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Evaluation of biofortified spring wheat genotypes for yield and micronutrient contents

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ABSTRACT

Genetic biofortification, a way to improve micronutrients mainly zinc (Zn) and iron (Fe) concentrations, in bread wheat (*Triticum aestivum* L.) that could reduce the malnutrition in the developing world. This study was conducted at the research field of Hill Crops Research Program, Kabre, Dolakha, Nepal from December 2015 to May 2016 to evaluate biofortified wheat genotypes for grain zinc and iron concentrations, grain yield and associated component traits. Fifty biofortified spring wheat genotypes were evaluated using alpha lattice design with two replications. Highly significant variation was found among the wheat genotypes for days to heading, days to maturity, thousand grains weight, grain yield, grain zinc and iron concentrations. The value of grain zinc concentration varied from 18.85 to 69.98 ppm with a mean value of 34.12 ppm. The genotype 6HPYT405 had the highest grain zinc concentration (69.98 ppm) followed by 6HPYT432 (46.62 ppm) and 6HPYT404 (44.56 ppm). Similarly, the value of grain iron concentration varied from 35.05 to 56.06 ppm with a mean value of 44.65 ppm. The genotypes 6HPYT420 had the highest grain iron concentration (56.06 ppm) followed by genotype 6HPYT405 (55.94 ppm) and 6HPYT421 (55.33 ppm). The genotypes 6HPYT404, 6HPYT405, 6HPYT410, 6HPYT421, 6HPYT423, 6HPYT431, 6HPYT432, 6HPYT439, 6HPYT440 and 6HPYT450 had higher grain zinc and iron concentrations. So, these genotypes can be used as parents in future breeding programs to develop zinc and iron-enriched wheat varieties. The genotypes namely 6HPYT428, 6HPYT437, 6HPYT438, 6HPYT443, 6HPYT444, 6HPYT447 and 6HPYT448 were found high yielding genotypes which could be selected for varietal development program.

Keywords: Biofortification, zinc, iron, heritability, wheat

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1 Introduction

Wheat (*Triticum aestivum* L.) is grown globally and is the world's second most important cereal, representing 44% calorie of global cereal consumption (FAO, 2013). Thus, the bio-fortification with respect to zinc (Zn) and iron (Fe) could have a measurable impact on levels of malnutrition. Worldwide, wheat is grown on around 221 million hectares of land, with a production of 729 million tons and productivity of 3.28 t ha^{-1} (FAOSTAT, 2013). In Nepal, it is the third important staple crop next to rice (*Oryza sativa* L.) and maize (*Zea mays* L.) in production but ranks second in consumption after Rice. During 2017-2018, the area under cultivation, production, and productivity were

706843 ha, 1949001 tons and 2757 kg ha^{-1}, respectively (MoAD, 2017).

Zinc and iron are two of the well-known nutrients that are important for human health (Cakmak et al., 1999). Dietary deficiency of essential micronutrients, Zn and Fe affects more than two billion people worldwide; pregnant women and children below the age of five suffering from severe acute malnutrition being the major victims (White and Broadley, 2009). There is no zinc storage system containing in our bodies, so one has to eat every day for his/her own requirements (Rink, 2011). In developing countries, where there are high child and adult mortality, among the major risk factors, Zn and Fe deficiencies come as fifth and sixth, respectively, among the top 10 risk factors contributing to the burden of disease (WHO, 2002). The widespread deficiencies of Fe and Zn in developing countries is mainly the result of monotonous consumption of cereal-based foods with a low concentration and reduced bioavailability of Fe and Zn (Graham et al., 2001). For resource-poor people, zinc intake is largely dependent on cereal-based food, especially in South Asia where wheat contributes the majority dietary energy where more than 26% of the population have inadequate Zn intake (Graham et al., 2001; Velu et al., 2012).

Developing more nutrient-dense staple food crops could help reduce malnutrition (Calderini and Ortiz-Monasterio, 2003). A global biofortification initiative (HarvestPlus) within the Consultative Group on International Agricultural Research (CGIAR) was launched to breed and disseminate crops for better nutrition (Brief, 2006). The Harvest Plus project is working with national and international partners to alleviate deficiencies of zinc and iron by biofortifying staple food crops with essential minerals and vitamins. This approach is considered to be the most economical solution to human micronutrient deficiency (Bouis, 2007). The zinc and iron concentration in biofortified wheat genotypes range from 19-52 mg kg^{-1} and 23-52 mg kg^{-1}, respectively (Velu et al., 2011). Biofortified staple foods though do not deliver high levels of minerals and vitamins per day compared to supplements or fortified food products, they can increase micronutrient intake for the resource-poor people of developing countries who consume them daily (Bouis et al., 2011). So, biofortification of staple cereal crops with Zn and Fe is a high priority-global issue.

As in Nepal till now no zinc and iron-enriched wheat variety has been released, it is assumed that high percent of Nepalese population is at risk of zinc and Iron deficiency. Therefore, the International Maize and Wheat Improvement Center (CIMMYT) is focusing on to release zinc and Iron enriched wheat varieties in Nepal as well as in South Asia through the Harvest Plus project (Hao et al., 2014). The nutrient-rich high yielding wheat cultivars offer the most economical and feasible means for improving micronutrient nutrition in rural areas. The present study was carried out to evaluate the performance of CIMMYT developed biofortified wheat lines for grain zinc and iron concentration, grain yield and its associated traits under Nepalese mid hill environment.

