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Variability in Fe and Zn content among Indian wheat landraces for improved nutritional quality

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Abstract

The short-term agricultural tools like agronomic bio-fortification of available nutrient resources are an emerging cost-effective strategy to address global malnutrition, especially in developing countries. This strategy involves supplying of micronutrients such as iron and zinc in the staple foods by using conventional plant breeding and biotechnology methods. The present study was to estimate iron and zinc in 180 Indian wheat landraces obtained from National Bureau of Plant Genetic Resources, Delhi. Substantial variations among 180 lines existed for both iron and zinc contents. Iron concentration ranged from 32.7 μg/g to 54.5 μg/g and zinc concentration from 15.8 μg/g to 66.3 μg/g in wheat landraces. Iron and zinc concentration were positively correlated implying the chance for concurrent selection for both the micronutrients. Six potential landraces, namely, IC-82198, IC-532790, IC-534886, IC-532310, IC-82377 and IC-79062 having high amount of both Fe and Zn content have been identified. Micronutrient-rich genotypes identified in this study opens up the possibilities for the identification of genomic regions or QTLs responsible for mineral uptake and translocation that can be used as donor for developing nutrient enriched varieties.

Key words: Diversity, landraces, iron, zinc, nutrition

Introduction

The human population in the developing world, particularly women and children, continue to suffer from under nutrition. The poor especially, often suffer from a basic lack of protein and energy, the adverse health effects of which are frequently compounded by deficiencies in micronutrients. Micronutrients are dietary components, often referred to as vitamins and minerals. These components required by the human body in small amounts, are vital to development, disease prevention, and wellbeing. Above this, they are not produced in the body and must be derived from the diet (Sijbesma and Sheeran 2011). Deficiencies in micronutrients such as iron, iodine, vitamin A, folate and zinc can have devastating consequences. At least half of the children worldwide ages 6 months to 5 years suffer from one or more micronutrient deficiency and globally more than 2 billion people are affected (http://www.unitedcalltoaction.org). Zinc and iron are very crucial for good health, zinc being essential for a healthy immune system, whereas iron is needed for the formation of red blood cells that carry oxygen around the body. Deficiencies of these two micro-nutrients are the most common nutritional deficiencies particularly women and children in developing countries like India where diet is based on cereals like wheat, rice and maize having low levels of Fe and Zn (Welch and Graham 2004). In, India the problem is double fold, first because of prevalence of vegetarian diet among majority of population and secondly, very low concentrations and poor bioavailability of Zn and Fe in the commonly used cereals aggravate the micronutrient deficiencies. Breeding new cereal genotypes with high genetic capacity for grain accumulation of micronutrients is widely accepted and most sustainable solution to the problem. However, the breeding approach is a long-term process and may be affected from low chemical solubility of Zn and Fe in soils due to high pH and low organic matter (Cakmak 2008). Among various techniques available for quantitative estimation of micronutrients, some are destructive in nature like

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Atomic Absorption Spectrometry, Inductively Coupled Plasma Optical Emission Spectrometry and a few are non-destructive like X-Ray Fluorescence (XRF). XRF can be well used for estimation of Zinc and Iron concentrations in wheat (Rai et al. 2012).

Wheat is major source of calorie intake which is 60%; any increase in the level of Fe and Zn in wheat grains can have significant impact on reducing micronutrient deficiencies. The improvement in micronutrient content in cereals can be done by conventional method for which harnessing of germplasm is essential. Hence, micronutrient rich lines can be selected from the existing variations in germplasm of wheat which was the core idea behind this study. We obtained 180 genotypes of Indian origin from National Bureau of Plant Genetic Resources (NBPGR), New Delhi, India. Therefore, a study was conducted to assess the variability for iron and zinc contents in wheat grains of 180 wheat landraces using ED-XRF method for their utilization in micro-nutrient biofortification program.

