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Variabilities of Total and Phytate Phosphorus Contents as well as Phytase Activity in Wheat

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Abstract

In this investigation 17 wheat varieties from four locations (Hayn, Biendorf, Nossen and Forchheim) of the crop years 1995 and 1996 were included. The aim of this work was to analyse the total and phytate phosphorus contents of these varieties as well as their phytase activities, which are important factors for the availability of phosphorus. For all 136 wheat samples the total phosphorus content was 3.74 g/kg DM, a phytate phosphorus content of 2.76 g/kg DM and the ratio phytate/total phosphorus of 74 % were determined. The phytase activity was relatively high with a mean value of 802 U/kg. In this study possible influences of variety, location and crop year on the phosphorus contents and phytase activities were tested. It was possible to detect statistically significant differences caused by variety and crop year for all tested parameters. The location influenced total-P and phytase activity statistically significant.

1 Introduction

Wheat is an important cereal for human nutrition and animal feeding. Phosphorus, calcium, trace elements and protein are important components of wheat. However, phytate complexes, in which ca. 60-70 % of the total phosphorus is bound (PERNOLLET, 1978), may reduce their availability for humans and monogastric animals. They hardly contain any endogeneous phytase and cannot hydrolize phytate phosphorus in the intestinal tract to reach the sufficient phosphorus supply and thus their optimal level of performance. The bound phosphorus is the reason why inorganic phosphorus must be added. It is important to know the contents of total and phytate phosphorus and the native phytase activity in different varieties of wheat to optimize the addition of inorganic phosphorus or microbial phytase, especially since there have not been any systematic investigations so far. The analyses carried out by Jongbloed and Kemme (1990), Lantzsch (1990), Eeckhout and De Paepe (1994) concerning total and phytate phosphorus and by Berk and Schulz (1993), Rodehutscord et al. 1994 and Eeckhout and De Paepe (1994), referring phytase activity include only small numbers of samples from abroad without describing the tested varieties, locations and climatic conditions. Most et al. (1993) as-

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sumed e.g. that the reason for varying values could be found in different methods of selection and analyses. However, there have not been made many systematic experiments concerning variabilities of total, phytate phosphorus contents and phytase activity caused by variety, year or environment.

The aim of this study was to find out possible climatic, genetic and location influences on total and phytate phosphorus contents and phytase activity in wheat as well as effects which could explain the variability of these values. For this reason appropriate investigations into the phosphorus contents (total, phytate-P and phytase activity) of varietally pure sample material from four locations and two crop years were carried out.

2 Materials and Methods

136 samples from 17 varieties in two crop years 1995 and 1996 grown on four locations in Central Germany (Saxony and Saxony-Anhalt) have been chosen to determine their total, phytate phosphorus contents and phytase activity as well as possible influences by variety, location and crop year. The selection was taken according to the different parameters (see tab.1) like different geographic, climatic conditions and locations.

The phosphorus content in soil on four locations was analysed and the differences in phosphorus content were balanced with P-fertilization to reach the same level of soil nutrient provision.

Analytical methods

The content of total phosphorus was analysed according to the methods of VDLUFA (1976/1993). It was measured spectrophotometrically at a wave length of 420 nm (Spectrophotometer Pharmacias "LKB-Ultrospec III") after mineralizing the sampling by breaking down and converting it with Molybdat-Vanadat. The phytate phosphorus content was determined according to the AOAC-method (1990) by the exchange of anions. The phytate phosphorus was extracted out of doubled, dried samplings with diluted 2.5% hydrochloric acid, mixed with EDTA/NaOH solution and put into an anion-exchange-column. Phytate was eluated with 0.7 mol NaCl and broken down by a mixture of concentrated HNO_3 / H_2SO_4 to determine the total-P calorimetrically. The phytase activity was measured following the method described by VDLUFA (1997). The enzyme phytase was mixed with Na-phytate and developed inorganic phytate from that substratum. The addition of acidic Molybdat-Vanadat reagent stopped the incubation and a coloured complex developed with the phosphate.

Statistical analysis

The evaluations of the results as analyses of the variance were made with the help of the programme STATISTICA FOR WINDOWS ™ (Version 5.0, Stat. Soft. INC. 1995). For the

multiple analysis of the variance the Tukey-HSD-Test has been used, while simple comparisons of mean values were made by the t-Test. Significant effects or interactions are shown in the tables by the p-value (level of significance:*=p<0.05;**=p<0.01; ***p<0.001; ns = not significant). Significant differences of the mean values are indicated by different letters within the tables. The standard deviation (±) is also given in the tables. All results were subjected to an analysis of principal components, in order to study them graphically and to study the correlations between analysed criteria.