2 Materials and Methods

2.1 Experimental site

The field experiment was conducted at the research farm of Hill Crops Research Program (HCRP), Kabre, Dolakha, Nepal from December 2015 to May 2016. Geographically, HCRP, Kabre, Dolakha is located in the mid-hill region at 27°3′N Latitude and 86°3′E Longitude at an altitude of 1650 meters above sea level. The soil texture class of the research site was a sandy loam.

2.2 Soil properties

Composite soil sample was sampled from the soil after land preparation from the field at different depth (0-30) cm. The soil was dried, grounded, and sieved through 2 mm sieve. The chemical and physical properties were analyzed in Agriculture Technology Lab, Pulchowk, Lalitpur. The detail of the soil analysis is given in Table 1.

2.3 Climatic condition

The research location represents mid hill region of Nepal and characterized by a warm temperate climate. The maximum temperature recorded ranged from 16.06 °C in the first week of December 2015 to 25.54 °C in the third week of May 2016 while minimum temperature recorded ranged from 4.33 °C in December to 13.82 °C in the last week of May. The experimental crop received a total of 17.2 mm rainfall in February, 18.5 mm in March, 10.1 mm in April and 139.3 mm in May. The meteorological data for experiment period were obtained from meteorological station, HCRP, Kabre, Dolakha, Nepal.

2.4 Plant materials

The plant material was sixth Harvest Plus Yield Trial (6HPYT) comprised 50 wheat genotypes, among them 47 were advanced lines obtained from CIMMYT with significantly improved Zn and Fe concentrations and desirable agronomic traits, along with two commercial checks [BAJ#1 (6HPYT402) and KACHU#1 (6HPYT403)] and one local check (WK1204).

Table 1. Physico-chemical properties of the soil at the experimental field, Kabre, Dolakha

Property	Content	Method
Soil pH	4.20 (very acidic)	pH meter
Soil organic matter (%)	3 (medium)	Walkey and Black method
Nitrogen (%)	0.15 (medium)	Micro-Kjeldahl method
Phosphorus (kg ha^{-1})	75.35 (High)	Modified Olsens bicarbonate method
Potassium (kg ha^{-1})	227.8 (medium)	Flame Photometer method
Iron (ppm)	2.35 (Very low)	
Zinc (ppm)	0.82 (Low)	
Texture	Sandy loam	The textural triangle

2.5 Experimental design and procedure

Field experiment was designed following the Alpha Lattice design with two replications. Each replication, had 5 blocks consisting of 10 plots. Each experimental unit (plot) was 2× 2 m^2 in size. In each plot, there were 8 rows with a row to row distance of 25 cm and continuous seeding was done. A gap of 0.5 m was maintained between the blocks and plots were continuous within the block. Seeding was done on 3rd December 2015. The standard agronomic cultivation protocol recommended by National Wheat Research Program, Bhairahawa, Nepal was followed to raise the crop. Fertilizers were applied at the rate of 120:60:60 NPK kg ha^{-1}.

2.6 Micronutrient sampling and analysis

The 20 spikes from each plot were harvested by plucking with hand wearing gloves at physiological maturity. When plots were dry, they were threshed carefully and sampled for Zn and Fe analysis. Metal contamination was avoided. Grain samples weighing about 20 g for all entries were carefully cleaned to discard broken grains and foreign material and used for micronutrient analysis. Grain samples were analyzed with a bench-top, non-destructive, energy-dispersive X-ray fluorescence (EDXRF) machine (model x-supreme 800, Oxford Instruments plc, Abingdon, UK) that was standardized for high-throughput screening of GZnC and GFeC (unit: mg kg^{-1} or ppm) concentration in whole grain wheat (Paltridge et al., 2012) The grain Zinc Iron concentration analysis work was done at Banaras Hindu University (BHU), India.

2.7 Statistical analysis

Data entry and processing was carried out using Microsoft Office Excel 2007. Analysis of variance (ANOVA) and mean estimation were done with the software- R Studio. Correlation analysis was done with SPSS. The statistical significance (alpha) was declared at 5% level of probability.

3 Results

The analysis of variance showed that genotypes were significant for days to heading, days to maturity, plant height, thousand kernel weight, grain yield, grain zinc and iron concentrations.

3.1 Days to heading and maturity

Analysis of variance shows highly significant differences ($p \leq 0.01$) among the genotypes for days to heading and days to maturity. The days to heading among genotypes varied between 103 to 116 d, with an average value of 108 d (Table 2). The earliest heading was found in genotype 6HPYT404 in 103 d followed by genotype 6HPYT447 (104 d), 6HPYT426 (105 d) and 6HPYT442 (105 d). The Check Variety WK1204 headed last in 116 d. The days to maturity varied among genotypes from 149 to 164 d with a mean value of 158 d. Genotype 6HPYT414 was found early maturing genotype with 149.49 d to maturity followed by genotypes 6HPYT418 (150 d), 6HPYT415 (151 days) and 6HPYT402 (152 d). The Check variety WK1204 was found late maturing genotype with 164.01 d to maturity.

