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BREEDING OF WHEAT FOR LOW PHYTATE

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ABSTARCT

High concentration of phytate in wheat flour hinders the absorption and utilization of some essential nutrients such as Iron (Fe), Manganese (Mn), Magnesium (Mg), Calcium (Ca) etc. A 5 x 5 diallel field research was conducted at the research farm of Khyber Pakhtunkhwa, Agricultural University Peshawar during 2007-08 and 2008-09 to determine Phytate concentration F_1 hybrids and parent genotypes of wheat. All 20 F_1 hybrids and 5 parent genotypes were planted at research farm of Khyber Pakhtunkhwa, Agricultural University using Randomized Complete Block Design (RCBD) with three replications. At threshing, grains from each plot were collected and Phytic acid was determined at Nuclear Institute of Food and Agriculture (NIFA). The values for Phytic acid ranged between 0.81% to 3.91% among the hybrids while the parent genotypes ranged from 1.25% to 3.42% at NIFA. The hybrid genotypes gave a wider range for phytic acid concentration compared to parent genotypes, indicating transgression at both ends. This study identified some potential hybrid populations with significantly lower phytate concentration in wheat grains, thus providing useful material to wheat breeders. These hybrids are recommended to be included further in breeding programmes and should be screened in the coming generations in order to obtain a wheat genotype with low phytate concentration which should be a big breakthrough in the improvement of nutritional quality of wheat as Phytic acid is a potent inhibitor for micro and macronutrients which in turn cause nutritional deformities in human beings. Generation of low phytate genotype is a dire need in order to overcome on nutritional deformities such as Anemia, Rickets and Osteomalacia etc.

Key-words: Phytate, wheat, genotypes, breeding.

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., Ca^{2+}, $Fe^{3+,}$ and Zn^{2+}). 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.

Wheat (*Triticum aestivum* L.) is one of the most important food crops covering two-third of the acreage under cereals in the world. It ranks first both in production and consumption in Pakistan and is one of the richest sources of carbohydrates. Wheat is a staple food crop for the people of Pakistan and serves as a real backbone in the economy of the country. Although the total production of wheat in Pakistan has increased manifolds over the past few decades, and we have touched the level of self sufficiency in the recent past yet we need to produce more wheat for export to earn foreign exchange. For export, we need to concentrate on nutritional quality of wheat grain in order to compete in the international market. Phytic acid is believed to adversely affect the bioavailability of some essential micronutrients such as Iron (Fe), Calcium (Ca), Manganese (Mn), Zinc (Zn), Magnesium (Mg) etc.

Therefore, we need to concentrate on an improving the nutritional value of our wheat grains. Phytic acid is an essential micronutrient that inhibits the absorption of some other essential micronutrient. Phytic acid binds the Iron (Fe), Calcium (Ca), Manganese (Mn), Zinc (Zn), Magnesium (Mg) etc. and inhibits them to be absorbed by the body (Leah *et al*. (2005)

Phytic acid (Myo-inositol hexaphosphate) is a constituent of cereal grain which is abundantly located in the bran. In humans, diets high in phytate (phytic acid) can significantly decrease the absorption to the phytic acid content of the flour (Brune *et al*. 1992). The interaction of phytic acid with protein, vitamins and minerals are important factors which limit the nutritive value of wheat. Phytic acid forms complexes with divalent and trivalent metallic ions, which are not absorbed by gastrointestinal tract (GIT) and decrease the bioavailability of these elements leading to nutritional deficiency diseases (Nutritional disorders). The Iron deficiency causes anemia which results due to poor intake of diet

and mal-absorption of nutrients. The mal-absorption of micronutrients is mainly due to the presence of phytic acid in the diet, which reduces their bioavailability. It has been clearly demonstrated that wheat varieties differ in phytic acid accumulation. Therefore, identification of wheat with relatively low phytic acid would be a step towards development of wheat cultivars with low phytic acid.

Little is known about the phytases of plant seeds in spite of the fact that this group of enzymes is the primary determinant for the utilization of the major phosphate storage compound in seeds, phytic acid (Pedersen (2007).

High density of negatively charged phosphate groups, phytic acid (PA) forms very stable complexes with mineral ions rendering them unavailable for intestinal uptake. Indeed, the first step in mineral absorption requires that the mineral remains in the ionic state. As the PA content of the diet increases, the intestinal absorption of Zinc, Iron and Calcium decreases. Phytates are always present in vegetal matrix composed of fibers, minerals, trace elements and other phyto -micronutrients (Walter *et al*. 2002).

