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BIOAVAILABLE GRAIN IRON DECLINES FROM OLD TO CURRENT PAKISTANI BREAD WHEAT CULTIVARS

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ABSTRACT

Green revolution is a revolutionary landmark in history of mankind where high yielding wheat varieties were developed to tackle massive famines. However, the genetic base has become narrow in cultivated varieties with improved yields and less bioavailability of micronutrients. An experiment was conducted to check the change in yield production and micronutrients during previous breeding efforts. Land races (44), approved varieties (68) and advanced lines (32) were grown in field in triple test lattice design in experimental area of University of Agriculture Faisalabad during 2016. Statistical analyses revealed a significant difference in yield contributing traits and grain iron. Land races had broader range of all the traits compared to other two groups. Grain iron contents showed higher values in landraces, while grain yield were higher in varieties and advance lines. Correlation and path coefficient analysis revealed change in the relationship of traits and effects of yield contributors in all three groups. Some genotypes were selected and crossed. These selected genotypes and F_1 were grown in the same field following year in randomized complete block design. The results showed that breeding program could rescue declining iron contents in modern wheat cultivars. In conclusion, domestication and green revolution has changed genetic makeup of wheat crop and narrowed its genetic base. However, grain quality and yield can be improved simultaneously by breeding strategies.

Key words: Green revolution, landraces, narrow genetic base, domestication, bioavailable iron, triple test lattice.

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INTRODUCTION

Wheat (*Triticum* spp.) accounts for 30% of global grain production and 45% of cereal nutrition (Charmet, 2011). It is being cultivated round the globe and covers one-sixth part of total arable land in world.

The genetic makeup of wheat crop has started to change after domestication. The domestication of wheat was started around 10,000 years ago (Gupta, 2004). *T. monococum* was the first domesticated wheat species. The domestication of wild emmer (*Triticum dicoccoides*) was the fundamentals for the evolution of tetraploid durum and hexaploid bread wheat (Feldman and Kislev, 2007). Einkorn and emmer wheat were cultivated prior to bread wheat. The genetic relationships in these wheat species indicated the south-eastern part of Turkey as place of first cultivation (Kimber and Feldman, 1987). The origin of wheat has always been a point of controversy among scientists due to high genetic diversity in Mediterranean and different parts of Asia. A Russian botanist Vavilov (1940) studied wild wheat in Mediterranean region and Southwest Asia concluded as its ancestral homeland of wheat. While an archaeologist from the University of Chicago, Robert Braidwood claimed the Fertile Crescent, including central Asia and northern Africa through the Mediterranean to be the origin of wheat (Kiple and Ornelas, 2000). However, in Central Asia and North Africa, wheat was introduced through Mediterranean (McGovern *et al.*, 2003).

The domestication can modify plant traits in two ways; i) by conscious and unconscious selection from wild types and later conscious selection of plants for specific location and use (Harlan, 1992), ii) intentional selection of plants which have some traits suitable for domestication (Abbo and Gopher, 2017). Upon domestication, it was estimated that initial diversity was reduced by 84% in durum wheat and by 69% in bread wheat (Jaradat, 2006).

Green revolution is a next big event in wheat history, which modified the genetic makeup of crop considerably. Norman Borlaug a pathologist, while working in agricultural modernization project in Mexico set the stone of green revolution. Borlaug developed Norin 10, semi dwarf wheat strain, by crossing adapted varieties to dwarf wheat strain of Japan. Norin 10 was fertilizer responsive, short stature variety, which resisted the lodging problem and thus become first high yielding wheat variety of green revolution (Hesser, 2006). The increase in the production due to development of high yielding varieties in green revolution has helped in avoiding widespread famines (Shiva, 1993).

However, it is a widespread notion that green revolution after domestication has also resulted in narrowing the genetic base of cultivated crops and changing the plant traits on average (Smale, 1997). Wheat is rich in nutrition but with the increased selection pressure, the genetic base has gone narrow for quality and yield related traits (Bouis and Saltzman, 2017). To test this hypothesis, a study was planned to investigate the change in yield and grain iron contents and their relationship in land races, approved varieties and advance lines. This study was aimed to identify suitable group of genotypes for improving iron contents, bioavailable iron, grain yield and decreasing the phytase content in grains of new varieties during breeding program.

MATERIALS AND METHODS

Experimental Materials: The experimental material was collected from Cereal Research Programme, Department of Plant Breeding and Genetics, University of Agriculture Faisalabad, Pakistan and Wheat Research Institute, Faisalabad, Pakistan. The plant material consists of 144 wheat accessions including 44 land races , 68 approved varieties and 32 advanced lines (Table 1S). Some genotypes and their crosses were also grown in next year (Table 2S).

Experimental design: The study was conducted at the experimental area of Department of Plant Breeding and Genetics, University of Agriculture Faisalabad (31^0 - 44' N, 73^0 - 06' E), Pakistan. The experimental design was a twelve-by-twelve triple test lattice (Cox *et al.*, 1940) with three replications. The genotypes were sown on 26 November 2016 following Goulden (1937) and Yates (1936). Each experimental unit consisted of two of 3 feet length 30 cm spaced rows with 10 cm space between plants. Some genotypes were selected on the basis of yield potential and grain iron bioavailability and crossed to get F_1 seeds.

