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THE LEVELS OF Ca, Mg AND PHYTATE PHOSPHORUS PRESENT IN SOME X TRITICOSECALE WITTMACK WITH AEGILOPS SP. HYBRIDS AND THEIR TRITICALE PARENTAL FORMS TRITICALE PARENTAL FORMS

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Abstract

The investigations aimed at the determination of total and phytate phosphorus content as well as concentration of Ca and Mg bound in the phytic acid complexes in hybrid kernels of triticale forms with *Aegilops* sp. and of triticale parental components. The research objects consisted of kernels of 4 strains, of which 2 had *Aegilops* sp. as a maternal form and triticale: *Ae. crassa* 4x x(Panda x Dańkowskie Złote) and *Ae. juvenalis* 6x x [(Lanca x L 506/79)x CZR 142/79] as a paternal form, whereas 2 other strains were obtained by reciprocal crossbreeding:[(Jana x Tempo) x Jana]x *Ae. juvenalis* 6x. The highest total phosphorus content occurred in triticale kernels of (Jana × Tempo) × Jana as well as in two hybrid strains created on the basis of this form (strains No 6 and 7); higher level was found in a strain of the previous generation. The percentage of phytic acid phosphorus in the total phosphorus contained in the kernels varied from 32.8 up to 69.4%. Among the parental components we compared, triticale (Lanca x L 506/79) x CZR 142/79 was characterized by the highest phytate phosphorus percentage in the total phosphorus, which was not confirmed in the hybrid kernels. The phytate Mg share in kernels of the hybrid strains appeared higher compared to the parental components, except no 5 cross combination *Ae. juvenalis* 6x x [(Lanca x L 506/79)x CZR 142/79]. As for Ca, the kernels of the strains were characterized by a lower content of this element in the phytate complexes compared with the parental forms. Among the strains analyzed, no 4, a cross-combination of *Ae. Cras-*

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sa 4x × (Panda × Dańkowskie Złote) and No 6 – [(Jana × Tempo) × Jana] × *Ae. Juvenalis* 6x, deserves special attention as its kernels contained higher Ca and Mg content, lower phytate phosphorus level and more advantageous Ca/P tot and Mg/P tot ratios.

Key words: X *Triticosecale* Wittmack, X *Triticosecale* Wittmack with *Aegilops* sp. hybrids, phytate phosphorus, phytate complexes calcium and magnesium.

POZIOM Ca, Mg I FOSFORU FITYNOWEGO W WYBRANYCH MIESZAŃCACH PSZENŻYTA Z KOZIEŃCAMI I ICH PSZENŻYTNICH FORMACH RODZICIELSKICH

Abstrakt

W badaniach określono zawartość fosforu ogólnego, fitynowego oraz poziom Ca i Mg związanego w kompleksach fitynowych w ziarniakach mieszańcowych form pszenżyta z kozieńcami oraz pszenżytnich komponentów rodzicielskich. Obiektem badań były ziarniaki 4 rodów, w tym 2, w których formą mateczną był kozieniec, a ojcowską pszenżyto: *Ae. crassa* 4x x (Panda x Dańkowskie Złote) i *Ae. juvenalis* 6x x [(Lanca x L 506/79)x CZR 142/79] oraz 2 rody otrzymane w wyniku krzyżowania odwrotnego: [(Jana x Tempo)x Jana]x *Ae. juvenalis* 6x. Największą zawartość fosforu ogólnego stwierdzono w ziarniakach pszenżyta (Jana x Tempo) x Jana i dwóch rodach mieszańcowych powstałych w oparciu o tę formę (rodach nr 6 i 7); wyższy poziom wykazano w rodzie wcześniejszej generacji. Udział fosforu fitynowego w całkowitym fosforze zawartym w badanych ziarniakach był zróżnicowany od 32,8 do 69,4%. Z porównywanych komponentów rodzicielskich pszenżyto (Lanca x L 506/79)x CZR 142/79 odznaczało się największym udziałem fosforu fitynowego w odniesieniu do całkowitego, co nie znalazło potwierdzenia w ziarniakach mieszańcowych. Udział Mg fitynowego w ziarniakach badanych rodów mieszańcowych był większy w porównaniu z komponentami rodzicielskimi, z wyjątkiem nr 5 kombinacji krzyżówkowej *Ae. juvenalis* 6x x [(Lanca x L 506/79)x CZR 142/79]. W ziarniakach badanych rodów, w porównaniu z formami rodzicielskimi, stwierdzono mniejszy udział Ca w kompleksach fitynowych. Spośród analizowanych rodów na uwagę zasługują nr 4 kombinacji krzyżówkowej *Ae. crassa* 4x x (Panda x Dańkowskie Złote) i nr 6 [(Jana x Tempo)x Jana]x *Ae. juvenalis* 6x], w których ziarnie stwierdzono większą zawartość Ca i Mg oraz mniejszy udział fosforu fitynowego, a także korzystniejsze wskaźniki Ca/P całkowitego i Mg/P całkowitego.

