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CULTIVARS FOR SPIKE AND OTHER TRAITS

ABSTRACT

The objective of the present study was to determine the extent and pattern of genotypic diversity for six spike quantitative characters and two other traits in 36 winter triticale cultivars released in Poland, to classify the cultivars into similarity groups (clusters) and to identify those traits, among the studied ones, which mostly discriminated distinguished groups of cultivars. The 36 cultivars, released in the period from 1982 to 1999, were evaluated across three years 2002–2004 at the Experimental Field Station in Czesławice near Nałęczów, Poland. The experiments were carried out on the brown soil with loess subsoil. In each year the one-replicated experimental design was used with 2 m² plots, rows 20 cm apart, and dense sowing using about 2 cm spacing of seeds. Analyses of variance for each trait data according to the random model (both cultivars and years were assumed to be random factors) were done. To classify and characterize genotypic diversity of the cultivars for the eight traits, the pattern analysis was used. It involved both cluster analysis using Ward's procedure with a measure of the multivariate similarity among cultivars being Squared Euclidean Distance and canonical variate analysis (CVA) on the basis of cultivar *BLUPs* for the original traits. Quite different groups of cultivars for the studied traits were found, specially one group was substantially distanced to the others. As it was shown by CVA, spike length and number of spikelets per spike as negatively correlated with number of grains per spikelet in the studied set of the cultivars relatively largest contributed to overall differentiation of the distinguished eight groups and then, these traits best discriminated among the eight cultivar groups in the term of Mahalanobis distance for the considered traits. The 1000 grain weight and grain protein content much less contributed to overall discrimination of the cultivar groups than the previous four traits. The most important agronomic traits characterizing productivity of the spike grain weight and its two components, e.g. number of grains per spikelet and number of grains per spike had least discriminating power for the groups of cultivars. Grain yield per unit area of cereals is a result of spike grain yield and the number of spikes per unit area. In these studies of winter triticale cultivar diversity only grain spike yield and its components were included. Thus, the presented study are a primary evaluating of phenotypic diversity in the cultivars. The further study on the cultivar diversity evaluation for grain yield per unit area and its components is necessary.

Key words: canonical variate analysis, cluster analysis, cultivars, principal component analysis, spike traits, winter triticale

INTRODUCTION

Knowledge of genetic diversity and variation provides breeders better insight for selecting parents in a breeding program in terms of identifying diverse genotypes for traits of adaptation and productivity (Autrique *et al.* 1996, Cui *et al.* 2001, Mohammadi and Prasanna 2003, Jaradat *et al.* 2004). Mating parents with complementary traits combinations may increase the chance of genetic gains for yield and yield stability. Study of diversity may also be interesting for crop management in order to match cultivars with specific production environments (Schlösser *et al.* 2000).

Studies of genotypic diversity among cereal germplasm, including cultivars, for adaptive and yield contributing traits, components of grain yield per plant and/or other morphological, physiological and phenological traits, is recognized as the first step for their classification and description (Jaradat 1991, Royo *et al.* 1995, Schlösser *et al.* 2000, Mohammadi and Prasanna 2003, Jaradat *et al.* 2004, Persson *et al.* 2006, Rawashdeh *et al.* 2007). Objectives of such assessments are to classify the accessions into homogenous groups for mentioned traits, to characterize (describe) obtained groups, to analyze the relationships among the groups of accessions and to identify a subset of traits that best distinguished differences among these groups

Assessment of grain yield per plant components and other productive traits instead yield *per se* when studying phenotypic diversity of cereal germplasms, including cultivars, could be justified by:

- 1) usually larger heritability of yield components than grain yield (Yan and Wallace 1995, Annicchiarico and Pecetti 1998) and
- 2) existing relationships between yield and the mentioned traits.