Parents along with their crosses were sown on 20 November 2017 in Randomized Complete Block Design in triplicate. One row of 3 feet length represented one genotype at 30 cm distance and 10 cm space between plants. Seeds were planted with dibbler keeping two seeds per hole. Standard agronomic practices were followed during crop season in field.

Data recording: Data was collected for flag leaf area (cm^2), plant height (cm), spike length (cm), spikelets per spike, number of productive tillers, spike weight (g), straw yield (g), grain yield per plant (g), harvest index, 1000-grain weight (g), grain iron contents ($\mu g\ g^{-1}$), grain phytate contents ($mg\ g^{-1}$) and bioavailable iron contents.

Grain Micronutrient Analysis: Grain micronutrient analysis was carried out in Allelopathy and Hitech Lab of University of Agriculture Faisalabad. Grain Iron (Fe) contents were estimated by using atomic absorption spectrophotometer (AAS). Oven dried samples were finely ground to powder form and sieved to get even finer powder. Wet digestion (Jones and Case, 1990) was carried out of 0.2 g sample with 5 ml di-acid digestion mixture (HNO_3: $HClO_4$ in 2:1 ratio) in labeled flask and digested on hot plate (250 ^0C) till solution become clear. After digestion the material was cooled and diluted to 50 ml by adding de-ionized water. The digest was then filtered and stored in airtight plastic bottles for determination of iron contents ($\mu g\ g^{-1}$) using atomic absorption spectrophotometer (Hitachi Polarized Zeeman AAS, Z-8200, Japan). The conditions described in AOAC (1990) were followed for using the instrument.

Grain phytate contents (PA) in wheat grains were determined indirectly using Haug and Lantzsch (1983) method. Finely grounded 0.02 g sample of wheat grains was extracted in 10 ml of 0.2 N HCl solutions. The extract (0.5 ml) was pipetted into screw cap test tube. One ml ferric solution (0.2 g $Fe(NH_4)_2(SO_4)_2.6H_2O$+ 100 ml 2N HCl + deionized water = 1000 ml with) was added and mixture was heated in water bath for 30 minutes and cooled in ice water for 15 minutes and allowed to adjust at room temperature. Then 2 ml of 2,2-bipyridine solution (10 g of 2,2-Bipyridine + 10 ml of thioglycolic acid + deionized water = 1000 ml) was added to mixture and the contents were mixed. The absorbance was read in spectrophotometer at 519 nm against deionized water after 30-60 seconds because bipyridine reacts with iron phytate and color changes with time. Reference solutions were prepared for phytate determination using Sodium phytate as described by Haug and Lantzsch (1983). The phytate content was calculated by the following formula

$$\text{Phytate (mg g}^{-1}\text{)} = \frac{\text{Micro gram from graph} \times \text{dilution factor}}{\text{Wt of sample} \times \text{ml sample taken} \times 1000000} \times 0.354 \times 100$$

Bioavailable iron was determined by phytate to iron molar ratio. If PA/Fe molar ratio is 1 or more, the bioavailability of iron gets poorer (Hurrell and Ines, 2010).

$$\text{Bioavailable iron (PA/Fe)} = \frac{\text{moles of Phytate}}{\text{moles of Iron}}$$

Statistical analysis: The collected data were subjected analysis of variance proposed by Goulden (1937) and Yates (1936) in 2016 and in next year as recommended by Steel *et al.* (1997). The landraces, varieties and advance lines were compared for averages and range of each trait. Pearson's rank correlation coefficients (ρ) were computed for traits according to Kwon and Torrie, (1964) for each group of genotypes using STATISTIX 8.1.

$$r = Cov.of\ a.b/\sqrt{(var.a)}(var.b)$$

The significance of the correlation was estimated using student t test.

$$t = r\sqrt{(n-2)}/(1-r2)$$

Path coefficient analysis was performed according to method given by Dewey and Lu (1959) using SPSS v. 23.0. Correlation and path coefficient analysis was repeated in next year.

RESULTS

Genetic Variability and Comparison of genotypes: The analysis of variance indicated significant differences for all the traits under consideration except for grain phytate contents in 2016 (Table 1). Differences among replications and blocks in replication were non-significant. All the traits showed high heritability. Heritability estimates of spike weight, grain yield, straw yield, harvest index and 1000-grain weight showed an increasing trend in F_1 and selected genotypes (Table 3S). Flag leaf area, plant height, productive tillers, spike length, grain iron contents, grain phytate contents and PA/Fe showed decreasing trend in heritability estimates of selected genotypes and crosses. Most of the yield contributing traits showed medium to high genetic advance in germplasm and medium to low genetic advance in selected genotypes and crosses. However, some traits showed increased genetic advance in selected parents and crosses (Table 3S).

All the groups of genotypes differed in average and range of traits under consideration. Flag leaf area, spike length, spike weight and harvest index showed increasing trend in advanced lines and varieties compared to landraces on average. Productive tillers, plant height, spikelets per spike, straw yield and grain iron contents showed decreasing trend in lines and varieties on average (Table 2). However, grain yield, phytate contents and PA/Fe have showed improved values in varieties as compared to advance lines and landraces. All the traits showed an increase in average values in selected genotypes and crosses during second year accept for straw yield, 1000-grain weight and PA/Fe.

