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IDENTIFICATION AND ISOLATION OF LOW PHYTIC ACID WHEAT (*TRITICUM AESTIVUM* L.) INBRED LINES / MUTANTS

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Abstract

Research studies were carried out for identification and isolation of low phytate bread wheat mutants / cultivars in Pakistan. Sixty six mutants / cultivars collected from different parts of the country were assayed for phytic acid contents. The phytic acid contents ranged from 0.98% (Parvaz-94) to 2.17 % (NRL-0443). The frequency distribution curve showed that most of the genotypes had seed phytic acid contents in the range of 1.28 - 1.72%. Pooled analysis of revealed that seed phytic acid contents have significant correlations with seed width ($p<0.001$), seed thickness ($p<0.01$) and seed volume ($p<0.05$). Seven genotypes viz., Bakhtawar-92, Parwaz-94, NRL-0406, NRL-0416, NRL0431, NRL-0439 and NRL-0441 were identified as having low phytic acid contents. The selected lines were grown at 5 locations differed for soil types and environmental condition to determine G x E interaction on phytic acid contents. Cultivar Parvaz-94 gave consistently low and mutant NRL-0431 gave the highest concentration of phytic acid across the location. The effects of genotypes, environments (locations) and their interactions on phytic acid content were all highly significant, with the location having the largest effect. The highly significant interaction between genotype and environment suggests that the correct evaluation of wheat germplasm by phytic acid content should be conducted in multi-environments.

Introduction

Phytic acid (*myo*-inositol-1, 2, 3, 4, 5, 6-hexa*kis*phosphate) is considered to be the most abundant storage form of phosphorus present in food grains. The phytic acid accounts for 65-85% of seed total phosphorus (Raboy, 1997). The accumulation site of phytic acid in monocotyledonous seeds (wheat, barley, rice etc.) is the aleurone layer, particularly the aleurone grain. It interferes with the absorption or utilization of Fe and Zn in staple food grains by forming insoluble precipitates with a number of polyvalent mineral cations (e.g., Ca2+, Fe3+, and Zn2+). It is considered to have an adverse effect on metabolism of minerals and causing micronutrient deficiency.

Through out the developing world, poor people subsist on diets mainly consisting of staple foods such as wheat or rice and little else. Populations that depend on wheat as staple food, consume diets rich in phytic acid, the storage form of phosphorus in seeds, present in substantial amounts in wheat (Lott, 1984). The lack of diversity in the foods they eat often lead to micronutrient deficiencies which cause severe consequences such as anaemia, complication in pregnancy and poor growth.

Bread wheat is the staple food grain in Pakistan. It is cultivated on 8.5 million hectares and is the cheapest source of protein and calories. The estimated average consumption is 340 gram per day per capita and constitutes 60% of the average daily diet of the common man. The phytic acid content is influenced by cultivar, climatic conditions and growing seasons. The presence of phytic acid in wheat and its products renders the mineral contents of the diet unavailable to the human body and binds to mineral nutrients such as iron and zinc, forming salts that are largely excreted and ultimately causes mineral depletion and deficiency.

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For a plant breeder, selecting among existing wheat varieties is the simplest approach. Results of a study at CIMMYT show that of the wheat varieties released by that organization of over 40 years of breeding, the best were about 20% higher in iron and zinc concentration in grain than the lowest (Ortiz-Monasterio, 1998; Graham *et al.*, 1999). A 20% difference is likely to be important to deficient consumers of this staple, provided they are capable of absorbing the iron. Surprisingly, the overall iron concentrations in grain were not depressed by the increased yield achieved over 40 years of breeding. Non-lethal recessive mutations that decrease seed phytic acid concentration have been isolated in maize (Raboy & Gerbasi., 1996 & 2000) barley (Larson *et al.*, 1998., Rasmussen & Hatzack., 1998), rice (Larson *et al.*, 2000) and soybean (Hitz *et al.*, 2002; Wilcox *et al.*, 2001).

Although the current available wheat varieties in Pakistan fairly meet the international quality standards, but the important role of seed phytic acid in human and animal nutrition has not yet received befitting attention. Like many other developing countries, iron deficiency (ID) and iron deficiency anemia (IDA) are widely prevalent in various age, sex and socio-economic groups in Pakistan, leading to substantial losses in terms of medical expenses and work productivity of the affected population. Therefore, searching genetically low phytic acid trait in wheat and subsequently development of low phytate varieties seems to be very important and can play major role for the remedy of iron and other micronutrient deficiencies in the country.

