PL ISSN 1429-3862
E-ISSN 2083-599X

Plant Breeding and Seed Science

82
Volume

PLANT BREEDING AND ACCLIMATIZATION INSTITUTE- NATIONAL RESEARCH INSTITUTE
RADZIKÓW 2021

PL ISSN 1429-3862
E-ISSN 2083-599X

Plant Breeding and Seed Science

82
Volume

PLANT BREEDING AND ACCLIMATIZATION INSTITUTE- NATIONAL RESEARCH INSTITUTE
RADZIKÓW 2021

Founded in 1957 as „Hodowla Roślin Aklimatyzacja i Nasiennictwo” in Plant Breeding and Acclimatization Institute (since 2010 Plant Breeding and Acclimatization Institute — National Research Institute). Edited by Jadwiga Lekczyńska (1957—1982), Stanisław Starzycki (1983), Henryk J. Czembor (1984—1999), Edward Arseniuk (2000-2021) and since 2021 by ... . Starting from Volume 40 (in 1996) published in English as Plant Breeding and Seed Science.

Since 2005 our Journal is citted in Thomson Master Journal List. Since 2009 the version online is on the Metapress Platform by Versita Ltd.
Digital version is also available on our website.

EDITORIAL OFFICE
Plant Breeding and Acclimatization Institute— National Research Institute,
Department of Scientific Information and Editorial Board, Radzików,
05-870 Błonie, Poland, Phone: +48 (22) 733 46 11,
e-mail: wydawnictwo@ihar.edu.pl

Managing Editor: Aleksandra Pindor, e-mail: a.pindor@ihar.edu.pl

DOI: 10.37317/pbss-2021-0001

Dariusz R. Mańkowski [1]*, Dorota Jasińska [2], Magdalena Anioła [2], Tadeusz Śmiałowski [1], Monika Janaszek-Mańkowska [3], Wioletta M. Dynkowska [4]

[1] Plant Breeding and Acclimatization Institute – National Research Institute, Radzików, Poland. Department of Seed Science and Seed Technology, Laboratory of Seed Production and Plant Breeding Economics.
[2] Poznańska Hodowla Roślin Sp. z o. o. Plant Breeding Station Nagrodowice, Poland.
[3] Warsaw University of Life Sciences, Warsaw, Poland. Department of Engineering
[4] Plant Breeding and Acclimatization Institute – National Research Institute, Radzików, Poland. Department of Biochemistry and Plant Physiology

*Corresponding author's e-mail: d.mankowski@ihar.edu.pl

ONE- AND MULTIVARIABLE CHARACTERISTICS OF SPRING BARLEY (*HORDEUM VULGARE* L.) GENOTYPES CROPPED IN 2017–2018 YEARS AT THE NAGRADOWICE PLANT BREEDING STATION

ABSTRACT

The aim of this study was to evaluate the yield variability of spring barley families grown at the Nagradowice Plant Breeding Station of Poznan Plant Breeding against other families studied in years 2017–2018 in Team Breeding Experiments. Research material included 250 spring barley families cultivated in 2017 and 2018 in 6 locations. Selection of spring barley families for preliminary experiments was based on synthesis of results obtained in inter-plant experiments established in 2016 and 2017 in 5 locations. Combined (due to location) analysis of variance for experimental data was performed for each year and each series of experiments separately. Best Weighted Linear Unbiased Estimators (BWLUE) for the effects of individual sources of variation were included in ANOVA model. Significant effect of location on mean yield was observed in each research year and each series of experiments. Crucial differences were also observed between tested varieties and breeding lines. Moreover, significant interaction between locations and varieties or breeding families was also observed. Self-organising map (SOM) was applied to develop multivariable characteristic of tested families and cultivars of spring barley. Analyses results, i.e. ranking of BWLUE effects as well as SOM segmentation revealed seven breading lines from Breeding Station Nagradowice, which may be considered for further breeding process.

Key words: barley, yield, cultivars scoring, BWLUE, SOM

Communicated by Grzegorz Żurek.

INTRODUCTION

Barley is one of the most important cereals in the world. The cultivation of barley has ranks fourth both in production quantity and cultivated area among grain crops (FAO, 2018). Most of the barley grain is used for animal feed (about 70%). A significant part is used for brewing and malting purposes and less than 6% is used for human nutrition (Tricase et al., 2018). In Poland spring barley is an important cereal within crop structure, too. High nutritional value of barley grain was confirmed by numerous studies on animal nutrition as well as research on characteristics of barley used for brewing purposes (Marquardt et al., 1994; Boros et al., 1996; Cyran et al., 2002; Anderson et al., 2008; Gołębiewski et al., 2014, Boros et al., 2015, Wiśniewska et al., 2020). Most cultivars of spring barley listed in Polish National List (PNL) were bred by Polish breeding companies. The PNL contains only a few foreign but very fertile cultivars (COBORU, 2019). Morever, the proportion of spring barley cultivars relative to other cereal species included in PNL is negligible. Therefore, it is important to further develop domestic barley farming and maintain high-quality barley grain. Especially since there is a growing interest in this grain as a potential raw material for the production of functional food (Boros et al., 2015; Wirkijowska et al., 2016; Grochowicz et al., 2017; Sakellariou & Mylona 2020.

Five Polish breeding companies, i.e. Malopolska Plant Breeding, Poznanska Plant Breeding, Plant Breeding Strzelce, Plant Breeding Danko and Plant Breeding Smolice conduct intensive work aimed at breeding new cultivars of spring barley. Obtaining fertile breeding lines that are resistant to changing environmental conditions can be significantly hasten by incorporating plant genetics into breeding research (Grzywa et al., 2002; Węgrzyn et al., 2002; Nadolska-Orczyk et al., 2017). However, other studies indicate that breeding progress is also conditioned by diversity of breeding materials both in terms of fertility and qualitative characteristics (Ploch et al., 2005, Gołębiewski et al., 2013; Boros et al., 2015).

The Team Breeding Experiments (TBE) carried out for many years at the stage of preliminary tests allowed for identification of spring barley lines which are the most valuable in terms of yield and qualitative traits. Selection of the best breeding lines is most often determined by scores for yields obtained during TBE. Nonetheless, there is still a lack of a consistent methodology for evaluation of breeding lines that considers all characteristics affecting the diversity of studied objects. Progress in experimental design as well as application of multivariate characteristic

enabled a more precise assessment of feature diversity of tested spring barley families and cultivars (Wójcik & Laudański, 1989; Laudański, 1996; Mańkowski et al., 2014).

The aim of this study was to evaluate the yield variability of spring barley families grown at the Nagradowice Plant Breeding Station of Poznan Plant Breeding against other families studied in years 2017–2018 in TBE. Modern statistical and data-mining tools were applied to achieve this goal.

MATERIAL AND METHODS

Research material included 250 spring barley families cultivated in 2017 and 2018 in 6 locations: Bąków – BKH (Opole Voivodeship), Nagradowice – NAD (Greater Poland Voivodeship), Polanowice – POB (Lesser Poland Voivodeship), Strzelce – STH (Greater Poland Voivodeship) Modzurów – MOB (Śląskie Voivodeship), Radzików – RAH (Mazowieckie Voivodeship). This group included also reference cultivars: *Planet*, *Radek* and *Soldo* which was replaced by *Runner* in 2018 (Tab. 1). Each year, studied objects were divided as follows: 2 series of 62 objects in 2017 (2017_S1 and 2017_S2) and 2 series of 63 objects in 2018 (2018_S1 and 2018_S2). Spring barley families cultivated in Nagradowice included the F8 (27 families) and F9 (13 families) generations. Selection of spring barley families for preliminary experiments was based on synthesis of results obtained in inter-plant experiments established in 2016 and 2017 in 5 locations. These families characterised with high yielding potential and were strongly resistant to the most important barley diseases: powdery mildew, barley rust and leaf net blotch. Most of families were also highly resistant to lodging of plants before harvest. Families selected for preliminary experiments in 2017 were obtained from lines derived from crosses made in 2010. For better identification, subsequent numbers of breeding lines from Breeding Station Nagradowice were marked with NAD prefix and the remaining ones had prefix LNE.

Field experiments in each location were carried out in an incomplete balanced block design, on 10 m^2 plots in 3 blocks, using mechanical seeding, with a sowing standard of 300 grains per 1 m^2. Detailed conditions of individual field experiments are presented in Table 2. Plant condition was assessed after germination. Meteorological conditions were monitored during spring and summer period. The grain was harvested mechanically using a plot harvester. After weighing, the crop expressed in kg per plot was converted to 15% dry matter according to Śmiałowski et al. (2017) methodology.

Table 1

List of spring barley breeding lines and reference cultivars tested in Team Breeding Experiments in 2017 and 2018

Name	Breeding Company – Breeding Station	2017		2018		Sum
		Series S1	Series S2	Series S1	Series S2	
Breeding lines						
NAD (1–40)	Poznańska Hodowla Roślin Sp. z o. o. [PL] – Breeding Station Nagradowice	10	10	10	10	40
LNE (1–10, 50–59, 120–129, 170–179)	Małopolska Hodowla Roślin Sp. z o. o. [PL] – Breeding Station Polanowice	10	10	10	10	40
LNE (11-20, 60-69, 100-109, 150-159)	Hodowla Roślin Smolice Sp. z o. o. Grupa IHAR [PL] – Experimental Station Bąków	10	10	10	10	40
LNE (21–29, 70–78, 130–139, 180–189)	Plant Breeding and Acclimatization Institute [PL] – Experimental Station Radzików	9	9	10	10	38
LNE (30–39, 79–88, 140–149, 190–199)	Hodowla Roślin Strzelce Sp. z o. o. Grupa IHAR [PL] – Breeding Station Strzelce	10	10	10	10	40
LNE (40–49, 89–98, 110–119, 160–169)	DANKO Hodowla Roślin Sp. z o. o. [PL] – Breeding Station Modzurów	10	10	10	10	40
Reference cultivars						
Planet	Ragt Semances [FR]	1	1	1	1	4
Soldo	Saaten Union [DE]	1	1			2
Runer	Saaten Union [DE]			1	1	2
Radek	Hodowla Roślin Strzelce Sp. z o. o. Grupa IHAR [PL]	1	1	1	1	4
In total		62	62	63	63	250

Table 2

Conditions for carrying out field experiments in 2017 and 2018

Specification	Bąków (BKH)		Polanowice (POB)		Modzurów (MOB)		Nagradowice (NAD)		Radzików (RAH)		Strzelce (STH)	
	2017	2018	2017	2018	2017	2018	2017	2018	2017	2018	2017	2018
Soil	mugwort		less on limestone substrate		mold weakly eroded		brown on the basis of boulder clay		brown		*NA*	
Forecrop	—	oilseed rape	beet	fodder beet	beet	—	sugar beet	white mustard	beet	beet	*NA*	*NA*
Fertilization												
N	—	76	2.18	10.5	26	—	69	90	18	*NA*	*NA*	*NA*
P	—	50	80	30	20	—	46	58	46	*NA*	*NA*	*NA*
K	—	80	120	42	60	—	40	100	60	*NA*	*NA*	*NA*
Sowing date	—	m 3 d 24	m 3 d 30	m 4 d 9	m 4 d 1	—	m 3 d 30	m 4 d 6	m 3 d 31	*NA*	*NA*	*NA*
Harvesting date	—	m 8 d 10	m 8 d 7	m 7 d 24	m 7 d 6	—	m 7 d 30	m 7 d 21	m 7 d 25	*NA*	*NA*	*NA*
Chemical protection	Mustang Forte 195 SE, Bi 58 400 EC, Fastac Active		Mustang Forte 195 SE, Basfoliar 36 Extra, Bi 58 400 EC		RSM 28, Biathlon 4D + Dasch HC, Granstar Ultra SX 50 SG + Trend + Fenoxinn 110 EC, Danadim 400 EC		Mustang Forte 195 SE, Axial 50 EC, Granstar Ultra, Puma Uniwersal 069EW Danadim		Mustang Forte 195 SE, Foxtrot 069 EW, Pyrinex 480 EC		*NA*	

NA – data not available

A combined (due to location) analysis of variance for experimental data was performed for each year and each series of experiments separately, according to the following fixed model (Wójcik & Laudański, 1989; Laudański, 1996; Mańkowski et al., 2014; Śmiałowski et al., 2017):

$$y_{ijk} = \mu + \alpha_i + \chi_k(\alpha_i) + \beta_j + \gamma_{ij} + \varepsilon_{ijk} ,$$

where y_{ijk} is a value of dependent variable in the *i*th location, for the *j*th family/cultivar in the *k*th block, μ is an overall mean for dependent variable in population, α_i means the effect of the *i*th level of factor A (location), $\chi_k(\alpha_i)$ stands for the effect of the *k*th block nested in the *i*th location, is the effect of the *j*th level of factor B (family/cultivar), γ_{ij} means the interaction between the *i*th level of factor A and the *j*th level of factor B and ε_{ijk} stands for the random error.