There are much experimental evidence of genotypic relationships between cereal grain yields (per plant or unit area) and its components. Grain yield of durum wheat genotypes was related rather closely to spike length and grain weight per spike (Villegas *et al.* 2007). Wang *et al.* (2002) identified similar relations for spring wheat cultivars. Giunta *et al.* (1999) found grain numbers m^{-2} as more important yield component than single grain weight in grain yield formation of triticale genotypes. Similar results were also reported for other small grain cereals (Evans 1993, Yan and Wallace 1995, García del Moral *et al.* 2003, 2005, Moragues *et al.* 2006, Peltonen-Sainio *et al.* 2007). The number of grains m^{-2} is determined through both spike number m^{-2} and spike fertility (grain number per spikelet). Dominating contribution of grain numbers m^{-2} in affecting genetic variation of grain yield was depended on a cereal species. Grain yield in spring oat and winter rye was associated at the genotypic level with higher numbers of set grain, while spring barley and winter wheat responded to yield in favored conditions of Finland also through higher single grain weight (Peltonen-Sainio *et al.* 2007).

Polish released triticale cultivars have not been studied to analyse the extent

of genetic diversity for yield-contributing traits, including yield components. There are two main special needs to assess genotypic diversity of this germplasm for these traits. The first, triticale is a rather newly-synthesized crop, then evaluation of germplasm in this species can not be focused, as commonly in many traditional crops, on a survey of genetic resources (Royo *et al.* 1995). The second, contribution of Polish triticale breeders to world's genetic gains in this species is large and it should be known how much and what genetic diversity for phenotypic traits has been created by the breeders and how to use this diversity and enriched it further. To fulfill a gap of knowledge of genetic diversity in Polish triticale cultivars for yield-contributing and other agronomic traits, some researches have been established at Institute of Genetics, Breeding and Plant Biotechnology of University of Life Sciences in Lublin, Poland.

The objectives of the present study were:

- 1) determining of patterns of genotypic variation and diversity for six spike quantitative characters and two other traits in 36 winter triticale cultivars released in Poland,
- 2) classifying the cultivars into groups of similarity (clusters),
- 3) identification of such traits, which in the set of studied ones, mostly discriminated distinguished groups of cultivars,
- 4) using of three multivariate methods: principal component analysis, cluster analysis and canonical variate analysis, illustration of integrated to comprehensive evaluating multi-traits variation and diversity in a plant germplasm collection.

MATERIALS AND METHODS

Thirty six (36) Polish winter triticale cultivars (Table 1), released 1982 - 1999, were evaluated from 2002-2004 at the Experimental Field Station of University of Life Sciences in Czesławice near Nałęczów, Poland. The experiments were carried out on the brown soil with loess subsoil. In each year the not replicated experimental design was used with 2 m² plots, rows 20 cm apart, and dense sowing using about 2 cm spacing of seeds.

The following quantitative traits were evaluated: plant height (X_1), spike length (X_2), number of spikelets per spike (X_3), number of grains per spikelet (X_4), number of grains per spike (X_5), 1000 grain weight (X_6), grain weight per spike (X_7), grain protein content (X_8). Plant height was measured in three random places on plots. All spike quantitative characters were determined on a sample of 50 spikes randomly taken from each plot by uprooting certain number of plants. Grain protein content was measured on a grain sample from each plot using Kjeld-Tec apparatus (N \times 5,7). Data for each trait were arranged in a two way classification (according to the cultivar and year) with one observation in a sub-class.

