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Possibility of utilization of two *Aegilops* sp. to enhance the nutritive value of triticale

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ABSTRACT

Study was undertaken to determine the possibility of improving the nutritive value of triticale by crossing it with two species of *Aegilops*. Materials for this study comprised of 5 triticale recombinants with *Aegilops crassa* Boiss 4x and *Aegilops juvenalis* (Thell) and 4 parental triticale forms. Parental species of *Aegilops* were not analysed because of difficulties in obtaining the needed quantity and quality of the grains. As compared to parental triticale forms the recombinants of triticale with *Aegilops* sp. characterized with a greater thousand kernel weight and volume weight, higher content of protein and dietary fibre, especially of its soluble non-starch polysaccharides fraction. *Aegilops* sp. had not a negative effect on digestibility of protein. The higher content of protein in triticale recombinants resulted however, in poorer values of protein quality indicators, such as lysine content, chemical score, essential amino acid index, biological value and net protein utilization. The other qualitative traits in hybrids did not differ from that received for parental forms. This preliminary study has shown that *Aegilops crassa* might be utilized for the improvement of triticale grain designated rather for food production, while *Aegilops juvenalis* for feeding purposes. Selection of appropriate triticale forms it seems also very important in this respect.

KEY WORDS: triticale, *Aegilops* sp., chemical composition, dietary fibre, nutritive value

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INTRODUCTION

Qualitative cereals are becoming more and more important as well as desired products for intensively developing various branches of cereal industry. These facts open new challenges for breeders to create cultivars that will satisfy specific requirements for different end-uses of cereals. In nutrition, cereals besides being a main source of energy and a substantial source of protein, constitute also the important source of bioactive components such as dietary fibre, phenolics, sterols, tocols, vitamins, minerals, all having positive effects on human health (Liu, 2007; Topping, 2007). Many breeding programmes presently conducted world-wide have been carried out to select or develop cereals with changes in the content of arabinoxylans, β-glucans, i.e. the main constituents of dietary fibre, or amylose as the precursor of resistant starch formation during heat processing (Monari et al., 2005; Saulnier et al., 2007). In contrary to human nutrition, dietary fibre is considered as anti-nutrient for monogastric animals, especially their soluble components due to high viscous properties when in aqueous solution (Choct and Annison, 1990). It has been well known that high viscosity of ileum digesta is associated with low digestibility and low availability of all nutrients and in consequence with poor feed utilization and decreased body weight (Bedford et al., 1991; Langhout et al., 2000). Triticale up to now is almost exclusively utilized as feed for animals but it has also a great potential to be exploited as an alternative cereal in human nutrition (Boros, 2006; Marciniak et al., 2006). Further improvement its nutritive value is thus highly important.

Various *Aegilops* species, such as *Ae. ventricosa*, *Ae. speltoides* L. *Ae. squarrosa* L., *Ae. umbellulata* Zhuk., *Ae. comosa* Sibth. et Sm. and *Ae. markgrafii* L. are widely exploiting as new genetic donors of agronomically important traits. Using these wild species, throughout distant hybridization many wheat breeding lines have been developed with the improved resistance to powdery mildew and various types of rust diseases (Delibes et al., 1987; Kerber and Dyck, 1990; Strzembicka and Gruszecka, 1997). Quality characters are also targeted, such as high molecular weight glutenins present in *Ae. umbellulata* or *Ae. tauschii,* utilized for improving bread-making quality of wheat (Liu et al., 2003). The impact of *Aegilops* on the content of dietary fibre and its major constituents has not been studied yet in their recombinants with triticale. No information is also available on the nutritive usability of such hybrids.

Aim of the study was to determine the possibility of using two *Aegilops* species, *Ae. crassa and Ae. juvenalis*, to enhance the nutritive value of triticale.

MATERIAL AND METHODS

Material comprised of three recombinants of triticale with *Aegilops crassa* Boiss 4x, two with *Aegilops juvenalis* (Thell.) Eig. and of four different triticale parental forms produced at the same environmental conditions. All hybrid forms of triticale were genetically stabilized at the hexaploid level. Parental species of *Aegilops* were not included in the study due to difficulties in obtaining the needed quantity and quality of the grains.