3.2 Plant height and grains $spike^{-1}$

Highly significant differences among genotypes were found for plant height and number of grains $spike^{-1}$. The plant height varied between 57 and 96 cm with a mean value of 81 cm (Table 2). The highest plant height was found in genotype 6HPYT410 (96 cm) followed by genotypes 6HPYT417 (96 cm) and 6HPYT414 (95 cm) while the shortest plant height was found in genotype 6HPYT402 (57 cm). The number of grains $spike^{-1}$ varied among genotypes from 17 to 49 with a mean value of 38. The highest number of grains $spike^{-1}$ was found in genotype 6HPYT412 (49) followed by genotypes 6HPYT441 (48) and 6HPYT409 (48) while the lowest one was found in 6HPYT405 (17).

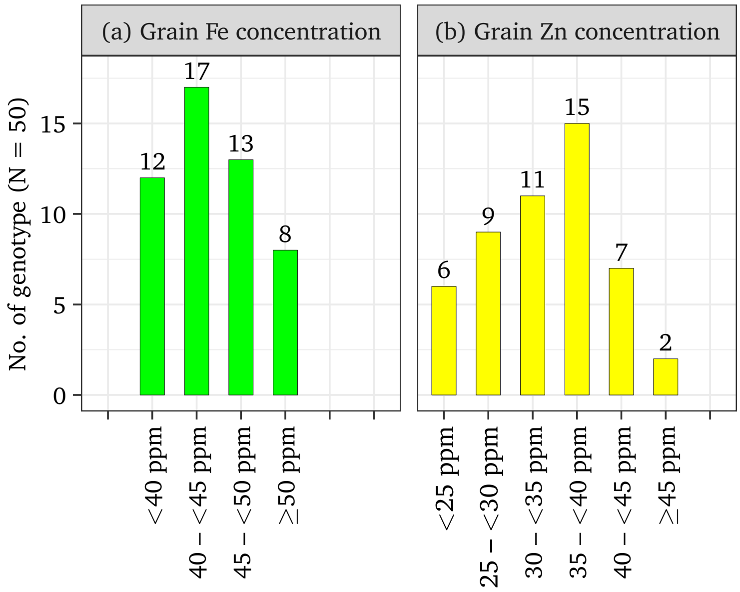

Figure 1. Frequency distribution for grain Fe and Zn concentrations of 50 biofortified wheat genotypes

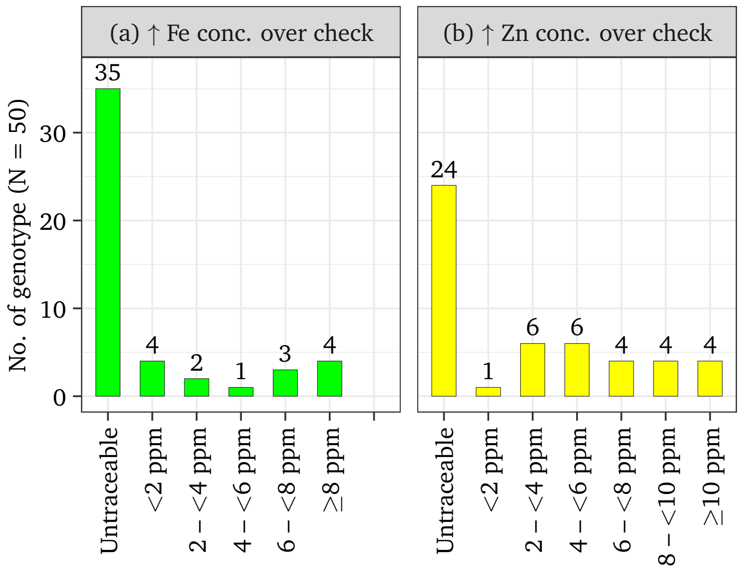

Figure 2. Increased grain Fe and Zn concentrations of 6th HPYT entires over local check

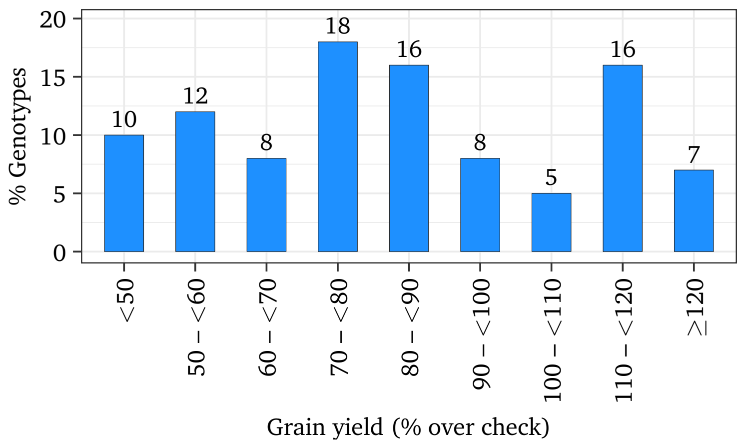

Figure 3. Increased grain yield of 6th HPYT entires over local check

3.3 Grain zinc and iron concentration

Highly significant differences among genotypes were found for grain zinc and iron concentration. The grain zinc concentration varied among genotypes from 18.85 to 69.98 ppm with a mean value of 34.12 ppm (Table 2 and Fig. 1). The grain zinc concentration was found highest in genotype 6HPYT405 (69.98 ppm) followed by genotype 6HPYT432 (46.62 ppm) and 6HPYT404 (44.56 ppm). The lowest grain zinc concentration was found in 6HPYT412 (18.85 ppm). The value of grain iron concentration varied between 35.05 to 56.06 ppm with a mean value of 44.65 ppm. The grain iron concentration was found highest in genotype 6HPYT420 (56.06 ppm) followed by 6HPYT405 (55.94 ppm), 6HPYT421 (55.33 ppm) and 6HPYT406 (54.12 ppm). The lowest grain iron concentration was found in 6HPYT412 (35.05 ppm). Among all 50 biofortified wheat genotypes, 25 genotypes (50% genotypes) shows grain zinc concentration higher than check variety WK1204 (Fig. 2). On the other hand, among all 50 biofortified wheat genotypes, 14 genotypes (28% genotypes) showed grain iron concentration higher than the check (WK1204) (Fig. 2).