Rice bread is a potential alternative to wheat bread for gluten-sensitive individuals. Incorporation of rice bran into bread made from white rice flour adds flavor but also phytic acid, which can reduce the bioavailability of minerals (Kadan and Phillippy, 2007).

Phytic acid is a potent inhibitor of mineral and trace element absorption occurs in all cereal grains and legume seeds. The possibility to increase phytase activity or reduce the phytic acid content by soaking and germination was investigated in a wide range of grains ands seeds. Germination increased phytase activity 3 to 5-fold in some cereal grains and legume seeds, while the influence on phytic acid content was insignificant in most materials tested. High apparent phytase activity was found in untreated whole grain rye, wheat, triticale, buckwheat, and barley (Szkudelski. 2005).

Human subjects have shown that diets high in phytic acid can cause Zinc deficiency, and that the phytate content is negatively correlated to Zinc absorption. Suboptimal Zinc status has been shown to cause increased morbidity, poor pregnancy outcome, impaired growth, immune competence and cognitive function, emphasizing the need to optimize Zinc bioavailability. (Linnerdal, 2000)

The amount of total P (Phosphorus) in plants increases with increasing plant dry weight. The seed P (phosphorus) content increased sharply from anthesis, and the P (Phosphorus) distribution percentage in the seed reached 35% of the absorbed P (Phosphorus) at the full-maturity stage. The phytate concentration negatively correlated with Ca, Mg, Zn, Mn and Fe concentrations in the seed; however, the total P (Phosphorus) concentrations in the seed correlated positively and the K concentration did not correlate with the phytate concentration. The Ca, Mg, Zn, Mn and Fe accumulated greatly at the early maturity stage of seeds; however, phytate accumulated with the increase in total P (phosphorus) concentration with seed maturity (Saneoka *et al*., 2006).

Keeping in view the importance of Phytic acid as a potent inhibitor for the bioavailability of micronutrients viz Iron (Fe) , Calcium (Ca), Maganase (Mn), Zinc (Zn), Magnesium (Mg) etc, to gastrointestinal tract . Therefore dephytinizatoin of phytic acid is an essential and for the said purpose we will breed wheat varieties with idea to develop low phytate wheat lines in order to decrease the inhibitory effect of phytic acid for the enhanced bioavailability of micronutrients to humans.

MATERIALS AND METHODS

This study was conducted at Research Farm of Khyber Pukhtunkhwa Agricultural University Peshawar, Pakistan during 2008 and 2009. Ten lines were screened for Phytic acid. Uqab (2.2%), Tatara (2.40%), Advanced line-5000, (2.75%) Ghaznavi-98 (1.06%), Saleem-2000 (2.43%), Pirsabak-2004 (1.77%), Fakhre sarhad (1,64%), Pirsabaq-2005 (2.89%), AUP-4606 (3.67%) and AUP-4006 (2.83%). Two contrasting groups (high phytate (Pirsabak-2005 (2.89%), AUP-4606 (3.67%) and AUP-4006 (2.83%) and low phytate (Pirsabaq-2004 (1.77%) and Ghaznavi-98 (1.06%) were identified. The two contrasting groups were crossed in 5 x 5 full diallel fashion. Fifteen to twenty spikes were emasculated and F_1 hybrids were developed. In the succeeding year, F_1 hybrids (20 F_1) along with five parents were sown. Phytic acid content in grain was determined. The experiment was laid down in Randomized Complete Block design (RCBD) with three replications. Each plot was consisted of with one row with a row length of 3 m and row to row and plant to plant distances of 30 cm and 25 were kept respectively. Standard cultural practices including hoeing, weeding, irrigation etc were carried out for the experiment to reduce experimental errors throughout the season. After maturity crops were harvested and Phytic Acid in F_1 and parents was determined.

Phytic Acid

At threshing random samples of grain was drawn from each plot for phytic acid determination. The sensitive method of Haug and Lantzsch, (1983) was adopted for the determination of phytate contents in the whole wheat flour samples.

Determination of Phytic Acid

The sample extract (with 0.2 N HCL) was heated with an acidic Iron-III solution of known Iron content. The decrease in the Iron content was the measure of free phytate in supernatant.