The model mentioned above introduces a factor design for a series of experiments accounted for the genotype-location combination also called cross-hierarchical design (Wójcik & Laudański, 1989; Laudański, 1996; Mańkowski et al., 2014; Śmiałowski et al., 2017). Application of such a model allowed for a synthetic analysis of series of experiments carried out in different locations. This analysis took into account the effect of blocks nested in locations, which meant that, according to the actual state, the effect of blocks was dependent on the location where a single experiment was conducted. The matrix notation of this model is then as follows:

$$\mathbf{y} = \mathbf{X\theta} + \boldsymbol{\varepsilon} = [\mathbf{N} \quad \mathbf{A} \quad \mathbf{B} \quad \mathbf{A} \otimes \mathbf{B} \quad \mathbf{C}] \begin{bmatrix} \mu \\ \boldsymbol{\alpha} \\ \boldsymbol{\beta} \\ \boldsymbol{\gamma} \\ \boldsymbol{\chi} \end{bmatrix} + \boldsymbol{\varepsilon},$$

where $\mathbf{y}$ is the vector of dependent variable observations, $\mathbf{X}$ means the matrix consisting of $\mathbf{N}$, $\mathbf{A}$, $\mathbf{B}$, $\mathbf{A} \otimes \mathbf{B}$, and $\mathbf{C}$ submatrices, composed of linearly independent vectors representing the experimental design, $\boldsymbol{\theta}$ stands for the vector of model parameters, μ is the global mean, $\boldsymbol{\alpha}$ means the vector of factor A (location) effects, $\boldsymbol{\beta}$ is the vector of factor B (family/cultivar) effects, $\boldsymbol{\gamma}$ stands for the vector of interactive effects, $\boldsymbol{\chi}$ is the vector of block effects, $\boldsymbol{\varepsilon}$ means the vector of unknown random deviations (experimental error), and $\mathbf{A} \otimes \mathbf{B}$ is the Hadamard-Kronecker product of matrices $\mathbf{A}$ and $\mathbf{B}$.

Assuming that

$$E(\mathbf{y}) = \mathbf{X\theta} = \mathbf{N}\mu + \mathbf{A}\boldsymbol{\alpha} + \mathbf{B}\boldsymbol{\beta} + (\mathbf{A} \otimes \mathbf{B})\boldsymbol{\gamma} + \mathbf{C}\boldsymbol{\chi}$$

and the vector of random deviations meets the condition (according to ANOVA assumptions)

$$\boldsymbol{\varepsilon} \sim NID(\mathbf{0}; \sigma_e^2 \mathbf{I}_n),$$

the imposed conditions of traceability are:

$$\mathbf{P_N A\alpha} = 0 \left(\sum_i n_i \alpha_i = 0 \right), \mathbf{P_N B\beta} = 0 \left(\sum_j n_j \beta_j = 0 \right), \mathbf{P_A C\chi} = 0 \left(\bigwedge_i \sum_k n_{ik} \chi_{ik} = 0 \right),$$

$$\mathbf{P_{(A,B)}}(\mathbf{A} \otimes \mathbf{B})\boldsymbol{\gamma} = 0 \left(\bigwedge_j \sum_j n_{ij} \gamma_{ij} = 0 \text{ and } \bigwedge_i \sum_j n_{ij} \gamma_{ij} = 0 \right)$$

The subspace of estimated parameters may be represented as

$$R\left[\mathbf{N}, (\mathbf{I}_n - \mathbf{P_N})\mathbf{A}, (\mathbf{I}_n - \mathbf{P_C})\mathbf{B}, \left(\mathbf{I}_n - \mathbf{P_{B,C}}\right)(\mathbf{A} \otimes \mathbf{B}), \left(\mathbf{I}_n - \mathbf{P_{A\otimes B}}\right)\mathbf{C}\right].$$

The determination of vector $\hat{\boldsymbol{\theta}} = [\hat{\mu}, \hat{\boldsymbol{\alpha}}, \hat{\boldsymbol{\beta}}, \hat{\boldsymbol{\gamma}}, \hat{\boldsymbol{\chi}}]$leads to calculation of the following unbiased estimators:

$$\hat{\mu} = (\mathbf{N}'\mathbf{N})^{-1}\mathbf{N}'\mathbf{y}$$

$$\hat{\boldsymbol{\alpha}} = \mathbf{P}_{\mathbf{A}^*}^{\mathbf{B}}\mathbf{y}$$

$$\hat{\boldsymbol{\beta}} = (\mathbf{I}_n - \mathbf{P_C})\mathbf{P}_{\mathbf{B}^*}^{\mathbf{C}}\mathbf{y}$$

$$\hat{\boldsymbol{\gamma}} = \left(\mathbf{I}_n - \mathbf{P}_{(\mathbf{B,C})}\right)\mathbf{P}_{(\mathbf{A}\otimes\mathbf{B})^*}^{(\mathbf{B,C})}\mathbf{y}$$

$$\hat{\boldsymbol{\chi}} = \left(\mathbf{I}_n - \mathbf{P}_{(\mathbf{A}\otimes\mathbf{B})}\right)\mathbf{P}_{\mathbf{C}^*}^{(\mathbf{A}\otimes\mathbf{B})}\mathbf{y},$$

which are called BWLUE (Mańkowski, 2013; Mańkowski et al., 2014; Śmiałowski et al., 2017) – *best weighted linear unbiased estimators* for the effects of individual sources of variation included in ANOVA model. These estimators are unbiased (they are weighted by the number of cases). This is especially important if assessing the effects of factors studied in incomplete experiments with different case rates within subclasses, since arithmetic means are incomparable in such a case. Furthermore, effect assessments obtained for tested cultivars and families might have characterised them directly, since they were not biased by any other main or interactive effects.

Families and cultivars of spring barley considered in the experiment were compared on the basis of the ranking of ratings obtained for BWLUE estimators. However, no detailed comparisons using multiple comparison procedures were made, since an interpretation of obtained results would be unreliable with such a number of examined objects (over 60 in each series). The effects' assessments obtained for families and cultivars of spring barley were then used to create multivariable characteristic of examined objects.

For the analysis purpose, locations were treated as the fixed factor, since they were selected subjectively and were not a random representation of a wider population (in this case the area of Poland). Thus, the analysis was consisted with ANOVA fixed model.

We applied a Kohonen neural network, known also as a self-organising map (SOM), to develop the multivariable characteristic of tested families and cultivars of spring barley (Kohonen, 1982). SOM rearranges multi-dimensional data

by mapping them onto low-dimensional hyperplane and enabling object clusterisation on the basis of similarity between input vectors (analysed variables). The basic advantages of this solution are simplification of representation of non-linear relationships and solving problems for which one cannot accurately define the nature of relationships between objects (Lasek & Myzik, 2012), detecting relations which would be missed if using traditional approaches (Tadeusiewicz, 2001), resistance to outliers (Vensanto, 2000). SOM is also successfully applied as a pattern recognition tool since it discovers multidimensional similarities between samples and their references and map them close together building areas of the possibly highest homogeneity called Voronoi cells (Trajer & Świderski, 2009; Janaszek & Trajer, 2011).

In our studies two separate SOM models were built. These models will be further referred as SOM17 and SOM18. The former SOM was trained using data collected in 2017. And the latter was trained on data obtained in 2018. The number of nodes within both SOMs was determined as proposed by Vesanto (2000). Each feature map consisted of 121 nodes arranged in a square of dimensions of 11×11 nodes. The input data was divided into the training set and test set which consisted of 80% and 20% of all input vectors respectively. The WTA (the winner takes all) algorithm was applied for SOMs training. After training process nodes corresponding to the reference samples were discovered and Voronoi cells covering the first order neighbourhoods of these nodes were considered in further analyses. All calculations were made using the Statistica software ver. 13.3 (TIBCO Software Inc., 2017).

RESULTS AND DISCUSSION

The ANOVA results obtained for experimental data are presented in Table 3. Significant effect of location on mean yield was observed in each research year and each series of experiments. Crucial differences were also observed between tested varieties and breeding lines. Moreover, significant interaction between locations and varieties or breeding families was also observed.

The comparison of reference varieties with tested breeding families should be considered the most important result of this experiment since the ranking position of breeding lines and their relation to reference varieties is more valuable information for a breeder than joining them into homogeneous groups. Considering that, comparisons based on the BWLUE estimator turn out to be more reliable than those simply based on arithmetic means. The ranking of varieties and breeding lines, created in this analysis, is presented in Figure 1.

ANOVA results according to cross-hierarchical model for two years of experiment carried out in two series

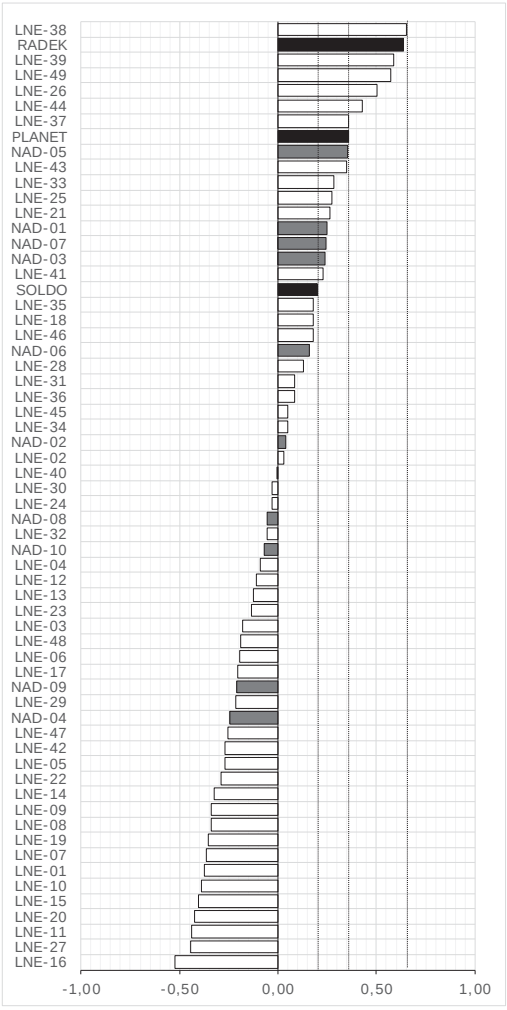
Source	Series I					Series II				
	df	SS	MS	F	p	df	SS	MS	F	p
2017										
Location	4	1120.215	280.054	1032.976	0.0000	4	803.816	200.954	853.933	0.0000
Block (Location)	10	139.386	13.939	51.412	0.0000	10	112.342	11.234	47.738	0.0000
Cultivars and lines	61	84.605	1.387	5.116	0.0000	61	76.906	1.261	5.357	0.0000
Interaction: Location × Cultivars and lines	244	118.656	0.486	1.794	0.0000	244	128.636	0.5270	2.240	0.0000
Error	610	165.379	0.271			610	143.550	0.235		
2018										
Location	4	632.280	158.070	418.120	0.0000	4	1402.006	350.502	742.445	0.0000
Block (Location)	10	26.549	2.655	7.023	0.0000	10	151.781	15.178	32.151	0.0000
Cultivars and lines	62	90.910	1.466	3.879	0.0000	62	99.931	1.612	3.414	0.0000
Interaction: Location × Cultivars and lines	248	157.014	0.633	1.675	0.0000	248	198.881	0.802	1.699	0.0000
Error	620	234.391	0.378			620	292.696	0.472		

df – degrees of freedom, SS – sum of squares; MS – means square; F – F-statistics; p – p-vaule

Figure 1. The size of BWLUE ratings for the tested lines and cultivars in the years 2017 and 2018

Year 2017

A) Series I



B) Series II

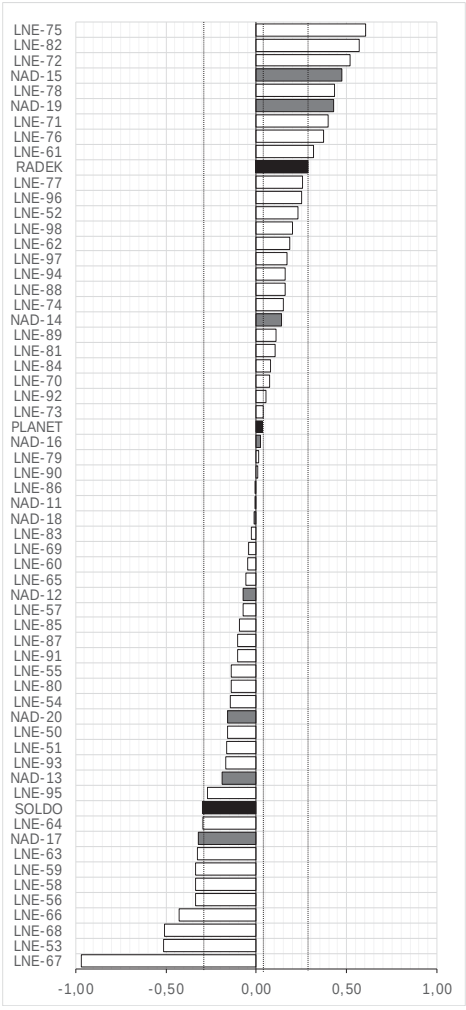
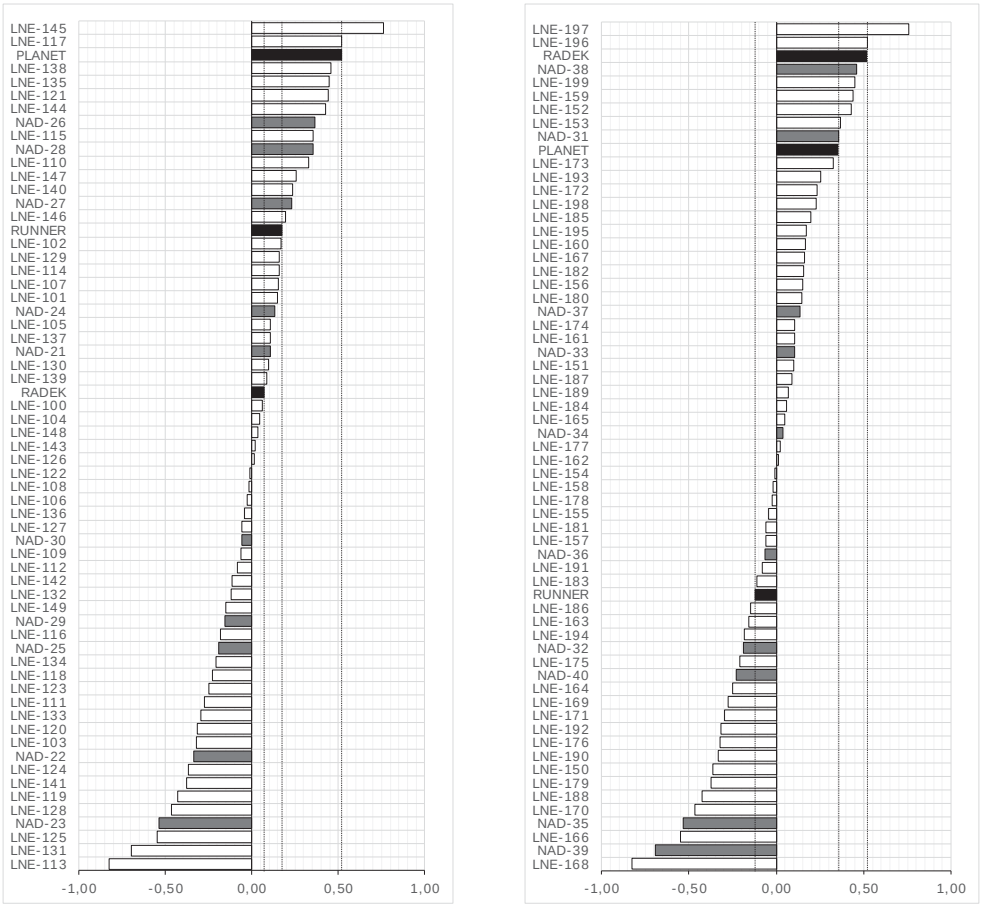