Słowa kluczowe: pszenżyto, mieszańce pszenżyta z kozieńcami, fosfor fitynowy, kompleksy fitynowe wapnia i magnezu.

INTRODUCTION

In cereal grains and seeds of leguminous crops or oil plants, 2/3 of total phosphorus is made up of phytates, (phytic acid salts) (myo-inositol hexakisphosphate IP6), which are accumulated chiefly in the aleuron grains and globoids to be utilized by plants during germination as a source of phosphorus and some other elements, such as Mg, K, Ca, Mn, Fe and Zn (HARALD, MORIS 1995, TROSZYŃSKA et al. 1992).

Phytate complexes are poorly available to monogastric animals and possess several antimetabolic properties. Owing to its chemical structure, phytic acid demonstrates high affinity for polyvalent cations. Being a potent chelator of macro- and microelements, this acid substantially reduces the availability of nutrients to monogastric animals, thus effectively limiting their resorption from the digestive tract (HURRELL 2003, LOPEZ et al. 2000). Phytic acid strongly binds to important mineral nutrients such as iron and zinc, forming salts which are largely excreted. Phytic acid-derived P in animal waste can contribute to water pollution, a significant problem in the world (SHARPLEY et al. 1994). Because phytic acid is a chelator of various chemical components and inhibits some digestive enzymes, it lowers the availability of protein, starch and minerals essential for the proper functioning of the animal organism (HARALD, MORIS 1995, HEINDL 2000, HURRELL 2003). With respect to poultry and swine production, since much of grain P is phytic acid P and as such it is excreted, in order to provide for animals' nutritional requirement for P and sustain optimal productivity, animal feeds must be supplemented with either an available form of P or the enzyme phytase (HEINDL 2000, RUTHERFURD et al. 2004, TROSZYŃSKA et al. 1992). As one possible approach to solving the problems associated with seed-derived dietary phytic acid, the U.S. Department of Agriculture and other institutions have isolated cereal and legume low-phytic acid mutations and have used them to breed first-generation low-phytate hybrids, cultivars and lines. Seed phytic acid is reduced in these crops by 50-95% (LARSON et al. 2000, RABOY et al. 2000, RABOY 2002).

The genetic breeding research conducted on triticale hybrids with *Aegilops* sp. aim at obtaining genotypes of a far wider economic importance than the initial forms. Some new forms developed through transfer of beneficial genes from *Aegilops* sp. to triticale and selection may improve yield components, technological value, plant resistance to take-all and root diseases, rusts and powdery mildew as well as their adaptability to soil acidity (ACHREMOWICZ et al. 2002, ARSENIUK et al.1998, GRUSZECKA et al. 1996a,b, GRUSZECKA et al. 2004, MASŁOWSKI et al. 1997, STRZEMBICKA, GRUSZECKA 1997).

Introduction of new triticale forms to crop production requires performing qualitative analyses of grains, including the content of phytate phosphorus and phytates, the compounds considered as antinutritional ones.

The objective of the present research was to determine the phytic acid content and the concentrations of Ca, Mg bound in the phytate complexes in relation to the total content of these minerals in hybrid kernels of the triticale strains with *Aegilops* sp. and their triticale parental components.

MATERIAL AND METHODS

The research material comprised kernels of 4 hybrid strains obtained through distant or wide hybridization of triticale with *Aegilops* sp. and their triticale parental components. The experiments were performed on two strains of cross combinations *Ae. crassa* 4x x (Panda x Dańkowskie Złote) (No 4) and *Ae. juvenalis* 6x [(Lanca x L 506/79) x CZR 142/79] (No 5) with *Aegilops* as a maternal form, and triticale as a paternal one, and two other strains of different generations of a combination [(Jana x Tempo) x Jana] x *Ae. juvenalis* 6x (nos 6 and 7) obtained through a reciprocal cross (Table 1). The hybrids strains were developed by two- or threefold back pollination of F1 hybrids with respective triticale parental form (B_2, B_3 or B_4) to be afterwards four- or fivefold reproduced by self-pollination (F_4 or F_5, respectively). In the subsequent generations, positive selection was performed including resistance to pathogens and environmental stressors followed by negative selection regarding some unfavorable traits of wild-*Aegilops* forms.