The following physicochemical parameters were determined: thousand kernel mass (TKM), hectolitre mass (HM), content of protein, ash and lipids that were analysed according to the AACC methods (2000). N-free extractives (NFE) was calculated with slighty modified methods as by difference of protein, ash, lipids and Klason lignin (Theander et al., 1995) analyses. The amino acids content was determined using ion-exchange chromatography on an AAA-400 amino acid analyzer (INGOS, the Czech Republic) in accordance to the procedure of Moore and Stein (1963), in which proteins were hydrolysed with 6N HCl for 24 h at 100˚C, after earlier oxidation of cystine and methionine with performic acid (Mason et al., 1980). Basing on amino acid content chemical score (CS) and essential amino acid index (EAA) were calculated as the protein quality indicators.

Grain samples were also analysed for the content of total dietary fibre (TDF) by the Uppsala method (AACC 32-25) as a sum of non-starch polysaccharides (NSP) and Klason lignin. The monosaccharide composition of the soluble and insoluble NSP polymers was analysed as alditol acetates by gas chromatography, as described by Englyst and Cummings (1984). A Hewlett-Packard 5890, Series II gas chromatograph was used, equipped with a flame ionization detector and Rtx 225 capillary column (30 m length and 0.53 mm id; cat #14049 from Restek Corporation, 110 Benner Circle, Bellefonte, USA). Temperature of the detector was 250°C and that of the injector 220°C. The column oven programme started at 190°C increasing by 5°C/min up to 215°C and holding this temperature for 5 min. Helium was used as a carrier gas. Total and insoluble fractions of NSP were calculated as the sum of appropriate monosaccharide residues and presented as anhydrous polymers. Soluble fraction of NSP is calculated from the difference between total and insoluble content of NSP. Viscosity was measured in water extracts prepared from wholemeal samples shaken with distilled water at a ratio 1:10 w/v on Brookfield Cone/Plate Digital Viscometer, with a 0.8° cone spindle and shear rate of 225 sec applied at 30°C (Boros et al. , 1993). All analyses were performed in duplicate and expressed on dry matter basis.

The balance trial was performed on rats using the general procedure of Eggum (1973). Ten experimental groups, each comprised of five male Wistar rats with an initial weight of 72.5 ± 3.0g were used to evaluate digestibility of dry matter (DMD) and protein (TPD), its biological value (BV) as well as net utilization (NPU). The rats were kept individually in metabolic cages and fed a fixed amount of diet, equals 10 g of DM/day, with a free access to water. A preliminary period lasted 4 days and a balance period 5 days. Body weight and feed intake were recorded at the end of the each period. During the balance period urine and faeces were collected separately. The diets contained equal amount of tested grains (80%) and were supplemented with vitamin (1%) and mineral (3.5%) mixtures, soyabean oil (5%) and wheat starch (10.5%). Casein diet containing 9.4% of protein was used as a reference diet. The experiment was conducted in compliance with European Union regulations concerning the protection of experimental animals and the study protocol was approved by the local ethics committee.

One-way analysis of variance was carried out with the GLM Procedure of SAS® not? and means were compared using Duncan's multiple range test. Pearson's correlation coefficients were calculated for all qualitative indices.

RESULTS AND DISCUSSION

All kernels of triticale hybrid lines with *Aegilops* sp. were well developed and plumped as compared to kernels of their parental forms (Table 1). The average TKM of hybrids was 50.7 g, exceeding their parental forms by 19%, except for the progeny of cultivar Presto, with no changes observed (46.0 vs 46.3 g). The differences between hybrids and triticale parental forms in HM were not so clearly visible, on average only 4%, but there were found high positive influence of *Ae. juvenalis* on their offspring (higher by 11%), independent on the parental form of triticale used. Recombinants of triticale and *Aegilops* sp. were also superior to the parental forms in the respect of protein content. An average amount of protein was 125 g/kg in the parental forms and 137 g/kg in the hybrid lines. Hybrids of *Ae. crassa* with two triticale forms, cultivar Presto and primary line of wheat Panda and rye Dańkowskie Złote showed protein content higher even by 14 and 17%, respectively. Values of the remaining basic grain components, i.e. ash, lipids and nitrogen free extractives in hybrids of triticale with *Aegilops* sp. were similar to the parental forms with the exception of lipid content in the hybrid of *Ae. crassa* and line [(Lanca ×L506/79) × CZR 142/79] exceeding by 22% parental forms.