3.4 1000-grain weight and grain yield

Highly significant differences among genotypes were found for grain yield and thousand grains weight. The grain yield varied among genotypes from 0.98 - 5.10 t ha^{-1} with a mean value of 2.81 t ha^{-1} (Table 2). The grain yield was found highest in genotype 6HPYT437 (5.10 t ha^{-1}) followed by 6HPYT443 (4.47 t ha^{-1}) and 6HPYT428 (4.17 t ha^{-1}) while the lowest one was found in 6HPYT405 (0.98 t ha^{-1}). The value of thousand grains weights varied between 40.20 and 60.07 g with a mean value of 51.32 g. The highest thousand grains weight was found in genotype 6HPYT433 (60.10 g) followed by genotypes 6HPYT444 (60.07 g) while the lowest one was found in 6HPYT419 (40.20 g). Among all 50 biofortified wheat genotypes, 13 genotypes (i.e. 26% of genotypes) yielded more than check WK1204. The grain yield percentage of all genotypes over check is given in Fig. 3.

3.5 Correlations

The correlation of various traits among the studied biofortified wheat genotypes is shown in Fig. 4. Days to heading showed a highly significant positive correlation with days to maturity (0.556**) and significant positive correlation with grain zinc concentration (0.302*). The plant height showed a significant negative correlation with grain iron concentration (−0.324*) and nonsignificant negative correlation with grain zinc concentration(−0.141). Grain zinc concentration showed a highly significant positive correlation with grain iron concentration (0.596**) While, it showed a significant negative correlation with grain yield (−0.433**), number of grains spike^{-1} (−0.472**) and grain iron yield (−0.293*). Grain iron concentration showed a significant negative correlation with grain yield (−0.486**), number of grains spike^{-1} (−0.365**) and plant height (−0.324*). Grain zinc yield showed highly significant positive correlation grain iron yield (0.825**) and significant positive correlation with number of grains spike^{-1} (0.285*). Similarly, grain iron yield showed a significant positive correlation with number of grains spike^{-1} (0.455**) and plant height (0.314*). Thousand-grain weight showed a non significant positive correlation with grain yield (0.161) while it showed a negative correlation with number of grains spike^{-1} (−0.188). Grain yield showed a highly significant positive correlation with number of grains spike^{-1} (0.509**), plant height (0.371 **), zinc yield (0.753**) and grain iron yield (0.950**). Similarly, it showed a non significant positive correlation with thousand grains weight (0.161).

3.6 Heritability estimate

In this study, heritability estimate ranged from 55.08% for grain iron concentration to 99.24% for plant height (Table 3). Robinson et al. (1907) classified values as high (>60%), moderate (30%-60%) and low (<10%). Accordingly, high heritability values were observed for days to heading, days to maturity, plant height, thousand grains weight, and grain yield. Moderate heritability value was observed for grain zinc and iron concentration and number of grains per spike (Table 3).

4 Discussion

Micronutrient deficiency is a growing serious problem in developing countries like Nepal. The reason for widespread deficiencies of Fe and Zn in developing countries is mostly due to monotonous consumption of cereal-based foods with a low concentration and reduced bioavailability of Fe and Zn (Graham et al., 2001). This study is focused on the evaluation of biofortified wheat lines for grain zinc and iron concentration, grain yield and associated component traits. Highly significant difference among the 50 biofortified wheat genotypes was observed for all traits as revealed by ANOVA. Genotypes differed significantly for days to heading and days to maturity with the range of 96-111 d for heading and 149-164 d for maturity. The results are in conformity with the findings of Thapa et al. (2009). The correlation between days to maturity and grain yield was found to be positive but nonsignificant (r = 0.054). Similar findings have been reported by Asif et al. (2004).

The plant height showed a significant positive correlation with grain yield and showed a negative correlation with the grain zinc and iron concentration.

Table 2. Mean value of 6th HPYT entries for days to heading, days to maturity, grain Fe and Zn concentrations, number of grains spike^{-1}, 1000-grain weight, and grain yield of wheat