Materials and methods

Plant materials and experimented site

A total of one hundred and eighty wheat landraces were taken for study which were obtained from the National Bureau of Plant Genetic Resources, NBPGR, New Delhi (Table 1). A field experiment was conducted during *rabi* 2012-13 and 2013-14 in two replications at Indian Agricultural Research Institute, Delhi. This area is situated at latitude of 28.08°N and longitude of 77.12°E with clayey soil of pH 8.4 (Table 1). The experiment was laid out in randomized complete block design with a spacing of 20 × 20 cm. Normal cultural practices were followed as per standard recommendation.

Table 2. Soil (0–15 cm deep) properties of the experimental plots at the time of sampling

Texture (sandy loam)

Soil properties	Value
pH (1: 2.5)	8.4
Organic carbon	5.2 g/kg
Available nitrogen	183 kg/ha
Available phosphorus	22.4 kg/ha
Available potassium	188 kg/ha
DTPA-Zn	3.1mg/kg
DTPA-Fe	4.8 mg/kg
DTPA-Cu	3.5 mg/kg
DTPA-Mn	25.9 mg/kg

Iron and zinc content estimation

Iron and zinc contents were estimated in the Division of Genetics, IARI, Delhi using an energy-dispersive X-ray fluorescence spectrometry (EDXRF) instrument (model X-Supreme 8000, Oxford Instruments plc, Abingdon, UK), which has 10 place auto sampler holding 40 mm Aluminum cups for high throughput screening of Zn and Fe in whole grain wheat (Paltridge et al. 2012). Measurement conditions for Zn and Fe were identical to those reported previously for the analysis of these elements in rice and pearl millet (Table 1) (Paltridge et al. 2012). Wheat grains was cleaned and 5g of each sample was weighed and transferred to sample cups. The sample cups were gently shaken for uniform distribution of samples and kept for analysis. Analysis was conducted in sample cups lined with 30 mm polypropylene inner cups sealed at one end with 4μm Poly-4XRF sample film. Data was taken from two replicas of landraces and concentration was expressed in microgram per gram (μg/g).

The statistical analysis was done with average of two years data using software Past 3.016. Amount of Fe and Zinc was grouped into low, medium and high. ANOVA was conducted by GenStat v12.1 (Payne et al. 2009).

Results and discussion

Wheat in India is the staple food for nearly half of the country's population, therefore, current scientific studies are focusing their attention towards enriching the nutritional status of wheat grain. Crop bio-fortification programs require fast, accurate and cost-effective methods for identifying nutrientrich genotypes. Therefore, we used energy dispersive X-ray fluorescence spectrometry (EDXRF) tool for the measurement of zinc (Zn) and iron (Fe) concentrations in the whole grain wheat. Many researchers have studied the feasibility of breeding for enhancing bio-available micro nutrients in grains by increasing the concentrations of metal binding proteins (Velu et al. 2014). The pre-requisite for initiating a breeding program to develop micronutrientrich genotypes is to screen the available germplasm and identify the source of genetic variation for the target trait and to understand the basis of micronutrient uptake process. Iron and Zinc contents in grains also depend on the micronutrient

Table 1. Average value of Iron (Fe) and Zinc (Zn) contents over consecutive years (2012-13 and 2013-14) in 180 wheat landraces (LR) obtained from NBPGR, New Delhi, India.