Materials and Methods

Sixty six bread wheat cultivars / stable mutants / recombinants were collected from different growing areas of Pakistan and were planted during 2003-04. The experimental site is situated at the research farm of Nuclear Institute for Food and Agriculture (NIFA), Tarnab (longitude $71^0$50, latitude $34^0$01), Peshawar, Pakistan. Mean annual rainfall ranged from 380-550 mm with slightly summer dominance. Each entry was grown in a non-replicated nursery comprise of two rows, 2.5m in length and 30cm apart. Land was thoroughly prepared and fertilized @ 50 kg N per acre in the form of urea and 30 kg P per acre in the form of DAP. Normal cultural practices were carried out during the growing season. At maturity, spikes were harvested from the genotypes with similar mature dates, threshed, dried, milled to pass through a 1.54 mm screen, and stored in a desiccator until Phytic acid analysis. Phytic acid contents were determined by the procedure of Haug & Lantzsch, (1983). The sample extract (with 0.2N HCl) was heated with an acidic Iron III solution of known iron content. The decrease in iron content (determined colorimetrically with 2, 2-bipyridine) in the supernatant was the measure of the phytate phosphorus. This method relies on binding of added iron by the phytic acid followed by back determination of the unbound free iron using 2, 2 bipyridine. Seed physical parameters of all the 66 genotypes were studied in detail. Seed weight was measured by taking average weight of 20 seeds, weighed individually. Seed volume was determined by displacement of a known volume of water by 100 kernels in triplicate for each entry and calculated to volume per single seed. Seed density was calculated as ratio of mass to volume. Seed length, width and thickness were measured using a micrometer and taking average values of 15-20 seeds. Ratios between various measurements were calculated. The seven selected relatively low phytate lines were tested across 5 different environments i.e., Tarnab, Malakandher, Pirsabak, Mansehra and Kaghan in N.W.F.P. during 2004-05. These locations were different for soil types, precipitation and temperature. The experiments were laid out according to randomized complete block design with three replications at each location. Four rows of 5m length per each entry were sown at 30 cm apart. Normal cultural practices were carried out during the growing season. At maturity, two central rows (plot size 3 m^2) were harvested at each location to determine grain yield and phytic acid contents.

Mean, minimum and maximum observed values, standard deviation and CV% for all parameters were determined. Coefficients of correlation were determined between various grain parameters with its phytic acid contents. Frequency distribution of phytic acid content in 66 genotypes was also determined.

Results and Discussion

Wheat cultivars / mutants were greatly different for phytic acid contents (Table 1). Phytic acid contents of 66 wheat genotypes was determined that ranged from 0.98 (Parwaz-94) to 2.17% (NRL 0443) with an average value of 1.50% and inter genotypic variability of 14.95%. Among different physical parameters of seeds, the highest variability was noted for seed density (37.43%), followed by seed volume (32.80%). The large variation in phytic acid content among wheat genotypes indicates the possibility of identifying and developing cultivars with low phytic contents in seed. Seven entries, i.e., Bakhtawar-92, Parvaz-94, NRL 0406, NRL 0416, NRL 0431, NRL 0439 and NRL 0441 were initially identified as having low phytic acid (≤1%) contents (Table 2). The frequency distribution of 66 wheat genotypes was worked out. In the present study, 17% genotypes were found in the range, 67% in the medium and 16% in the higher range of phytic acid contents (Table 3). The frequency distribution curve showed that most of the genotypes had seed phytic acid contents in the range of 1.43–1.72% (Fig. 1). Similar frequency distribution for phytic acid contents was also reported in rice (Liu *et al*., 2005).

Significantly positive correlation was observed between seed phytic acid and seed thickness (Table 4). Seed weight and density were negatively correlated with seed phytic acid contents. These parameters can be used as physical markers for phytic acid contents in wheat seed after further confirmation.

