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Full Length Research Paper

The screening wheat (*Triticum aestivum* L.) plants for moderate phytate content using colorimetric values of semi-grain seeds

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To select wheat cultivars with moderate phytate content, in this experiment the authors found out a new method quickly screening individual plant in huge offspring. Twenty-three wheat cultivars were twice planted at two places. For one part of the seeds harvested, they randomly cut them into two semi-grain seeds one by one, the semi-grain seeds without embryos were assayed by colorimetric values in some micro-test plates, and the semi-grain seeds with embryos were reserved temporarily; whereas for another part seeds, they determined their phytate content and inorganic phosphorus content, respectively. The results indicated that, there were significantly negative correlations (p = 0.01) not only between the phytate content and the inorganic phosphorus content but also between the phytate content and the colorimetric value, whereas there was a significantly positive correlation (p = 0.01) between the inorganic phosphorus content and the colorimetric value. In conclusion, using colorimetric value of one semi-grain seed without embryo, wheat breeders might appraise phytate content of another semi-grain seed with embryo; if the colorimetric value of the semi-grain seeds without embryo is fit for moderate phytate content, the corresponding semi-grain seed with embryo would be reserved for next generation planting.

Key words: Colorimetric method, individual selection, inorganic phosphorus, phytate, semi-grain seed, *Triticum aestivum* L.

INTRODUCTION

Phytate, inositol hexaphosphate, an anti-nutrition factor, has been excreted into the environment leading to phosphorus waste and global phosphorus pollution (Raboy, 2001; Oatway et al., 2001; Turner, et al., 2002). To solve the problem, selecting crop cultivars of low or moderate phytate content might be an efficient solution. However, we are still using the HPLC (High Performance Liquid Chromatography) or HPIC (High Performance Ion Chromatography) as the main methods of assaying phytate content. With these methods reported, it is very difficult to quickly screen a large sample before next wheat breeding season. Breeders need a new method to quickly determine phytate content of individual plants. In this study, the authors applied an assay of the inorganic phosphorus content as a criterion to quickly screen wheat seeds instead of phytate content.

MATERIALS AND METHODS

Materials preparation

Twenty-three wheat cultivars were planted in October at Anhui and in September at Tianjin, China, respectively. The experimental layout was a randomized complete blocks design with three replications. The sowing was done by an experimental drill in 1.0 × 2.0 m plots. The seeding rate was 300 seeds m^{-2} for each plot. Fertilizer application was 90 kg N ha^{-1} and 60 kg P_2O_5 ha^{-1} at

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Table 1. Phytate contents, inorganic phosphorus contents, colorimetric values of twenty three wheat cultivars and their correlation coefficients.

Place and year		PC (mg g^{-1})	IPC (mg g^{-1})	CV	Correlation coefficient		
					PC-IPC	PC-CV	IPC-CV
Anhui, 2004	Range	0.23 - 2.16	0.16 - 2.41	0.05 - 3.78			
	Mean±Sd	0.81±0.26	0.73±0.29	0.94±0.31	-0.7571**	-0.6538**	0.7242**
	F-value	24.22**	27.43**	25.01**			
Anhui, 2005	Range	0.17 - 2.29	0.18 - 2.44	0.07 - 3.83			
	Mean±Sd	0.78±0.34	0.77±0.18	0.96±0.22	-0.7427**	-0.6944**	0.7265**
	F-value	31.32**	18.36**	20.07**			
Tianjin, 2004	Range	0.25 - 2.57	0.12 - 2.30	0.07- 3.83			
	Mean±Sd	0.86±0.21	0.67±0.30	0.84±0.15	-0.7176**	-0.7035**	0.6894**
	F-value	25.43**	36.18**	20.32**			
Tianjin, 2005	Range	0.15 - 2.10	0.14 - 2.66	0.04 - 3.70			
	Mean±Sd	0.74±0.26	0.83±0.34	0.91±0.16	-0.6372**	-0.6687**	0.6744**
	F-value	26.28**	21.29**	17.05**			