3 Results and Discussion

The mean total phosphorus content was 3.74 ± 0.31 g/kg DM, the phytate phosphorus content 2.76 ± 0.39 g/kg DM and the ratio phytate/total-P 74 ± 8 %. The range of average values was as follows: for total-P 2.86 - 4.56 g/kg DM, for phytate-P 1.93 - 3.73 g/kg DM and the ratio phytate/total-P 57 - 91 %. These results are little lower than the values reported by Lolas et al.(1976) [total-P: 4.10 g/kg DM; phytate-P: 2.96 g/kg DM], Jongbloed and Kemme (1990) [total-P: 4.10 g/kg DM; phytate-P: 2.90 g/kg DM], Özkaya and Özkaya (1998) [total-P: 3,92 g/kg DM; phytate-P: 2,96 g/kg DM]. The values of this study are in agreement with those ascertained by Lantzsch (1990), who determined total-P with 3.80 g/kg DM; phytate-P with 2.77 g/kg DM and the ratio phytate/total-P: 73 % and by Pierce et al. (1977) with 3.80 g/kg DM total-P and 2.60 g/kg DM phytate-P and the values found by Barrier-Guillot et al. (1996) with 3.64 g/kg DM total-P and 2.18 g/kg DM phytate phosphorus. In contrast to this other authors described lower contents, e.g. Eeckhout and De Paepe (1994) [total-P: 3.30 g/kg DM; phytate-P: 2.20 g/kg DM] or Simons (1981) [total-P: 3.35 g/kg DM; phytate-P: 2.55 g/kg DM].

There were significant varietal differences for total-P only for Aron, Toronto and Zentos in comparison with Tambor which had a lower phosphorus content. The same statistical analysis (table 3) revealed that phytate phosphorus was also influenced by variety, which means that its variability depends on the genotype. The influence of variety on the total phosphorus content was also reported by Carré et al. (1997). The mean value of the phytase activity of the selected samples was 802 ± 189 U/kg. It can be compared with the values given by Berk and Schulz (1993) [815 ± 210 U/kg], by Rodehutscord et al. (1994) [700 U/kg] and by Pointillart et al. (1994) [878 U/kg]. The present investigation showed that the effect of variety on the phytase activity was highly significant (table 3), its variation ranged from 540 to 1360 U/kg. This result is in agreement with Carré et al. (1997).

The variability of total phosphorus and phytase activity can also be caused by location (table 4). Only the phytate phosphorus did not vary significantly on the four different locations. This effect could be directly attributed to the environmental conditions of each location. Barrier-Guillot et al. (1996) also proved a highly significant influence of the location on phytase activity.