Figure 1 cont. The size of BWLUE ratings for the tested lines and cultivars in the years 2017 and 2018

Year 2018

Series I **Series II**



Considering reference varieties in 2017_S1 group, the Radek variety had the highest BWLUE effect (Fig. 1A). Slightly lower effect was observed for Planet variety while Soldo variety characterised by the lowest value of the BWLUE estimator. The only breeding line with BWLUE effect higher than Radek was the LNE-38. Five other LNE- breeding lines had values of the BWLUE estimator on intermediate level between Radek and Planet varieties. The effect very similar to the Planet variety was observed in case of NAD-05 line, while lines NAD-01, NAD-07 and NAD-03 characterised by effects not much higher than that observed for Soldo

variety. The intermediate effect between Planet and Soldo varieties was characteristic for 5 LNE- lines, while other 38 LNE- remaining lines, as well as other NAD- lines had BWLUE effect lower than Soldo variety.

Taking into account 2017_S2 group, the Radek variety characterised by the highest BWLUE effect (Fig. 1B). Higher effects were observed only in case of 9 breeding lines, including NAD-15 and NAD-19 lines. As in previous group, also in this group Planet variety had lower BWLUE effect than Radek and intermediate effects between these varieties were observed for 16 tested lines, including NAD-14. The NAD-16 breeding line had the value of the BWLUE estimator not much lower than Planet variety. The lowest value of the BWLUE estimator had Soldo variety and intermediate effects between Planet and Soldo were observed for 24 breeding lines, including NAD-11, NAD-18, NAD-12, NAD-20 and NAD-13. The other lines, with NAD-17 included, had BWLUE effect lower than Soldo variety.

In 2018_S1 group, the highest values of the BWLUE estimator were noted for LNE-145 and LNE-117 breeding lines (Fig. 1C). Regarding reference varieties, the highest BWLUE effect was observed in case of Planet variety, lower for Runner variety and the lowest for Radek variety. The BWLUE intermediate effects between Planet and Runner were noted in case of 12 lines, including NAD-26, NAD-28 and NAD-27 while intermediate effects between Runner and Radek were observed for 11 tested lines, including NAD-24 and NAD-21. Remaining 30 NLE- and 4 NAD- breeding lines had BWLUE effect lower than Radek variety.

Considering 2018_S2 group, the Radek reference variety had the highest BWLUE effect (Fig. 1D). Line LNE-197 as well as LNE-196 turned out to be better in terms of BWLUE effect. The effects lower than Radek variety but higher than the reference variety Planet were observed for 6 lines, with NAD-38 and NAD-31 included. The Runner reference variety turned out to have the lowest BWLUE effect. The intermediate effect between Planet and Runner varieties was characteristic for 32 tested lines, including NAD-37, NAD-33, NAD-34 and NAD-36, while other 16 LNE- and 4 NAD- remaining lines characterised by the effect lower than Runner variety.

In order to identify breeding lines which were most closely related to reference varieties in terms of yield, self-organizing maps were built where results of experiments (yield) in subsequent locations were treated as input variables (Fig. 2). This approach allowed to consider the level of yield as and the existing G×E interaction at the same time. Training and testing errors of SOM17 were 0.776229 and 1.485188 respectively whereas errors for SOM18 were 0.771346 and 1.412580.

The analysis of spatial distribution of nodes which mapped lines provided by Nagradowice Breeding Station within SOM17 model revealed that five of these

lines i.e. NAD-01, NAD-03, NAD-07, NAD-08 and NAD-10 had the yielding profile similar to Radek reference variety (Fig. 2A). Moreover, seven other LNE lines were related to this variety, too. In the same model, three lines included in LNE group represented a yield profile comparable to Soldo variety, and the other three to Planet variety.

Considering a spatial distribution of nodes in SOM18 we observed that there were up to twelve breading lines with yielding profile and the G×E interaction similar to Planet reference variety, including NAD-21 and NAD-24 lines (Fig. 2B). Furthermore, five breading lines characterised by a profile comparable to Radek reference variety, along with NAD-37 line, whereas four other tested lines were definitely more related to Runner reference variety.

Figure 2. Kohonen self-organizing map (SOM) for the results from 2017 and 2018. Gray color indicates inactive neurons, numbers in parentheses mean families other than NAD, dark color marked Woronoj's areas around the control cultivars.

A) Year 2017

	1	2	3	4	5	6	7	8	9	10	11
1	**NAD-20** [6]	**NAD-13** [1]			**NAD-11 NAD-12** [2]	[1]	[1]	[3]	[1]	**NAD-14 NAD-18** [4]	**NAD-15** [4]
2	[1]	[2]			[1]			**NAD-19**			
3			[1]		[1]	[1]	[1]				[1]
4	[1]		**NAD-09**	**NAD-17**					[1]	[1]	
5	[2]				[1]		[1]	[1]			[1]
6	[3]				*SOLDO* [1]					[1]	
7	[7]		[1]			[1]	*PLANET*				[3]
8	[3]	[1]					[2]		**NAD-05**		**NAD-16** [1]
9	[1]				[2]		[2]				**NAD-02** [3]
10				[2]			*RADEK* [3]				
11	[5]	[1]	[2]	[1]	**NAD-04** [4]	**NAD-03 NAD-08** [1]	**NAD-01 NAD-07** [1]	**NAD-10**		[1]	**NAD-06** [4]

Figure 2 cont. Kohonen self-organizing map (SOM) for the results from 2017 and 2018. Gray color indicates inactive neurons, numbers in parentheses mean families other than NAD, dark color marked Woronoj's areas around the control cultivars.

B) Year 2018

	1	2	3	4	5	6	7	8	9	10	11
1	[3]	NAD-21 [4]	*PLANET*	[3]	[1]	*RADEK* [1]	NAD-37 [2]	[1]	[1]	[1]	NAD-39 [6]
2	NAD-27 [3]	NAD-24 [3]				[1]				[1]	[1]
3	[2]										
4								[1]			[1]
5	NAD-28				[1]			[1]		[1]	NAD-32 NAD-35 [7]
6	[2]			*RUNNER*		NAD-31		[1]	[1]	[1]	NAD-36
7	NAD-26 [3]	[2]	[2]	[1]			NAD-38 [2]				
8	[1]		[1]	[2]							[1]
9	[5]	NAD-30	[1]				[1]				NAD-40 [2]
10	NAD-22 NAD-25 [2]		[1]	[1]				[1]		[1]	NAD-34
11	[4]	[2]	[1]	NAD-23 [2]		NAD-29 [2]	[3]		[1]	NAD-33 [1]	[4]

CONCLUSIONS

Varietal ranking derived from BWLUE effects (estimators) gives a great possibility to realise breading selection on the basis of any quantitative variable, e.g. yield. Lines selected hereby may be addressed to further stages of breeding process or to registration experiments. Data-mining tools and methods enable a holistic and synthetic view of field experiments. This paper is the best example how a specific usage of self-organizing maps may be applicable to indicate varieties similar to reference ones characterised by a comparable reaction to changing environmental conditions (location of the experiment).

Analyses results, i.e. ranking of BWLUE effects as well as SOM segmentation, revealed seven breading lines (NAD-15, NAD-19, NAD-05, NAD-26, NAD-28, NAD-38 and NAD-31), from Breeding Station Nagradowice, which can be

considered the best and most promising of the best chance of registration as a cultivar. These lines had high positions in BWLUE effects ranking (Fig. 1).

Regarding similarity to reference varieties, NAD-01, NAD-03, NAD-07, NAD-08, NAD-10, NAD-21, NAD-24 and NAD-37 deserve attention too. On created SOM planes these lines were located within Voronoi areas appointed around reference varieties (Fig. 2).

The methodological approach presented in this paper is a good solution in comparative breeding experiments, such as TBE realised by PBAI-NRI in cooperation with Polish breeding companies. This is confirmed by the presented research.

ACKNOWLEDGEMENTS

This research was financed within statutory topic No 2-6-00-3-01 titled "Biological progress and its components, including effects of crop cultivation in Poland, as well as selection and optimisation of statistical data analysis methods in evaluation of breeding effects."

LITERATURE

Andersson, A.A.M., Lampi, A-M., Nyström, L., Piironen, V., Li, Li., Ward, J., Gebruers, K., Courtin, C.M., Delcour, J.A., Boros, D., Fraś, A., Dynkowska, W., Rakszegi, M., Bedo, Z., Shewry, P.R., Åman, P. (2008). Phytochemical and fibre components in barley varieties in the HEALTHGRAIN diversity screen. *J. Agric. Food Chem., 56*, 9767–9776.

Boros, D., Rek-Ciepły, B., Cyran, M. (1996). A note on the composition and nutritional value of hulless barley. *J. Anim. Feed Sci., 5*, 417–424.

Boros, D., Fraś, A., Gołębiewska, K., Gołębiewski, D., Paczkowska, O., Wiśniewska, M. (2015). Nutritional value and health-promoting properties of grains of cereal and rape seed varieties recommended for cultivation in Poland. Monographs and Scientific Dissertations PBAI-NRI, 49, Radzików, Poland. *(in polish)*

COBORU (2019). Polish National List of Agricultural Plants Varieties. COBORU, Słupia Wielka, Poland.

Cyran, M., Izydorczyk, M., MacGregor, W. (2002). Structural characteristics of water-extractable nonstarch polysaccharides from barley malt. *Cereal Chem., 79*, 359–366.

FAO (2018). World Food and Agriculture – Statistical Pocketbook 2018; FAO: Rome, Italy.

Gołębiewski, D., Kamińska, B., Burek, J., Boros, D. (2014). Influence of the genotype and locality on the features determining the brewing value of grains of new spring barley lines. *Biuletyn IHAR, 274*, 3–14. *(in polish)*

Gołębiewski, D., Myszka, K., Fraś, A., Boros, D., Burek, J., Mańkowski, D. (2013). Genotypic and environmental diversity of characteristics of the brewing value of barley spring clones from pre-registration experience of 2012. *Biuletyn IHAR, 268*, 47–58. *(in polish)*

Grochowicz, J., Dominik, P., Fabisiak, A. (2017). Possibilities of using natural food as an effect of the global trend in the demand for pro-health food. *Zeszyty Naukowe UW, 54 (3)*, 223–240. *(in polish)*

Grzywa, M., Śmiałowski, T. (2002). Application of translocation to the localization of short-chain genomes in the barley Delisa mutant. *Biuletyn IHAR, 221*, 57–67. *(in polish)*

Janaszek, M.A., Trajer, J. (2011). Method of evaluating diversity of carrot roots using a self-organizing map and image data. *Computers and Electronics in Agriculture, Vol. 79, Issue 1*, 75–81.

Kohonen, T. (1982). Self-organized formation of topologically correct feature maps. *Biological Cybernetics, Vol. 48, Issue: 1*, 59–69.