Variance analysis of phytic acid contents of seven wheat cultivars / mutants, grown at five locations in Pakiatan, showed that the effect of genotypes, location and their interaction were all highly significant (Table 5). Locations shared a greater contribution to variation than genotypes, indicating that phytic acid variable, depending on the environment. Highest mean value of 2.341% for phytic acid was at Kaghan and the lowest of 1.435% at Pirsabak (Table 6). Parvaz-94 with the lowest mean value of 1.595% was found consistent across locations for phytic acid contents. Genetic and environmental variations in the phytic acid contents of cereals and legumes have been reported in several studies (Batten, 1986; Feil & Fossati, 1997; Liu *et al*., 2005; Miller *et al*., 1980; Raboy *et al*., 1984, Zeb *et al*., 2006). In the present study, significant genetic and environmental effects were also found, but the environmental effect appears to be predominant in determining phytic acid content. The lowest value of 1.17% for PAC was obtained by cultivar Parvaz-94 at Tarnab location while the highest value of 2.483% was noted for NRL 0416 at Kaghan. In this study, we found significant interaction between location and genotypes in terms of PAC, indicating the importance of using suitable cultivars for a given location, so as to control phytic acid content. In addition, it is suggested that the comprehensive evaluation of wheat germplasm for phytic acid content should be conducted in multi-environments. It would suggest that phytic acid contents of seed is greatly influenced by environments, therefore suitable cultivars should be grown at specific locations.

Table 1. Physio-chemical characteristics of 66 wheat cultivars / mutants.

Entries	Phytic acid (%)	S. length (mm)	S. width (mm)	S. thickness (mm)	S. weight (g)	S. volume (cc)	S. density
NRL 0116	1.79	6.20	2.90	2.92	0.039	0.025	1.563
NRL 0228	1.61	6.12	2.79	2.92	0.039	0.035	1.105
NRL 0332	1.31	5.99	2.84	2.97	0.038	0.02	1.903
NRL 9930	1.61	6.05	3.00	2.67	0.036	0.02	1.79
Bakhtawar	1.15	6.15	3.05	2.57	0.035	0.02	1.755
Takbeer	1.47	6.30	3.07	2.79	0.04	0.05	0.796
NRL 2005	1.79	6.81	3.25	2.74	0.035	0.05	0.694
NRL 0316	1.53	6.53	3.07	2.97	0.042	0.04	1.04
Tatara	1.51	6.83	3.10	2.95	0.043	0.03	1.426
Chakwal	1.52	7.39	3.40	3.00	0.041	0.04	1.025
NRL 0331	1.45	6.20	3.18	2.77	0.04	0.04	0.991
NRL 9918	1.38	6.15	3.33	2.69	0.033	0.02	1.631
Inqilab-91	1.56	6.22	3.43	2.82	0.037	0.02	1.86
NRL 2009	1.59	6.65	3.45	2.90	0.034	0.04	0.843
NRL 0307	1.66	6.40	3.33	2.87	0.041	0.04	1.027
NRL 0328	1.74	6.96	3.53	3.15	0.045	0.03	1.491
Pirsabak-85	1.81	6.88	3.45	2.97	0.04	0.05	0.795
LU-26	1.49	7.42	3.33	2.97	0.05	0.05	0.998
NRL 0227	1.68	6.91	3.43	2.97	0.039	0.06	0.649
NRL 2017	1.49	6.68	3.40	2.90	0.041	0.04	1.034
NRL 0401	1.45	6.65	3.18	2.74	0.037	0.05	0.741
NRL 0402	1.5	6.45	3.53	2.77	0.042	0.04	1.05
NRL 0403	1.6	6.17	3.35	2.79	0.037	0.03	1.246
NRL 0404	1.61	6.54	3.56	3.00	0.049	0.05	0.975
NRL 0405	1.51	6.45	2.90	2.59	0.04	0.05	0.796
NRL 0406	1.11	6.22	3.25	2.69	0.038	0.04	0.942
NRL 0407	1.55	6.35	3.43	2.69	0.034	0.02	1.693
NRL 0408	1.24	6.40	3.68	3.07	0.036	0.05	0.728
NRL 0410	1.67	6.60	3.40	3.15	0.045	0.05	0.901
NRL 0411	1.4	6.63	3.53	3.18	0.038	0.05	0.755
NRL 0412	1.57	6.50	3.30	3.05	0.041	0.03	1.379
NRL 0413	1.37	6.88	3.58	2.90	0.047	0.04	1.168
NRL 0414	1.22	6.48	3.38	2.90	0.043	0.04	1.087
NRL 0415	1.38	6.12	3.23	2.69	0.043	0.02	2.185
NRL 0416	1.04	6.10	3.30	2.54	0.043	0.02	2.156

Table 1. (Cont'd.)