Hybrids of triticale and *Aegilops* sp. characterized also, on average, by the increased content of total DF and its components, especially soluble fraction of NSP, mainly arabinoxylans (by 8, 32 and 52%, respectively) as compared to triticale parental forms (Table 2). Higher contents of soluble fraction of NSP in

Table 1. Physicochemical characteristic of triticale hybrids with *Aegilops* sp. as compared to their parental forms, g/kg DM

Cereal form	TKM*	HM*	Protein	Ash	Lipids	NFE*
Parental hexaploid triticale						
A. [(Lanca × L506/79) × CZR 142/79]	44.6	67.9	133 ± 0.3	19.4 ± 0.3	22.7 ± 0.3	799
B. Presto	46.3	73.9	124 ± 0.3	19.6 ± 0.1	22.7 ± 0.4	814
C. (Panda × Dańkowskie Złote)	41.3	70.8	115 ± 0.3	18.5 ± 0.5	23.2 ± 0.2	820
D. [(Jana × Tempo) × Jana]	38.7	67.5	129 ± 0.3	21.2 ± 0.1	25.3 ± 0.3	795
Hybrids						
Aegilops crassa 4x × A	53.6	66.7	142 ± 0.3	19.9 ± 0.1	28.0 ± 0.1	781
Aegilops crassa 4x × A) x B	46.0	73.8	142 ± 0.7	17.6 ± 0.4	24.4 ± 0.6	791
Aegilops crassa 4x × C	50.7	74.7	135 ± 0.3	18.8 ± 0.1	21.5 ± 0.3	804
Aegilops juvenalis 6x × A	53.6	75.6	130 ± 0.0	18.7 ± 0.2	21.6 ± 0.2	808
D × *Aegilops juvenalis* 6x	49.7	74.6	133 ± 0.0	19.8 ± 0.0	24.9 ± 0.2	795
Parental hexaploid triticale	42.7	70.0	125	19.6	23.5	807
SD	3.4	3.0	7.6	1.1	1.2	12
hybrids	50.7	73.1	137	19.1	24.1	796
SD	3.2	3.6	5.4	0.7	2.7	11
SEM*	0.80	0.52	0.21	0.14	0.20	0.58

*TKM - thousand kernel mass, g; HM – hectolitre mass, kg; NFE - N-free extractives; SEM - standard error of the mean hybrids, arabinoxylans in particular, were not accompanied by the proportional increase in water extract viscosity (WEV) values. This findings is in contrast with the results of earlier studies, that showed a high relationship between the level of soluble arabinoxylans and the viscosity of water extract in such cereals as: wheat, triticale and rye, in which these polysaccharides constitute the major soluble component of DF (Boros et al., 1993). Viscous properties of soluble arabinoxylans depend on many factors, among them, concentration in the grain, degree of polymerization, molecular weight, association with other grain components, that acting synergistically (Bengtsson et al., 1992; Izydorczyk and Biliaderis, 1995). Lack of significant relationship between viscosity and content of soluble fractions of NSP might indicate the involvement of other factors, possibly preharvest sprouting in some samples analysed, resulting in hydrolysis of arabinoxylans and changing their structure to less viscous oligosaccharides polymers. It might also indicate differences in the structure and properties of dietary fibre and its constituents in *Aegilops* species and triticale forms used. Different structure and properties of dietary fibre constituents could be envisaged by contrasting impact of *Ae. crassa* and *Ae. juvenalis* on their recombinants with the same triticale line [(Lanca × L506/79) × CZR 142/79]. Total contents of DF and NSP and above all its soluble fractions were considerably increased in the recombinant with *Ae. crassa* (by 24, 27 and 81%, respectively). In this recombinant threefold increase in content of soluble arabinoxylans was even observed, while viscosity of water extract of their grain was only slightly higher (by 6%). Recombinant with *Ae. juvenalis* showed similar to triticale line level of TDF (110 g/kg), slightly greater amount of total NSP (89 vs 84 g/kg) and its soluble fraction (15 vs 13 g/kg), with decreased (by 5%) WEV value.