Table 1

Research material

Sample No	Forms	Generation
	triticale	
1	Panda x Dańkowskie Złote	-
2	(Lanca x L 506/79) x CZR 142/79	-
3	(Jana x Tempo) x Jana triticale hybrids with *Aegilops* sp:	-
4	*Ae. crassa* 4x x (Panda x Dańkowskie Złote)	B_4/F_5
5	*Ae. juvenalis* 6x x [(Lanca x L 506/79) x CZR 142/79]	B_4/F_5
6	[(Jana x Tempo) x Jana] x *Ae. juvenalis* 6x [1]	B_2/F_4
7	[(Jana x Tempo) x Jana] x *Ae. juvenalis* 6x [2]	B_3/F_5

After the selection, the kernels were examined for the total phosphorus content by the spectrophotometric method (λ=365 nm) (Shimadzu Corp. 160 A) after the phosphorus and ammonium molybdate complex color had been developed in the presence of sulfuric acid.

For determination of phytic acid, the samples were extracted with trichloroacetic acid (TCA) and prepared for determination of the Fe content according to the method of LATTA, ESKIN (1980).

The level of minerals (Mg, Ca) in phytates complexes after extraction with trichloro acetic acid was determined by the atomic absorption spectrometry (AAS) method (Unicam 939).

All the data were expressed on the dry weight basis.

RESULTS AND DISCUSSION

Our previous studies on triticale hybrids with *Aegilops* had demonstrated that an exogenous phytase additive to the investigated triticale forms, under *in vitro* conditions, increased a level of phosphorus released from the phytate complexes. The most favourable dephosphorilation effects were obtained during 5-hour incubation at pH 3 (MAKARSKA, GRUSZECKA 2001).

In the present research, the highest total phosphorus content was determined in the kernels of triticale (Jana x Tempo)x Jana and two hybrid strains (No 6 and 7) formed on the basis of this triticale. It is noteworthy that a higher phosphorus concentration was recorded in strain no 6 of the previous generation as compared to strain no 7 (Table 2).

Table 2

Content of total and phytate phosphorus in kernels of triticale hybrids with *Aegilops* sp. and their triticale parental forms

Sample No	Forms	Phosphorus total (%)	Phosphorus phytate (mg·g-1)	Phosphorus phytate/ /Phosphorus total (%)
	triticale			
1	Panda x Dańkowskie Złote	0.287±0.004	1.429±0.012	49.79
2	(Lanca x L 506/79) x CZR 142/79	0.234±0.008	1.396±0.009	59.65
3	(Jana x Tempo) x Jana triticale hybrids with *Aegilops* sp	0.377±0.008	1.336±0.005	35.43
4	*Ae. crassa* 4x x (Panda x Dańkowskie Złote)	0.217±0.010	1.507±0.011	69.44
5	*Ae. juvenalis* 6x x [(Lanca x L 506/79) x CZR 142/79]	0.358±0.007	1.512±0.007	42.23
6	[(Jana x Tempo) x Jana] x *Ae. juvenalis* 6x [1]	0.375±0.005	1.232±0.005	32.85
7	[(Jana x Tempo) x Jana] x *Ae. juvenalis* 6x [2]	0.360±0.004	1.468±0.003	40.78

The content of phytate in the grain of the test triticale strains ranged from 1.232 mg·g^{-1} (sample 6) to 1.512 mg·g^{-1} (sample 5). In comparison with phytate in wheat grain, these results were nearly fivefold lower (HERNANDEZ et al. 2003).

The percentage of phytate phosphorus in total phosphorus varied in kernels of the test forms from 32.85 up to 69.44% (Table 2). The values were lower or close to those reported by MATYKA et al. (1993), who studied numerous triticale cultivars. Among the parental components we compared, Lanca x L 506/79) x CZR 142/79 triticale distinguished itself by the highest phytate phosphorus content in total phosphorus. However, this result was not confirmed for the hybrid strain kernels. In hybrid strains nos 6 and 7, the phytate phosphorus percentage was close to a level recorded in the triticale parental form. It should be emphasized that a higher phytate content was determined in the kernels of the later generation (strains nos 6 and 7), whereas in grain of strain no 4 (*Ae. crassa* 4x x (Panda x Dańkowskie Złote) a substantially higher phytate phosphorus level was noticed versus the parental form: Panda x Dańkowskie Złote.