Entries	Phytic acid (%)	S. length (mm)	S. width (mm)	S. thickness (mm)	S. weight (g)	S. volume (cc)	S. density
NRL 0417	1.38	6.58	3.30	2.84	0.041	0.03	1.355
NRL 0418	1.38	6.32	3.28	2.77	0.033	0.03	1.105
NRL 0419	1.24	6.32	3.18	2.59	0.031	0.03	1.026
NRL 0420	1.31	6.43	3.38	2.87	0.032	0.05	0.639
NRL 0421	1.38	6.38	3.30	2.79	0.034	0.04	0.856
NRL 0422	1.41	6.30	3.30	2.90	0.031	0.03	1.048
NRL 0423	1.44	6.43	3.40	3.15	0.039	0.03	1.292
NRL 0424	1.59	7.32	3.43	3.02	0.042	0.06	0.7
NRL 0425	1.57	7.67	3.56	3.30	0.046	0.07	0.662
NRL 0426	1.56	6.60	3.18	3.00	0.038	0.05	0.755
NRL 0427	1.74	6.12	3.20	3.10	0.038	0.02	1.883
NRL 0428	1.36	6.76	3.30	2.82	0.04	0.04	0.989
NRL 0429	1.54	6.60	3.45	3.00	0.038	0.03	1.267
NRL 0430	1.53	6.65	3.53	2.90	0.041	0.05	0.824
NRL 0431	1.02	7.14	3.66	3.35	0.041	0.05	0.816
NRL 0432	1.59	7.65	3.58	3.25	0.054	0.07	0.777
NRL 0433	1.91	6.58	3.51	2.90	0.039	0.05	0.781
NRL 0434	1.74	6.53	3.58	2.92	0.038	0.05	0.759
NRL 0435	1.62	6.60	6.12	2.95	0.033	0.04	0.825
NRL 0436	1.7	6.55	3.48	2.92	0.042	0.04	1.047
NRL 0437	1.4	7.11	3.56	3.33	0.04	0.05	0.806
NRL 0438	1.25	6.55	3.53	2.97	0.037	0.06	0.616
NRL 0439	1.1	6.25	3.66	2.92	0.041	0.05	0.815
NRL 0440	1.43	6.55	3.76	3.28	0.04	0.03	1.343
NRL 0441	1.18	6.83	3.48	2.90	0.037	0.06	0.611
NRL 0442	1.77	7.04	3.89	2.90	0.041	0.06	0.68
NRL 0443	2.17	6.65	3.58	3.15	0.045	0.06	0.753
NRL 0444	1.81	6.83	3.63	3.02	0.039	0.06	0.645
Parwaz 94	0.98	Nd	Nd	Nd	Nd	Nd	Nd
NRL 9912	1.61	Nd	Nd	Nd	Nd	Nd	Nd
Mean	**1.50**	**6.58**	**3.41**	**2.92**	**0.04**	**0.04**	**1.08**
Min	**0.98**	**5.99**	**2.79**	**2.54**	**0.03**	**0.02**	**0.61**
Max	**2.17**	**7.67**	**6.12**	**3.35**	**0.05**	**0.07**	**2.19**
SD	**0.22**	**0.38**	**0.41**	**0.18**	**0.00**	**0.01**	**0.40**
CV	**14.95**	**5.79**	**12.10**	**6.33**	**11.25**	**32.80**	**37.43**

Table 2. Selected wheat cultivars / mutants with parentage for low phytic acid contents.

Vars./mutants	Parentage	Phytic acid %
Bakhtawar-92	KAUZ “S”	1.15
Parwaz-94	CNO'S'/LR64//SON64/3/SON/4/PRL'S'	0.98
NRL-0406	INTISAR-5	1.11
NRL-0416	ALMAZ-14	1.04
NRL-0431	ALTAR84/AE.SQUARROSA(224)//YACO/3/AL TAR84/ AE.SQUAROSSA(19)//OPATA	1.25
NRL-0439	ATTILA*2/PBW65	1.16
NRL-0441	ATTILA*2/4/CAP//KAL/BB/3/NAC	1.18

Table 3. Frequency distribution of phytic acid content in grains of 66 wheat cultivars / mutants.

