J. Agr. Sci. Tech. (2015) Vol. 17: 747-756

Screening and Characterization of Wheat Germplasms for Phytic Acid and Iron Content

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ABSTRACT

Phytic acid is a major storage form of phosphorous in cereals that acts as food inhibitor by chelating micronutrients and prevents it to be bioavailabe for monogastric animals, including humans. Ninety three wheat (*Triticum aestivum* L.) germplasms, including cultivars from India, were characterized for phytic acid and Fe contents. Phytic acid contents ranged from 0.59 (IITR 92) to 2.08% (IITR 25). The Fe contents of all wheat germplasms ranged from 9.97 (IITR 25) to 45.77 mg kg^{-1} (IBW 1133) while historical cultivars from India contain an average of 21.7 mg kg^{-1} Fe. This initial screening facilitated the identification of diversity in germplasms for this trait that can be exploited for genetic improvement in wheat. Forty eight F_2 wheat lines from (WL711×IITR 19) were also evaluated, which demonstrated considerable variation in phytic acid content. Phytic acid contents ranged from 0.58 to 2.01% in F_2 lines with an average of 1.52%. The genotypes showed significant differences in phytic acid and Fe contents. F_2 lines of WL711×IITR19 also illustrated variation in phytic acid content that were significant. The progenies having lower phytic acid content compared to parents are useful for further crop improvement. A relatively high broad sense heritability (93.4%) and genetic advance (32.3%) of phytic acid showed that progenies of this cross would be useful for reducing phytic acid.

Keywords: Food and nutrition, Genetic improvement, Wheat genotypes.

INTRODUCTION

Wheat (*Triticum aestivum*) is the most important food crop of the world and is the cheapest source of protein and calories. Wheat contributes approximately with 30% of the total cereal production worldwide and is a major source of minerals for many people (McKevith, 2004). There are many human diseases that are related to micronutrition (Guo *et al*., 2012), hence it is important to improve the micronutrient in foods. In bread wheat, Fe content range from 21–32 mg kg^{-1} and Zn content 15–22 mg kg^{-1} (Rawat *et al*., 2009), but a very small portion of the existing amount is retained during processing and has low bioavailability due to the presence of phytic acid food inhibitors. Compared to cultivated wheat, wild and primitive wheat are a better genetic resource for high Fe and Zn concentrations. Wild emmer wheat, *Triticum turgidum* ssp. *dicoccoides*, showed prominent genetic variation in the concentrations of Zn ranging from 14 to 190 mg kg−1 (Cakmak *et al*., 2010; Gupta *et al*., 2013). Phytic acid is the major storage form of phosphorous in cereals. Approximately 65 to 85% of total seed phosphorus is stored as phytic acid (Vats and Banerjee, 2004). Phytic acid chelates micronutrients and prevents it to be bioavailable for monogastric animals, including humans, because they lack phytic acid hydrolyzing enzyme in their digestive tract (Schroder *et al*., 1996; Boling *et al*., 2000; Singh *et al*., 2011). Reducing the amount of phytic acid in seed is one way to increase the micronutrient in diet and develop cultivars with low phytic acid content. Selection among the existing low phytic acid wheat varieties is the simplest approach for a plant breeder, but non-lethal recessive

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mutations can also be used to decrease seed phytic acid concentration. Low phytic acid mutations have been isolated in barley (Larson *et al*., 1998; Rasmussen and Hatzack, 1998), maize (Raboy and Gerbasi, 2000), rice (Larson *et al*., 2000) and soybean (Wilcox *et al*., 2001; Hitz *et al*., 2002; Guttieri *et al*., 2004). Hence, screening genetically low phytic acid trait in wheat and afterward development of low phytate varieties is very important. Low phytic acid varieties can be used to fulfill the deficiencies caused by iron, zinc, and other micronutrients (Khan *et al*. 2007). Wheat breeding in most world regions is mainly focused on improving grain yields, decrease farming cost, increasing resistance to diseases, and lodging (Rengel and Romheld, 2000; Bouis and Welch, 2010; Hussain *et al*., 2012).