Lasek, M., Myzik, A. (2012). Kohonen's neural networks in data analysis. An attempt to use data in the exploration of territorial units. *Problemy Zarządzania, 3 (38)*, vol. 10: 124–147. *(in polish)*

Laudański, Z. (1996). Application of projection operators in analysing non-orthogonal data – theory, algorithms and examples. Wydawnictwo SGGW, Warsaw, Poland. *(in polish)*

Mańkowski, D.R. (2013). Structural equations models SEM in agricultural research. Monographs and Scientific

Dissertations PBAI-NRI, 42, Radzików, Poland. *(in polish)*
Mańkowski, D.R., Śmiałowski, T., Laudański, Z., Janaszek-Mańkowska, M. (2014). Application of cultivar scoring for incomplete data (case study: field experiment with × *Triticosecale* Witt.). In: Laudański, Z., Mańkowski, D.R. (Eds.), International Conference — The 44th International Biometrical Colloquium and IV Polish-Portuguese Workshop on Biometry – Conference Papers. IHAR-PIB, Radzików, p. 39.
Marquardt, R.R., Boros, D., Guenter, W., Crow, G. (1994). The nutritive value of barley, rye, wheat and corn for young chicks as affected by use of a *Trichoderma reesei* enzyme preparation. *Anim. Feed Sci. Technol., 45*, 363–378.
Nadolska-Orczyk, A., Rajchel, I.K., Orczyk, W., Gasparis, S. (2017). Major genes determining yield-related traits in wheat and barley. *Theor Appl Genet, 130,* 1081–1098.
Ploch, M., Cyran, M., Kasztelowicz, K., Boros, D., Burek, J. (2005). Variability and interdependence of malting barley quality characteristics from the 2004 collection. *Biuletyn IHAR, 235*, 155–162. *(in polish)*
Sakellariou, M., Mylona, P. (2020). New Uses for Traditional Crops: The Case of Barley Biofortification. *Agronomy, 10 (12)*, 1964.
Śmiałowski, T., Cieplicka, A., Mańkowski, D.R. (2017). One- and multivariable characterization of spring barley (*Hordeum vulgare* L.) cultivars grown in Smolice Plant Breeding and tested in field breeding experiments in 2016. *Biuletyn IHAR, 282*, 63–78. *(in polish)*
Tadeusiewicz, R. (2001). Introduction to neural networks. StatSoft Polska, Cracow, Poland. *(in polish)*
TIBCO Software Inc., (2017). Statistica (data analysis software system), version 13, http://statistica.io
Trajer, J., Świderski, A. (2009). Using cluster analysis for examining carrot varieties diversity. *Annals of Warsaw University of Life Sciences – SGGW, Agriculture, 54,* 71—76.
Tricase, C., Amicarelli, V., Lamonaca, E., Leonardo Rana, R. (2018). Economic Analysis of the Barley Market and Related Uses. In: Grasses as Food and Feed. IntechOpen, London, UK.
Vesanto, J., (2000). Using SOM in Data Mining. Helsinki University of Technology, Espoo, Finland.
Węgrzyn, S., Grzywa, M., Śmiałowski, T. (2002). Evaluation of the combinational capacity of varieties and strains of spring barley for yield and resistance to powdery mildew. *Biuletyn IHAR, 221*, 45–55. *(in polish)*
Wirkijowska, A., Rzedzicki, Z., Sobota, A., Sykut-Domańska, E., Zarzycki, P., Bartoszek, K., Kuzawińska, E. (2016). Barley in human nutrition. *Polish Journal of Agronomy, 25*, 41–50. *(in polish)*
Wiśniewska, M., Boros, D., Zych, J. (2020). Nutritional value of selected mixtures of spring cereals with legumes. *Biuletyn IHAR, 289*, 51–62. *(in polish)*
Wójcik, A.R., Laudański, Z. (1989). Planning and statistical inference in experiments. PWN, Warsaw, Poland. *(in polish)*

DOI: 10.37317/pbss-2021-0002

Magdalena Wiśniewska*, Anna Fraś, Agnieszka Dmoch

Plant Breeding and Acclimatization Institute – National Research Institute
*Corresponding author's e-mail: m.wisniewska@ihar.edu.pl

VARIABILITY OF SELECTED QUANTITATIVE TRAITS IN NEW SPRING BARLEY GENOTYPES.

ABSTRACT

The research included 19 breeding lines and 4 cultivars of spring barley from the preliminary field experiments harvested in 2020 in Radzików. All barley samples were characterized for the content of protein, non-starch polysaccharides (NSP) with soluble (S-NSP) and insoluble (I-NSP) fractions and β-glucan. Additionally, viscosity of water extracts (WEV) was measured to determine the functional properties of the grain. It was the most diverse parameter (CV = 27%) and was significantly correlated with β-glucan content ($r = 0.50$; for $p < 0.05$). This dependence is shown by the results obtained for the grain of the Avatar cultivar and the RAH 744/19 breeding line, in which the content of β-glucan (5.3% and 4.8%, respectively), as well as the WEV (3.3 mPa.s and 3.0 mPa.s, respectively) were the highest. The lowest content of β – glucan (3.5%) and one of the lowest WEV values (1.4 mPa.s) were observed for KWS Jessie cultivar. Principal component analysis (PCA) showed a substantial impact of the two components PC1 and PC2 on the variability of the analyzed material showing significant variability of the 5 barley genotypes and confirmed the previous results of biochemical analyzes. Our results made it possible to indicate several genotypes that may constitute a source of variability in breeding works aimed at improving the quality of barley. Presented study also show that the grain of some new barley genotypes, with a favorable chemical composition from a fodder and brewing perspective, is a good material for future use in industry.

Key words: barley, β – glucan, dietary fiber, non-starch polysaccharides, utility value of barley

INTRODUCTION

Barley (*Hordeum vulgare* L.) is one of the first cereals cultivated by humans. Until the improvement and widespread cultivation of wheat and rice, barley was widely used for consumption and fodder as well as for production of beverages or medicinal purposes. Currently, the importance of barley as a food grain is low, but still in some parts of the world (Morocco, Tibet, Ethiopia) it remains the main source of food (Newman and Newman, 2006). In highly developed countries, barley is primarily used as an animal feed and as a raw material for the production of malt

Communicated by Grzegorz Żurek.

and beer (Arngren et al., 2011; Alazmani et al., 2015; Sorour et al., 2021). This situation is slowly changing due to new nutritional trends. Consumers are interested not only in foods with high nutritional value, but also in foods with functional properties (Narina et al., 2012; Grochowicz et al., 2017). Barley grain contains high amount of dietary fiber, both soluble and insoluble, and a number of other biologically active and nutritious compounds, therefore it is a very good raw material for production of such food (Boros et al., 2015; Grochowicz et al., 2017; Prasadi et al., 2020). The use value of barley grain is determined by the content of basic chemical components, mainly protein and dietary fiber complex. The quality requirements for barley raw material intended for nutritional and fodder purposes and malt are described in the Polish Standard (PN – R – 74109), which in the case of consumer grain specifies them to a minimum degree (Wirkijowska et al., 2016). The protein content is the basic criterion for the selection of cultivars in all directions of use. Grain used in human nutrition and feeding animals should contain as much protein as possible. Spring cultivars of two-rowed barley are particularly useful for the production of groats, as they are characterized by a well-filled grain with a large amount of protein (Gąsiorowski, 1997; Noworolnik, 2014). Cultivars with a protein content in the range between 9.5% and 11.5% are selected for the malt production (Gąsiorowski, 1997; Klockiewicz – Kamińska, 2005; Kunze, 2010), what ensures appropriate grain modification and the desired, high malt extractivity and higher beer yield by weight unit of barley. The amount of protein and the amino acid profile are key factors determining the nutritional value of plant raw material in the food and feed industry. Cereal protein is partially defective due to the insufficient amount of essential amino acids, mainly lysine. However, barley protein is second only to rye and oat proteins in lysine content. The additional advantage of barley protein is also the high content of exogenous amino acids, at the level of 34% – 38% (Biel and Jacyno, 2013; Sterna et al., 2015; Wiśniewska et al., 2020). Protein bioavailability depends not only on its quantity and biological value, but also on the presence of other substances that limit its absorption, hence they are referred to as anti-nutritional factors. This group includes such components as the dietary fiber complex, especially its water-soluble fraction, rich in arabinoxylans and β-glucan. According to the research previously described by Izydorczyk and Dexter (2008), the amount of arabinoxylans in barley grain is in the range of 3.5% – 6.1%, and β – glucan 2.5% – 11.3%. Arabinoxylans due to their solubility are divided into water-extractable (WE – AX) and water- unextractable (WUE – AX) and the latter fraction accounts for more than 90% of the total barley AX. Compared to these compounds, β-glucan is characterized by a much higher degree of solubility. Therefore its content is one of the most important parameters

for assessing the suitability of barley varieties in a malt house or brewery, because it causes many problems in during the entire technological process. These polysaccharides also limit the process of germination and hydrolysis of compounds contained in the kernel, especially starch (Gamlath et al., 2008). As a result, they contribute to the reduction of malt extractivity, and in wort and beer they cause their high viscosity, which leads to problems related to their filtration (Jadhav et al. 1998; Izydorczyk et al., 2000, Jin et al., 2004). In animal nutrition, the soluble fraction of fiber adversely affects the production rates of fed animals by reducing body weight gain, worse feed utilization, and even digestive system ailments (Jadhaw et al., 1998, Boros et al., 2015, Wiśniewska et al., 2020). In human nutrition, dietary fiber is a desirable component in the diet because of its numerous documented pro-health properties (Zieliński i in., 2012; Mudgil i Barak, 2013; Perczyńska i in., 2017, Idehen i in., 2017; Fraś i in, 2018; Henrion i in., 2019). For this reason, barley varieties with a higher β-glucan content are preferred in the groats industry.

The ability of non-starch polysaccharides, especially β-glucan, to form viscous solutions was investigated many years ago (Greenberg and Whitmore, 1973; Aastrup, 1979; Bhatty, 1987). With regard to arabinoxylans, these studies have been very scarce. The influence of these compounds was ignored, suggesting their low solubility in water, and thus little influence on the viscous properties of the solutions in which they are located. Nevertheless, considering both of these polysaccharides, it was found that their quantity, structure, molecular weight and activity of endoenzymes: β – glucanase and xylanase significantly determine the viscosity of the grain extract. The results of the research conducted so far indicate that the measurement of the viscosity of the water or acidic grain extract can be successfully used as an indicator of the functional properties of the grain in relation to barley as well as other cereals (Izydorczyk et al., 2000; Cyran et al., 2002; Caprita et al., 2011a, Boros et al., 2015; Cyran i in., 2019). For this reason, the water extract viscosity (WEV) was included in the evaluation of the studied barley genotypes.

The aim of the study was to characterize new spring barley breeding lines in terms of protein content, non-starch polysaccharides and the related water extract viscosity, and to indicate genotypes that stand out in terms of the analyzed characteristics, taking into account the possible direction of its use.

MATERIALS AND METHODS

The research material comprised of 19 spring barley breeding lines including 14 forage and 5 brewers lines and 4 standard cultivars: two forage (Avatar, Rekrut) and two brewers (KWS Jessie, RTG Planet). The selected cultivars constitute

a comparative pattern for the brewing and forage families. These cultivars were selected for preliminary tests by Research Centre for Cultivar Testing. All samples were harvested in 2020 in Radzików (Poland) and came from the preliminary field experiments.

The averaged samples of barley grains were ground prior chemical analysis in the Cyclotec™ (Foss, Denmark) laboratory mill passing through a 0.5 mm sieves. All samples were stored in the fridge, in sealed plastic cups until analysis. The moisture content of the grain was determined according to the AACC method 46–16.01 (AACC, 2003). Protein content was analyzed using the Kjeldahl method (AOAC 955.04) on a Kjeltec Auto 1030 Analyzer (Foss, Denmark), using N x 6.25 as a conversion factor (AOAC, 1995). Non-starch polysaccharides (NSP) content with its fractionation to insoluble (I-NSP) and soluble (S-NSP) fraction was determined using gas chromatography (GS) as previously described by Englyst and Cummings (1984). In this method, the NSP of each fraction is a sum of individual monomers: arabinose, xylose, mannose, galactose and glucose. Based on this analysis, the arabinoxylans (AX) content in each fraction (WUE-AX – water unextractable arabinoxylans and WE-AX – water extractable arabinoxylans) was calculated as the sum of arabinose and xylose. β-glucan content was analysed with colorimetric method, using the Megazyme (Bray, Ireland) procedure in accordance with the AACC 32–23.01 method (2011). The water extract viscosity (WEV) of the grain was analyzed using a Brookefield model LVDV-II Cone/Plate Digital Viscometer (Brookefield, Stoughton, MA), according Boros et al. (1993). All analyses were performed in duplicate and results reported on a dry weight basis [% of d.w.]. To study the variability of the chemical components within different genotypes, a one way analysis of variance (ANOVA) and Tukey's contrast analysis were performed. The coefficients of variation (CV%) and the Pearson correlation coefficients (r) between analysed traits were also calculated. Principal component analysis (PCA) was carried out to obtain an overview of differences in analyzed components between each barley varieties. All statistical analyses were performed using Statictica (data analysis software system), version 13.3 *(TIBCO Software Inc., 2017).*

RESULTS AND DISCUSSION

The analyzed barley lines and cultivars differed significantly in terms of all traits, and the obtained results are presented in Table 1. The variability of most of the analyzed features was low, in the range of 8–13%, only the WEV was characterized by a high coefficient of variation at the level of 27%. Protein is the main nutrient

Table 1. Chemical composition (% of d.w.) and water extracts viscosity (mPa.s) in the analyzed barley grain.