Genotypes	DH	DM	PH	GZnC	GFeC	ZY	IY	NGPS	TGW	GY	YR Score
WK1204	116 a	164a	70wx	32.9b-m	46.1d-l	111.9c-h	157.1d-i	44 a-f	52c-k	3.41f-j	0
6HPYT402	105r-w	152ij	57A	35.2b-l	43.2g-n	63.5l-q	83.0u-x	37 c-l	51f-p	2.00s-y	0
6HPYT403	110d-h	158e-g	64yz	41.2b-f	48.6a-h	62.6l-q	74.2w-y	32 j-m	54b-h	1.56 x-z	tr
6HPYT404	103w	152ij	78p-u	44.6bc	52.9a-f	82.9g-p	99.5q-w	34 e-m	49j-q	1.89u-y	60 mr-ms
6HPYT405	116ab	158efg	76t-v	69.9a	55.9ab	64.8l-q	52.8yz	17 n	51f-q	0.98 A	5mr
6HPYT406	107j-v	154hi	69wx	30.9d-n	54.1a-d	63.3l-q	111.1n-t	36 c-l	55b-g	2.10r-w	60 ms-s
6HPYT407	107i-u	163ab	82j-p	26.0g-n	43.3g-n	53.2o-q	89.5s-x	34 g-m	55b-g	2.09r-x	30 ms
6HPYT408	109e-o	160b-e	80m-t	37.1b-i	45.5e-m	59.5m-q	78.2v-y	36 c-l	52c-l	1.72v-y	30 mr-ms
6HPYT409	109e-o	159c-g	82i-p	28.2f-n	47.3c-k	69.9j-q	117m-r	48 ab	49i-q	2.55n-r	20 mr-ms
6HPYT410	113b-d	159c-g	96a	41.9b-e	52.6a-f	64.5l-q	79.8u-x	25 mn	57a-c	1.57w-z	tr
6HPYT411	114abc	158efg	76t-v	37.9b-i	39.7j-n	38.5 q	42.6z	25 mn	53b-j	1.02zA	10mr
6HPYT412	110d-f	160b-e	88c-f	18.9n	35.0n	51.8pq	101.7q-v	49 a	50h-q	2.82k-p	10 mr
6HPYT413	106o-v	156gh	77q-v	20.6mn	37.8l-n	75.8i-p	146.2f-l	37 c-l	46q-s	3.78c-f	40 mr-ms
6HPYT414	107l-v	149j	95ab	21.9l-n	37.6mn	63.9l-q	116.4m-r	34 f-m	47m-r	3.12h-l	80 s
6HPYT415	106p-v	151ij	83h-o	22.5k-n	47.8b-j	61.5m-q	136.7h-n	43 a-h	55b-f	2.81k-p	tr
6HPYT416	108f-s	157e-g	79o-t	35.9b-k	43.3g-n	83.1g-p	104.1p-v	33 i-m	52c-l	2.43o-u	80 s
6HPYT417	109e-n	156f-h	96ab	34.1b-m	44.1g-m	86.8f-o	117.1m-r	34 e-m	54b-h	2.58m-r	30 mr-ms
6HPYT418	106q-v	150j	69xy	29.2e-n	39.1k-n	69.3k-q	95.2r-x	30 k-m	53c-k	2.43o-u	0
6HPYT419	112c-e	156gh	81k-p	36.7b-j	43.6g-m	83.1g-p	100.2q-w	33 i-m	41 t	2.21q-v	30 mr-ms
6HPYT420	111c-f	157e-g	64yz	31.1c-n	56.1 a	56.5n-q	101.9q-v	40 a-k	52d-n	1.82v-y	tr
6HPYT421	110d-g	157e-g	62 z	40.4b-f	55.3a-c	69.8j-q	95.3r-x	40 a-k	46p-s	1.82v-y	80 s
6HPYT422	110e-l	158d-g	74u-w	26.3g-n	38.8l-n	71.9j-q	105.4p-u	43 a-g	55b-f	2.78k-p	tr
6HPYT423	105s-w	156gh	85d-l	39.9b-g	41.3h-n	145.6a-c	148.4e-k	43 a-i	51f-q	3.48e-i	0
6HPYT424	107m-v	158e-g	87c-h	37.3b-i	44.2g-m	93.4f-m	112.2n-t	41 a-j	46p-s	2.53n-s	tr
6HPYT425	107k-v	156f-h	86d-j	24.9i-n	38.9l-n	90.3f-n	142.1g-m	44 a-e	55b-g	3.59d-h	30 ms
6HPYT426	105u-w	158e-g	81l-q	29.5e-n	45.4e-m	72.8i-q	110o-t	42 a-j	42st	2.38p-u	0
6HPYT427	110e-j	157e-g	85d-l	32.6b-n	41.5h-n	94.9f-m	122.5k-q	33 h-m	56a-d	2.95i-o	0
6HPYT428	109e-p	158e-g	91bc	25.8h-n	39.9i-n	107.3d-i	168.6c-g	41 a-j	50f-q	4.17bc	0
6HPYT429	110d-g	158d-g	81l-r	25.7h-n	39.6j-n	77.6h-p	120.9l-r	34 e-m	51f-q	3.14h-l	0
6HPYT430	110d-g	159c-g	79n-t	37.6b-i	45.7e-m	74.3i-q	88.9t-x	30 k-m	50g-q	1.97t-y	tr
6HPYT431	107n-v	158e-g	84f-n	41.6b-f	42.3h-n	116.2b-g	119.7l-r	44 a-f	56a-e	2.84k-p	tr
6HPYT432	107m-v	160c-f	86d-j	46.6 b	43.8g-m	145.0a-c	136.7h-n	45 a-d	48k-r	3.05h-n	0
6HPYT433	109f-q	159c-g	86d-h	22.9j-n	38.7l-n	87.1f-o	151.1e-j	47 ab	60a	3.86c-f	5r-mr
6HPYT434	110d-i	162a-d	84f-o	29.3e-n	41.7h-n	105.9d-j	152.7e-j	43 a-i	47n-r	3.76c-g	0
6HPYT435	108f-r	160c-f	87c-g	39.2b-h	45.6e-m	97.6e-l	113.0n-t	41 a-j	49j-q	2.51n-t	0
6HPYT436	110e-l	162a-c	80m-s	36.7b-i	44.9f-m	101.1e-k	122.6k-q	41 a-j	50h-q	2.67k-q	20mr-ms
6HPYT437	108f-s	159d-g	76r-v	31.3c-n	39.9i-n	158.2a	206.5ab	44 a-f	52e-o	5.10 a	20ms
6HPYT438	107j-v	159d-g	88c-f	35.7b-l	45.5e-m	138.6a-d	179.8cd	45 a-c	54b-i	4.02b-e	tr
6HPYT439	107j-u	158d-g	76s-v	39.3b-h	41.2h-n	120.9b-f	129.4j-p	43 a-g	48l-r	3.12h-m	tr
6HPYT440	107j-v	160b-e	73v-x	43.9b-d	53.4a-e	108.0d-i	132.3i-o	38 b-l	47o-r	2.50o-t	40 mr-ms
6HPYT441	109e-o	159c-g	86d-i	32.4c-n	39.6j-n	84.5g-p	100.2q-w	48 a	44r-t	2.62l-r	5r-mr
6HPYT442	105t-w	159c-g	85e-m	32.2c-n	47.6b-j	93.9f-m	136.6h-o	32 j-m	54b-h	2.90j-p	tr
6HPYT443	110e-k	159c-g	85d-l	32.6c-n	47.8a-i	158.7a	216.7 a	40 a-k	58ab	4.47b	tr
6HPYT444	107g-u	157f-h	83g-o	36.7b-j	46.0d-l	157.2 a	187.6bc	35 d-m	60a	4.02b-d	10mr
6HPYT445	108f-t	159d-g	86d-k	31.8c-n	51.1a-g	100.9e-k	159.6d-h	38 b-l	51e-o	3.21g-k	60 ms-s
6HPYT446	109fg-q	160c-f	87c-h	27.8f-n	40.6h-n	104.6d-j	150.1e-j	45 a-d	53c-j	3.86c-f	60 ms-s
6HPYT447	104vw	152 ij	85d-l	32.7b-n	43.3g-n	131.7a-e	171.6c-f	29 lm	55b-g	4.00b-e	60 ms-s
6HPYT448	110e-m	160c-f	89c-e	37.6b-i	43.7g-m	151.2ab	173.6c-e	34 e-m	52c-m	3.99b-e	0
6HPYT449	107h-u	161b-e	89cd	37.0b-i	42.7h-n	102.4e-k	115.9m-s	43 a-g	47o-r	2.73k-q	0
6HPYT450	108f-s	160c-f	83h-o	41.4b-f	48.2a-i	63.1l-q	71.5xy	28 lm	55b-g	1.51yzA	0
Grand mean	108	158	81	34.1	44.65	91.8	122.9	38	51.32	2.81	
CV (%)	1.26	0.99	2.95	19.2	8.73	18.6	10.8	12.8	4.76	9.34	
F-value	7.86	7.57	24.78	3.81	3.45	6.79	15.4	3.83	5.97	24.55	
F-test	**	**	**	**	**	**	**	**	**	**	
SEM±	0.97	1.11	1.69	4.64	2.757	12.09	9.41	3.45	1.73	0.18	