Correlation analysis: The correlation between yield contributing and other traits showed differences in land races, varieties and advanced lines (Table 3). These differences may have arisen due to directional and targeted selection by the breeders. Grain yield showed positive correlation with straw yield, harvest index, 1000-grain weight and negative correlation with flag leaf area in land races. In varieties, grain yield showed positive correlation with plant height, productive tillers, spike length, spike weight, spikelets per spike, 1000-grain weight, harvest index, straw yield and phytate contents. Productive tillers, 1000-grain weight, harvest showed positive correlation with grain yield in advance lines. In the parents and F_1 grain yield showed positive significant correlation with flag leaf area, plant height, spike weight, harvest index and straw yield. While, 1000-grain weight showed positive correlation with grain yield in land races, varieties and advance lines. Selected genotypes and crosses showed positive correlation of 1000-grain weight with plant height, productive tillers and grain iron contents.

Path coefficient analysis: Direct and indirect effect of characters were computed using path coefficient analysis. It revealed high positive direct effect of straw yield, 1000-grain weight and harvest index on grain yield per plant in land races (Figure 1). In varieties 1000-grain weight, productive tillers, harvest index and straw yield had moderate direct effect on grain yield (Figure 2). While in selected genotypes and their F_1 grown next year grain yield had low direct effect of 1000-grain weight, productive tillers, and high direct effect of harvest index and straw yield (Figure 3). Grain iron contents had low direct effect on grain yield in all the groups of genotypes. Indirect effect of spike weight and phytate contents through 1000-grain weight was high on grain yield in land races. Spikelets per spike and productive tillers had low indirect effect on grain yield through 1000-grain weight in land races. In varieties, indirect effects of spike weight and productive tillers were low and that of spikelets per spike and phytate contents were moderate through 1000-grain weight. Similarly, spike weight had low effect and spikelets per spike and productive tillers had moderate indirect effect on grain yield through 1000-grain weight in selected genotypes and crosses. Phytate contents showed negative and moderate indirect effect on grain yield through 1000-grain weight.

Table 1. Mean squares of some yield contributing and grain iron related traits.

Traits	Year 1(Triple test lattice design)			Year 2 (RCBD)	
	Replication	Block in Replication	Treatment	Replication	Treatment
Flag leaf area (cm^2)	120.22	62.65	79.41^{*}	24.70	150.82^{**}
Plant height (cm)	3.70	435.90	516.70^{**}	30.19	180.89^{**}
Productive tillers per plant	6.25	5.61	6.55^{*}	45.14	18.84^{**}
Spike length (cm)	0.06	2.40	4.44^{**}	7.28	40.80^{**}
Spikelets per spike	4.06	2.77	6.00^{**}	34.07	43.84^{**}
Spike weight (g)	0.22	0.47	0.66^{**}	2.54	1.68^{**}
Grain yield per plant (g)	14.99	19.10	25.56^{*}	14.30	78.23^{**}
Straw yield per plant (g)	903.60	525.60	840.30^{**}	58.25	467.88^{**}
Harvest index	125.80	75.14	91.79^{**}	57.00	644.49^{**}
1000-grain weight (g)	4.96	43.56	127.30^{**}	41.23	69.48^{**}
Grain iron ($\mu g\ g^{-1}$)	0.57	671.40	1274^{**}	12.70	407.90^{**}
Grain phytate ($mg\ g^{-1}$)	4.63	4.67	4.70^{ns}	0.50	5.7^{**}
PA/Fe	0.91	5.88	14.35^{**}	1.17	13.0^{**}

Note: Degree of Freedom (D.F) for replication was 2, block in replication was 33 and that for treatment was 143; ** stands for highly significant at ∞ P = 0.01% and * stands for significant and ns = nonsignificant at ∞ P = 0.05%; Genetic advance (GA) at 10% selection intensity and 5% selection intensity.

Table 2. Highest, Lowest and average values of yield contributing and grain iron related traits in advanced lines, varieties and landraces of wheat.

	Year 1									Year 2		
	Local land races			Varieties			Lines			Parents + Crosses		
	Highest	Lowest	Average	Highest	Lowest	Average	Highest	Lowest	Average	Highest	Lowest	Average
FLA	48.69	14.63	21.74	34.46	15.9	23.87	44.49	15.7	24.55	45.1	6.5	31.63
PH	133.68	96.04	117.71	110.25	57	94.49	107.15	80.29	92.13	118	33.67	102.37
PT	13.25	6.67	9.88	12.53	5.8	8.63	13	6	8.31	19	4	10.57
SL	13.24	5.74	10.38	13.66	9.96	12.03	13.24	11.16	12.24	17	5.2	13.06
SPS	23.3	12.67	20.17	32.5	14.67	20.76	73.67	13.48	21.42	39	15.7	25.2
SW	3.77	1.8	2.86	10.02	2.25	3.42	4.06	2.05	3.49	5.2	1.8	3.68
SY	126.13	66.27	90.62	123.3	50.83	88.05	103.87	54.13	76.8	70.8	34.7	68.71
GY	21.47	8.4	9.79	23.6	11.53	22.37	27.83	11.73	20.11	64	12.5	40.03
HI	28.26	10.96	17.78	33.95	12.06	20.38	35.37	14.54	22.71	96	18.4	49.34
TGW	55.67	27.67	43.86	59.61	35.88	46.8	59.61	36	46.31	48.66	27.01	39.33
Fe	77.75	6.2	28.02	86.7	3.1	21.32	84.4	4.85	20.42	152.5	43.37	65.86
PA	0.62	0.32	0.42	1.01	0.33	0.43	0.51	0.38	0.42	2.91	0.32	1.06
PA/Fe	5.98	0.46	2.00	15.7	0.34	3.28	7.90	0.42	3.26	4.70	0.52	1.42

Where FLA = flag leaf area, PH = plant height, PT = productive tillers, SL = spike length, SPS = spikelets per spike,SW = spike weight, SY = straw yield, GY = grain yield, HI = harvest index, TGW = 1000-grain weight, Fe = grain iron contents, PA = grain phytate contents and PA/Fe = bioavailable iron contents.