Evaluation of the mineral components in the examined triticale hybrid strains, presented in our previous paper, indicated a beneficial influence of the wild forms of *Ae. crassa* 4x and *Ae. juvenalis* 6x on their level and univalent/divalent cations ratio shown in the hybrid strains (MAKARSKA, GRUSZECKA 2003).

Generally, the phytate Mg content in kernels of the strains researched in this work proved higher as compared to the respective triticale parental components (except strain no 5) – Table 3, while the Ca concentration was usually lower in the phytate complexes of the strains investigated than in their triticale parental components (except strain no 5).

Among the strains analyzed, attention should be given to strains nos 4 and 6, whose grains contained more Ca and Mg but less phytate phosphorus and had superior indices of Ca/P total and Mg/P total compared to the respective triticale parental forms (Tables 3 and 4).

Table 3

Content of Ca and Mg in phytate complexes in kernels of triticale hybrids with *Aegilops* sp. and their triticale parental forms

Sample No	Ca ($g \cdot kg^{-1}$)	Phytate Ca ($mg \cdot g^{-1}$)	Phytate Ca /Total Ca (%)	Mg ($g \cdot kg^{-1}$)	Phytate Mg ($mg \cdot g^{-1}$)	Phytate Mg /Total Mg (%)
1	0.22	0.0254	11.54	1.32	34.04	13.33
2	0.24	0.0258	10.75	1.42	42.59	15.35
3	0.27	0.0259	9.59	1.60	31.70	10.25
4	0.25	0.0219	8.75	1.41	41.17	14.96
5	*0.24*	0.0252	9.00	1.49	33.84	11.74
6	0.31	0.0283	9.13	1.74	35.37	10.49
7	0.25	0.0248	9.93	1.50	35.87	12.33

Table 4

Percentage of Ca and Mg to total and inorganic phosphorus in kernels of triticale with *Aegilops* sp. hybrids and their triticale parental forms

Sample No	P inorganic/P total (%)	Ca/P total	Ca/P inorganic	Mg/ P total	Mg/P inorganic
1	50.17	0.07	0.15	0.46	0.92
2	40.17	0.10	0.26	0.61	1.51
3	64.46	0.07	0.19	0.42	0.66
4	30.41	0.12	0.38	0.65	2.13
5	57.82	0.07	0.12	0.42	0.72
6	59.73	0.08	0.14	0.46	0.78
7	59.17	0.07	0.12	0.42	0.70

CONCLUSION

1. Strains of triticale with *Aegilops* sp. hybrids, as compared to parental components, were usually characterized by increased Ca and Mg content in grain as well as lower Ca and higher Mg concentration in phytate complexes.

2. Kernels of cross combinations between *Ae. crassa* 4x x (Panda x Dańkowskie Złote) [B_4/F_5] and [(Jana x Tempo)x Jana] x *Ae. juvenalis* 6x [B_2/F_4] distinguished themselves by a higher Ca and Mg content alongside lower phytate phosphorus and more favourable Ca/P total and Mg/P total ratio.

3. Distant or wide hybridization of triticale and *Aegilops* sp.may yield hybrids of more beneficial nutritional parameters compared to their triticale parental components.

REFERENCES

ACHREMOWICZ B., GRUSZECKA D., KORNARZYŃSKI K., KULPA D., PIETRUSZEWSKI S. 2002. *Vigor variability in hybrid kernels of triticale with Aegilops under the influence of biostimulation.* Int. Agrophysics, 16 (2): 91- 96.

ARSENIUK E., GRUSZECKA D., TARKOWSKI C. 1998. *Analysis of Stagonospora nodorum blotch resistance in hybrids of triticale, wheat, rye, Aegilops sp., Agrotriticum sp. and Dasypyrum sp*. Proc. of the 4th Int. Triticale Symp., 26-31 July 1998, Red Deer, Alberta, Canada, 1: 303-311.

GRUSZECKA D., TARKOWSKI C., STEFANOWSKA G., MARCINIAK K. 1996a. *The influence of parental components on amino-acid composition of Aegilops sp. with X Triticosecale Wittmack hybrids.* 10th Int. Cereal and Bread Congress. „Cereals 96. The source and the future of civilization". Porto Carras (Chalkidiki), Greece, 9-12 June 1996, Book of Abstracts, H 14, 174.