Frequency	Phytic acid %	Frequency %
1	>0.98 <1.13	1.52
4	>1.13 <1.28	6.06
6	>1.28 <1.43	9.09
12	>1.43 <1.58	18.18
19	>1.58 <1.72	28.79
13	>1.72 <1.87	19.70
8	>1.87 <2.02	12.12
1	2.02	1.52
1	>2.02	1.52

Table 4. Correlation coefficients of phytic acid contents with seed physical parameters of 66 wheat cultivars / mutants.

Parameters	Phytic acid (%)
Seed Length (mm)	0.160
Seed Width (mm)	0.093
Thickness (mm)	0.186
Seed Weight (g)	-0.152
Seed Volume (cc)	0.152
Density (g/cc)	-0.113

* p<0.05, ** p<0.02, *** p<0.01

Table 5. Analysis of variance of phytic acid in grains of 07 varieties planted at 05 locations.

Source	Df	SS	MS	F. value
Locations	4	11.488	2.872	120.3357**
Varieties	6	0.642	0.107	4.4852**
Location X Varieties	24	1.929	0.080	3.3681**
Error	70	1.671	0.024	
Total	**104**	**15.730**		

Table 6. Phytic acid contents (%) of 07 selected wheat cultivars/mutants at 05 locations.

Entries	Locations					Mean
	Kaghan	Pirsabak	Tarnab	Malakandher	Mansehra	
Parwaz	2.093	1.390	1.157	1.410	1.923	1.595 b
Bakhtawar	2.197	1.493	1.453	1.610	1.760	1.703 b
NRL-0406	2.360	1.310	1.660	1.483	1.840	1.731 ab
NRL-0416	2.483	1.320	1.523	1.557	1.447	1.666 b
NRL-0431	2.457	1.770	1.820	1.443	1.860	1.870 a
NRL-0439	2.407	1.450	1.607	1.537	1.737	1.747 ab
NRL-0441	2.393	1.310	1.860	1.280	1.623	1.693 b
Mean	**2.341 a**	**1.435 d**	**1.583 c**	**1.474 cd**	**1.741 b**	

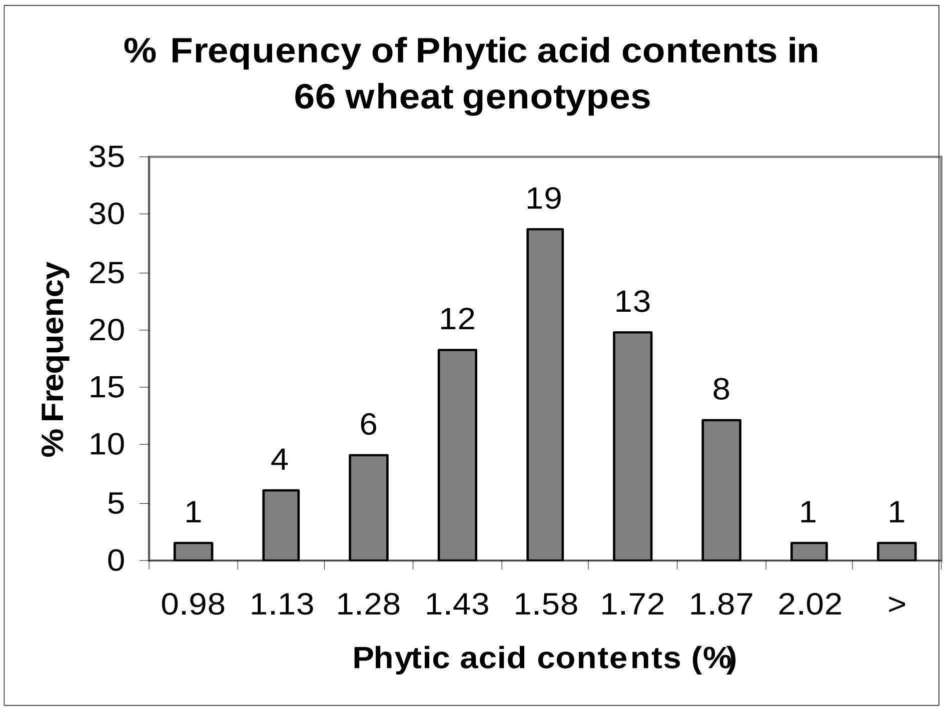

Fig. 1. Frequency distribution of phytic acid content in grains of 66 wheat cultivars / mutants.

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