Table 1S. Genotypes used in experiment.

Varieties		Advance Lines	Landraces	
Iqal-2000	FSD-2008	10113	T3(T. durum)	C518(T. astivum)
PBW-222	Bathoor-2008	9886	T4(T. sphaerococcum)	C271(T. astivum)
Moomal-2002	SH-2002	9970	T5(T. sphaerococcum)	C247(T. astivum)
Marvi-2002	Aas-11	9889	T2(T. durum)	C273(T. astivum)
Zincol-15	Dawar-97	10119	T1(T. durum)	C591(T. astivum)
Bahawalpur-2000	Hashm-2008	10116	T18(T. astivum)	T25(T. astivum)
Gomal-2008	MH-97	9880	T19(T. astivum)	T11(T. astivum)
FD-85	Watan	10129	T24(T. astivum)	T12(T. astivum)
Miraj-2008	Chakwal-86	10132	T23(T. astivum)	C250(T. astivum)
Pirsabak-2005	AS-2002	10114	T22(T. astivum)	Bouni Wheat
Shafaq-2006	LU-26S	10130	T21(T. astivum)	C258(T. astivum)
GA-2002	NifaBarat-2009	10060	T20(T. astivum)	Local wheat Queta
Pirsabab-2008	Lasani-2008	10065	T17(T. astivum)	C228 (T. astivum)
Saleem-2000	Ufaq-2002	10111	T16(T. astivum)	C256 (T. astivum)
Shaheen-94	Sindh-81	9885	T15(T. astivum)	Local Wheat Faisalabad
Khyber-89	MaxiPAK65	10117	T14(T. astivum)	9D (Selection)
Bakhtawar-94	PB-2011	10137	T13(T. astivum)	8A (Selection)
FD-83	SKD-1	10128	T10(T. astivum)	C217(T. astivum)
Kohinoor-83	Sarsabz	9930	T9(T. astivum)	C245(T. astivum)
Pakistan-13	Wafaq-2001	9874	T8(T. astivum)	Local Tall Chakwal
Pak-81	AARI-11	10115	T7(T. sphaerococcum)	C248 (T. astivum)
Auqab-2000	Pasban-90	9884	T6(T. sphaerococcum)	C288 (T. astivum)
Millit-2011	Anmol-91	9878		
Kaghan-93	Kohsar-95	10136		
Nowsher-96	Glaxy-13	10125		
Inq-91	Abadagad-93	9940		
Rohtas-90	Bakhatwar-93	10118		
Shaheen-94	NARC-2008	10067		
Pirsabab-2004	FakharShrad	9875		
Chakwal-97	Shahkar-2013	9882		
Sariab-92	Parwaz-94	10112		
Sulman-96	Bars-2009	9877		
Mehran-89	Fareed-2006			
Bakhar-2002	Margalla-99			

Table 2S .Selected genotypes and crosses.

Genotypes	Crosses	Crosses	Crosses
Sarsabz	Sarsabz × Zincol-16	Mexipak-65 × Zincol-16	SKD-1 × Zincol-16
9884	Sarsabz × AAS-11	Mexipak-65 × AAS-11	SKD-1 × AAS-11
Iqbal-2000	Sarsabz × PB-2011	Mexipak-65 × PB-2011	SKD-1 × PB-2011
Mexipak-65	SArsabz × FSD-2008	Mexipak-65 × FSD-2008	SKD-1 × FSD-2008
9886	9884 × Zincol-16	9886 × Zincol-16	Anmol-91 × Zincol-16
NARC-2009	9884 × AAS-11	9886 × AAS-11	Anmol-91 × AAS-11
SKD-1	9884 × PB-2011	9886 × PB-2011	Anmol-91 × PB-2011
Anmol-91	9884 × FSD-2008	9886 × FSD-2008	Anmol-91 × FSD-2008
Zincol-16	Iqbal-2000 × Zincol-16	NARC-2009 × Zincol-16	
AAS-11	Iqbal-2000 × AAS-11	NARC-2009 × AAS-11	
PB-2011	Iqbal-2000 × PB-2011	NARC-2009 × PB-2011	
FSD-2008	Iqbal-2000 × FSD-2008	NARC-2009 × FSD-2008	

Table Error! Use the Home tab to apply 0 to the text that you want to appear here.**3S. Heritability and genetic advance for some yield contributing and grain iron related traits at 10% and 5% selection intensity.**