Molecular markers can be used in breeding programs, especially to improve traits relating to wheat grain (Goutam *et al*., 2013). Ram *et al*. (2011) reported the importance of Glu-3 alleles in evaluation of wheat germplasm and breeding. They identified most of low molecular weight (LMW) glutenin alleles by combined SDS-PAGE and PCR based methods. One hundred eighty two Indian bread wheat cultivars were characterized using allele specific marker for LMW-GS for quality breeding.

Improvement in crop plants mostly depends on the magnitude of genetic variability. Selection of germplasms having groups of desired traits can be obtained by study of associations among various traits. In this work, variability of phytic acid and Fe contents were evaluated for 93 wheat genotypes from India.

MATERIALS AND METHODS

Plant Materials

The study was carried out at experimental field of Biotechnology Department, Motilal Nehru National Institute of Technology, Allahabad U.P. The experimental material of the study comprised 85 hexaploid wheat wild germplasms and eight cultivars. The germplasms were collected from Department of Biotechnology, IIT Roorkee and Department of Genetics and Plant Breeding, Sam Higginbotton Institute of Agriculture Technology and Science Allahabad, India. (Table1)

Table 1. List of cultivars and germplasms studied.

Cultivars					
Lok1			WL711		
Halna			S701		
K7			Kedar		
HD2733			PBW343		
Germplasms					
AAI 13	IBW 1050	AAI/IBW 1035	IITR 17	IITR 33	IITR 82
AAI 15	IBW 1074	AAI/IBW 1033	IITR 18	IITR 34	IITR 83
AAI 16	IBW 1078	AAI/IBW 1084	IITR 19	IITR 38	IITR 88
AAI 23	IBW 1103	AAI/IBW 1083	IITR 20	IITR 65	IITR 89
AAI 25	IBW 1113	K-9533	IITR 21	IITR 66	IITR 91
AAI 28	IBW 1133	GW-03-12	IITR 22	IITR 67	IITR 92
AAI 29	IBW 1115	GAW-94	IITR 23	IITR 69	IITR 95
AAI 47	AAI/IBW 1081	WR-1451	IITR 24	IITR 70	IITR 96
AAI 344	AAI/IBW 1075	CS	IITR 25	IITR 71	IITR 102
AAI 347	AAI/IBW 1064	IITR 8	IITR 26	IITR 72	IITR 103
IBW 1038	AAI/IBW 1097	IITR 9	IITR 27	IITR 73	
AAI-03-12	AAI/IBW 1016	IITR 10	IITR 28	IITR 74	
IBW 1014	AAI/IBW 1104	IITR 11	IITR 29	IITR 75	
IBW 1026	AAI/IBW 1129	IITR 13	IITR 30	IITR 76	
IBW 1046	AAI/IBW 1036	IITR 15	IITR 31	IITR 79	

The experiment was laid out in a randomized block design with three replications. The standard agronomical practices were used to grow the plants. The genetic study was conducted utilizing a cultivar, the WL711 having 1.71% phytic acid and a genotype IITR 19 having 0.68% phytic acid. Morphological characters such as seed color, seed structure, days of flowering and maturity were also taken in consideration. WL-711 has amber color bold seed and early maturity while IITR 19 has red color shrink seed with late maturity. Crosses were made between WL711 and IITR 19; the resulting F_1 plants were grown in field during 2010-2011 growing season, while the resulting F_2 plants were grown in 2011-2012 and F_3 plants in 2012-2013. Data from plants recorded for days upto 50 percent flowering, days to maturity, plant height, peduncle length, number of tillers per plant, spike length, number of spikelet per spike, 1,000 grain weight, grain color, grain size and phytic acid contents. A sub-sample of seed from each plant was analyzed for phytic acid concentration. Triplicate estimates were made for each sample. The determination of phytic acid was based on the modified Wade assay.