S.No.	IC.No.	Fe (ppm)	Zn (ppm)	S.No.	IC.No.	Fe (ppm)	Zn (ppm)	S.No.	IC.No.	Fe (ppm)	Zn (ppm)
LR-001	IC-532787	36.0	43.7	LR-062	IC-532476	39.0	51.5	LR-123	IC-532156	46.1	38.7
LR-002	IC-82263	40.5	52.2	LR-063	IC-532482	35.8	46.9	LR-124	IC-532148	37.5	42.9
LR-003	IC-79052	39.6	41.0	LR-064	IC-532483	38.3	44.5	LR-125	IC-532145	44.0	43
LR-004	IC-82217	41.7	52.7	LR-065	IC-534554	37.6	44.3	LR-126	IC-532155	49.0	47.3
LR-005	IC-82210	35.9	48.4	LR-066	IC-534555	38.0	49.9	LR-127	IC-532790	51.4	44.2
LR-006	IC-82247	37.3	47.2	LR-067	IC-534553	42.7	42.4	LR-128	IC-532147	46.9	43.5
LR-007	IC-79055	43.9	45.3	LR-068	IC-534549	41.6	52.2	LR-129	IC-532151	41.7	42.4
LR-008	IC-79043	35.8	48.2	LR-069	IC-534556	45.8	57.2	LR-130	IC-82377	53.8	50
LR-009	IC-79046	40.0	47.8	LR-070	IC-532485	34.8	40.7	LR-131	IC-534480	41.2	41.8
LR-010	IC-82206	36.1	50.5	LR-071	IC-82185	35.0	40.4	LR-132	IC-534524	39.8	42.4
LR-011	IC-79047	36.6	45.0	LR-072	IC-82192	38.3	47.7	LR-133	IC-534481	40.5	37.9
LR-012	IC-532794	42.4	45.7	LR-073	IC-82180	38.9	61.0	LR-134	IC-534543	41.9	41
LR-013	IC-82371	36.2	43.5	LR-074	IC-82190	38.7	46.8	LR-135	IC-534534	42.3	42.1
LR-014	IC-532292	41.2	51.1	LR-075	IC-532149	37.2	47.5	LR-136	IC-82187	41.8	37.1
LR-015	IC-82425	34.9	46.7	LR-076	IC-82393	38.3	42.9	LR-137	IC-82198	50.1	43.1
LR-016	IC-532777	41.7	51.5	LR-077	IC-532153	37.6	32.4	LR-138	IC-82193	42.5	30.6
LR-017	IC-532788	42.9	50.0	LR-078	IC-82381	36.6	46.0	LR-139	IC-82189	41.8	52.9
LR-018	IC-532784	43.4	59.4	LR-079	IC-532146	38.7	43.4	LR-140	IC-82200	43.0	43.4
LR-019	IC-532779	40.7	15.8	LR-080	IC-532150	42.4	46.8	LR-141	IC-532258	44.9	50.5
LR-020	IC-82398	42.8	50.4	LR-081	IC-79065	38.2	43.3	LR-142	IC-532699	47.6	53.1
LR-021	IC-532868	41.4	53.9	LR-082	IC-79090	39.1	53.6	LR-143	IC-82440	43.1	43.6
LR-022	IC-532886	41.8	56.4	LR-083	IC-532492	41.8	36.6	LR-144	IC-532261	43.5	40.3
LR-023	IC-532891	46.7	60.4	LR-084	IC-79067	36.1	37.0	LR-145	IC-532262	42.0	57.3
LR-024	IC-532241	44.9	66.3	LR-085	IC-79080	43.3	46.4	LR-146	IC-532265	38.4	48.7
LR-025	IC-532238	49.4	50.2	LR-086	IC-532497	35.3	41.3	LR-147	IC-532263	43.7	42
LR-026	IC-532242	47.4	46.1	LR-087	IC-532495	39.9	39.0	LR-148	IC-532264	39.2	59.1
LR-027	IC-532880	38.0	41.9	LR-088	IC-532487	37.2	41.4	LR-149	IC-532268	36.5	42.4
LR-028	IC-532232	39.7	43.6	LR-089	IC-532490	40.2	43.2	LR-150	IC-532259	45.9	44
LR-029	IC-534883	38.7	48.9	LR-090	IC-532489	38.6	36.0	LR-151	IC-532243	39.3	44.8
LR-030	IC-532284	35.9	36.6	LR-091	IC-532089	37.1	39.1	LR-152	IC-532240	35.6	44.4
LR-031	IC-532297	40.5	37.4	LR-092	IC-78899	46.7	43.5	LR-153	IC-532231	39.1	49.7
LR-032	IC-532298	36.0	44.9	LR-093	IC-532095	41.5	45.3	LR-154	IC-532237	49.7	56
LR-033	IC-532309	32.7	36.8	LR-094	IC-78877	46.8	43.6	LR-155	IC-532244	46.3	60
LR-034	IC-532318	35.9	54.0	LR-095	IC-532094	35.2	35.2	LR-156	IC-532486	45.8	51.6
LR-035	IC-532310	54.5	48.2	LR-096	IC-532096	43.8	45.5	LR-157	IC-532486	41.7	50.7
LR-036	IC-532286	43.7	58.3	LR-097	IC-532098	38.3	38.9	LR-158	IC-532473	39.6	49.7
LR-037	IC-532267	43.0	55.6	LR-098	IC-532093	33.1	43.5	LR-159	IC-532474	42.9	49.1
LR-038	IC-532271	42.8	49.5	LR-099	IC-532090	38.4	44.4	LR-160	IC-532475	37.6	46.5
LR-039	IC-532697	36.3	52.8	LR-100	IC-532091	46.7	37.3	LR-161	IC-534802	39.6	44.2