Effects		Protein		I-NSP*		S-NSP*		NSP*		WUE-AX*		WE-AX*		AX*		β-glucan		WEV*	
Barley fodder lines	RAH 36/19	12,1	bc	8,3	h	4,8	efgh	13,1	j	4,5	I	0,5	d	5,0	j	4,5	def	2,3	*cd*
	RAH 97/19	11,1	hij	8,6	gh	4,6	gh	13,3	j	4,9	gh	0,5	cd	5,4	hi	4,0	hij	2,0	*e*
	RAH 281/19	12,3	b	9,7	cde	5,9	b	15,7	cde	5,1	fgh	0,5	bcd	5,6	ghi	4,7	cde	2,3	*cd*
	RAH 337/19	11,4	fgh	9,0	fg	5,7	bc	14,7	fghi	4,9	H	0,5	d	5,4	i	4,9	bc	2,3	*cd*
	RAH 411/19	11,8	cde	9,8	cd	4,2	h	14,0	ij	5,3	defgh	0,5	d	5,8	defgh	3,7	jkl	1,9	*ef*
	RAH 419/19	14,0	a	11,9	a	5,4	bcdef	17,3	ab	6,7	A	0,6	ab	7,3	a	3,6	l	1,6	*h*
	RAH 420/19	10,8	j	9,5	def	7,0	a	16,5	bc	5,3	def	0,6	abc	6,0	cdefg	5,3	a	1,9	*fg*
	RAH 426/19	11,2	ghi	9,1	efg	5,7	bc	14,8	efghi	5,1	fgh	0,5	cd	5,6	fghi	4,9	bc	1,7	*g*
	RAH 474/19	10,8	j	9,1	fg	5,4	bcdef	14,4	hi	4,9	gh	0,5	bcd	5,5	hi	3,7	jkl	1,2	*k*
	RAH 493/19	11,4	fgh	10,3	c	5,0	cdefg	15,3	efgh	5,6	bcd	0,5	d	6,1	cd	3,8	ijkl	1,3	*jk*
	RAH 503/19	10,9	ij	9,7	cdef	5,6	bcd	15,2	efgh	5,3	defgh	0,5	d	5,8	defgh	4,3	fgh	1,4	*ij*
	RAH 532/19	12,1	bc	11,0	b	6,8	a	17,8	a	5,9	b	0,6	abc	6,6	b	5,1	ab	1,6	*h*
	RAH 615/19	11,4	fgh	10,0	cd	4,7	fgh	14,7	fghi	5,8	bc	0,5	d	6,3	bc	3,7	jkl	1,3	*jk*
	RAH 620/19	11,7	def	9,9	cd	5,6	bc	15,5	def	5,4	def	0,5	cd	5,9	cdefg	4,5	ef	1,6	h
Barley brewing lines	RAH 691/19	10,8	j	10,3	c	4,8	efgh	15,1	efgh	5,5	cde	0,5	d	6,0	cdef	4,1	ghi	2,4	*c*
	RAH 744/19	10,8	j	9,9	cd	5,6	bcd	15,5	def	5,4	def	0,5	d	5,9	defg	4,8	bcd	3,0	*b*
	RAH 832/19	9,8	k	10,2	c	5,5	bcde	15,7	cde	5,6	bcde	0,5	cd	6,1	cde	4,4	fg	1,8	*g*
	RAH 885/19	10,8	j	9,9	cd	4,9	defgh	14,8	efghi	5,3	def	0,5	d	5,8	defgh	4,0	hijk	2,0	*e*
	RAH 889/19	10,1	k	9,6	def	5,3	bcdefg	14,8	efghi	5,3	defgh	0,5	d	5,8	defgh	4,1	ghi	2,0	e
Pattern of fodder varieties	AVATAR	12,0	bcd	9,4	def	6,9	a	16,3	cd	5,3	defg	0,7	a	6,0	cdef	5,3	a	3,3	*a*
	REKRUT	10,2	k	10,0	cd	4,7	fgh	14,7	fghi	5,3	def	0,5	d	5,8	defgh	3,7	kl	2,2	cd
Pattern of brewing varieties	KWS JESSIE	9,3	l	9,7	cdef	4,8	efgh	14,5	ghi	5,2	efgh	0,5	d	5,7	efghi	3,5	l	1,4	*i*
	RGT PLANET	11,5	efg	9,9	cd	5,5	bcde	15,4	efg	5,2	efgh	0,5	bcd	5,8	defgh	4,3	fgh	2,2	d
Statistics F; F		241,6		41,7		35,07		46,5		38,1		13,6		46,3		93,8		617,5	
p - value		0,000		0,000		0,000		0,000		0,000		0,000		0,000		0,000		0,000	

*I-NSP– insolubel nonstarch polysaccharides; S-NSP- solubel nonstarch polysaccharides; NSP nonstarch polysaccharides
*WUE-AX – water unextractable arabinoxylans; WE-AX – water extractable arabinoxylans; AX – arabinoxylans;
* WEV – water extract viscosity

of cereal grains and the content of this component in the analyzed material was determined at an average level of 11.2%. The lowest content of protein was obtained in the grain of the brewing cultivar KWS Jessie (9.3%), and the highest in the forage breeding line RAH 419/19 (14.0%). Higher average amounts of protein in the range of 11.6% – 15.5% were obtained by Biel and Jacyno (2013), Boros et al. (2015), and Haverlentova et al. (2020). In the grain of any of the studied brewing breeding lines, the obtained amount of protein did not exceed 11%, that is, it was within the 9.5% – 11.5%, preferred by malt houses and breweries (Klockiewicz – Kamińska, 2005; Kunze, 2010). Fodder breeding lines contained an average of 11.7% protein, which is 1.2% more than in the brewing genotypes. Only the grain of 6 breeding lines was characterized by a higher level of this component than the presented average. Similar in the case of standard cultivars, the forage cultivars contained an average of 0.7% more protein compared to the brewing cultivars.

The non-starch polysaccharides (NSP) was the next analyzed component. The highest amount of NSP was obtained in the grain of RAH 532/19 (17.8%), RAH 419/19 (17.3%) whereas the least in RAH 36/19 (13.1%) and RAH 97/19 (13.3%). Similar NSP values in the range of 12.6% and 18.6% were described by Bach Knudsen (2014) and Boros et al. (2015). Wiśniewska et al. (2020) obtained a lower content of NSP, at a level of 11.9%. In the above studies, the quantity of S-NSP and I-NSP was 33% – 35% and 65% – 67% of the total amount of non-starch polysaccharides, respectively. A similar amount of particular fractions of the NSP was obtained in the presented study. The S-NSP consisted on average 36% of the total amount of NSP in the analyzed barley grain, whereas the average content of this fraction was at a level of 5.4%, with a variability of 13%. Two genotypes, the forage cultivar Avatar and the RAH 420/19 breeding line were characterized by significantly higher (over 42%) than the average content of S – NSP. The content of S-NSP in these samples was also the highest, at a level of 6.9% and 7.0%, respectively. The breeding lines with the lowest (about 31%), proportion of the soluble NSP fraction in the total amount of these compounds are RAH 419/19, RAH 691/19 and RAH 411/19, containing 5.4%, 4.8% and 4.2% of S – NSP, respectively. The insoluble fraction of NSP (I-NSP) accounted for an average of 64% of the total NSP, and its extreme values were obtained for forage breeding lines and ranged from 8.2% for the RAH 36/19 to 11.9% for the RAH 419/19 breeding line.

Arabinoxylans (AX) are the predominant part among the non-starch polysaccharides in barley grain. The average content of AX in analyzed material was at a level of 5.9% and extreme values were obtained for lines with the highest amount of I-NSP and ranged from 5.0% for RAH 36/19 to 7.3% for RAH 419/19 breeding line. The same lines also contained extreme levels of WUE-AX ranging

from 4.5% (RAH 36/19) to 6.7% (RAH 419/19) and an average content of these compounds at the level of 5.3%. The presented range of WUE-AX content differed from the range described by other authors, who obtained the content of these compounds in the amount of 3.5% – 6.1% (Izydorczyk and Dexter, 2008; Boros et al., 2015). The average content of the water-extractable arabinoxylan fraction (WE – AX) in the analyzed genotypes was 0.52%, which accounted for about 9.0% of the total amount of AX in the grain. The highest amount of these compounds was found in the following breeding lines: RAH 419/19, RAH 420/19 and RAH 532/19 (0.6%) and the cultivar Avatar (0.7%), in which the amounts of AX in total were also one of the largest (7.3%, 6.0%, 6.6%, 6.0%, respectively). The amount of WE-AX described previously by Izydorczyk and Dexter (2008) was slightly higher, in the range of 0.4% – 1.0%, in comparison to values obtained in our research 0.45% (RAH-337/19) – 0.72% (Avatar). The barley grain studied by Zhang et al. (2013) was characterized by a much lower content range from 0.25% to 0.39% WE – AX. This fraction averaged 7.8% of the total arabinoxylans, which was found to be an average of 4.10%.

Another important polysaccharide in barley grain, included in the NSP is β-glucan. The average content of this component in the analyzed genotypes was at a level of 4.3%. The grain of the brewing variety KWS Jessie characterized by the lowest β-glucan level (3.5%), whereas the highest content (5.3%) was observed for the forage variety Avatar. Among the breeding lines, the most noteworthy are those with the extreme content of β – glucan in the grain – the highest: RAH 420/19 (5.3%), RAH 532/19 (5.1%), RAH 337/19 (4.9%), RAH 426/19 (4.9%)) and those with the smallest: RAH 419/19 (3.6%), RAH 411/19 (3.7%), RAH 474/19 (3.7%) and RAH 615/19 (3.7%). The first of these genotypes are a desirable raw material for the food production, while breeding lines with a low β-glucan content are used in the fodder industry, as well as in the malt house and brewery. The analyzed fodder and brewing lines contained a similar average amount of β-glucan (4.3% vs. 4.2%, respectively), but the variability of forage breeding lines in terms of this trait was higher than that of the brewing lines (13% vs. 8%). Similar content of β – glucan, at the level of 3.8%, was described by Nishantha et al. (2018) who analyzed the content of this polysaccharide in 28 barley cultivars grown in different places around the world. They found that about 90% of the tested cultivars contained from 3.0% to 5.0% of β-glucan. In other studies, Bach Knudsen (2014) reported the average amount of barley β-glucan at the level of 4.1%.

According to the literature data, the polysaccharides of barley grain are significantly related to the viscosity of the water extract (Lazaridou et al., 2004, Caprita et al., 2011a, b), which allows to determine the physiological properties

of the grain with high probability, significantly related to its chemical composition. The obtained viscosity values differed significantly and ranged from 1.2 mPa.s for the RAH 474/19 breeding line to 3.3 mPa.s for the reference variety Avatar. Lower WEV values in the range between 1.2 mPa.s and 1.9 mPa.s were presented by Boros et al. (2015). On the other hand, Caprita et al. (2011a) obtained higher values of WEV of barley grain at the level of 3.32 cP. In the presented study, the average value of the viscosity for the brewing lines was 2.2 mPa.s, and for the forage lines 1.7 mPa.s. The water extracts of 40% of brewing lines and 50% of forage lines were characterized by a higher viscosity than the obtained average values. The lowest WEV was obtained for the grain of the following breeding lines: RAH 493/19 (1.3 mPa.s), RAH 615/19 (1.3 mPa.s), RAH 503/19 (1.4 mPa.s), and the brewing cultivar KWS Jessie (1.4 mPa. s), while the highest WEV was characteristic for the grain extracts of the following genotypes: RAH 744/19 (3.0 mPa.s), RAH 691/19 (2.4 mPa.s) and RAH 36/19 (2.3 mPa.s), RAH 281/19 (2.3 mPa.s) and RAH 337/19 (2.3 mPa.s). The viscous properties of the analyzed extracts were determined primarily by the presence of β-glucan, what was confirmed by the significant correlation obtained for these features ($r = 0.50$ for $p < 0.05$). The same relationship, at the level of $r = 0.86$, was obtained by Boros et al. (2015). In another study Caprita et al. (2011) showed a significant relationship between the viscosity of the aqueous extract and the molecular weight of the soluble fraction of dietary fiber contained in the grain of barley, oats, triticale and wheat ($r = 0.917$).

On the basis of the obtained results, it is worth emphasize the chemical profile of several barley genotypes. First of all the RAH 419/19 breeding line with the highest protein (14%) and non-starch polysaccharides (17.3%) content in which the amount of insoluble fraction (11.9%), as well as the level of non-extractable arabinoxylans (WUE – AX) (6.7%) were also the highest, whereas the amount of β-glucan was the lowest (3.6%). The chemical composition of two another breeding lines RAH 36/19 and RAH 97/19 was very similar and characterized by the lowest content of non-starch polysaccharides (13.1% and 13.3%, respectively) and similar values of particular fractions of NSP and AX, but differed significantly in the content of proteins (12.1% vs. 11.1%). The grain of the RAH 420/19 breeding line and the forage cultivar Avatar was also similar in terms of the content of analyzed components. In both cases it contained the highest β-glucan content at a level of 5.3% and WE – AX at a level of 0.6% and. 0.7%, respectively, whereas the amounts of S – NSP constituted 42% and 43% of the total content of NSP in these genotypes, respectively. However, both genotypes differed in the protein content, (10.8% vs. 12.0%) as well as the WEV (1.9 mPa.s vs. 3.3 mPa.s). The grain of the RAH 532/19 breeding line was a rich source of bioactive ingredients and characterized of the highest

content NSP (17.8%) and also their individual fractions (I – NSP – 11.0%, S – NSP – 6.8%), as well as arabinoxylans (6.6%) and β – glucan (5.1%), with relatively low viscosity value (1.6 mPa.s). The results obtained in the presented study for the RAH 744/19 breeding line and the KWS Jessie cultivar showed the relationship between the amount of β-glucan and WEV. In case of breeding line the WEV was at a level of 3.0 mPa.s, and the polysaccharide content was one of the highest among studied barley genotypes at a level of 4.8%. The grain of the brewing cultivar KWS Jessie had one of the lowest viscosity value (1.4 mPa.s) and the lowest level of β – glucan (3.5%). Additionally, the RAH 744/19 breeding line was characterized by a 1.5% higher protein amount as compared to the KWS Jessie cultivar (10.8% vs. 9.3%, respectively).