Statistical Analysis

The statistical analyses were carried out by analysis of variance (ANOVA) for all characters to test the level of significance among the 93 genotypes. The phytic acid content was also measured. Basic statistics for all parameters of 48 F_2 lines were determined. The Data obtained from plants were subjected to statistical analysis to calculate heritability and genetic advance. Heritability in broad sense (H) was computed according to Lush (1940).

$H= \sigma^2_g / \sigma^2_p$

Where, $\sigma^2_{g=}$ Genotypic variance, $\sigma^2_{p=}$ Phenotypic variance.

Genetic advance (GA) was calculated following Johnson *et al*. (1955).

$GA= (\sigma^2_g / \sigma_p) \times K$

Where, $\sigma_{p=}$ Phenotypic standard deviation; $\sigma^2_{g=}$ Genotypic variance, K= Selection intensity

(The value of K= 1.755 in this study at 10% selection pressure).

Genetic advance percent= (GA/Mean)×100

RESULTS AND DISCUSSION

Screening of Wheat Genotypes

Phytic acid content, in the 93 wheat germplasms, ranged from 0.59% (IITR 92) followed by IITR19 (0.68) to 2.08% (IITR25). Lok1, WL711, Halna, K7, Kedar and S701 are some popular cultivars in India which contain phytic acid in between 1.52-1.71%, but there are great variations in phytic acid content in wheat germplasms. Phytic acid content in grains of 93 wheat genotypes showed that the maximum phytic acid was in the range of 1.3-1.5% (Figure 1). Two genotypes i.e. IITR 92 and IITR 19 were initially identified as having low phytic

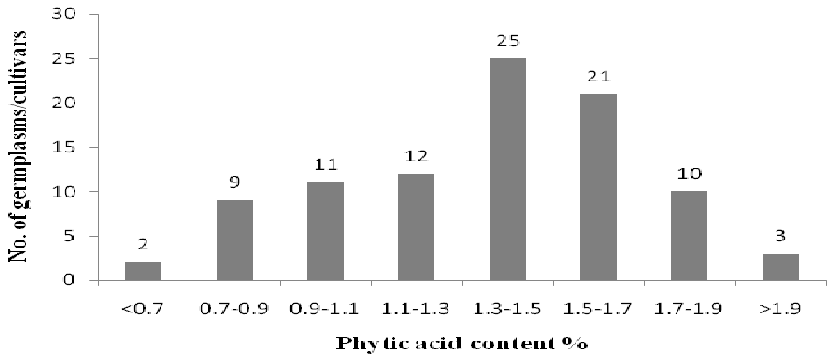

Figure 1. Range of phytic acid content in grains of 93 wheat germplasms and cultivars.

acid (< 0.7%). Khan *et al*. (2007) reported frequency distribution for phytic acid content in the range of 1.43-1.72% in 66 wheat genotypes. Ahmad *et al*. (2013) reported 1.06 to 3.67% phytic acid in parental wheat genotype while the value among F_1 hybrids ranged from 0.56% to 3.43%.The analysis of variance showed that the difference among germplasms for phytic acid content was highly significant (Table 2). The great variation in phytic acid content among the wheat germplasms indicates that there are possibilities of identifying and developing cultivars with low phytic acid contents in grain. The Fe content of all wheat germplasms ranged from 9.97 (IITR25) to 45.77 mg kg^{-1} (IBW 1133) while popular Indian cultivars contain an average of 21.7 mg kg^{-1} iron (Table 3).

Genetic Study of Low Phytic Acid Wheat Genotypes

The segregation result of F_2 population shows that there are wheat plants which contain low phytic acid with better morphological traits. The result of segregation analysis revealed that, out of 48 F_2 lines, lines no. 2, 7, and 41 had lower phytic acid content. Phytic acid content in all lines ranged from 0.58 to 2.01%. Seeds of line no.2 had 0.58% phytic acid, lower than both parents with amber color (Figure 2). Therefore, this line may be used further for breeding programs (Table 4). The characters can be used as morphological marker for phytic acid content

Table 2. Analysis of variance (ANOVA) of phytic acid in grains of 93 genotypes.