S.No.	IC.No.	Fe (ppm)	Zn (ppm)	S.No.	IC.No.	Fe (ppm)	Zn (ppm)	S.No.	IC.No.	Fe (ppm)	Zn (ppm)
LR-040	IC-532290	40.5	37.7	LR-101	IC-41597	38.7	37.3	LR-162	IC-534806	37.3	41.8
LR-041	IC-534886	50.4	44.3	LR-102	IC-41504	36.6	54.3	LR-163	IC-534822	36.1	38.3
LR-042	IC-212142	47.9	53.3	LR-103	IC-55507	43.3	44.9	LR-164	IC-534808	47.2	45.8
LR-043	IC-212145	40.6	53.3	LR-104	IC-55636	45.5	48.9	LR-165	IC-534814	42.0	39.4
LR-044	IC-534885	43.4	57.8	LR-105	IC-532139	39.7	52.6	LR-166	IC-534819	35.0	38.2
LR-045	IC-532144	38.6	50.1	LR-106	IC-79056	41.9	51.4	LR-167	IC-534805	47.2	44.9
LR-046	IC-212185	38.7	35.8	LR-107	IC-79050	38.6	44.3	LR-168	IC-534820	41.5	38.0
LR-047	IC-534884	39.8	42.7	LR-108	IC-79062	51.3	56.0	LR-169	IC-534823	42.5	35.0
LR-048	IC-532097	43.3	51.9	LR-109	IC-79063	37.4	47.6	LR-170	IC-82410	39.1	44.9
LR-049	IC-532092	34.4	44.1	LR-110	IC-79053	41.5	37.8	LR-171	IC-532137	42.5	43.1
LR-050	IC-534887	33.7	42.2	LR-111	IC-532773	39.9	39.9	LR-172	IC-78895	43.8	46.6
LR-051	IC-532282	41.9	39.2	LR-112	IC-75547	41.5	45.2	LR-173	IC-532141	42.5	44.4
LR-052	IC-532274	36.5	42.8	LR-113	IC-79079	43.6	47.4	LR-174	IC-532138	45.5	42.2
LR-053	IC-532272	42.2	49.0	LR-114	IC-79085	38.7	32.7	LR-175	IC-532136	44.8	51.3
LR-054	IC-532276	33.7	39.0	LR-115	IC-79066	40.9	35.2	LR-176	IC-532134	40.2	43.7
LR-055	IC-532273	35.7	41.9	LR-116	IC-79068	41.7	36.2	LR-177	IC-532140	33.5	34.7
LR-056	IC-532285	34.9	40.5	LR-117	IC-79083	38.0	43.0	LR-178	IC-532142	35.8	34.7
LR-057	IC-532281	38.4	42.7	LR-118	IC-79077	48.7	32.8	LR-179	IC-78891	42.3	41.5
LR-058	IC-532279	39.4	38.3	LR-119	IC-532184	43.3	32.7	LR-180	IC-532143	35.3	42.1
LR-059	IC-78897	40.2	41.8	LR-120	IC-532175	42.9	44.8				
LR-060	IC-532277	37.7	45.4	LR-121	IC-82372	43.4	40.6				
LR-061	IC-532478	37.7	47.2	LR-122	IC-532289	48.4	43.2				