Table 1

**Description of 36 Polish winter triticale cultivars tested in the study.
The registration year is given in parenthesis**

Breeding company	Cultivar
DANKO Hodowla Roślin Sp z o.o.	Lasko (1982), Grado (1984), Dagro (1985), Domino (1988), Presto (1989), Moniko (1990), Ordo (1990), Tewo (1991), Moreno (1992), Prego (1992), Purdy (1992), Vero (1992), Nemo (1995), Eldorado (1996), Pinokio (1996), Disco (1997), Fidelio (1997), Lamberto (1998), Mundo (1998), Piano (1998), Kitaro (1999), Pronto (1999)
Hodowla Roślin STRZELCE Sp z o.o.	Malno (1987), Ugo (1988), Almo (1989), Bogo (1993), Tornado (1996), Alzo (1997), Marko (1997), Prado (1998)
Cultivars bred in Poland, but registered in other countries	Debo, Ego, Falko, Origo (in the Netherlands), Chrono (in France), Typo (in Austria)

Statistical analysis

Analyses of variance for each trait data according to the random model (both cultivars and years were assumed to be random factors) were done (Hooks *et al.* 2006). Variance components for all effects in the model were estimated separately for each trait by *REML* method (Searle *et al.* 1992) using SAS[®] PROC MIXED (User's Guide 2002). Coefficients of repeatability of three-year phenotypic means for the traits studied were estimated on the basis of the variance components (Yan and Holland 2010). Estimates of random genotypic values for cultivars were obtained using *the best linear unbiased predictor, BLUP* (Searle *et al.* 1992, Hooks *et al.* 2006). The principal components analysis (PCA) from the sample correlation matrix calculated on *BLUPs* for the eight traits was employed to detect structure of correlation among the studied traits, to identify the traits contributing mostly to the overall genetic variation among the cultivars and to deliver data for cluster analysis which would be unbiased less by noise as compared to original data (Harch *et al.* 1997, Casler and van Santen 2000, Mohammadi and Prasanna 2003).

Pattern analysis was used to classify cultivars based on eight traits (Harch *et al.* 1997). This methodology involved two stages. Firstly, in order to classify cultivars into homogenous groups hierarchical cluster analysis was carried out by Ward's procedure (SAS[®] PROC FASTCLUS; SAS/STAT User's Guide 2002) using the Squared Euclidean Distance on scores of the first three principal components which accounted for 82% of the overall variation among cultivars (instead standardized cultivar *BLUPs* for the eight traits because they could have been biased by a noise) as a measure of dissimilarity. An indication of the final number of groups (clusters) was obtained on the base of the pseudo F and pseudo t^2 statistics (Caliński and Harabasz 1974, Khattree and Naik 2000,

Mohammadi and Prasanna 2003). Canonical variate analysis was carried out on the base of the cultivar *BLUPs* for the all traits (SAS® PROC CANDISC (SAS/STAT User's Guide 2002) to analyze the relationships among the cultivar groups and to identify a subset of traits that best distinguished differences among these groups. Phenotypic correlations between original variables and canonical variate on a group means basis can then be used to assess which variables drive the differentiation among clusters, e.g to assess cluster discriminating power of the studied traits (Krzanowski 1988, Jaradat *et al.* 1991, Ruiz de Galarreta and Alvarez 2001, Crossa *et al.* 2002, Gutiérrez *et al.* 2003, Mohammadi and Prasanna 2003, Hooks *et al.* 2006).

Table 2
Mean values, variance components, coefficient of genotypic variation and heritability coefficient for eight traits in a set of 36 winter triticale cultivars

Traits	Mean	SE	Range of means for cultivars	$\hat{\sigma}_G^2$	$\hat{\sigma}_Y^2$	$\hat{\sigma}_e^2$	CV_G	h^2
Plant height [cm] – X ₁	106.25	1.33	91.5 – 121.8	46.58	145.48	52.06	6.42	0.73
Spike length [cm] – X ₂	9.80	0.13	8.5 – 11.7	0.44	0.50	0.50	6.74	0.72
Number of spikelets per spike – X ₃	24.42	0.24	22.7 – 28.2	1.17	2.46	2.47	4.43	0.59
Number of grains per spikelet – X ₄	1.89	0.04	1.4 – 2.2	0.02	0.13	0.08	7.33	0.43
Number of grains per spike – X ₅	46.26	0.90	38.2 – 60.7	14.33	67.87	44.25	8.18	0.49
1000 grain weight [g] – X ₆	51.61	0.87	41.0 – 60.0	19.99	10.89	21.76	8.66	0.73
Grain weight per spike [g] – X ₇	2.38	0.06	1.7 – 3.4	0.08	0.16	0.13	11.66	0.63
Grain protein content [%] – X ₈	10.37	0.13	9.0 – 11.9	0.40	1.71	0.58	6.14	0.68