Traits	**Year 1**			**Year 2**		
	H^2	**GA (10%)**	**GA (5%)**	**H^2**	**GA (10%)**	**GA (5%)**
Flag leaf area (cm^2)	89.65	18.36	21.49	57.07	6.66	7.80
Plant height (cm)	99.48	23.82	27.88	89.98	12.10	14.16
Productive tillers per plant	99.48	34.75	40.67	94.40	4.13	4.83
Spike length (cm)	98.79	40.28	47.14	83.45	5.27	6.17
Spikelets per spike	97.46	2.23	2.61	95.95	6.41	7.51
Spike weight (g)	92.19	13.38	15.66	98.13	1.29	1.51
Grain yield per plant (g)	99.47	16.56	19.39	99.79	8.97	10.49
Straw yield per plant (g)	97.46	74.81	87.56	98.26	21.53	25.20
Harvest index	93.14	2.96	3.46	96.93	24.88	29.12
1000-grain weight (g)	90.33	19.30	22.59	99.60	8.43	9.87
Grain iron (µg g^{-1})	98.45	7.70	9.02	67.33	13.12	15.36
Grain phytate (mg g^{-1})	98.28	9.61	11.25	94.23	2.26	2.65
PA/Fe	99.47	2.54	2.98	93.97	3.41	3.99

Where H^2 is broad sense heritability, GA(10%) is genetic advance at 10% selection intensity and GA (5%) is genetic advance at 5% selection intensity.

Table 3. Correlation analysis of yield contributing and grain iron related traits in advanced lines, varieties and landraces of wheat (n=15).

Land Races	**FLA**	**PH**	**PT**	**SL**	**SW**	**SPS**	**TGW**	**HI**	**SY**	**Fe**	**PA**	**PA/Fe**
1000-grain weight (g)	0.02	0.05	0.03	-0.15	0.13	0.12						
Grain iron (µg g^{-1})	-0.06	0	-0.06	0.29**	0.03	0.1	-0.12	-0.05	-0.07			
Grain phytate (mg g^{-1})	0.04	0.09	-0.03	0.06	-0.15	-0.03	0.08	0.16	-0.12	0		
PA/Fe	0.06	0.02	-0.03	-0.09	-0.1	-0.11	0.08	0.09	0.01	-0.76**	0.25*	
Grain yield (g)	-0.18*	0.02	-0.02	0.04	-0.01	0.07	0.17*	0.40**	0.26**	-0.06	0.02	0.03
Varieties	**FLA**	**PH**	**PT**	**SL**	**SW**	**SPS**	**TGW**	**HI**	**SY**	**Fe**	**PA**	**PA/Fe**
1000-grain weight (g)	0.12	0.05	-0.02	0.15	0.1	0.08	`					
Grain iron (µg g^{-1})	0.1	0.07	0.09	0.12	0.12	0.19**	0.08	-0.09	0.17**			
Grain phytate (mg g^{-1})	0	-0.01	-0.08	0.11	0.12	-0.03	0.21**	0.17**	-0.04	-0.06		
PA/Fe	-0.03	-0.01	-0.08	-0.13	-0.09	-0.08	0.01	0.09	-0.11	-0.72**	0.22**	
Grain yield (g)	0.07	0.32**	0.39**	0.18**	0.32**	0.38**	0.21**	0.33**	0.36**	0.13	0.17**	-0.02
Advanced Lines	**FLA**	**PH**	**PT**	**SL**	**SW**	**SPS**	**TGW**	**HI**	**SY**	**Fe**	**PA**	**PA/Fe**
1000-grain weight (g)	0.05	-0.11	-0.04	0.08	0.14	0.03						
Grain iron (µg g^{-1})	0.04	0	-0.23	-0.08	0.03	-0.02	-0.08	-0.11	-0.09			
Grain phytate (mg g^{-1})	-0.05	-0.05	-0.1	-0.12	-0.06	0.08	0.27**	-0.12	0	0		
PA/Fe	0.03	0.09	0.15	0.09	0.02	0.09	0.22*	0.07	0.21*	-0.71	0.20*	
Grain yield (g)	-0.08	0.18	0.21*	0.15	-0.01	0.03	0.54**	0.44**	0.15	-0.15	0.08	0.32**
Crosses + Parents	**FLA**	**PH**	**PT**	**SL**	**SW**	**SPS**	**TGW**	**HI**	**SY**	**Fe**	**PA**	**PA/Fe**
1000-grain weight (g)	0.04	0.19*	0.37**	-0.05	-0.04	-0.05						
Grain iron (µg g^{-1})	0.1	0.08	0.21*	-0.19*	-0.22*	0.13	0.28**	0.20*	-0.11			
Grain phytate (mg g^{-1})	0.09	0.02	0.17*	0	-0.1	-0.08	0.28**	0.04	0.01	0.33**		
PA/Fe	0.05	-0.03	0.05	0.12	0.03	-0.19*	0.14	-0.06	0.07	-0.24**	0.82**	
Grain yield (g)	0.17*	0.31**	0.52	0.16	0.50**	-0.02	0.13	0.39**	0.49**	0.12	0.04	-0.02

Where FLA = flag leaf area, PH = plant height, PT = productive tillers, SL = spike length, SPS = spikelets per spike, SW = spike weight, SY = straw yield, GY = grain yield, HI = harvest index, TGW = 1000-grain weight, Fe = grain iron contents, PA = grain phytate contents and PA/Fe = bioavailable iron contents and “*” is for significant correlation and “**” is for highly significant correlation.