(i.) PC: Phytate content, IPC: inorganic phosphorus content, CV: colorimetric value.** showed p = 0.01; (ii.) Anhui (31.4°N and 117.5°E at 240 m above sea level) locates the East in Chine, and Tianjin (41.1°N and 117.3°E at 130 m above sea level) locates the North in China; (iii.) 3×96 seeds from 10 individual plants were randomly selected and assayed by colorimetric values for a three-micro-test-plate trial per plot, so three plots per cultivars were counted as three 'mean's to act as three replications; the residual seeds per plot were mixed and determined by phytate contents and phosphorus contents, there were also three replications according to three plots, respectively; (iv.) The correlation coefficients were counted according to 23 wheat cultivars consisting of three replications (three plots).

planting. Annually, the field materials were divided into two parts, one part, five random individual-plant samples per wheat genotype, was harvested by individual plant to be air-dried in May at Anhui and in June-July at Tianjin, and they were used to assay the colorimetric value of the semi-grain seeds without embryo of six seeds per spike, and another part was collected by plot to be milled into wheat meal (filtered by 60 screen mesh) in order to assay those phytate content and inorganic phosphorus content. The data analyses of the samples were shown in the notes of Table 1.

HPLC analysis of phytate content

Phytate content was determined using the method of Prachuab and Joe (2005).

Assay of inorganic phosphate content

Inorganic phosphorus content was determined as described by Ames (1966) and Xiao et al. (2005).

Colorimetric values determination on semi-grain seeds

A semi-grain seed (one seed cut into two semi-grain seeds with one part having embryo and the other part without embryo, Figure 1) was ground into powder, weighed and transferred into a cell of a 96-cell micro-test plate (Figure 1, route I to II), and 10 µL HCl (0.4 M) mg^{-1} sample (dry weight) was added into the cell, and then the micro-test plate was placed in icebox at 4°C for about 24 h. The next day, 10 µL sample solution of the cell, 90 µL distilled water and 100 µL display reagent (the composition was explained at next paragraph) were extracted to a cell of another micro-test plate in turn, mixed, and then saved at room temperature for one day to be used to compare with criterion phosphorus solutions.

The display reagent (5 mL) contained 1 mL of 3 M H_2SO_4, 1 mL of 2.5% $(NH_4)_6MoO_{24}$ (w/v), 1 mL of 10% L-ascorbic acid (w/v, stored at 4°C) and 2 mL of distilled water, which were instantly confected as they were used.

Standard phosphorus was used as criterion solution. Five 100-µL standard phosphorus solutions with 0.0, 0.54, 1.08, 1.62 and 2.70 µg of KH_2PO_4 per 100 µL distilled water, were put into five cells of a micro-test plate. After this, 100 µL of the display reagent was added to the cells again. The next day, chroma (blue by originally colorized figure) of the cells was directly observed, moreover, they turned thicker and thicker with accretion of criterion phosphorus contents (Figure 1, standard phosphorus). We named colorimetric values of the standard phosphorus solutions by 0, 1, 2, 3 and 4 in turn. Compared with the chroma of the standard phosphorus solutions, all samples might also be scored by 0 to 4 based on identical or adjacent principle. Colorimetric value 0, 1, 2, 3 and 4 contained 0.0, 0.54, 1.08, 1.62 and 2.70 mg of inorganic phosphorus per 1.0 g sample (dry weight), respectively.

Statistical analysis

Data were subjected to analysis of variance (ANOVA), mean comparisons were done using the Newman Keuls test (p = 0.01), and Pearson correlations were also preformed. The statistic software package version 5.0 was used for the analysis.