SE = $\sqrt{(\hat{\sigma}_G^2/I + \hat{\sigma}_e^2/nI)}$ is the standard error of mean, *n* is the number of years studied, *I* is the number of cultivars studied (in this experiment *n*=3, *I*=36, respectively), $\hat{\sigma}_G^2$, $\hat{\sigma}_Y^2$, $\hat{\sigma}_e^2$ are estimates of variance components for cultivars (genotypes), years and residual (including both experimental error and GEI effects), respectively, $CV_G = \sqrt{\hat{\sigma}_G^2} / \text{mean} \times 100\%$ is coefficient of genotypic variation, $h^2 = \hat{\sigma}_G^2 / (\hat{\sigma}_G^2 + \hat{\sigma}_e^2/n)$ is the repeatability coefficient of three-year phenotypic means

RESULTS AND DISCUSSION

Variation of the traits. The genotypic variances ($\hat{\sigma}_G^2$) were significant for all traits as tested by F ANOVA test (results not included). The cultivars were characterized by a substantial variability of the spike yielding traits. The greatest genotypic variation was detected for number and weight of grains per spike as well as for 1000 grain weight (Table 2). The results are similar to those obtained by Giunta *et al.* (1999), Zych (2002, 2003) and Cyfert (2004). They show that among winter triticale germplasm included in the Polish cultivars one could select valuable genotypes as parents useful in recombination breeding for yield improving (Kociuba 2000). The repeatability coefficients of three-year

phenotypic means for the traits were mostly moderate, ranging from 0,43 for number of grains per spikelet to 0,73 for plant height, spike length and 1000 grain weight (Table 2). Thus, the observed differences (variability) in three-year phenotypic means of cultivars for the traits were not entirely due to genetic effects, although they were predominantly determined genetically (Yan and Holland 2010).

Table 3
Percent of overall variation among cultivars explained by the first three principal component analysis and correlation coefficients between these components and eight original traits obtained on the base of BLUP's of 36 winter triticale cultivars

Traits	Correlation coefficients		
	PC1	PC2	PC3
Plant height [cm] – X ₁	-0.05	0.28	0.75
Spike length [cm] – X ₂	0.52	-0.75	-0.02
Number of spikelets per spike – X ₃	0.67	-0.47	-0.31
Number of grains per spikelet – X ₄	0.46	0.86	-0.01
Number of grains per spike – X ₅	0.76	0.61	-0.11
1000 grain weight [g] – X ₆	0.48	-0.57	0.49
Grain weight per spike [g] – X ₇	0.95	0.13	0.23
Grain protein content [%] – X ₈	-0.14	-0.05	0.80
Percent of variation between clusters explained	33.40	28.77	19.94

Principal component analysis (PCA).

The first three principal components accounted for 82% of the overall variation among cultivars (Table 3). The first principal component (PC1) was positively, rather closely, correlated with grain weight per spike and its two contributing characters: number of spikelets per spike and number of grains per spike. Then PC1 synthesized information about variation in cultivars for these three traits changing simultaneously in the same direction and contributing to a large part of the overall genetic variation among the cultivars. PC1 performs also the major gradient of the multivariate variation in a set of the cultivars. Then, it may be recognized as a common factor called 'grain productivity of spike'. The second principal component (PC2) was mostly correlated positively with number of grains per spikelet and negatively with spike length. Importance of the both traits in the multivariate inter-cultivar variation is less than those traits related mainly to PC1. Contrasting signs of the correlation coefficients for the both traits related mainly to PC2 indicates that simultaneous changing in cultivars for these traits is in the contrasting direction. PC2 may be treated as second common factor called 'architecture of spikelets in spike'. Plant height and grain protein content as correlated closely positively with PC3 contributed least, among the eight traits, to the total variation of cultivars. Then, PC3

constitutes third common factor having least contribution to the overall variation among cultivars. This factor could be called ‘grain protein performing’.