DH: days to heading, DM: days to maturity, PH: plant height, GZC: Grain Zinc Concentration, GIC: Grain Iron Concentration, ZY: Zinc yield, IY: Iron yield, TGW: Thousand grain Weight, NGPS: Number of grains spike^{-1}, GY: Grain Yield and YR Score: Stripe rust/yellow rust score. Means within the column with the same letters are not significantly different. (**) indicates highly significantly different at 1% level of significance

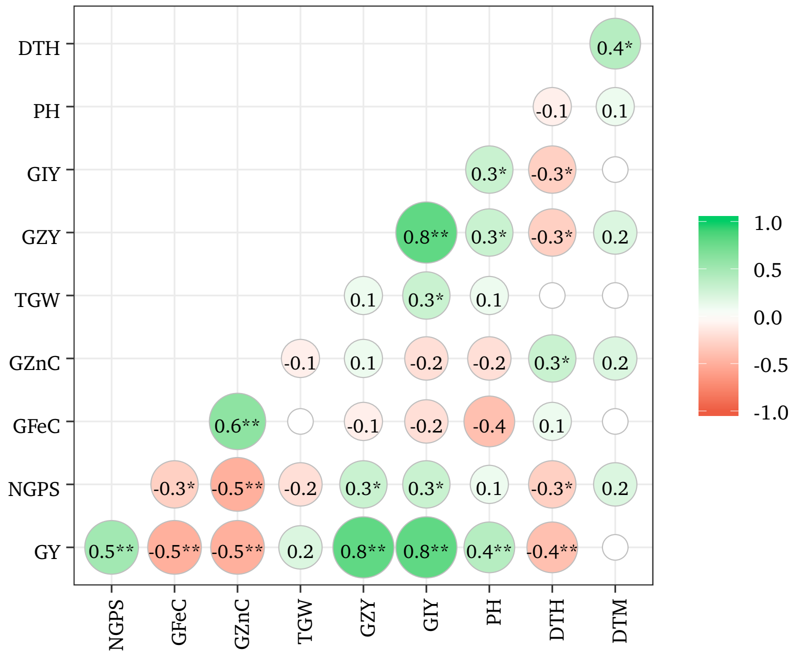

Figure 4. Pearson's Correlation coefficient among different traits for 6th HPYT biofortified wheat genotypes