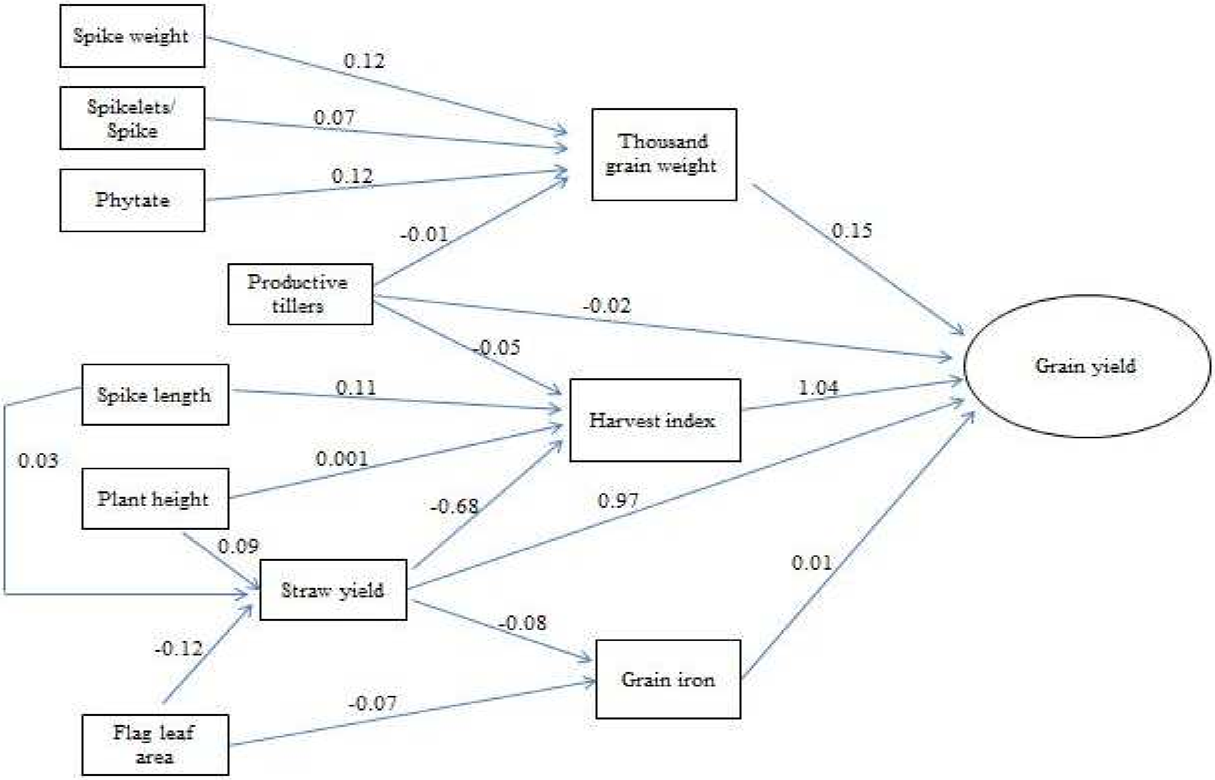

Figure 1. Path coefficient analysis of Land races

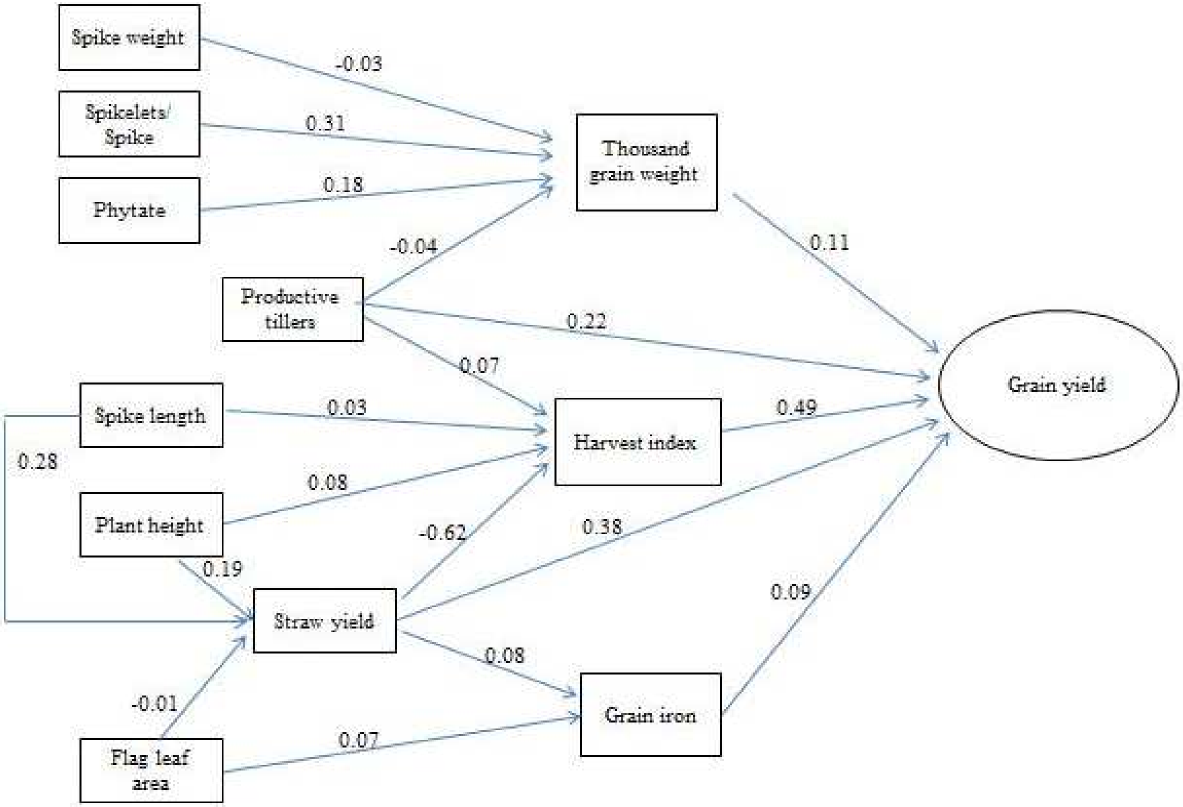

Figure 2. Path coefficient analysis of varieties.

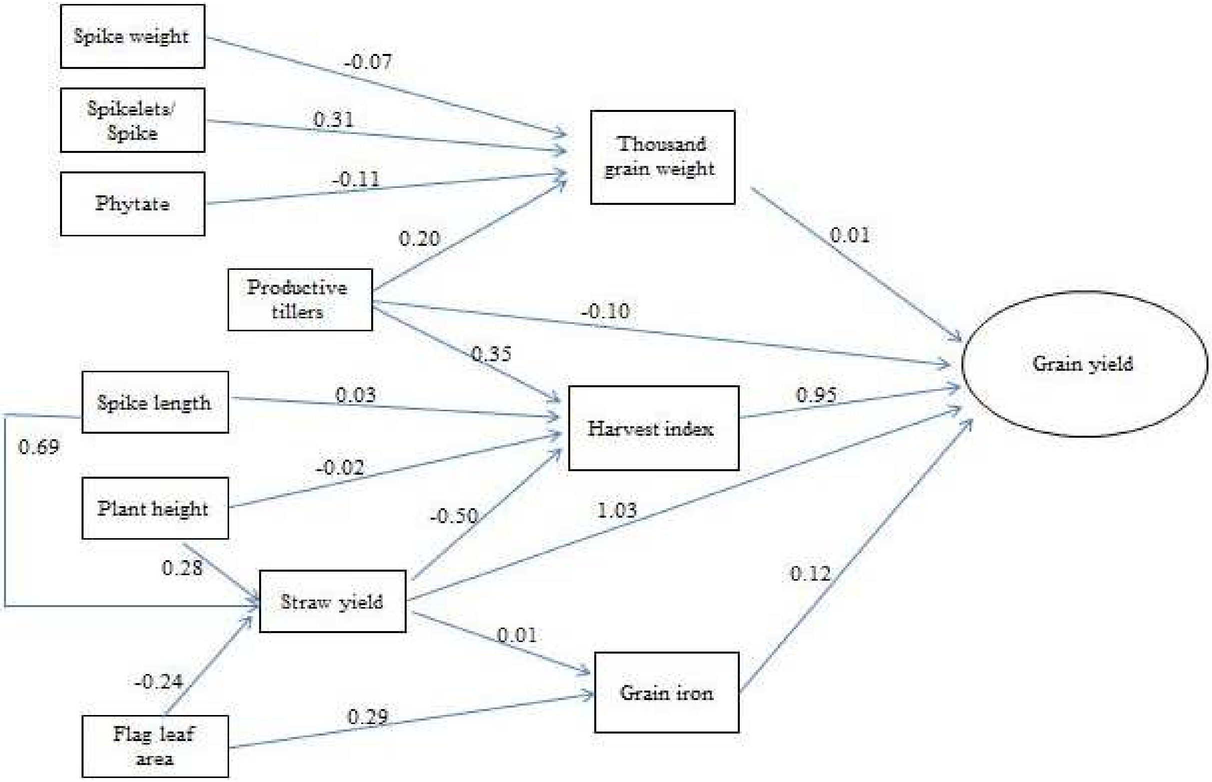

Figure 3. Path coefficient analysis of parents and F$_1$.

DISCUSSION

The source to sink relationship of wheat crop has changed after domestication and green revolution. A crop may be limited by source and sink or a combination of both (Borras *et al.*, 2004). The results of this study showed that some morphological traits have increased values in varieties released after green revolution and advance lines. These include flag leaf area, spike length, spike weight, harvest index and 1000-grains weight (Table 2). Earlier study of wheat indicated a decrease in number of leaves and phyllochron period in modern wheat varieties showing a rapid growth system in modern wheat varieties and higher canopy cover of old cultivars compared to modern cultivars (Siddique *et al*., 1989). It appeared as if the decrease in number of leaves was compensated by increasing the area of leaves. Flag leaf area was negatively correlated with grain yield in land races and became positively correlated in F$_1$ population. However, the direct effect of flag leaf area to grain iron contents became higher in varieties and F$_1$ compared to landraces indicating the possibility of agronomic biofortification through foliar application as shown in Figure 1 and 3 (Zhang *et al.*, 2010). Spike length and spike weight increased in varieties and advanced lines indicating the increase in sink size in modern genotypes compared to land races. Increase in allocation of photo-assimilates to spikes in modern cultivars of wheat compared to older ones, suggests increased sink size in modern cultivars (Joudi *et al.* 2017). The correlation analysis revealed spike length and spike weight positive correlations with grain yield and other yield contributing traits in approved varieties. However, the positive correlation of spike length became highly significant with grain yield in F$_1$ population. The direct effect of spike length to harvest index was high in land races compared to other groups (Ali *et al.* 2008). The increase in the grain weight is apparently compensated by decrease in the number of spikelets in spike. However, the correlations of 1000-grain weight remained significant in all three groups with grain yield indicating that it has been a vital contributor to grain yield per plant.