IC = Indigenous collection

uptake and translocation efficiency from root to grains (Velu et al. 2014). In the samples, Iron concentration ranged from 32.7 μg/g to 54.5 μg/g whereas zinc concentration varied from 15.8 μg/g to 66.3 μg/g (Fig. 1, Table 2). The mean value of iron content in the germplasm lines is 40.6 μg/g and zinc is 45 μg/g. These values were comparative with previous studies those analyzing wheat landraces for Fe and Zn content (Heidari et al. 2016). Further, it was reported that Zn^{+2} content varies between 5 and 30 μg/g depending upon soil Zn amount (Heidari et al. 2016) however, in present study, maximum 66 μg/g of Zn is estimated indicating that wheat landraces have higher Zn^{+2} concentration than commercial cultivars. The lowest concentration of iron was recorded in IC-532309 and the lowest zinc concentration was found in IC-532779. Among the landraces studied, IC-532310 had highest iron content of 54.5 μg/g and the IC-532241 possessed the highest zinc content of 66.3 μg/g (Fig. 1). Standard error +0.31 and +0.5 was obtained for iron and zinc contents (Fig. 2). The ellipse shows the area where 95% of the total genotypes fall based on its iron and zinc content. Convex hull indicates the genotypes which have extreme variation for iron and zinc content (Fig. 3). A non-significant but positive correlation (+0.247) was observed between iron and zinc contents in 180 genotypes indicating the possibility of simultaneous effective selection for both the micronutrients. However, a significant positive correlation was measured between grain Fe and Zn content (Badigannavar et al. 2016). The difference may be due to the difference in Fe and Zn contents of soil. As per previous studies, Fe and Zn content varied across location and soil type. Range of Fe and Zn content among different set of cultivars across the location and soil type varied from 11 to 60 μg/g for Zn and 11 to 80 μg/g for Fe, with an average amount of 30 μg/g (Badigannavar et al. 2016; Velu et al. 2014). Therefore, based on the iron and zinc contents in the present study, these 180 genotypes were classified into three categories, low (0-30 μg/g), moderate (30.1 to 40 μg/g) and high (>40 μg/g). For iron content, 84 genotypes

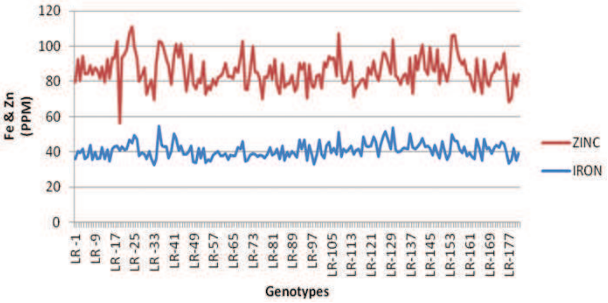

Fig. 1. Iron and Zinc contents in 180 genotypes

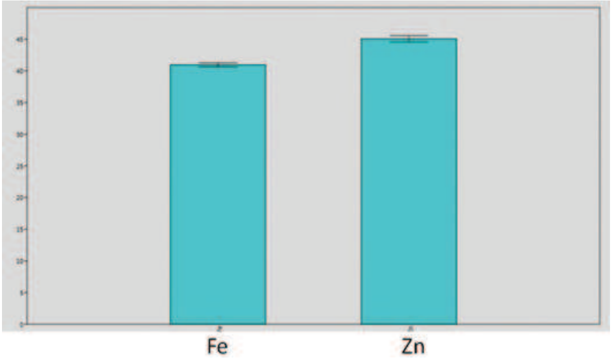

Fig. 2. **Bar diagram showing standard error in iron and zinc content among 180 wheat landraces**

