.85	78.30	77.13	97.7	157.02	109.01	104.72	92.3	207.26

PCV: Phenotypic coefficient of variation; GCV: Genotypic coefficient of variation; $h^2_{b.s}$: Broad sense heritibility; GAM: Genetic advance as percent mean

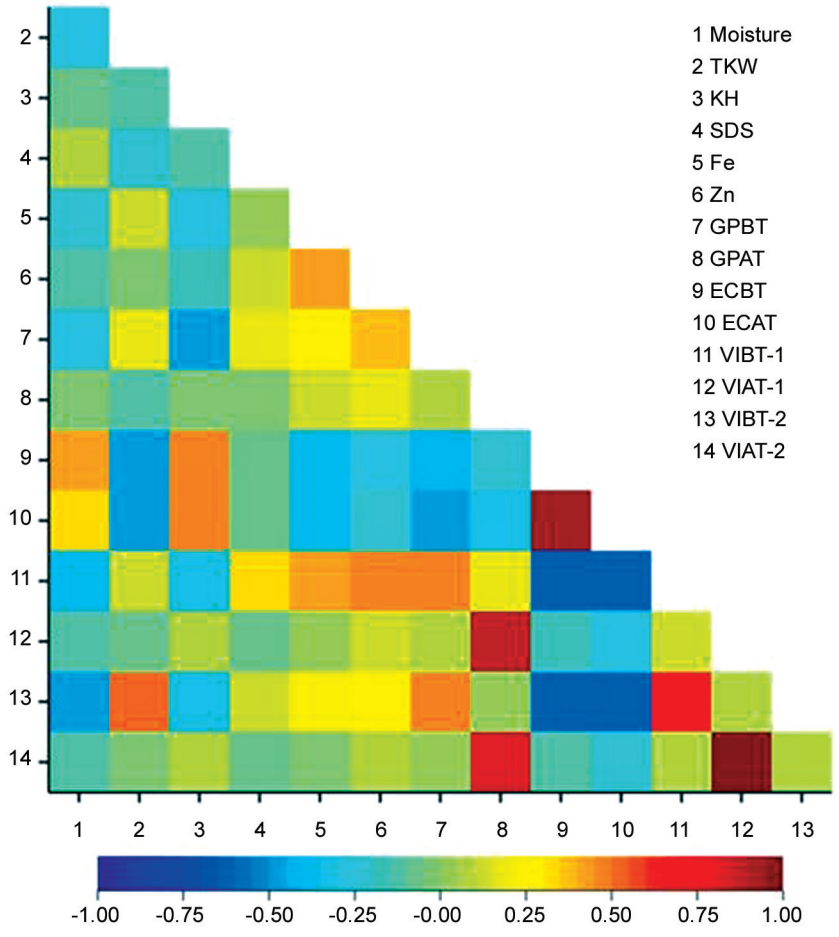

Fig 1 Graphical representation of correlation coefficients of 53 wheat genotypes for different quality and longevity related traits.

high heritability and GAM for both SV and TKW was reported by Hokrani *et al*. (2013).

Association studies of 53 wheat genotypes for different quality and longevity related traits are depicted in Fig 1. Association study reveals that genotypes of all the three sets studied, exhibited almost a similar pattern with respect to association of TKW with ECACD which was significantly negative; SV with vigor index, Fe with Zn, and Zn with VIBCD-1 which were significantly positive; GPACD with ECACD and vigor index which was significantly positive, and ECACD with VIACD-1 and 2 which was significantly negative. The negative association between TKW and ECACD could be due to the greater compactness of starch grains in high TKW genotypes, thereby resulting in lower leakage of leachates during soaking. Association between Fe and Zn was strong and positive in all the three sets of genotypes studied. This corroborates with earlier findings in bread wheat (Hugo *et al*. 2010), durum wheat (Ficco *et al*. 2009, Badakshan *et al*. 2013) and emmer wheat (Peleg *et al*. 2008). There was no significant association between micronutrients and vigor index after controlled deterioration, therefore

the role of micronutrients in seed longevity is doubtful. The association between GPACD and vigor index was significant and positive. EC was significant and negatively associated with vigor index. Tajbakhsh (2000) reported a similar association between EC and vigor, wherein, higher the EC lower was the seed vigor.

In this study we identified six genotypes (IC 542394, IC 542391, IC 542416, IC 542431, IC 542426, and IC 542387) with high Fe and Zn, high GP and vigor index-1 and 2 before and after controlled deterioration, low EC before and after controlled deterioration. These genotypes are expected to store well in gene banks also. These genotypes also contain high grain Fe and Zn concentration, which can be used in breeding programs to improve seed storability and Fe and Zn concentration. Seed longevity of durum varieties, bread wheat germplasm lines, and bread wheat varieties, respectively, was good, intermediate and poor. The close association observed between Fe and Zn may help in improving both the traits simultaneously. Electrical conductivity (EC) of the seed leachates can be used as a surrogate trait for indirect selection for seed longevity of genotypes due to its significant negative association.

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