Grain phytate contents showed no correlation with 1000-grains weight and grain yield in landraces. Grain phytate contents had highly significant correlation with 1000-grain weight in varieties, advance lines and F$_1$ population. Path analysis also suggested high contribution of phytate contents to 1000-grain weight in all the groups. This finding suggested a relationship of 1000-grain weight with phytate contents. Both 1000-grain weight and grain phytate contents have increased in varieties and advance lines but the negative correlation of these two in land races has been lost in modern cultivars indicating the possibility of increase of phytate contents (anti-nutrient) by improving 1000-grain weight. Spikelets per spike showed positive correlation with spike weight, spike length in approved varieties. Spikelet in a spike showed significant positive correlation with grain iron contents in varieties indicating that by increasing spikelets per spike, grain iron contents also has increased in varieties. However in F_1 population spikelets per spike showed negative correlation with PA/Fe indicating that by improving spikelets per spike we can improve bioavailability of grain iron likewise.

Plant height, productive tillers, straw yield and grain iron contents have shown a decreasing trend in advance lines and varieties. These differences may be due to difference in time pattern and location and genetic makeup of accessions. It is a general notion that plant height has decreased after green revolution and selection of dwarf genotypes. The correlation analysis revealed that productive tillers became positively correlated with grain yield in varieties and advance lines while, productive tillers and plant height both became major contributors to straw yields in modern genotypes. Grain iron contents showed significant negative correlation with PA/Fe estimates for bioavailability in land races, varieties and advance lines. It indicated that by increasing the amount of iron in grains, bioavailability of grain iron will also improve. Grain iron contents showed correlation with spike related traits in all the groups of genotype and with straw yield in varieties. However, the F_1 population had significant correlation of grain iron contents with 1000-grain weight and grain phytate contents indicating the possibility of improving grain yield along with grain iron contents. Previous study in wheat also concluded positive correlation of grain iron contents with bioavailable contents and no correlation with phytate in grains (Salunke *et al.,* 2011; Younas *et al*., 2020).

Phytate contents, grain yield and PA/Fe showed an increasing pattern in varieties compared to land races and then a decreasing trend in advance lines compared to varieties. F_1 population had increased phytate contents and decreased PA/Fe due to increased iron contents. The grain yield had an increasing trend from landraces to F_1 population. This may be due to the positive correlation of grain phytate with grain yield. Phytate being a major storage component also showed same trend as that of grain yield per plant. The changing pattern of bioavailability from highest in land races to lowest in varieties may be due to decrease in iron contents and increase of grain phytate contents compared to the landraces. The negative correlation of grain yield with flag leaf area has lost in varieties and productive tillers, spike length, harvest index, 1000-grain weight and straw yield per plant became major contributor to grain yield in varieties. The positive correlation of grain yield with PA/Fe in advance lines indicates that further improvement in grain yield would result in decreasing the iron bioavailability. Previous studies in wheat also reported positive correlation of grain quality related to grain yield in some varieties (Chatrath *et al.,* 2018; Sokoto *et al.,* 2012). While other studies of wheat also reported non-significant or negative correlation of grain yield contributing characters with grain micronutrient content (Badakhshan *et al.,* 2013; Gregorio, 2002; Joshi *et al.,* 2010).

The germplasm is useful for improving grain yield and grain iron contents via selection. Grain yield per plant and 1000-grain weight can be improved through direct selection from the population as they have high heritability and medium to high genetic advance at 10% and 5% selection intensity. Increased selection intensity up to 5% seems promising for improving grain iron contents, grain phytate contents and grain iron bioavailability. Earlier study in wheat also indicated low heritability for grain iron contents (Joshi *et al.,* 2010). While for development of low phytate lines, Gupta *et al.* (2015) reported high heritability and high genetic advance. The difference in the results may be due to different genetic backgrounds of germplasm in both the studies. Thus, germplasm has considerable potential for improving wheat nutritive value and grain yield per plant. While in F_1 population, the genetic advance became medium to low for all traits indicating the need to increase the selection pressure in F_2 for improving the traits.

In conclusion green revolution and variety development procedure, from last few decades has considerably improved the grain yield of wheat. The results indicated that grain yield per plant has improved in recent cultivars but bioavailability of grain iron has decreased. However, the F_1 and parent population clearly shown the possibility of improving the bioavailability of grain iron and phytate along with grain yield. Although phytate is anti-nutrient compound but it also serves to prevent diabetes mellitus, heart diseases, and anti-carcinogenic properties (Kumar *et al.* 2010). Thus mineral malnutrition can be countered by improving grain yield and grain iron contents so that grain phytate contents increase remain harmless and desirable.

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