Cluster analysis.

The cultivars were classified into 8 groups (Fig. 1) including entries characterized by similar their three-year phenotypic means for the eight studied traits within each group (Table 4).

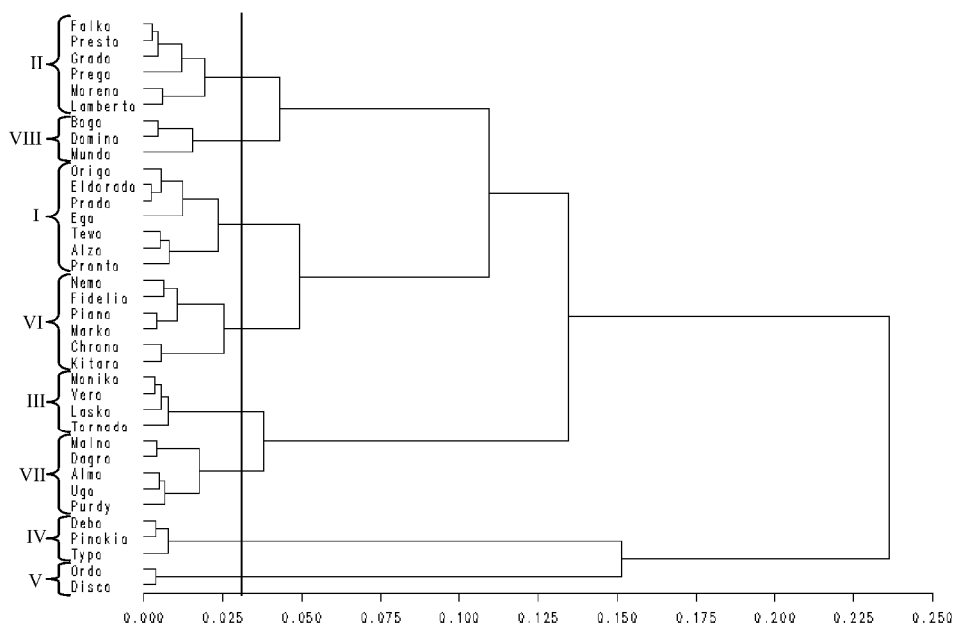


Fig. 1. Dendrogram of 36 winter triticale cultivars using Ward’s method on squared Euclidean distance for standardized BLUP values for eight spike and two other agronomic traits (cutting line shows 8 homogenous groups)

Cultivars in each group (except group V) have been developed by different breeding company. It shows that there is no systematic differences for profiles of spike yielding and two other agronomic traits in cultivars coming from the companies. Those cultivars assigned to groups II, III and V had largely been developed by the same breeding company, DANKO Hodowla Roślin Sp z o.o. The cultivars in groups II, III were very similar one to the other for all studied traits and cultivars in group V were substantially distinctive from the both previous groups, mainly for those traits mostly correlated with PC1 (X_3 , X_5 , X_7) and, then, greatly discriminating cultivars. The cultivars Debo and Typo (registered in foreign countries) and Pinokio (developed by DANKO) assigned to group IV were those most distinctive for each of the studied traits in comparison to remaining groups (Table 4). The cultivars in group IV are

characterized by the lowest plant height (95.31 cm), long spikes (11.49 cm), much spikelets per spike (27.12) with few grains (1.50) affecting very small number of grains per spike (40.63) but very high 1000 grain (59.11g). As a result of such combination of the spike yield component levels in these three cultivars, their mean of grain weight per spike was relatively high (2.42) and relatively low for grain protein content (9.49%). The relations detected between spike yield components and grain spike yield in the studied germplasm of the winter triticale cultivars confirm largely previous results obtained in cereal researches (Royo *et al.* 1995, Giunta *et al.* 1999, Kociuba 2000, Jaradat *et al.* 2004, Peltonen-Sainio *et al.* 2007).