Table 3. Estimation of heritability of different characters in 6th HPYT biofortified wheat genotypes

Characters	Variance			Heritability (%)
	Genotypic	Environmental	Phenotypic	
Days to heading	6.47	1.88	8.35	77.45
Days to maturity	8.03	2.45	10.48	76.67
Grain zinc concentration (ppm)	60.68	43.15	103.83	58.44
Grain iron concentration (ppm)	18.65	15.21	33.86	55.08
Plant height (cm)	68.39	5.75	74.14	92.24
Number of grains spike^{-1}	33.76	23.83	57.59	58.62
Thousand kernel weight (g)	14.87	5.98	20.85	71.32
Grain yield (t ha^{-1})	0.81	0.07	0.88	92.17

The results were consistent with the findings of Srinivasa et al. (2014). This means that dwarf plants take more zinc and iron than tall plants. The correlation between the number of grains/ spike and thousand grains weight was negative (r = −0.161). This finding is supported by previous findings obtained by Sharma (1993), Thapa et al. (2009) and Ojha et al. (1982) who found a negative relationship between number of grains spike^{-1} and thousand grains, and suggested that the thousand grains weight could not be increased only by increasing the number of grains spike^{-1}.

Thousand-grain weight showed no correlation with zinc and iron concentration which comply with the findings by Velu et al. (2012). In contrast, McDonald et al. (2008) and Pfeiffer and McClafferty (2007) reported zinc and iron-enriched genotypes had higher 1000 kernel weights. The correlation between thousand grains weight and grain yield was positive and non-significant (r = 0.161). This finding was found similar to the previous findings obtained by Ojha et al. (1982). Grain yield ranged between 0.98 and 5.10 t ha^{-1} with a mean value of 2.81 t ha^{-1} which is higher than national average of Nepal whereas lower than the yield reported by Velu et al. (2012) and Mishra et al. (2015). The higher grain yield of biofortified genotypes as compared to famous variety 'WK1204' indicates that there is a possibility to enhance Zn and Fe content of wheat without compromising for grain yield. The work of Velu et al. (2012) and McDonald et al. (2008) also indicated such a possibility.

In this study, the concentration of zinc in grain varied from 18.85 to 69.98 ppm. The range of 29-39.5 ppm as described by Velu et al. (2012) and 17-61 ppm

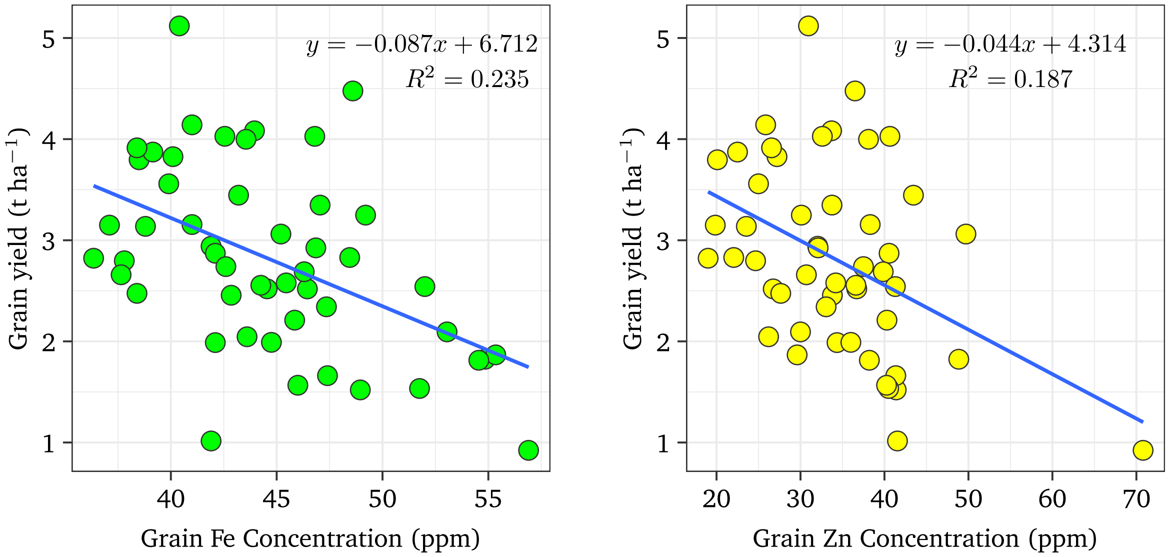

Figure 5. Association between grain Fe and Zn concentration with grain yield of biofortified wheat genotypes