As expected, the quality of protein in the hybrids due to increased content of protein was reduced (Table 3). It was illustrated by decreased content of lysine and other essential amino acids as well as EAA index. In all grains first limiting amino acid was lysine with CS from 37 to 45, while second limiting amino acid was isoleucine in 7 samples with CS in range from 56.8 to 62.4 or threonine in two samples with CS equal 60.8. There was not found any impact of *Aegilops* sp. on digestibility of protein in the grain of their offspring (on average 90.3 vs 89.7), except for the negative effect ($P<0.05$) of genes recombination of *Ae. crassa* with cultivar Presto (92.0 vs 89.1). However, the higher content of protein in recombinants as compared to triticale parental forms resulted in poorer values of protein quality indices, such as lysine content, CS, BV and NPU (Tables 3 and 4). Both, lysine content and EAA index were negatively correlated ($P<0.01$, $r=-0.86$ and -0.72) with TKM, confirming that the smaller grains have higher share of aleurone layer in total kernel mass. In cereals with starchy endosperm, the aleurone is proteinaceous part of the kernel reach in the essential

Table 2. Content of dietary fibre and constituted polysaccharides in triticale hybrids with Aegilops sp. as compared to parental forms, % DM

Cereal form	T-NSP	I-NSP	S-NSP**	Klason lignin	TDF	WEV, mP.s
Parental hexaploid triticale						
A. [(Lanca × L506/79) × CZR 142/79]	84.3 ± 5.2	71.1 ± 0.3	13.1 (5.5)	25.8 ± 0.15	110	1.58 ± 0.04
B. Presto	81.5 ± 3.8	69.9 ± 0.7	11.6 (6.4)	18.8 ± 0.11	100	1.49 ± 0.01
C. (Panda × Dańkowskie Złote)	91.7 ± 2.4	69.9 ± 0.5	21.8 (12.1)	23.7 ± 0.03	115	1.39 ± 0.01
D. [(Jana × Tempo) × Jana]	97.4 ± 1.4	84.2 ± 2.4	13.3 (8.0)	30.1 ± 0.01	127	1.67 ± 0.02
Hybrids						
(*Aegilops crassa* 4x × A)	106.9 ± 1.2	83.3 ± 1.7	23.7 (15.1)	29.2 ± 0.08	136	1.68 ± 0.03
[(*Aegilops crassa* 4x × A) × B]	91.4 ± 4.3	75.7 ± 2.2	15.7 (8.9)	24.7 ± 0.14	116	1.55 ± 0.07
(*Aegilops crassa* 4x × C)	100.3 ± 8.7	74.5 ± 2.7	25.8 (17.5)	20.9 ± 0.02	121	1.32 ± 0.01
(*Aegilops juvenalis* 6x × A)	88.9 ± 4.1	74.1 ± 1.5	14.8 (8.3)	20.9 ± 0.07	110	1.49 ± 0.07
(D × *Aegilops juvenalis* 6x)	100.3 ± 4.6	81.9 ± 4.5	18.4 (11.0)	27.5 ± 0.05	128	1.54 ± 0.04
Parental hexaploid triticale	88.7	73.8	15.0 (8.0)	24.8	114	1.53
SD	7.2	6.9	4.6	0.4	11.3	0.12
hybrids	97.6	77.9	19.7 (12.2)	24.6	122	1.52
SD	7.4	4.4	4.9	0.4	10.2	0.13
SEM*	2.8	1.3		0.5	3.3	0.02

* NSP - nonstarch polysaccharides, T-NSP, total NSP, I-NSP - insoluble NSP, S-NSP - soluble NSP, TDF - total dietary fibre, WEV - water extract viscosity; SEM - standard error of the mean

** values in parentheses present soluble arabinoxylans

Table 3. Content of limiting essential amino acids and qualitative protein indices of triticale hybrids with Aegilops sp. as compared to their parental forms,g/16 g N