Table 4

Means for studied traits in each of eight homogenous groups (clusters) of Polish winter triticale cultivars (sets of cultivars assigned to each group are given below)

Groups	X ₁	X ₂	X ₃	X ₄	X ₅	X ₆	X ₇	X ₈
I	108.45	9.22	23.82	2.11	50.54	50.06	2.53	9.85
II	111.48	9.74	24.02	1.77	42.59	47.32	2.03	10.18
III	107.83	9.93	24.68	1.91	47.26	52.67	2.48	11.67
IV	95.31	11.49	27.12	1.50	40.63	59.11	2.42	9.49
In	112.03	10.78	27.48	2.17	59.75	55.22	3.30	10.05
VI	97.78	9.93	23.72	1.99	46.61	52.79	2.43	10.34
VII	114.26	9.55	23.44	1.73	41.17	56.95	2.35	11.22
VIII	99.29	8.91	24.61	1.89	46.67	41.21	1.96	10.02

I-Alzo, Ego, Eldorado, Origo, Prado, Pronto, Tewo, II-Falko, Grado, Lamberto, Moreno, Prego, Presto, III-Lasko, Moniko, Tornado, Vero, IV-Debo, Pinokio, Typo, V-Disco, Ordo, VI-Chrono, Fidelio, Kitaro, Marko, Nemo, Piano, VII-Almo, Dagro, Malno, Purdy, Ugo, VIII-Bogo, Domino, Mundo

Canonical variate analysis (CVA).

The first canonical variable (CAN1) accounted for 46% of the overall variation of Mahalanobis distance between the groups and first three canonical variables explained 82% of this variation (Table 5). CAN1 was mainly correlated negatively with spike length and number of spikelets per spike and it was also correlated positively with number of grains per spikelet. These three traits relatively largest contributed to overall group differentiation and then, they best discriminated among the eight groups in term of Mahalanobis distance for the studied traits. The 1000 grain weight and grain protein content, as correlated positively rather strongly with CAN2, less contributed to overall discrimination of the groups than the previous four traits. Grain weight per spike and its two components: number of grains per spikelet and number of grains per spike as rather close related positively to CAN3 contributed relatively least to multivariate variation between the groups.

Table 5
Percent of variation between clusters explained by the first three canonical variables and correlation coefficients of 36 winter triticale cultivar BLUPs for eight original traits and cultivar values of these canonical variables

Traits	Correlation coefficients		
	CAN1	CAN2	CAN3
Plant height [cm] – X ₁	0.42	0.11	-0.36
Spike length [cm] – X ₂	-0.77	0.20	0.21
Number of spikelets per spike – X ₃	-0.59	-0.18	0.32
Number of grains per spikelet – X ₄	0.66	-0.12	0.70
Number of grains per spike – X ₅	0.40	-0.20	0.79
1000 grain weight [g] – X ₆	-0.46	0.79	0.19
Grain weight per spike [g] – X ₇	0.01	0.33	0.75
Grain protein content [%] – X ₈	0.36	0.63	-0.42
Percent of variation between clusters explained	46.42	22.79	13.79