Source	df	SS	MS	F
Genotypes	92	46.08	0.501	40.937**
Replication	2	0.01	0.005	0.388
Error	184	2.25	0.012	
Total	278	48.34		

** at 5% *P* value.

Table 3. Analysis of variance (ANOVA) of Fe in grains of 93 genotypes.

Source	df	SS	MS	F
Genotypes	92	22696.54	246.70	1035.85**
Replication	2	546.13	273.06	1146.55
Error	184	43.82	0.238	
Total	278	23286.49		

** at 5% *P* value.

Figure 2. Grain color of F_2 population of wheat.

Table 4. Morphological and chemical characteristic of 48 F_2 lines of wheat (WL711× ITR19).

Line No.	Days of flowering	Days of maturity	Plant height cm	No. of tillers	Peduncle length cm	Spike length cm	No. of spikelet per spike	1000 grain weight	Phytic acid (%)	Grain color	Grain structure
1	68	104	116	19	53	10	14	39.75	1.18	R [a]	B [b]
2	84	119	126	21	46	15	24	29.80	0.58	A [c]	S [d]
3	70	103	121	14	65	12	18	34.90	1.44	R	B
4	71	106	118	12	48	11	16	30.60	1.25	R	S
5	83	119	131	17	45	15	24	24.50	1.44	R	S
6	84	121	119	14	46	18	21	21.71	1.58	A	S
7	87	122	118	24	47	15	20	36.84	0.79	R	B
8	64	99	120	6	57	12	18	46.57	1.44	A	B
9	70	100	109	4	52	8	16	31.08	1.31	R	S
10	68	104	122	5	65	13	18	38.42	1.64	R	B
11	72	106	119	25	59	13	20	42.86	1.58	R	B
12	74	110	126	6	54	12	16	34.17	1.25	R	B
13	73	106	128	12	58	10	14	43.13	1.71	A	B
14	62	97	102	16	50	12	18	35.63	1.51	R	B
15	67	103	105	4	50	9	14	37.27	1.58	R	B
16	75	107	123	5	51	11	16	36.35	1.55	R	B
17	70	105	118	6	52	12	14	40.31	1.58	R	B
18	76	110	118	5	50	10	16	42.50	1.64	A	B
19	84	119	129	19	47	13	20	27.50	1.31	A	S
20	68	105	117	14	46	12	16	44.86	1.71	R	B
21	64	99	115	12	58	11	16	30.00	1.44	R	S
22	74	109	114	16	42	15	22	37.91	1.98	R	B
23	72	106	134	13	49	16	22	36.35	1.71	R	B
24	69	103	110	10	44	13	20	39.55	1.58	R	B
25	72	106	119	4	52	12	16	45.75	1.84	A	B
26	78	111	141	16	50	12	18	45.90	1.64	A	B
27	79	112	128	11	49	11	16	45.75	1.67	R	B
28	81	110	120	15	55	12	18	40.24	1.97	R	B
29	64	99	118	13	51	12	18	42.56	1.51	R	B
30	78	110	132	11	54	14	20	30.20	1.64	R	S
31	79	110	131	12	56	13	18	40.00	2.01	R	B
32	66	102	118	15	46	16	18	29.41	1.67	R	S
33	76	110	134	14	57	13	18	39.15	1.71	R	B
34	70	105	115	11	43	12	18	33.00	1.91	R	S
35	67	100	108	5	50	10	16	43.64	1.66	R	B
36	70	105	127	8	58	12	16	37.14	1.58	R	B
37	71	106	117	14	56	14	22	34.81	1.64	R	B
38	76	108	133	23	60	14	18	37.92	1.84	A	B
39	72	107	90	3	43	10	14	34.55	1.18	R	B
40	68	104	102	15	46	12	18	36.88	1.44	R	B
41	84	120	125	24	35	15	24	28.91	0.88	R	S
42	70	105	118	23	52	12	20	34.09	1.25	R	B
43	72	105	125	22	40	10	18	29.71	1.18	R	S
44	63	96	98	6	39	11	18	29.26	1.31	R	S
45	68	105	116	10	51	12	18	42.59	1.91	A	B
46	74	106	112	25	44	11	18	31.75	1.74	R	S
47	70	104	100	4	48	9	14	39.32	1.51	A	B
48	86	119	138	24	36	14	22	29.75	1.64	R	S
(P_1)	65	104	100	15	40	11	18	45.00	1.71	A	B
(P_2)	82	119	130	9	57	14	18	34.42	0.68	R	S