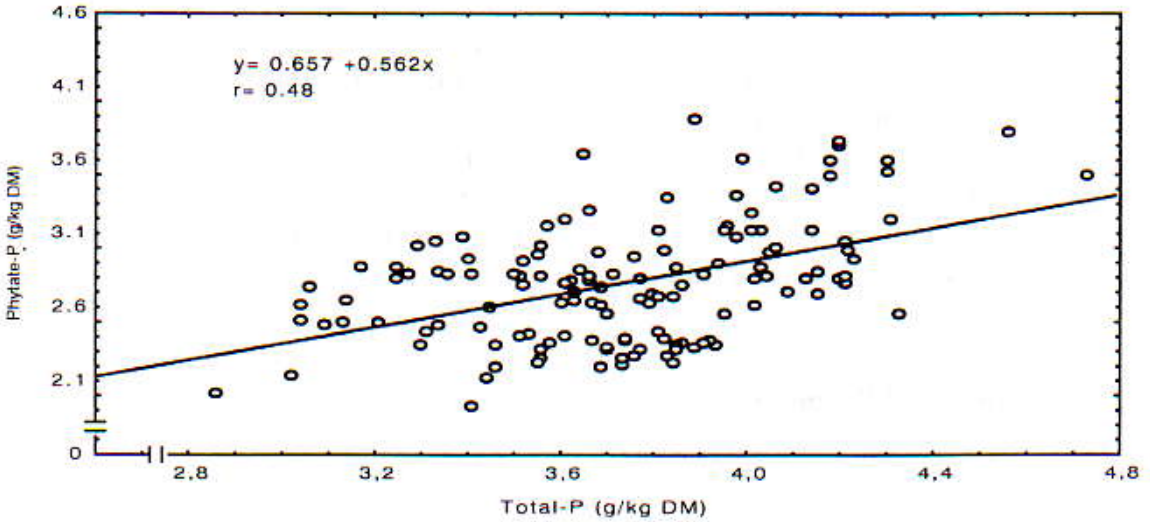

Fig. 1: Correlation between total-P and phytate-P (g/kg DM) in wheat

Table 1: Characteristics of the tested locations

Location	Year	Soil	Valuation index of field	Height above sea-level (m)	P_2O_5 content (mg/100g soil)	Soil nutrient provision	pH-value
Hayn	1995	loam	35-40	441	23	C	6.4
	1996				13	B	7.4
Biendorf	1995	loam	92-96	75	14	B	7.1
	1996				47	D	6.3
Forchheim	1995	sandy-loam	33	565	26	C	5.7
	1996				12	B	5.7
Nossen	1995	loam	65	225	24	D	6.1
	1996				17	C	5.6

Table 2: Climatic conditions in the crop years (1995 and 1996)*

Location	Average precipitation (mm)			Average temperature (°C)		
	long-term average**	1994/1995	1995/1996	long-term average**	1994/1995	1995/1996
Hayn	502	675	399	6.3	6.1	6.4
Biendorf	324	359	423	8.5	10.4	7.1
Forchheim	810	946	936	6.1	6.8	4.8
Nossen	584	793	713	8.0	8.4	7,0

*October - August **Over 10 years

Table 3: Total phosphorus, phytate phosphorus contents and phytase activity of 17 wheat varieties

	Total-P		Phytate-P		Phytate-P/ Total-P		Phytase activity		
	(g/kg DM)				(%)		(U/ kg)		
Aron	3.99^{b}	± 0.38	2.90^{ab}	± 0.36	73	± 6	814	bcde	± 119
Batis	3.80^{ab}	± 0.24	2.69^{ab}	± 0.21	71	± 4	772	abcde	± 99
Bussard	3.74^{ab}	± 0.18	2.64^{ab}	± 0.22	71	± 3	713	abcd	± 76
Contra	3.76^{ab}	± 0.26	2.69^{ab}	± 0.43	72	± 8	794	abcde	± 174
Ebi	3.73^{ab}	± 0.26	2.80^{ab}	± 0.23	75	± 8	859	bcde	± 181
Estica	3.76^{ab}	± 0.33	2.78^{ab}	± 0.28	74	± 4	758	abcd	± 82
Herzog	3.73^{ab}	± 0.58	2.98^{ab}	± 0.48	80	± 8	741	abcd	± 150
Jonas	3.78^{ab}	± 0.25	2.76^{ab}	± 0.14	73	± 6	529	a	± 29
Moldau	3.81^{ab}	± 0.30	2.87^{ab}	± 0.36	75	± 6	726	abcd	± 140
Pegassos	3.66^{ab}	± 0.25	2.64^{ab}	± 0.32	72	± 9	882	bcde	± 143
Piko	3.64^{ab}	± 0.38	2.61^{ab}	± 0.28	72	± 7	978	de	± 188
Ritmo	3.56^{ab}	± 0.29	2.64^{ab}	± 0.34	74	± 7	1044	e	± 197
Tambor	3.37^{a}	± 0.22	2.30^{a}	± 0.17	68	± 6	924	cde	± 165
Tarso	3.48^{ab}	± 0.19	2.51^{a}	± 0.27	72	± 8	615	ab	± 78
Toronto	3.99^{b}	± 0.23	2.98^{ab}	± 0.37	75	± 8	683	abc	± 154
Transit	3.89^{ab}	± 0.35	3.12^{b}	± 0.44	80	± 9	971	de	± 135
Zentos	3.93^{b}	± 0.22	3.08^{b}	± 0.43	78	±11	834	bcde	± 158
Mean val.	3.74 ± 0.31		2.76 ± 0.39		74 ± 8		802		± 189
Min.-Max.	3.04 - 4.56		1.93 -3.73		*57 - 92*		520-1390		
p-value	**		**		*0,70 ns*		***		