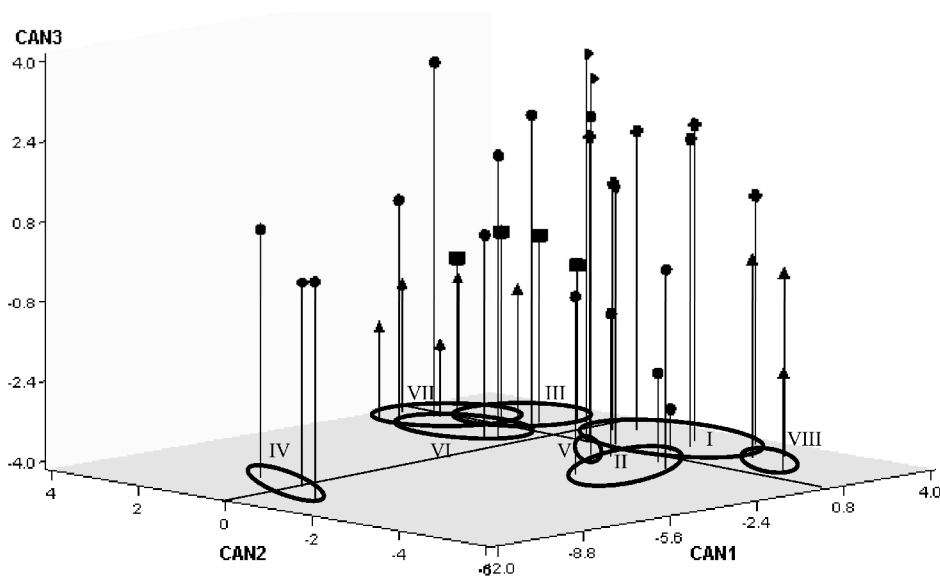


Fig. 2. Scatter plot of the first three canonical variable values of BLUPs for 36 winter triticale cultivars assigning to eight groups determined by Ward's cluster analysis

The plot of canonical *BLUP* values of cultivars shown in Fig. 2 clearly displays clustering of the original trait *BLUP* values and well approximates similarities between the cultivar groups. The approximation of the real relationships between the groups by three CV-axes was quite good because the first three canonical variables explained 82% of the Mahalanobis distance variation among the groups. CAN1 scores for cultivars mainly separated the cultivar groups IV and others. characterized

by very contrasting values of those traits related strongly with this canonical variates, then having the largest discriminating power, i.e. spike length, number of spikelets per spike and number of grains per spikelet and also by traits correlated not so closely with CAN1, i.e. plant height, number of grains per spike and grain protein content. The rest of seven groups are diverse relatively less one to the other. Configuration of cultivars in group IV for the first three canonical variates shows that these cultivars presented the highest spike length, number of spikelets per spike and 1000 grain weight, while the lowest number of grains per spikelet, plant height, number of grains per spike and grain protein content. This multivariate characterization (traits profile) of cultivars in group IV is closely reflected by means of traits for cultivars in the eight groups (Table 4). Cultivars assigned to group IV seemed to be larger distanced to the cultivars in group I, having contrasting values of all those traits correlated mainly and also less with CAN1.

CONCLUSIONS

Spike length and number of spikelets per spike as negatively correlated with number of grains per spikelet in the studied set of Polish winter triticale cultivars relatively largest contributed to overall differentiation of the distinguished eight groups and then, these traits best discriminated among the eight cultivar groups in the term of Mahalanobis distance for the considered traits. The 1000 grain weight and grain protein content much less contributed to overall discrimination of the cultivar groups than the previous four traits. The most important agronomic traits characterizing productivity of the spike grain weight and its two components, e.g. number of grains per spikelet and number of grains per spike had least discriminating power for the groups of cultivars. Grain yield per unit area of cereals is a result of spike grain yield and the number of spikes per unit area. In these studies of winter triticale cultivar diversity only grain spike yield and its components were included. Thus, we have recognized the presented study of the cultivar diversity of winter triticale as a primary evaluating of phenotypic diversity in the cultivars. Therefore, the further study on the cultivar diversity evaluation for all important agronomic traits (including grain yield per unit area and its components) is necessary in which also using multi-environment cultivar trials are required.

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