Principal Component Analysis (PCA) was used to illustrate any variations in the material and to identify correlations between analyzed traits. The results of PCA analysis are presented in Figure 1. The two principal components, PC1 and PC2 explained 47.67% and 29.92% of the variation, respectively. The PC1 component dependent mainly on the content of protein (correlation coefficient, r = -0.54) and NSP (r = -0.95), including I – NSP (r = -0.82), S – NSP (r = -0.59), as well as their main component AX (r = -0.90), together with WUE – AX (r = -0.85) and WE – AX (r = -0.69). The PC2 component was dependent on the content of the soluble fraction of dietary fiber: S-NSP (r = 0.72), including WE – AX (r = 0.52) and β-glucan (r = 0.90) as well as the WEV (r = 0.63). On the basis of the obtained results, 3 groups were distinguished among the examined facilities, depending mainly on WEV. The first of these were forage families RAH 281/19, RAH 337/19, RAH 426/19 and the brewery house RAH 744/19, which were characterized by high WEV values and high PC1 values. The second, most numerous group consisted of both brewery (RGT Planet, KWS Jessie, RAH 691/19, RAH 832/19, RAH 885/19, RAH 889/19) and forage breeding lines (Rekrut, RAH 97/19, RAH 411/19, RAH 474/19, RAH 503/19, RAH 620/19), with average viscosity and average values of parameters related to the first component. The third group consisted of two forage lines: RAH 493/19 and RAH 615/19, with one of the lower WEV, β – glucan and S – NSP values among all the analyzed samples and the average values of the parameters that constituted the PC1 component. Besides the described groups, five individual genotypes of forage origin were also distinguished, characterized by outstanding values for selected parameters. These include the Avatar cultivar with the highest WEV, WE – AX and S – NSP content among the analyzed barley samples.

The RAH 420/19 fodder line also distinguish in terms of WEV and β-glucan content. Furthermore, the RAH 419/19 line contained the highest amount of pro-

tein and NSP insoluble fraction, whereas the RAH 532/19 breeding line contained the highest amount of NSP among all samples and one of the highest protein content. The last distinguishing object was the RAH 36/19 fodder line, that contained the smallest amount of NSP, including AX among the studied samples.

The analyzed genetic material of barley described in the presented study, with such a diverse chemical profile of the grain, may be a good source of variability that can be used in breeding work for various directions of barley use.

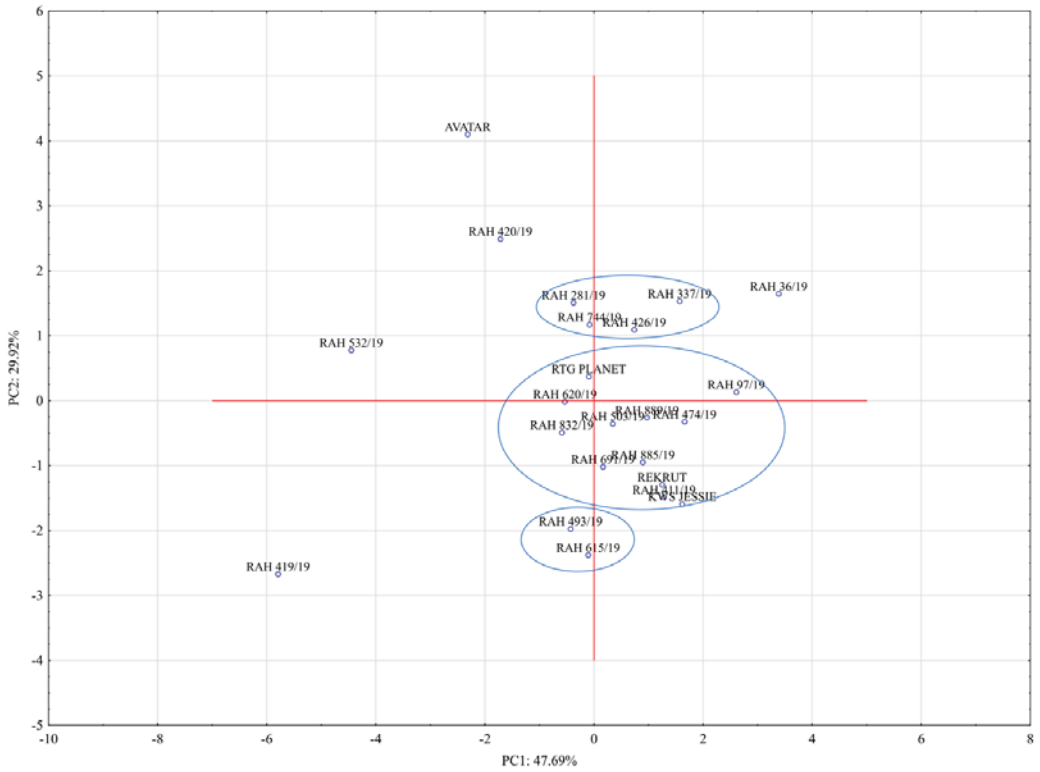


Figure 1. Score plot for the first two principal components from analysis of barley.

CONCLUSION

1. Barley genotypes showed low variability in the content of chemical components, expressed in coefficients of variation, which for most of the examined features did not exceed 10%. The exception was the WEV with CV = 27%.
2. The significant correlation obtained between WEV and β-glucan content confirmed the possibility of using the viscosity measurement as an indicator of the functional quality of barley, both for breeders and producers of fodder, malt houses, breweries and the food industry.

3. The results of chemical analyzes made it possible to select several breeding lines (RAH 36/19, RAH 97/19, RAH 419/19, RAH 420/19, RAH 532/19, RAH 744/19) as potential sources of variability, necessary in breeding works.
4. The obtained chemical results were confirmed by the principal components analysis, on the basis of which 5 breeding lines (RAH 36/19, RAH 419/19, RAH 420/19, RAH 532/19, Avatar) with the greatest variation among the parameters have been identified. These genotypes may be valuable for barley breeding in terms of specific functional characteristics.

REFERENCES

Aastrup S. 1979. The relationship between the viscosity of an acid flour extract of barley and its β – glucan content. Carlsberg Res.Commun.44: 289–304.

Alazmani A. 2015. Evaluation of yield and yield components of barley varieties to nitrogen. International Journal of Agriculture and Crop Sciences, 8 (1): 52- 54.

Arngren M., Hansen P. W.; Eriksen B.; Larsen J.; Larsen R. 2011. Analysis of pregerminated barley using hyperspectral image analysis. Journal of Agricultural and Food Chemistry, 59: 11385–11394.

Bach Knudsen K. E. 2014. Fiber and nonstarch polysaccharide content and variation in common crops used in broiler diets. Poultry Science, 93: 2380–2393.

Bhatty R. S. 1987.Relatioship between acid extract viscosity and total soluble and insoluble β – glucan contents of hulled and hulless barley. Canadian Journal of Plant Science. 67 (4): 997–1008.

Biel W., Jacyno E. 2013. Chemical composition and nutritive value of spring hulled barley varieties. Bulgarian Journal of Agricultural Science., 19 (4): 721–727.

Boros D., Marquardt R.R., Slominski B.A., Guenter W. 1993. Extract viscosity as an indirect assay for water-soluble pentosans content in rye. Cereal Chemistry, 70: 575–580.

Boros D., Fraś A., Gołębiewska K., Gołębiewski D., Paczkowska O., Wiśniewska M. 2015. Wartość odżywcza i właściwości prozdrowotne ziarna odmian zbóż i nasion rzepaku zalecanych do uprawy w Polsce. Monografie i Rozprawy Naukowe IHAR-PIB, 49, 1–119.

Caprita R., Caprita A., Cretescu I., Julean C. 2011a. Influence of temperature on the extractability of polysaccharides in barley. Animal Science and Biotechnologies, 44 (2): 81–84.

Caprita A., Caprita R., Simulescu V. O., Drehe R. M., 2011b. Water extract viscosities correlated with soluble dietary fiber molecular weight in cereals. Journal of Agroalimentary Processes and Technologies, 17 (3): 242–245.

Cyran M., Izydorczyk M. S., MacGregor A.W. 2002. Structural characteristics of waterextractable nonstarch polysaccharides from barley malt. Cereal Chemistry. 79 (3): 359–366.

Cyran M., Snochowska K., Śmiałowski T. 2019. Wysokocząsteczkowe arabinoksylany ziarna pszenicy: zawartość, masa cząsteczkowa oraz związek z poziomem lepkości ekstraktu. Biuletyn IHAR., 285: 287–288.

Englyst H.N., Cummings J.H. 1984. Simplified method for the measurement of total non-starch polysaccharides in plant foods by gas-liquid chromatography of constituent sugars as alditol acetates. Analyst. 109, 937–942.

Fraś A., Gołębiewski D. Gołębiewska K., Mańkowski D.R., Boros D. 2018. Content of nutrient and bioactive non-nutrient components in different oat products. Quality Assurance and Safety of Crops & Foods: 10: 307–313.

Gamlath J., Aldred G.P., Panozzo J. F. 2008. Barley (1–3; 1–4)-β – glucan and arabinoxylan content are related to karnel hardness and water uptake. Journal of Cereaal Science. 47: 365–371.

Gąsiorowski H. 1997. Jęczmień chemia i technologia. PWRiL, Poznań, wyd. I.

Greenberg D. C., Whitmore E. T. 1974. A rapid method for estimating the viscosity of barley extracts. J. Inst. Brew. 80: 31–33.

Grochowicz J., Dominik P., Fabisiak A. 2017. Możliwości wykorzystania żywności naturalnej jako efekt ogólnoświatowego trendu w zakresie zapotrzebowania na żywność prozdrowotną. Zeszyty Naukowe UV. 54 (3): 223–240.

Haverlentova M., Babulicova M., Koseva B., Dyulgerova B. K., Hendrichova J., Valcheva D. D., Valchev D. G., Hašana R. 2020. Grain quality of spring barley genotypes grown at agro-ecological conditions of the Slovak Republic and the Republic of Bulgaria. Journal of Central European Agriculture. 21 (4): 775–788.

Henrion M., Francey C, Lê K. – A., Lamothe L. 2019. Cereal β – glucans: The impact of processing and how it affects physiological responses. Nutrients. 11 (8): 1729–1742.

Idehen E., Tang Y., Sang S. 2017. Bioactive phytochemicals in barley. J. Food Drug Anal., 25 (1): 148–161.

Izydorczyk M. S., Storsley J., Labossiere D., MacGregor A. W., Rossnagel B. G. 2000. Variation in total and soluble β – glucan content in hulless barley: effects of thermal, physical and enzymic treatments. Journal of Agricultural

and Food Chemistry. 48: 982–989.
Izydorczyk M.S., Dexter J.E. 2008. Barley β – glucans and arabinoxylans: Molecular structure, physicochemical properties, and uses in food products – a Review. Food Research International 41: 850–868.
Jadhav S. J., Lutz S. E., Ghorpade V. M., Salunkhe D. K. 1998. Barley: chemistry and value-added processing. Critical Reviews in Food Science and Nutrition, 38 (2): 123–171.
Jin J. L., Speers R. A., Paulson A. T., Stewart R. J. 2004. Barley β – glucan and their degradation during malting and brewing. Technical Quarterly Master Brewers Association of the Americas. 41: 231–240.
Klockiewicz – Kamińska E. 2005. Metoda oceny wartości browarnej i klasyfikacja jakościowa odmian jęczmienia. Wiadomości odmianoznawcze, COBORU, Słupia Wielka, zeszyt 80.
Kunze W. 2010.Technology Brewing and Malting., 4th International Edition, VLB Berlin, Germany.
Lazaridou A, Biliaderis C.G., Micha-Screttas M., Steele B.R., 2004. A comparative study on structure-function relations of mixed-linkadge (1–3), (1–4) linear β-glucans. Food Hydrocolloids. 18: 837–855.
Mudgil D., Barak S. 2013. Composition, properties and health benefits of indigestible carbohydrate polymers as dietary fiber: reviev. Int. J. Biol. Macromol. 61: 1–6.
Narina S.S., Hamama A. A., Bhardwaj H. L. 2012. Nutritional and mineral composition of flax sprouts. Journal of Agricultural Science, 4 (11): 60–65.
Newman C.; Newman R.; Brief A. 2006. History of Barley Foods. Cereal Foods World, 51:4–7.
Nishantha M. D. L. C., Zhao X., Jeewani D. C., Bian J., Nie X., Weining S. 2018. Direct comparison of β – glucan content in wild and cultivated barley. International Journal of Food Properties, 21 (1): 2218–2228.
Noworolnik K. 2014. Agrotechnika w kształtowaniu plonu i jakości ziarna jęczmienia jarego na cele pastewne i spożywcze. Studia i raporty IUNG – PIB. 41 (15): 21–37.
Perczyńska A., Marciniak-Łukasiak K., Żbikowska A., 2017. Rola β-glukanu w przeciwdziałaniu chorobom cywilizacyjnym. Kosmos. 66: 379- 388.
Prasadi N. V. P., Joye I. J. 2020. Dietary fibre from whole grains and their benefits on metabolic health. Nutrients. 12: 3045–3066.
Sorour M. A., Ramadan B. R., Mehanni A. E., Kobacy W. 2021. Impact of soaking and germination process on starch and non – starch polysaccharides in some Egyptian barley cultivars. Journal of Food and Dairy Science, 12 (6): 147–151.
Sterna V., Zute S., Jâkobsone I. 2015. Grain composition and functional ingredients of barley varieties created in Latvia. Proceedings of the Latvian Academy of Sciences 69 (4): 158–162.
Wirkijowska A., Rzedzicki Z., Sobota A., Sykut-Domańska E., Zarzycki P., Bartoszek K., Kuzawińska E. 2016. Jęczmień w żywieniu człowieka. Polish Journal of Agronomy 25: 41–50.
Wiśniewska M., Boros D., Zych J. 2020. Wartość pokarmowa wybranych mieszanek zbóż jarych z roślinami bobowatymi grubonasiennymi. Biuletyn IHAR. 289: 51–62.
Zhang X., Xue D., Wu F., Zhang G. 2013. Genotypic and environmental variations of arabinoxylan content and endoxylanase activity in barley grains. Journal of Integrative Agriculture, 12 (8): 1489–1494.
Zieliński H., Achremowicz B., Przygodzka M. 2012. Przeciwutleniacze ziarniaków zbóż. Żywność. Nauka. Technologia. Jakość. 80 (1): 5–26.