[a] Red ; [b] Bold; [c] Amber; [d] Shrink.

in wheat grain after further confirmation.

The mean value of phytic acid content of F_2 lines was recorded and it was found 1.52% (Table 5). In the maximum plants of F_2 segregation lines, days of flowering, days of maturity, no. of tillers per plant, no. of spikelet per spike were in between 70-75 days, 105-110 days, 10-15, and 18-20, respectively. Plants height, peduncle length, and spike length were in between 110-120, 50-55, and 12-14 cm, respectively, in the maximum number of segregants in F_2 population. Variations in spike length of F_2 lines wheat are shown in Figure 3.There are great variations between spikes of F_2 population. The spike of WL711 is amber color, compact, and small, while the spike of IITR19 is red and comparatively large. The spikes of some F_2 segregants are larger than both parents with red or amber color (Table 4). In maximum no. of F_2 lines, 1000 grain weights were recorded between 35-40 g (Table 6).

The F_2 progeny from WL711×IITR19 indicated the 93.4% heritability in broad sense with 32.3% genetic advance for phytic acid. A quite high heritability and genetic advance of phytic acid indicated that progenies of this cross would be useful for reducing phytic acid. These results are in accordance with the findings of Ahmad *et al*. (2013).

In correlation study, a correlation between PA and Fe content in grain was not observed (r= -0.07). Among 93 genotypes, some contained higher concentration of phytic acid and low concentration of Fe (data not shown). Lower concentration of Fe may be due to the presence of high concentration of phytic acid, which is a chelating agent that binds minerals making them unavailable for dietary absorption (Akond *et al*., 2011; Hirschi,, 2009). A negative correlation between phytic acid and Fe contents was also reported in common bean by Akond *et*

Table 5. Range, mean, and standard error of means for nine quantitative characters of 48 wheat F_2 lines.

Character	Range	Mean	SE
Days of flowering	62-87	72.97	0.93
Days of maturity	96-122	107.22	0.92
Plant height (cm)	90-141	119.22	1.53
No. of tillers/plant	3-25	13.06	0.95
Peduncle Length (cm)	35-65	50.10	0.96
Spike Length (cm)	8-18	12.31	0.29
No. of spikelet/spike	14-24	18.06	0.39
1000 grain weight	21.71-46.57	36.35	0.86
Phytic acid %	0.58-2.01	1.52	0.04

Figure 3. Variability in spike length of F_2 population of wheat.

Table 6. Frequency distribution of morphological characters for F_2 progeny wheat.

Days of flowering	No. of plants	Days of maturity	No. of plants	Plant height	No. of plants	Peduncle length	No. of plants	No. of tillers/ plants	No. of plants	Spike length	No. of plants	1000 grain weight	No. of plants	No. of spikelet/F spike	No. of plants
60-65	5	95-100	5	90-100	2	35-40	3	0-5	5	08-10	3	20-25	2	14-16	6
65-70	9	100-105	9	100-110	6	40-45	7	05-10	9	10-12	12	25-30	7	16-18	11
70-75	18	105-110	19	110-120	19	45-50	11	10-15	16	12-14	21	30-35	11	18-20	17
75-80	8	110-115	7	120-130	13	50-55	15	15-20	9	14-16	10	35-40	14	20-22	7
80-85	6	115-120	5	130-140	7	55-60	9	20-25	7	16-18	2	40-45	10	22-24	4
85-90	2	120-125	3	140-150	1	60-65	3	25-30	2	18-20	1	45-50	4	24-26	3