Cereal form	Lys*	Thre*	Met + cys*	Sum of EAA*	CS*	Index EAA*
Parental hexaploid triticale						
A. [(Lanca × L506/79) × CZR 142/79]	2.88	3.09	3.94	32.6	41.2	70.4
B. Presto	2.95	3.00	4.09	31.3	42.1	68.1
C. (Panda × Dańkowskie Złote)	3.10	3.12	4.27	32.6	44.2	71.6
D. [(Jana × Tempo) × Jana]	3.16	2.96	4.17	31.6	45.1	69.1
Hybrids						
Aegilops crassa 4x × A	2.83	2.86	4.00	31.1	40.4	67.2
Aegilops crassa 4x × A) × B	2.82	2.86	3.46	30.6	40.3	65.6
Aegilops crassa 4x × C	2.60	2.92	3.65	29.8	37.2	64.3
Aegilops juvenalis 6x × A	2.62	2.95	4.05	32.1	37.4	65.3
D × *Aegilops juvenalis* 6x	2.66	2.75	4.17	31.6	38.0	64.9
Parental hexaploid triticale	3.0	3.0	4.1	32.0	43.2	69.8
SD	0.1	0.1	0.1	0.7	1.8	1.5
hybrids	2.7	2.9	3.9	31.0	38.7	65.5
SD	0.1	0.1	0.3	0.9	1.6	1.1

* Lys - lysine, thre - threonine, met+cys - methionine and cystein, EAA - essential amino acids, CS - chemical score

Table 4. Digestibility and utilization of protein and dry matter in triticale hybrids with *Aegilops* sp. as compared to their parental forms

Cereal form	TPD*	BV*	NPU*	DMD*
Casein - reference diet	*97.0*^{a}	*98.1*^{a}	*95.2*^{a}	*96.6*^{a}
Parental hexaploid triticale				
A [(Lanca × L506/79) × CZR 142/79]	90.6^{bc}	70.6^{c}	63.9^{c}	89.8^{cd}
B Presto	92.0^{b}	74.9^{b}	68.9^{b}	90.7^{b}
C (Panda × Dańkowskie Zł.)	88.9^{c}	66.2^{d}	58.9^{de}	89.6^{cd}
D [(Jana × Tempo) × Jana]	89.8^{bc}	73.5^{b}	66.0^{bc}	89.0^{de}
Hybrids				
Aegilops crassa 4x × A	89.3^{c}	65.7^{d}	58.7^{de}	88.5^{e}
(*Aegilops crassa* 4x × A) × B	89.1^{c}	65.4^{de}	58.3^{de}	89.1^{de}
Aegilops crassa 4x × C	89.7^{c}	64.1^{de}	57.5^{de}	90.3^{bc}
Aegilops juvenalis 6x × A	90.3^{bc}	66.7^{d}	60.3^{d}	90.4^{bc}
D × *Aegilops juvenalis* 6x	90.0^{bc}	62.7^{e}	56.5^{e}	89.0^{de}
Pooled SEM	0.79	1.01	1.22	0.32

*TPD - true protein digestibility, BV -biological value, NPU - net protein utilization, DMD - dry matter digestibility

amino acids, therefore their share in the kernel, significantly influences quality of the whole grain proteins. Since aleurone layer is built of thick-walled cells, tightly packed, liberation of amino acids due to enzymatic hydrolyses in the digestive tract of rats was hampered, resulting in decreased protein utilization.

At the time when triticale was introduced into field production it has been acknowledged to be of high nutritive potential for human (Hulse and Laing, 1974), although, poor gluten quality deprived it from being utilized for breadmaking. The poor gluten quality is the reason that until now triticale has been considered almost exclusively as a cereal component in animal feeds. The feeding value of modern triticale cultivars for monogastric animals is high, equal or sometimes even better than wheat cultivars (Myer, 1998; Boros, 2002). In our earlier study, Polish triticale cultivars of superior nutritive value were characterized by high TKM, low content of soluble arabinoxylans and low viscosity of grain water extract (Boros, 2002). To maintain or further improve the high quality of triticale for feeding purposes the above three criteria should be taken into account in breeding programmes. Another approaches that have already been undertaken to further improve feeding value of triticale is transfer of genes bearing valuable traits from other wild grasses and supplementation of feed mixtures with appropriate enzymes (Fabijańska et al., 2007).

CONCLUSIONS

Recombination of triticale and *Aegilops* sp. genes increased the range of variability in the content of protein and dietary fibre in their progenies. The preliminary study indicates that *Aegilops crassa* might be used for quality improvement of triticale and possibly other cereals designated for human consumption, while *Aegilops juvenalis* is better when enhancement of feeding value is expected. Due to increased protein content recombinants of triticale and *Aegilops* sp. characterized with the poorer chemical quality indicators of protein, its biological value and net utilization.

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