DOI: 10.37317/pbss-2021-0003

Zbigniew Laudański [1], Dariusz R. Mańkowski [1]*, Leszek Sieczko [2],
Monika Janaszek-Mańkowska [3]

[1] Plant Breeding and Acclimatization Institute — National Research Institute in Radzików, Poland
[2] Institute of Agriculture — Warsaw University of Life Sciences, Poland
[3] Institute of Mechanical Engineering — Warsaw University of Life Sciences, Poland
* Corresponding author: Dariusz R. Mańkowski; E-mail: d.mankowski@ihar.edu.pl

THE APPLICATION OF ORTHOGONAL CONTRASTS TO DETERMINE HOMOGENEOUS GROUPS

ABSTRACT

The paper presents a modified approach to analysis of data obtained from experiments carried out according to classical factorial designs. Four examples were discussed in order to present details of proposed method. Modification of the analysis of variance presented here enables more effective use of information on how studied factors affect the means of dependent variable. The specificity of this approach is based on alternative multiple comparison procedure incorporating orthogonal contrasts to determine homogeneous groups.

Key words: experiment, data analysis, linear model, ANOVA, multiple comparisons, orthogonal contrasts

INTRODUCTION

From the nineteenth century, comparative experiments were frequently used in various fields of science. However, results of such experiments may be affected by errors if groups of experimental units are not equivalent at the start of the experiment. R. A. Fisher pointed out that if the experimental units (plots) are randomly assigned into groups, their equivalence should be guaranteed at least in terms of arithmetic means (Fisher, 1925, 1935; Cochran & Cox, 1957). Thus, his experimental designs provide both comparisons and randomization which eliminates also an unconscious bias of the experimenter. Random selection guarantees an impartiality towards each factor, even though its meaning is not known to the experimenter.

Communicated by Grzegorz Żurek.

According to Fisher, consider the simplest randomized complete one-factor design, where each observation may be described by the following linear model:

$$y_{ij} = m + a_i + e_{ij} \text{ for } i = 1, \cdots, p; j = 1, \cdots, n_i; \sum_{i=1}^{p} n_i = n \quad (1)$$

where: y_{ij} – value of the dependent variable for the *i*-th level of factor A and the *j*-th replication; m – general mean; a_i – effect of *i*-th level of factor A; e_{ij} – experimental error for the *i*-th level of factor A and the *j*-th replication; p – number of levels of factor A; n_i – number of replications for *i*-th level of factor A; n – total number of observations. Assuming that $y_{ij} \sim N(m + a_i; \sigma^2)$ and $e_{ij} \sim N(0; \sigma^2)$.

The analysis of variance for such experimental data takes into account two types of variation: within object variation, arising from the variability (s_e^2) of the random deviations, and between object variation, arising from the variability (s_a^2) of tested effects a_i. The ratio of between-to-within object variation (called the *F* statistics) given as:

$$F_{emp} = \frac{s_a^2}{s_e^2} \quad (2)$$

has the *F* distribution under the null hypothesis written as:

$$H_0: \bigwedge_{1 \le i \le p} a_i = 0 \equiv H_0: \sum_{i=1}^{p} a_i^2 = 0. \quad (3)$$

If compared with the critical value of F distribution at certain significance level (α) the *F* statistics is the basis to confirm (if $F_{emp} \le F_{\alpha;p-1;n-p}$) or deny (if $F_{emp} > F_{\alpha;p-1;n-p}$) the veracity of the null hypothesis given by formula (3) (Fisher, 1925; Cochran & Cox, 1957; Elandt, 1964; Searle, 1971; Wójcik & Laudański, 1989; Laudański, 1996; Mańkowski, 2002; Box et al., 2005; Montgomery, 2005).

Considering comparative experiments the most familiar procedure following rejection of null hypothesis (compared objects differ significantly) involves multiple comparisons in order to determine exactly which levels of given effect (factor) are equivalent in terms of analyzed response (usually mean value). Such procedures, called sometimes *post hoc tests* or *mean separation tests*, allow to extract specific subgroups of compared objects, homogeneous in terms of mean values

of the response, i.e. subgroups within objects which do not differ significantly between each other considering mean value of dependent variable. There are many methods of conducting multiple comparisons which differ with kind of comparisons they make (pairwise or with control) as well as with the type of error they control (individual or interval error rates). The list of mean separation tests includes a lot of procedures, like those of Tukey, Tukey-Kramer/Spjotvoll-Stoline or Student-Newman-Keuls, each based on the distribution of studentized range, Duncan – based on the distribution proposed by the author and individual error rates, Bonferroni – based on a modified usage of Student's *t*-distribution or Scheffe – based on the *F* distribution (Tukey, 1953; Dunnett, 1955; Cornifield & Tukey, 1956; Schéffe, 1959; Elandt, 1964; Duncan, 1975; Biegun & Gabriel, 1981; Hochberg & Tamhane, 1987; Hochberg, 1988; Wójcik & Laudański, 1989; Hsu & Nelson, 1998; Rafter et al., 2002). Moreover, multiple comparisons may be realized using the method of minimized within-group sum of squares (Wagner, 1977) and procedures derived from cluster analysis (Caliński & Corsten, 1985).

Probably the most versatile and frequently used for multiple comparisons is Tukey's procedure and its variants. It can be used to compare group means derived from orthogonal designs characterized by the same number of observations for each object, as well as from non-orthogonal ones characterized by an uneven number of observations for objects. For example, Student-Newman-Keuls or Duncan procedures should not be used for comparison of group means obtained from non-orthogonal designs as generally standard errors of mean differences may vary for each pair of compared object means.

If the null hypothesis is not rejected, there is no basis to conclude that objects are significantly different which means that all of them form one homogeneous group of means. Thus, the experimenter may sometimes fail to formally confirm a guess about diversity of tested objects.

In this paper we present an alternative multiple comparison procedure incorporating orthogonal contrasts to formally confirm an assumption about diversity of tested objects and to determine homogeneous groups.

The presented analyzes were performed in the IBM®SPSS program.

EXAMPLES AND DISCUSSION

Example 1

In a preliminary experiment 17 lines and 3 cultivars of rye has been studied. The unbalanced experiment was performed in 20 incomplete blocks, each split into plots of 10 m^2. Rye yield expressed in kilograms per plot was a dependent variable.

Analysis of variance for this experiment (Table 1) showed no differences between studied objects in terms of mean yield obtained from plot ($p = 0.2175$). The experimental accuracy for comparisons of mean yields calculated for analyzed objects (percentage ratio of standard deviation for object means to overall mean – coefficient of variation) ranged from 8.26% to 9.48%, whereas mean comparison accuracy was 8.92%. These values indicate that the experiment was carried out properly.

Considering Tukey procedure, mean yields for tested objects did not differ significantly (mean value of honestly significant difference HSD at $\alpha = 0.05$ was equal to 2.712, which means that mean comparison accuracy was 33.8%), whereas *t*-test showed significant difference between objects 16th and 9th (least significant difference LSD = 1.446 < 9.057–7.399 = 1.658) at the significance level $\alpha = 0.05$ (mean comparison accuracy in this case, i.e. percentage ratio of LSD to overall mean, was 20.7%).

Splitting tested objects according to the results of the comparison by the Student's procedure into two subgroups and performing analysis of variance for such a dataset will be equivalent to performing analysis in a cross-hierarchical design: blocks×-objects within subgroups. Thus, it is possible to confirm the existence of differences between mean yields calculated for subgroups, even though variation of mean yields for objects within each subgroup is not significant.

Otherwise, if 20 objects are split into subgroups, group 1: (16, 13, 6, 20, 18, 17, 15, 8, 7, 10, 11) and group 2: (2, 4, 1, 14, 19, 5, 12, 3, 9), mean yields obtained for these subgroups will be 8.419 and 7.549 respectively (Table 2). The *F* test (Table 3) confirms significance of differences between subgroups in terms of mean yields (F_{emp}=18.2058), whereas differences of mean yields obtained for objects within each subgroup are not significant (F_{emp}=0.3927).

This example proved that the analysis of variance cannot give fully satisfactory results of multiple mean comparisons. This happens because ANOVA is based on comparison of all possible independent differences between pairs of means. If the analysis concerns of many small differences and only few large ones then global null hypothesis cannot be rejected, because sum of squares which measures these differences is too small relative to degrees of freedom corresponding to a number of comparisons. Such situation may occur quite frequently in practice, therefore modification of ANOVA technique to obtain homogeneous subgroups is justified. It should be noted that the analysis of variance described above (a comparison of two subgroups of analyzed objects) is nothing but a comparison known as a contrast between effects of tested objects.

Table 1

Analysis of variance for experimental data

Source	df	SS	MS	F_{emp}	p-value
Blocks	19	11.6468	0.6130	0.8830	0.6039
Objects	19	17.5452	0.9234	1.3303	0.2175
Residual	41	28.4613	0.6942		

Table 2

Postulated division into groups

Group I											
Object	16	13	6	20	18	17	15	8	7	10	11	$\bar{y}_I$
Mean	9.057	8.794	8.744	8.662	8.648	8.489	8.322	8.054	8.013	7.917	7.904	8.419
Group II											
Object	2	4	1	14	19	5	12	3	9			$\bar{y}_{II}$
Mean	7.796	7.701	7.611	7.597	7.470	7.458	7.454	7.453	7.399			7.549

Table 3

Complex analysis of variance for experimental data

Source	df	SS	MS	F_{emp}	p-value
Blocks	19	11.6468	0.6130	0.8830	0.60394
Objects	19	17.5452	0.9234	1.3303	0.21754
including:					
Between groups	1	12.6380	12.6380	18.2058	0.00011
Within groups	18	4.9072	0.2726	0.3927	0.98235
Residual	41	28.4613	0.6942		

ORTHOGONAL CONTRAST CONSTRUCTION

Consider a modified technique of analysis of variance for the model (1): randomized complete one-factor design. The hypothesis (2) for this design is that all mean values calculated for tested objects represent one homogeneous group centered around the estimated experimental mean (m).

Rephrase our problem as follows: there are subgroups of examined objects having estimated mean values centered around the subgroup mean (subgroup centroid) similar as in the model (1) all object means are centered around an overall mean. One can always guess the existence of such subgroups but they must be properly

identified to ensure rejecting the null hypothesis of equality between subgroup means (centroids) without rejecting the null hypothesis of differences between means within these subgroups.

We will use one of hierarchical cluster analysis methods known as centroid clustering to identify such object subgroups. In centroid clustering distance between two clusters is defined as distance between their centers of gravity (here: between means/average point in the multidimensional space defined by values of analyzed variables). Agglomeration procedure assumes that each object creates initially a separate cluster. Assuming that there is at most *p* object subgroups, agglomeration procedure results in subsequent divisions into separate subgroups of objects as number of subgroups is reduced from *p–1* to *2* based on an arbitrary distance measure, for example Euclidean or square Euclidean distance between means of each subgroup. Distances of Student or Fisher which take into account experimental design may be also used (Laudański, 1996; Mańkowski, 2002). The Fisher distance may be expressed as:

$$Q = \frac{n_s n_t (\bar{y}_s - \bar{y}_t)^2}{n_s + n_t} = n_s \bar{y}_s^2 + n_t \bar{y}_t^2 - \frac{(n_s \bar{y}_s + n_t \bar{y}_t)^2}{n_s + n_t} \tag{4}$$

whereas Student distance may be determined as:

$$\sqrt{Q} = \sqrt{\frac{n_s n_t (\bar{y}_s - \bar{y}_t)^2}{n_s + n_t}} \tag{5}$$

In both formulas *s* and *t* are indicators of subgroup means $\bar{y}_s$ and $\bar{y}_t$ respectively, n_s and n_t denote numbers of observations that correspond to subgroup means and are combined into one subgroup containing $n_s + n_t$ observations while reducing the number of subgroups from (*v* + 1) to *v*. Note that the formula (4) expresses sum of the squares of contrast between groups identified with subscripts *s* and *t*. In analysis of variance contrast is defined as linear function of object means of known constant coefficients sum of which is equal to 0. In other words, if vector $\mathbf{c} = [c_1, c_2, \cdots, c_p]'$, where $\sum_{s=1}^{p} c_s = 0$, expresses estimated contrast (comparison) between means (components of vector **a**) established as $\mathbf{c}'\mathbf{a}$ then sum of squares calculated for the following hypothesis:

$$H_0: \mathbf{c}'\mathbf{a} = 0 \tag{6}$$

is equal to

$$Q = \mathbf{a}'\mathbf{c}[\mathbf{c}'\mathbf{C}\mathbf{c}]^{-1}\mathbf{c}'\mathbf{a} \tag{7}$$

where **C** is a matrix such that the covariance matrix of vector **a** is equal to $s_e^2 \boldsymbol{C}$.

In particular case, if $c_s = 1$ and $c_t = -1$ while other coefficients are zero, then Q expresses the sum of squares of F statistic which tests equality of means calculated for subgroups s and t. Mindful of the relationship $t^2_{\alpha;n-p} = F_{\alpha,1,n-p}$ one may apply Student's t statistic instead of F statistic to test hypothesis expressed by formula (6). Note that Student's test may be one-sided, i.e. may verify hypothesis written as:

$$H_0: \mathbf{c}'\mathbf{a} \leq 0 \tag{8}$$

If so, then testing statistic takes the form of $t_{emp} = \sqrt{\frac{Q}{s_e^2}}$ and consequently the null hypothesis formulated in equation (8) should be rejected on the significance level α, if $t_{emp} > t_{2\alpha,n-p}$ or by analogy if $F_{emp} = \frac{Q}{s_e^2} > F_{2\alpha,1,n-p}$.