al. (2011). Nair and Iyenger (2009) reported that iron availability was negatively correlated with phytate content. The recommended level of phytate: Fe molar ratio is < 1.0 in food products (Roos *et al*., 2013). Lopez *et al*. (2002) reported that phytic acid accumulation was correlated with phosphorus accumulation, but not with total mineral content in seeds. Chiangmai *et al*. (2011) studied the correlation between phytic acid (PA) content and yield. They reported that there was positive correlation between PA content and yield that may be a hindrance to discover new high yielding potential corn varieties with reduced phytic acid content in F_1 hybrids.

CONCLUSIONS

The present study revealed that germplasms, cultivars, and inbred lines show significant differences in phytic acid and Fe content. Such variants of germplasms can be used for plant breeding programs for further improvement of wheat grain. F_2 lines of parents WL711 and IITR19 also illustrate variation in phytic acid content and are significant. Few progenies showed phytic acid content less than both parents and are useful for further crop improvement. A relatively high heritability and genetic advance of phytic acid showed that progenies of this cross would be useful for reducing phytic acid.

ACKNOWLEDGEMENTS

This work is financially supported by Ministry of Human Resource Development (MHRD) and Department of Science and Technology (DST), New Delhi, India. The authors sincerely acknowledge the coordination of Dr S. Marker, Genetics and Plant Breading, SHIATS, Allahabad, India, and Prof. H. S. Dhaliwal for providing the breeding material for this study.

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غربال گری و تشخیص ویژگی های ژرم پلاسم های گندم برای اسید فیتیک و آهن موجود در آنها

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چکیده

اسید فیتیک یک شکل عمده فسفر در غلات است که با کلات کردن(chelating) مواد غذایی کم مصرف و کاهش زیست دسترسی (bioavailability) آنها، به عنوان بازدارنده جذب بعضی مواد غذایی در حیوانات تک معده ای (از جمله انسانها) شناخته می شود. در این پژوهش، ۹۳ ژرم پلاسم گندم(Triticum aestivum L.) که شامل کولتیوارهایی از هندوستان بود از نظر محتوای اسید فیتیک و آهن بررسی شدند. مقدار اسید فیتیک در آنها بین ۰.۵۹٪ (در مورد IITR 92) تا ۲.۰۸٪ (در مورد IITR 25) تغییر میکرد. در این ژرم پلاسم ها مقدار آهن بین $9/97\ mg\ kg^{-1}$ (در IITR 25) تا $45/77\ mg\ kg^{-1}$ (در IBW 1133) بود در حالی که کولتیوار های قدیمی هند به طور میانگین $۲۱/۷ mg\ kg^{-1}$ آهن دارند. این غربالگری اولیه شناسایی تنوع این صفات را در ژرم پلاسم های گندم تسهیل کرده و می توان از آن برای بهبود ژنتیکی گندم استفاده کرد. همچنین، ۴۸ رگه F2حاصل از

(WL711 x IITR 19) ارزیابی شد که تنوع زیادی در مورد اسید فیتیک نشان داد. محتوی اسید فیتیک آن ها بین ۰/۵۸٪ تا ۲/۰۱٪ با میانگین ۱/۵۲٪ بود. این ژنو تیپ ها تفاوت معنی داری در مورد مقدار اسید فیتیک و آهن داشتند. نیز، رگه های F2حاصل از تلاقی WL711 x IITR19 تفاوت های معنی داری در فیتیک اسید نشان دادند. فرزندان و نتاجی که اسید فیتیکی کمتر از والد ها دارند برای اصلاح ژنتیکی مفید هستند. توارث پذیری زیاد (۹۳٪) و عریض و پیشرفت ژنتیکی(genetic advance) معادل ۳۲٪ گواهی داد که فرزندان این تلاقی برای اصلاح ژنتیکی در مورد اسید فیتیک مناسب اند.