Reagents

Phytate reference solution: Sodium salt of Phytic acid was used for reference. Stock solution was prepared which contained 0.15 grams sodium phytate in 100 ml distilled water. The reference solutions was prepared by diluting the stock solution with HCL in a range from 3 to 30 micro-grams (ug ml^{-1}) phytate phosphorus.

Ferric Solution

Ammonium Iron-III Sulphate. 12 H_2O (0.2 g) was dissolved in 100 ml of 2 N HCL and was made upto 1000 ml with distilled water.

2, 2-Bipyridine Solution

Ten grams of 2, 2-bipyridine and 10 ml of Thioglycolic acid was dissolved in distilled water and the volume was made up-to 1000 ml.

Procedure

The defatted and finally ground flour sample (0.06) was extracted with 10 ml of 0.1 N HCL for 1 hour. Form this extract 0.5 ml was taken into stopper test tube. Ferric solution (1 ml) was added to this and was covered with stopper. These tubes were heated in a boiling water bath for 30 minutes and were allowed to cool at room temperature. 2, 2-biphyridine solution (2 ml) was added to this and was mixed. The absorbance was measured within 4 minutes. A standard curve was made in phytic acid and was determined by the following formula:

Phytic acid = Phosphorus phytate x 4.97

Whereas 4.97 is ratio of phosphorus (Atomic weight) to the phytic acid (Formula weight)

4.97 = Phosphorous Phytic $acid^{-1}$

RESULTS AND DISCUSSION

Wheat genotypes were significantly different for phytic acid contents (Table-1 and Table-2). Phytic acid contents of 25 wheat genotypes (5 parents + 20 Hybrids) were determined. Concentration of Phytic acid in parents ranged from 1.25% to 3.42% while in hybrids genotypes the range of Phytic acid concentration was from 0.81% to 3.91 %. From the results it is clear that wheat genotypes (Hybrids) have a clear cut range of Phytic acid concentration in F1 generation. Since in F1 generation Segregation for a particular trait is rear and generally it appears in F2 and onward generations. Therefore there is dire need to handle the materials in the succeeding generations with the idea to release a low phytate line in wheat. This should be big breakthrough in the improvement of nutritional quality of wheat. Our results from Table 2 show that hybrids genotypes have a potential trend of decreasing concentration of Phytic acid. Our results of Phytic acid concentration are in line with Jabar *et al.* (2007) who also reported differences in Phytate concentration in different wheat varieties and lines. The large variation in phytic acid content among wheat genotypes indicates the possibility of identifying and developing cultivars with low phytic acid contents in caryopses.

Table 1 Phytic Acid levels in different Genotypes of wheat.

S. No.	Genotypes	Phytic Acid %
1	Advanced Line 5006	2.84%
2	Ghaznavi-98	1.06%
3	Saleem 2000	2.43
4	Uqab	2.2%
5	Tatara	2.40%
6	Pir Sabaq 2004	1.77%
7	Fakhre Sarhad	1.64%
8	Pir Sabaq 2005	2.89%
9	AUP 4606	3.67%
10	AUP 4006	2.83%

Table 2. Phytic Acid levels in different crosses of wheat.

S. No.	Genotypes	Phytic Acid %
1	G-98 x AUP 4606	1.5267
2	AUP 4006	3.42
3	AUP 4606 x Ps 2004	2.38
4	Ps 2004 x G-98	2.4833
5	AUP 4006 x Ps 2004	0.8067
6	Ps 2005	2.485
7	AUP 4006 x AUP 4606	2.83
8	Ps 2004 x AUP 4606	2.5333
9	Ps 2004 x Ps 2005	1.46
10	AUP 4606 x Ps 2005	2.3367
11	G-98 x Ps 2005	2.8133
12	AUP 4606 x AUP 4006	2.6567
13	AUP 4606 x Ps 2005	2.55
14	AUP 4606 x G-98	1.63
15	G-98	1.2533
16	AUP 4606	1.61
17	Ps 2005 x AUP 4006	2.83
18	Ps 2004	1.66
19	Ps 2005 x G-98	3.91
20	Ps 2005 x Ps 2004	1.5833
21	Ps 2004 x AUP 4006	2.6033
22	Ps 2005 x AUP 4606	2.7767
23	AUP 4006 x G-98	2.8133
24	G-98 x AUP 4006	3.43
25	G-98 xPs 2004	2.32

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