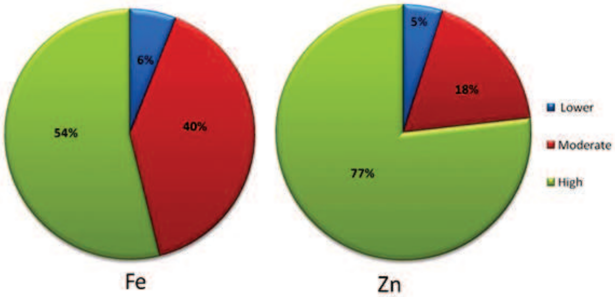

Fig. 4. **Genotype classification based on iron and zinc content**

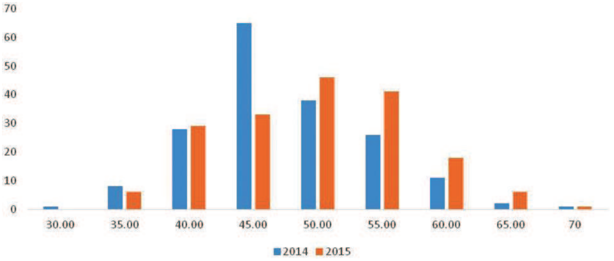

Fig. 5. **Roles of environment in the accumulation of Fe**

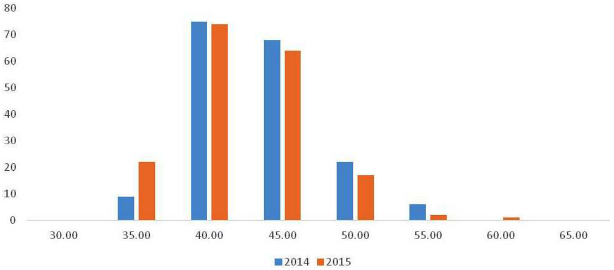

Fig. 6. **Roles of environment in the accumulation of Zn**

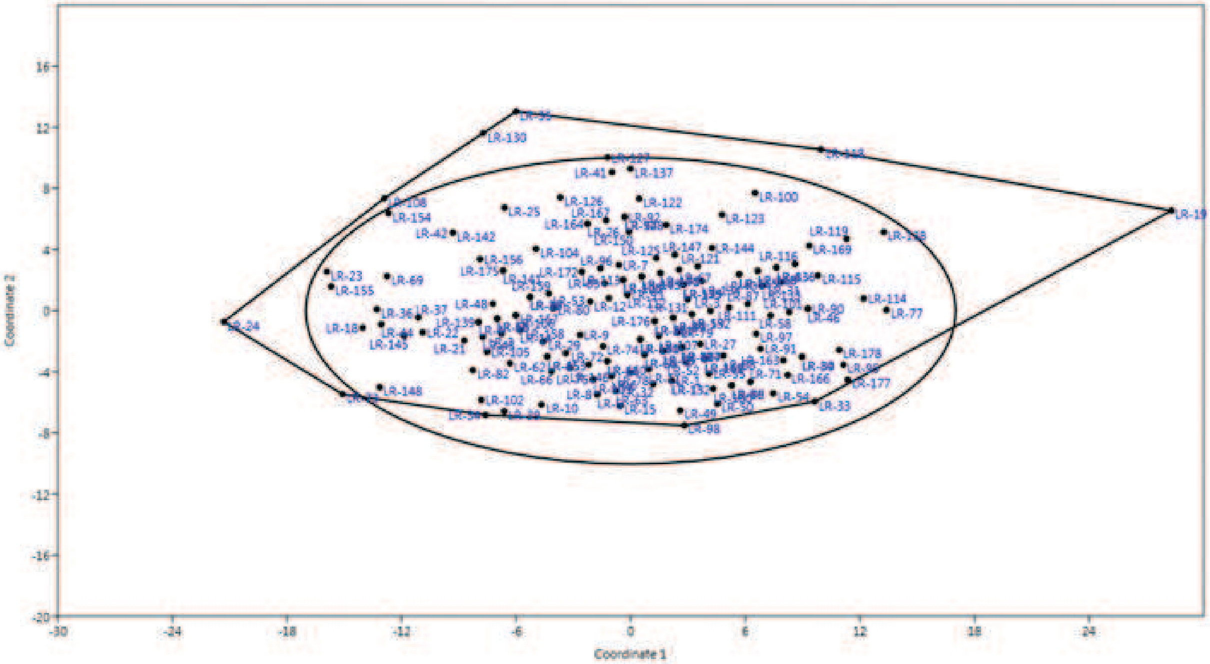

Fig. 3. Scatter diagram showing variation for iron and zinc in 180 wheat genotypes

were grouped in moderate and 91 placed in high category (Fig. 4). For Zn content, one genotypes was found with the low zinc content, 36 with the moderate zinc content and 144 were grouped in high zinc category under the same scale as of iron (Fig. 4). Maximum no. of genotypes are under high category while for iron maximum no. of landraces fell in moderate category. Out of 180, six landraces, namely, IC-82198, IC-532790, IC-534886, IC-532310, IC-82377 and IC-79062 showed higher Fe (> 50 μg/g in grain samples) and Zn content (>40 μg/g in grain samples). These genotypes were found promising and can be utilized in breeding programme.