RESULTS AND DISCUSSION

The results in Table 1 showed that, there were significant

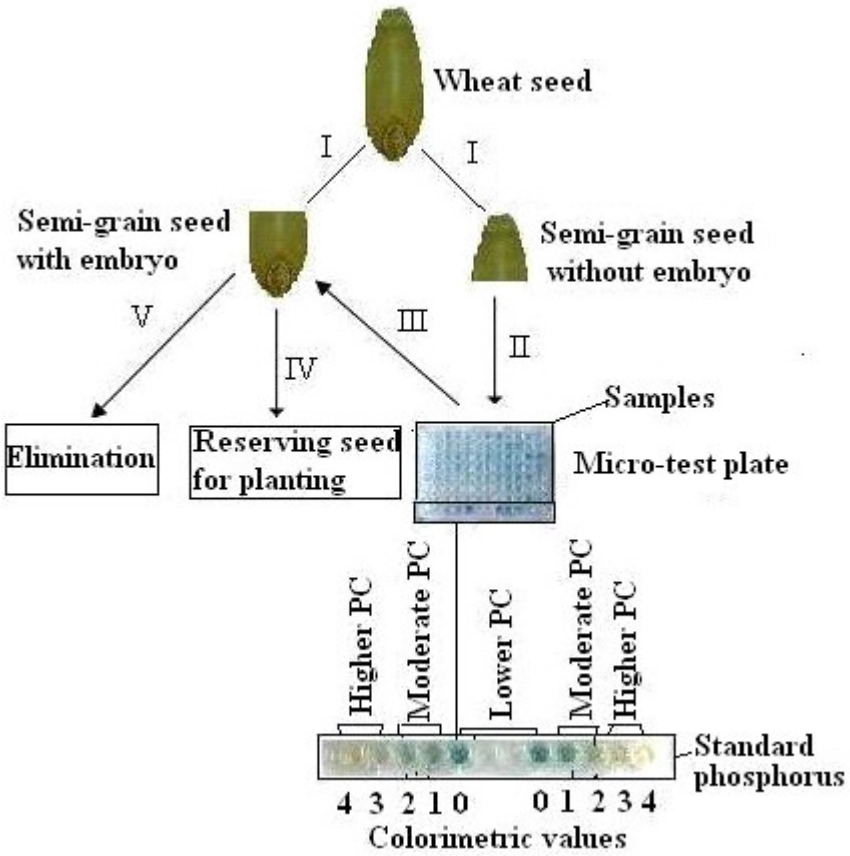

Figure 1. Routes of individual selection for moderate phytate wheat using semi-grain seeds. PC represented phytate content; "I" indicated that one seed was cut into a semi-grain seed with embryo and a semi-grain seed without embryo by knife; "II" indicated that the wholemeal of a semi-grain seed without embryo was placed into a cell of micro-test plate and its colorimetric value was assayed based on the chroma of criterion phosphorus; "III" indicated that the semi-grain seed with embryo was classified as a list of lower, moderate, and higher phytate content seed according to the colorimetric value of the corresponding semi-grain seed without embryo; "IV" and "V" indicated that the semi-grain seeds with embryo of moderate phytate contents might be planted in field, whereas the semi-grain seeds with embryo of lower and higher phytate contents should be eliminated.

($p = 0.01$) negative correlations not only between the phytate contents and the inorganic phosphorus contents but also between the phytate contents and the inorganic phosphorus colorimetric values. There was a significant ($p = 0.01$) positive correlation between the inorganic phosphorus contents and their colorimetric values on all trials. It suggested, the higher the inorganic phosphorus content (Vicky et al., 2007, Raboy et al., 2000) or its colorimetric value from the same seed, the lower the phytate content would be (Xinglin, 2007b); the colorimetric values of wheat seeds could indirectly express their phytate contents.

Also noted that, first, the phytate content of crops is too low to increase their yield (Vicky et al., 2007; Xinglin and Yuexin, 2006); second,, moderate phytate content may be more beneficial on human nutrition and reduce the risk of colon and breast cancer as an anti-oxidant (Evers et al., 1999; Graf and Eaton, 1993) and through control free iron (Thompson and Zhang, 1991). Raboy et al. (2000) classified crop seeds as two criterions of "low" and "normal" phytate. However, our breeding aim will not be to select "low" phytate cultivars but to select "moderate" phytate cultivars. Based on barley phytate content range (Vicky et al., 2007) and wheat phytate content ranges (Xinglin, 2007a), if 0.5 - 1.0 mg phytate per g of crop seeds is moderate content, the colorimetric value 1 or 2 of semi-grain seeds is fit for breeding aim (Figure 1: III, IV and V).

In addition, this method was high efficiency. If he or she need to assay 18 seeds of 3 spikes per individual plant, one micro-test plate could determine 5 individual plants at one times. According to the efficiency, one person could averagely assay 4 plates one day, so she or he could screen 180,000 seeds from 2,000 individual plants in 100

days before next breeding season. At early generation, the efficiency of such an individual selection might well meet the need of selecting modertae phytate cultivars during wheat breeding.

Furthermore, this method only need some simple apparatus to perform it such as a constant temperature boiler and an icebox, and it had an easy operation process, so it was fit for many breeders in the developing countries.

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