GRUSZECKA D., TARKOWSKI C., STEFANOWSKA G., MARCINIAK K. 1996b. *Cytological analyses of F_1 Aegilops hybrids with Triticosecale. Triticale: today and tomorrow.* Guedes Pinto (eds.). Developments in Plant Breeding, Kluwer Academic Publishers. 5: 195-202.

GRUSZECKA D., KULPA D., MAKARSKA E. 2004. *Evaluation of grain technological quality of X Triticosecale Wittmack with Aegilops sp. hybrids.* Int. Agrophysics, 18 (2): 127-132.

HARALD B.,F., MORIS E., R. 1995. *Phytate: a good or a bad food component?* Nutrit. Res., 15: 733-754.

HEINDL U. 2000. *The different aspects of applying phytase to animal feed. Phytase in animal nutrition*. Proc. of the Intern. Symp. on Phytase in Animal Nutrition., 11-20.

HERNANDEZ M., SOUSA V., MORENO A., VILLAPANDO S., LOPEZ-ALARCON M. 2003. *Iron bioavailability and utilization in rats are lower from lime-treated corn flour than from wheat flour when they are fortified with different sources of iron*. J. Nutr., 133: 154-159.

HURRELL R.F. 2003. *Influence of vegetable protein sources on trace element and mineral bioavailability*. J. Nutr., 133: 2973-2977.

LARSON S.R. RUTGER J.N., YOUNG K. A., RABOY V. 2000. *Isolation and genetic mapping of a non-lethal rice low phytic acid mutation.* Crop. Sci., 40: 1397-1405.

LATTA M., ESKIN M. 1980. *A simple and rapid colorimetric method for phytate determination.* J. Agric. Food Chem., 28: 1313-1315.

LOPEZ H.W., VALLERY H., LEVRAT-VERNY M.,COUDRAY CH., DEMIGNE CH., REMESY CH. 2000. *Dietary phytic acid and wheat bran enhance mucosal phytase activity in rat small intestine*. J. Nutr., 130: 2020-2025.

MAKARSKA E., GRUSZECKA D. 2000. *Wpływ pszenżytnich form rodzicielskich na zawartość mineralnych składników pokarmowych w mieszańcowych ziarniakach X Triticosecale Wittmack z Aegilops sp.* Biul. Magnezol., 5 (3): 171-176.

MAKARSKA E., GRUSZECKA D. 2001. *Badanie trwałości kompleksu fitynowego w warunkach in vitro w mieszańcowych ziarniakach pszenżyta z kozieńcami.* T. HABER, H. PORZUCEK, (red.) Wyd. PAN, Warszawa.

MASŁOWSKI J., GRUSZECKA D., TARKOWSKI C. 1997. *Tolerancyjność mieszańców pszenżyta z kozieńcami na toksyczne jony glinu*. Zesz. Nauk. AR Szczecin. Rol., 65 (175): 255-258.

MATYKA S., BOGUSZ G., KOROL W. 1993. *Fityniany w ziarnie zbóż, nasionach roślin strączkowych i rzepaku „oo"*. Biul. Inf. Przem. Pasz., 32 (1): 37-43.

RABOY V., GERBASI P.F., JUNG K.A., STONEBERG S.D., PICETT S.G., BARMAN A. T., MURTHY P.P., SHERIDAN W.F., TREL D. S. 2000. *Origin and seed phenotype of maize low phytic acid 1-1 and low phytic acid 2-1.* Plant Physiol., 124: 355-368.

RABOY V. 2002. *Progress in breeding low phytate crops*. J. Nutr., 132: 5035-5055.

RUTHERFURD S.M., CHUNG T.K., MOREL P.C.H., MOUGHAN P.J. 2004. *Effect of microbial phytase on ileal digestibility of phytate phosphorus, total phosphorus and amino acids in a low-phosphorus diet for broilers*. Poult. Sci., 83: 61-68.

SHARPLEY A.N., CHARPA S.C., WEDEPOHL R., SIMS J.Y., DANIEL T.G., REDDY K.R. 1994. *Managing agricultural phosphorus for protection of surface waters: issues and options*. J. Environ. Qual., 23: 437-451.

STRZEMBICKA A., GRUSZECKA D. 1997. *Characteristics of X Triticosecale Wittmack with Aegilops sp. hybrids with respect to their reaction to leaf rust.* J. Appl. Genet., 38 B: 129-134.

TROSZYŃSKA A., HONKE J., ZDUŃCZYK Z. 1992. *Fityniany w surowcach roślinnych.* Cz. 1. *Właściwości chemiczne fitynianów oraz sposoby ich usuwania.* Przem. Spoż., 3: 78-81.