Example 2

Consider data from Table 4 to introduce procedure described above. Table 4 presents ANOVA results obtained for experiment carried out for 5 corn cultivars. The experiment was performed in completely random design with 6 replications. Corn yield expressed in kilograms per plot (experimental unit) was a dependent variable. Mean yields per plot are presented in Table 5. Assuming the existence of 5 subgroups (each cultivar corresponds to separate subgroup) matrices of Fisher distances (tab. 6) between subgroup means may be determined according to the formula (4). As a final result two subgroups (Tab. 7) are obtained with Fisher distance equal to:

$$Q = \frac{24 \cdot 6 \cdot (99.4 - 71.75)^2}{24 + 6} = 3669.708 \tag{9}$$

Table 4

Analysis of variance for experimental data

Source	df	SS	MS	F_{emp}	p-value
Objects	4	5267.9284	1316.9821	190.3427	2.49E–18
Residual	25	172.9751	6.9190		

Table 5

Object means values ($n_i = 6$)

Objects	5	2	1	4	3	$\bar{\bar{y}}$
Means	107.75	105.65	97.15	87.05	71.75	93.87

Table 6

Matrix of Fisher distances

Step 1				
Object	2	1	4	3
5	**13.23**	337.08	1285.47	3888.00
2	×	216.75	1037.88	3447.63
1	×	×	306.03	1935.48
4	×	×	×	702.27
Step II				
Object	1	4	3	
5,2	364.81	1544.49	4886.01	
1	×	**306.03**	1935.48	
4	×	×	702.27	
Step III				
Object	1,4	3		
5,2	**1278.96**	4886.01		
1,4	×	1656.49		

Table 7

Mean values of groups

Groups	(1,2,4,5)	(3)	$\bar{\bar{y}}$
Means	99.40	71.75	93.87
n_i	24	6	30

Results presented in extended ANOVA table (tab. 8) for this experiment show that 5 corn cultivars form 4 homogeneous groups. Mean yields calculated separately for cultivar 5 and 2 do not differ significantly between each other, while mean yield representing these two cultivars differs significantly from mean yields observed in other three cultivars each of which forms a separate homogeneous group. Table 9 presents a series of contrasts which exhausts the set of all possible orthogonal contrasts available for this experiment. Fisher distance corresponds to the sum of squares calculated for the contrast of compared objects. Different distance measures eg. Euclidean distance allows to obtain the same or a different set of orthogonal

contrasts. For example, formula (9) for computing the square Euclidean distance takes the following form:

$$Q = (99.4 - 71.75)^2 = 764.5225. \quad (10)$$

Table 8

Complex/extended analysis of variance for experimental data

Source	df	SS	MS	F_{emp}	p-value
Objects	4	5267.928	1316.982	190.343	2.49E–18
H_0: Test subjects do not form a homogeneous groups					
2 groups	1	3669.708	3669.708	530.381	2.37E–18
3 groups	1	1278.960	1278.960	184.847	4.73E–13
4 groups	**1**	**306.0301**	**306.0301**	**44.230**	**5.72E–07**
5 groups	1	13.230	13.230	1.9121	0.179
Residual	25	172.975	6.919		

Table 9

Set of orthogonal contrasts

Objects	1	2	3	4	5	SS
Contrast 1	0	–1	0	0	1	13.230
Contrast 2	1	0	0	–1	0	306.030
Contrast 3	1	–1	0	1	–1	1278.960
Contrast 4	1	1	–4	1	1	3669.708

Example 3

Consider experiment conducted in randomized complete blocks where effect of corn cultivar on yield per plot was studied. Results of ANOVA for experimental data, extended by orthogonal contrasts, are presented in Table 10. The analysis showed that 8 corn varieties formed 4 homogeneous groups regarding mean yield per plot. Mean yields calculated for each cultivar, homogeneous groups obtained according to proposed method and well known multiple comparison procedures are summarized in Table 11. It should be noted that standard multiple comparison procedures resulted in inseparable homogeneous groups. Complete separation of homogeneous groups is rarely attainable in practice, particularly if a large number of analyzed objects (means) is taken into account. The application of orthogonal contrasts enables complete separation of homogeneous groups (mutually independent) in each case.

Table 10

Complex analysis of variance for experimental data

Source	df	SS	MS	F_{emp}	p-value
Blocks	2	5.643	2.821	0.0595	0.94247
Cultivars	7	2347.247	335.321	7.0776	0.00099
Test subjects do not form a homogeneous groups					
2 groups	1	1476.056	1476.056	31.1549	0.00007
3 groups	1	601.142	601.142	12.6882	0.00313
4 groups	**1**	**223.414**	**223.414**	**4.7156**	**0.04757**
5 groups	1	43.867	43.867	0.9259	0.35226
6 groups	1	2.160	2.160	0.0456	0.83398
7 groups	1	0.327	0.327	0.0069	0.93497
8 groups	1	0.282	0.282	0.0059	0.93986
Residual	14	663.291	47.378		

Table 11

Homogeneous groups

Cultivar	$\bar{y}_i$	Orthogonal contrasts method	Tukey	Newman–Keuls	Duncan	Bonferroni	Scheffe	Student
1	104,87	**a**	a	a	a	a	a	a
4	104,40	**a**	ab	ab	ab	ab	ab	ab
8	94,43	**b**	abc	abc	b	abc	ab	b
6	93,23	**b**	abc	abc	b	abc	ab	b
2	87,73	**c**	abc	bc	bc	abc	ab	bc
7	87,30	**c**	bc	bc	bc	abc	ab	bc
3	82,83	**c**	c	c	c	bc	b	c
5	73,60	**d**	c	c	c	c	b	c

Example 4

An experiment discussed by Wagner (1977) will be used to present direct comparison of method based on minimal orthogonal contrasts with procedure based on minimal within-group sum of squares. The experiment concerned 14 cultivars of sugar beet and was realized in completely randomized block design with 6 replications. Sugar yield was a dependent variable and mean yields (dt/ha) obtained for each cultivar are presented in Table 12. Table 13 presents results of ANOVA,

extended by orthogonal contrasts for experimental data. Comparison procedure based on minimum within-group sum of squares resulted in 3 homogeneous groups of object means, while application of Tukey procedure allowed to obtain 2 homogeneous groups (Wagner, 1977). Method of minimal orthogonal contrasts based on Fisher distance between object means resulted in distinguishing 4 homogeneous groups (Tab. 14). Thus the most numerous group discussed by Wagner (1977) had been split into 2 separate subgroups (group 1 and 2 in Tab. 15). It is not difficult to note that the application of orthogonal contrasts resulted in considerable span of mean sugar yields observed between groups, whereas within each group object means calculated for cultivars were concentrated around group mean.

Table 12

Mean yield values of compared beet cultivars

Cultiv.	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Yield	98.36	100.87	107.58	102.32	105.11	106.13	102.03	102.29	100.58	84.11	95.52	101.91	96.44	103.05

Table 13

Complex analysis of variance for experimental data

Source	df	SS	MS	F_{emp}	p-value
Blocks	5	193.05	38.61	2.175	0.067615
Cultivars	13	2610.79	200.83	11.314	3.53E–12
H_0: Test subjects do not form a homogeneous groups					
2 groups	1	1725.174	1725.174	97.193	1.56E–14
3 groups	1	569.553	569.553	32.087	3.62E–07
4 groups	**1**	**244.935**	**244.935**	**13.799**	**0.000425**
5 groups	1	22.658	22.658	1.276	0.262796
Within groups	9	48.470	5.386	0.303	0.971258
Residual	65	1153.75	17.75		

Table 14

Fishers distances matrix

Object	11,13	1	2,4,7,8,9,12,14	3,5,6
10	563.588	609.188	1654.880	2210.453
11;13	×	**22.658**	323.167	762.855
1	×	×	64.471	281.791
2,4,7,8,9,12,14	×	×	×	244.935
Object	1,11,13	2,4,7,8,9,12,14	3,5,6	
10	721.616	1654.880	2210.453	
1,11,13	×	326.570	812.250	
2,4,7,8,9,12,14	×	×	**244.935**	
Object	1,11,13	2,3,4,5,6,7,8,9,12,14		
10	721.616	1985.187		
1,11,13	×	**569.553**		
Object	1,2,3,4,5,6,7,8,9,11,12,13,14			
10	**1725.174**			

Table 15

Means division into homogenous groups

Group 1								
Cultivar	3	6	5					
Yield	107.58	106.13	105.11					106.27
Group 2								
Cultivar	14	4	8	7	12	2	9	
Yield	103.05	102.32	102.29	102.03	101.91	100.87	100.58	101.86
Group 3								
Cultivar	1	13	11					
Yield	98.36	96.44	95.52					96.77
Group 4								
Cultivar	10							
Yield	84.11							84.11

CONCLUSIONS

The alternative multiple comparison procedure incorporating orthogonal contrasts to determine homogeneous groups of objects undergone analysis of variance enables complete separation of analyzed object means that means within homogeneous groups do not differ significantly between each other but between-group means (centroids) are significantly different. Moreover, significant association of group variation relative to the total object variation ensures optimal separation of object means into distinct homogeneous groups. Proposed procedure may be applied for each linear ANOVA model and analysis of covariance of classified data.

Commonly used multiple comparison procedures are based generally on comparing the distances between means calculated for pairs of objects relative to the appropriate error that results from covariance matrix of these means (thus they correspond to the matrix of experimental design). Although these procedures are very useful for comparison selected objects to each other (answering the question: does cultivar A differ significantly from cultivar B in terms of mean value of studied feature) applying them to split objects into homogeneous subgroups results in an approximate picture of possible separation, especially if number of objects is large. The procedure discussed in this paper consists in determination of orthogonal contrasts between means according to the criterion of minimum contrast and it seems to meet the expectations of practitioners.

REFERENCES

Biegun, J.M., Gabriel, K.R. (1981). Closure of the Newman-Keuls multiple comparisons procedure. *Journal of the American Statistical Association,* **76**, 374.

Box, G.E.P., Hunter, J.S., Hunter, W.G. (2005). Statistics for Experimenters — Design, Innovation, and Discovery. Second Edition. Wiley and Sons Inc., New Jersey, USA.

Caliński, T., Corsten, L.C.A. (1985). Clustering means in ANOVA by simultaneous testing. *Biometrics*, **41**, 39—48.

Cochran, W.G., Cox, G.M. (1957). Experimental design. John Wiley & Sons Inc., New York, USA.

Cornfield, J., Tukey, J.W. (1956). Average values of mean squares in factorials. *Annals of Mathematical Statistics*, **27**, 907—949.

Duncan, D.B. (1975). T test and intervals for comparisons suggested by the data. *Biometrics*, **31**, 339—359.

Dunnett, C.W. (1955). A multiple comparisons procedure for comparing several treatments with a control. *Journal of the American Statistical Association*, **50**, 1096—1121.

Elandt, R. (1964). Mathematical statistics applied to agricultural experimentation. PWN, Warsaw, Poland. (*in polish*)

Fisher, R.A. (1925). Statistical Methods for Research Workers. Oliver and Boyd, Edinburgh and London, UK.

Fisher, R.A. (1935). The Design of Experiments. Oliver and Boyd, Edinburgh and London, UK.

Hochberg, Y. (1988). A sharper Bonferroni procedure for multiple test of significance. *Biometrika*, **75**, 800—802.

Hochberg, Y., Tamhane, A.C. (1987). Multiple Comparison Procedures. John Wiley & Sons Inc., New York, USA.

Hsu, J.C., Nelson, B. (1998). Multiple comparisons in the General Linear Models. *Journal of Computational and Graphical Statistic*, **7**, 23—41.

Laudański, Z. (1996). Application of projection operators for analysing of non-orthogonal data. Theory, algorithms and examples. *Treatises and Monographs*, **208**. Warsaw University of Life Science Publishing, Warsaw, Poland. (*in polish*)

Mańkowski, D.R. (2002). Proposal of division into subgroups some similar one-dimensional populations. Master's thesis, Warsaw University of Life Science, Warsaw, Poland. (*in polish*)

Montgomery, D.C. (2005). Design and analysis of experiment. 6th edition. John Wiley & Sons Inc., Hoboken, NJ, USA.
Rafter, J.A., Abell, M.L., Braselton, J.P. (2002). Multiple Comparison Methods for Means. Society for Industrial and Applied Mathematics: 259—278.
Scheffe, H. (1959). The analysis of variance. John Wiley & Sons Inc., New York, USA.
Searle, S.R. (1971). Linear models. John Wiley & Sons Inc., New York, USA.
Tukey, J.W. (1953).The problem of multiple comparisons. Princeton University.
Wagner, W. (1977). A posteriori grouping varieties or objects based on analysis of variance from single experiment. Variety Assessment Bulletin, 2 (10). (*in polish*)
Wójcik, A.R., Laudański, Z. (1989). Planning and statistical inference in experimentation. PWN, Warsaw, Poland. (*in polish*)

Content

List of reviewers in the year 2021
Plant Breeding and Seed Science

Dariusz Załuski	Uniwersytet Warmińsko-Mazurski w Olsztynie
Habibur Rahman	University of Alberta, Edmonton, AB, Canada
Janusz Wątroba	StatSoft Polska
Laurencja Szała	The Plant Breeding and Acclimatization Institute (IHAR) - National Research Institute
Magdalena Wiśniewska	The Plant Breeding and Acclimatization Institute (IHAR) - National Research Institute
Urszula Piechota	The Plant Breeding and Acclimatization Institute (IHAR) - National Research Institute
Wioletta Dynkowska	The Plant Breeding and Acclimatization Institute (IHAR) - National Research Institute