Analysis of variance between years, genotypes and year x genotype interaction showed significant variation for both iron and zinc across both the years (Tables 3 and 4; Figs. 5 and 6). Substantial genetic variation for grain Fe and Zn concentrations were reported in wheat germplasm that included landraces and its wild relatives (Cakmak et al. 2000). More than 3000 germplasm accessions, including hexaploid, tetraploid, and diploid sources from the International Maize and Wheat Improvement Center (CIMMYT) gene bank have been screened for Zn and Fe variation (Monasterio and Graham, 2000). Materials with the highest Zn and Fe concentrations are progenitors of wheat such as einkorn and wild emmer wheat and landraces (Ortiz-Monasterio et al. 2007). *Triticum dicoccoides, Aegilops tauschii*, *Triticum monococcum*, and *Triticum boeoticum* were among the most promising sources of high Fe and Zn grain concentration (Cakmak et al. 2000). Krishnappa et al. (2018) reported that some of the accessions of hexploid wheat *T. spelta* also possessed high Zn content (~60 mg/kg), whereas synthetic wheat involving *T. dicoccoides* displayed >50 mg/kg contents. However, best line for Zn and Fe showed highly unstable performance across the year and locations (Velu et al. 2014). Molecular mapping of QTLs in wheat has also been done to enhance iron and zinc level (Yunfeng et al. 2012). Unfortunately, little variation exists in improved adapted wheat varieties and researchers, therefore, focused on a more in depth evaluation of wheat landraces (Monasterio and Graham 2000). In this study, we have identified six potential landraces namely, IC-82198, IC-532790, IC-534886, IC-532310, IC-82377 and IC-79062 having high amount of both Fe and Zn contents which may play significant role in future breeding programs for enhancement of grain Zn and Fe.

Table 3. Analysis of variance showing significant difference for Fe concentration between two different years and also significant interaction between years and genotypes

Source of variation	d.f.	s.s.	m.s.	v.r.	F value
Year	1	160.78	160.777	83.63	<.001
Geno	178	16755.76	94.133	48.97	<.001
Year x geno	178	2332.98	13.107	6.82	<.001
Residual	722	1387.98	1.922		
Total	1079	20637.49			

d.f. = degree of freedom, s.s. = total sum of squares; m.s. = mean sum of square; v.r. = variance ratio

Table 4. Analysis of variance showing significant difference for Zn concentration between two different years and also significant interaction between years and genotypes

Source of variation	d.f.	s.s.	m.s.	v.r.	F value
Year	1	1769.984	1769.984	673.4	<.001
Geno	178	42807.460	240.491	91.5	<.001
Year x geno	178	7815.399	43.907	16.7	<.001
Residual	722	1897.733	2.628		
Total	1079	54290.570			

d.f.= degree of freedom, s.s.= total sum of squares, m.s.= mean sum of square, v.r.= variance ratio

Previous studies and the present study have indicated that there is no consistent value of iron and zinc for a genotype. The variation depends on different factors such as micronutrient homeostasis, sampling method, grain nature, soil properties, analytical methods, environment, genotype and genotype x environment interaction (Anuradha et al. 2012). As micronutrient malnutrition poses a significant global challenge, the development of micro-nutrient enriched genotypes serve as the need of the hour. The extreme genotypes identified in this study will be useful for selecting and breeding lines with enriched micronutrient wheat in future.

Authors' contribution

Conceptualization of research (SG, NKS); Designing of the experiments (SG, RSJ); Contribution of experimental materials (SG, BS); Execution of field/ lab experiments and data collection (SG, RSJ); Analysis of data and interpretation (SG, BS); Preparation of manuscript (SG, BS).

Declaration

The authors declare no conflict of interest.

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