Table 4: Influence of the location on total phosphorus, phytate phosphorus and phytase activity

Location	Total-P (g/kg DM)	Phytate-P (g/kg DM)	Phytate-P of Total-P (%)	Phytase activity U/kg
Hayn (n=34)	3.94^{b} ±0.19	2.75 ±0.48	70^{a} ± 9	891^{b} ±211
Biendorf (n=34)	3.66^{a} ±0.27	2.62 ±0.37	72^{ab} ± 5	757^{a} ±159
Forchheim (n=34)	3.77^{b} ±0.26	2.85 ±0.38	76^{bc} ± 7	737^{a} ±157
Nossen (n=34)	3.59^{a} ±0.21	2.83 ±0.33	79^{c} ±7	823^{ab} ±167
p-value	***	*0.08*	***	**

Tab. 5: Mean total phosphorus, phytate phosphorus contents and phytase activities of the crop years 1995 and 1996

Crop year	Total phosphorus (g/kg DM)	Phytate phosphorus (g/kg DM)	Phytate phosphorus (% total phosphorus)	Phytase activity U/kg
1995 (n= 68)	3.62a ±0.37	2.86a ±0.40	79a ±9	752a ±169
1996 (n = 68)	3.87b ±0.24	2.66b ±0.37	69b ±8	853b ±208
p-value	***	**	***	***

Besides an influence of the crop year on total phosphorus, phytate phosphorus, on its portion in total phosphorus and on phytase activity could be statistically determined. There was a remarkable difference between the mean values ascertained for 1995 and 1996 (see table 5). The reason was probably that the two crop years were very different concerning the amount of precipitation and the time of dry and wet periods. NAHAPETIAN and BASSIRI (1976) tested the influence of the crop year on total and phytate-P and found significant differences as well.

The relation between total phosphorus and phytate phosphorus of all samples (n = 136) in both crop years was a positive, significant correlation (fig.1). The function of correlation means that the phytate phosphorus increases by 0.56 g/kg DM when the total phosphorus content rises by 1 g/kg DM. The correlation coefficient of r = 0.48 can be compared with the coefficient of r = 0.56 found by CASADO et al. (1993) [n=55] and BARRIER-GUILLOT et al. (1996) [n=56].

4 Conclusions

This study showed that there are significant effects of genotype and crop year on both total and phytate phosphorus contents. There are relatively high phytase activities in the different varieties of wheat which can vary considerably. Besides an influence of the variety one could also ascertain influences of location and crop year. However one should carry out further studies to prove the effects caused by environmental conditions. The investigations should include other cereals as well.

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6 Zusammenfassung

Variabilität von Gesamt- und Phytatphosphorgehalt sowie Phytaseaktivität in Weizen

Für diese Untersuchung wurden 17 mitteldeutsche Weizensorten von vier Standorten in den Erntejahren 1995 und 1996 hinsichtlich ihres Gesamt-, Phytataphosphorgehaltes und ihrer Phytaseaktivität ausgewählt. Es sollte analysiert werden, ob auftretende Variabilitäten der Gesamt- und Phytatphosphorgehalte bzw. der Phytaseaktivität sorten-, standort- oder witterungsbedingt sind.

Folgende Ergebnisse der vorliegenden Analyse lassen sich zusammenfassen:

- Für die Erntejahre 1995 und 1996 wurden drei bzw. vier Weizensorten ermittelt, die hinsichtlich ihrer Gesamt- bzw. Phytatphosphorgehalte von den übrigen Sorten stärker abwichen. Diese Differenzen konnten als statistisch signifikante Unterschiede gesichert werden.
- Der Standort beeinflußt den Gesamtphosphorgehalt hoch signifikant und hatte eine ebenso starke Wirkung auf den prozentualen Anteil des Phytat-P am Gesamt-P. Der Phytatphosphorgehalt wurde durch den Standort nicht signifikant verändert.
- Auf die native Phytaseaktivität hatte der Standort ebenfalls einen statistisch signifikanten Einfluss.
- Die klimatischen Bedingungen, die in den beiden in die Untersuchung einbezogenen Erntejahren sehr unterschiedlich waren, hatten statistisch signifikante Einflüsse auf Gesamt-, Phytatphosphorgehalt, den prozentualen Anteil von Phytat-P am Gesamt-P und auf die Phytaseaktivität.

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