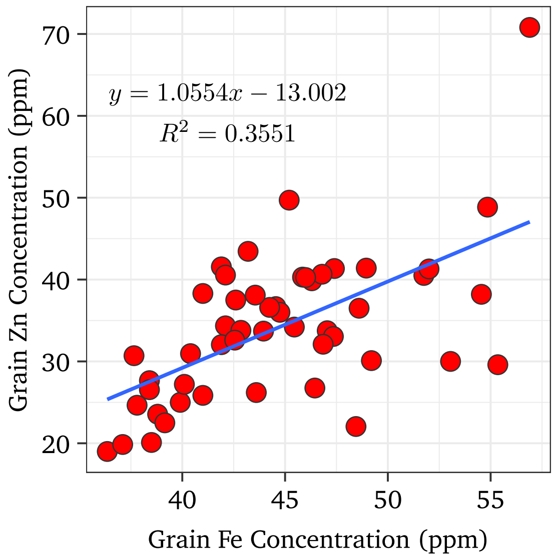

Figure 6. Association between grain Zn concentration and grain Fe concentration of biofortified wheat genotypes

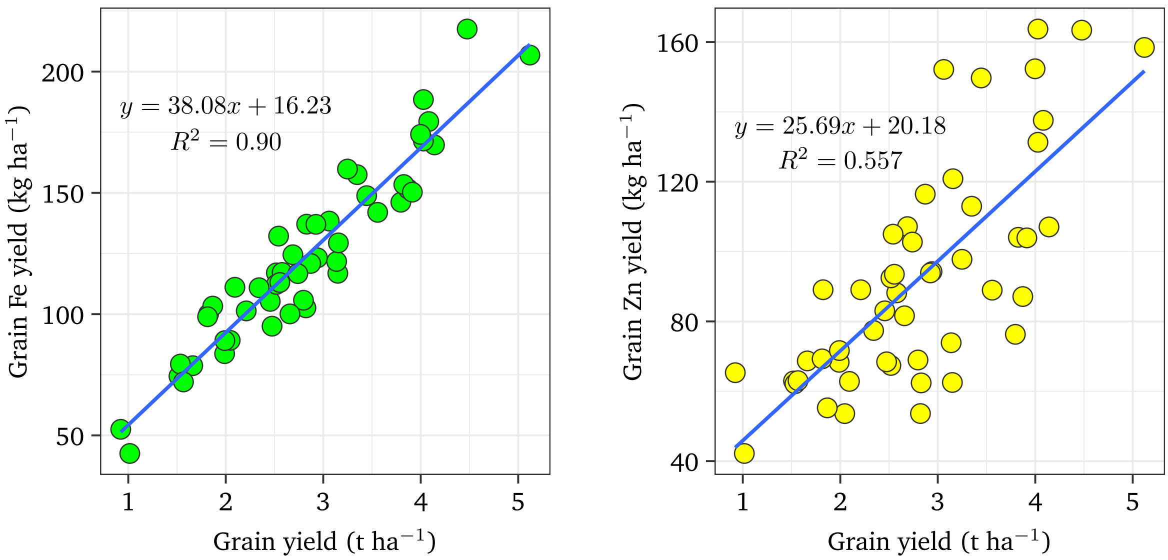

Figure 7. Association between grain Fe and Zn concentration with grain yield of biofortified wheat genotypes

was reported by Velu et al. (2011). There is a high genetic variation for Zn in the primitive and wild relatives of cultivated wheat as documented by Velu et al. (2012). Correlation between grain yield and Zn concentration was negative (r = −0.433**), which was also reported by Gomez-Becerra et al. (2010) and Velu et al. (2012). Grain Fe concentration in this study ranged from 35.05 to 56.06 ppm. Similar results were reported in previous studies by Velu et al. (2011) and Velu et al. (2012). There was significant negative correlation between grain iron concentration with grain yield (−0.486**) and number of spikes m^{-2} (−0.324*), which is in accordance with the results by various authors (Gomez-Becerra et al., 2010; Velu et al., 2012). The positive significant association between zinc and iron concentration (r = 0.355**) suggest that it is feasible to simultaneously improve both. This is in line with the result reported by Velu et al. (2012).

For days to heading, days to maturity, plant height, thousand kernel weight and grain yield, high heritability values were observed. High heritability values in these characteristics have shown that the observed variability is dependent mainly under genetic control and less on the environment and the possibility of progress in selection. The result is in accordance with the results of Ali et al. (2008). A moderate heritability was observed for grain zinc and iron concentration and number of grains $spike^{-1}$. Genotype × environmental interactions are not an important problem, depending on the indirectly high heritability of zinc and iron content in the grains as reported by Velu et al. (2012). The correlation coefficient between grain zinc yield and grain yield was positive and significant (r = 0.75).The association between grain zinc yield and the grain yield showed a positive linear trend, indicating that there was no yield penalty when entries has high Zn; at the same time no concentration effect when entries had lower grain yield. In case of Fe, the positive significant correlation coefficient was observed between grain yield and grain iron yield (r = 0.95), indicating that some of the highest yielding entries also had a high Fe concentration. It is compatible with the result described by Velu et al. (2012) and McDonald et al. (2008).

5 Conclusions

Genotypic variation was found among all the tested genotypes for different agronomic traits. Grain Zn and Fe yield were highly positively correlated with grain yield. The genotypes 6HPYT404, 6HPYT405, 6HPYT410, 6HPYT421, 6HPYT431, 6HPYT432, 6HPYT440 and 6HPYT450 were found to have the highest grain zinc and iron concentration. So, these genotypes can be used as parents in breeding programs to develop zinc and iron-enriched wheat varieties. The genotypes namely 6HPYT428, 6HPYT437, 6HPYT438, 6HPYT443, 6HPYT444, 6HPYT447 and 6HPYT448 were found high yielding genotypes which could be selected for varietal development program.

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Conflict of Interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

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