Jan BOCIANOWSKI[1], Marek CICHOCKI[1], Piotr SZULC[2], Anna TRATWAL[3], Kamila NOWOSAD[4], Joanna KOBUS-CISOWSKA[5]
[1] Poznań University of Life Sciences, Department of Mathematical and Statistical Methods
ul. Wojska Polskiego 28, 60-637 Poznań, Poland; ORCID: 0000-0002-0102-0084, e-mail: jboc@up.poznan.pl
[2] Poznań University of Life Sciences, Department of Agronomy
ul. Dojazd 11, 60-632 Poznań, Poland, ORCID: 000-0002-9670-3231, e-mail: pszulc@up.poznan.pl
[3] Institute of Plant Protection – National Research Institute, Department of Pests Methods Forecasting and Plant Protection Economy:
ul. Władysława Węgorka 50, 60-318 Poznań, Poznań; ORCID: 0000-0001-9611-8799, e-mail: A.Tratwal@iorpib.poznan.pl
[4] Wroclaw University of Environmental and Life Sciences, Department of Genetics, Plant Breeding and Seed Production
pl. Grunwaldzki 24A, 53-363 Wrocław, Poland, ORCID: 0000-0001-6837-7806, e-mail: kamila.nowosad@up.wroc.pl
[5] Poznań University of Life Sciences, Department of Gastronomical Sciences and Functional Foods
ul. Wojska Polskiego 31, 60-624, Poznań, Poland; ORCID 00000003-2834-0405, e-mail: joanna.kobus@up.poznan.pl

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ANALYSIS OF THE NITROGEN AND MAGNESIUM DOSES EFFECTS OF TWO CULTIVARS OF MAIZE (*Zea mays* L.) USING MULTIVARIATE METHODS

Summary

The paper presents the results of a multivariable research regarding the evaluation of variability of selected quantitative traits in two cultivars of maize (Zea mays L.*): ES Palazzo and ES Paroli after using doses of nitrogen and magnesium. The study took into account 12 traits recorded for three years (2009-2011). The statistical analysis of obtained results was conducted using multivariable methods: multivariate analysis of variance, canonical variable analysis and Mahalanobis distances. The most varied objects were A4B1C2 and A1B2C1 (in 2009), A4B1C1 and A2B2C2 (in 2010), A4B2C1 and A1B2C2 (in 2011) and A4B2C1 and A1B2C2 (for all three years). The most similar objects (with regard to the 12 traits analyzed together) were A4B2C2 and A3B2C2 (in 2009), A4B2C2 and A3B1C2 (in 2010), A2B2C2 and A2B1C2 (in 2011) and A4B2C2 and A3B2C2 (for all three years). The Mahalanobis distances between particular objects in particular years of observations were positive and statistically significantly correlated.*
Keywords: *canonical variable analysis, maize, stay-green, nitrogen, magnesium, Mahalanobis distances*

ANALIZA EFEKTÓW DAWEK AZOTU I MAGNEZU DWÓCH ODMIAN KUKURYDZY (*Zea mays* L.) Z UŻYCIEM METOD WIELOWYMIAROWYCH

Streszczenie

W pracy przedstawiono wyniki badania zmienności wybranych cech ilościowych dwóch odmian kukurydzy (Zea mays L.*): ES Palazzo i ES Paroli po zastosowaniu różnych dawek azotu i magnezu stosując wielowymiarowe metody statystyczne. W badaniach wzięto pod uwagę 12 cech ilościowych obserwowanych w doświadczeniu trzyletnim przeprowadzonym w latach 2009-2011. Analiza statystyczna uzyskanych wyników została przeprowadzona z użyciem metod wielowymiarowych: wielozmiennej analizy wariancji, analizy zmiennych kanonicznych i odległości Mahalanobisa. Najbardziej zróżnicowanymi obiektami były A4B1C2 i A1B2C1 (w 2009), A4B1C1 i A2B2C2 (w 2010), A4B2C1 i A1B2C2 (w 2011) oraz A4B2C1 i A1B2C2 (dla wszystkich trzech lat). Natomiast, najbardziej podobnymi obiektami (pod względem wszystkich 12 cech traktowanych łącznie) były A4B2C2 i A3B2C2 (w 2009), A4B2C2 i A3B1C2 (w 2010), A2B2C2 i A2B1C2 (w 2011) oraz A4B2C2 i A3B2C2 (dla trzech lat). Odległości Mahalanobisa pomiędzy poszczególnymi obiektami w poszczególnych latach prowadzenia obserwacji były dodatnio i istotnie statystycznie skorelowane.*
Słowa kluczowe: *analiza zmiennych kanonicznych, kukurydza, stay-green, azot, magnez, odległości Mahalanobisa*

1. Introduction

The exchange between the biophysical environment and a cultivated plant happens mainly through the leaf's assimilation area, so the size of the leaf (leaf area index – LAI) is an important trait determining productivity [10]. The development of leaves and their longevity influence the amount of captured sunlight, which later influences the amount of accumulated biomass and final yield. Therefore, the area of leaves and their count per plant are important elements in evaluating the process of photosynthesis of maize genotypes [7]. According to Pandey et al. [13] maize genotypes vary in terms of the number of leaves, the speed of growth and production of biomass in conditions differing in terms of available water and nitrogen. The analysis of growth of organs actively involved in photosynthesis of two different maize cultivars was presented in a previous study [19]. There, the research material was analyzed in terms of quantitative traits examined independently.

It would seem interesting to analyze the variability of two maize cultivars in terms of all 12 traits analyzed together and how combinations of nitrogen and potassium fertilization influence them. Thus, next to one-dimensional statistical methods of evaluating the results it is recommended that multivariate methods also be used, so that correlations of analyzed traits can be taken into consideration as well [17].

The aim of the study was multi-trait evaluation of phenotype variability of two maize cultivars (ES Palazzo and ES Paroli of the "stay-green" type) related to nitrogen and magnesium fertilization. To analyze the traits, the research applied the canonical variable analysis [1, 8, 11, 21], based on a multivariate analysis of variance (MANOVA), in an experiment conducted in the configuration of complete blocks.

2. Material and Methods

2.1. Experimental field

Field experiments were conducted at the Department of Agronomy at Poznań University of Life Sciences on the fields of the Research Institute in Swadzim (52°26'20''N, 16°44'58''E) in the years 2009-2011. They were conducted using the split-plot×split-block system with three research factors, in four field repetitions. The experiment evaluated the influence of four doses of urea (A1 – 0, A2 – 50, A3 – 100, A4 – 150 kg N ha^{-1}), two doses of magnesium (B1 – 0, B2 – 25 kg MgO ha^{-1} in the form of kieserite) on the analysis of growth of organs actively involved in the process of photosynthesis of two maize cultivar types [C1 – ES Palazzo (FAO 230-240, flint type) and C2 – ES Paroli „stay-green" (FAO 250, flint-dent type)]. Both examined maize cultivars are single cross cultivars (SC). Total effective temperatures in the cultivation of ES Palazzo for grain amount to 1600°C, and of ES Paroli, to 1665°C.

The nitrogen was applied according to the scheme of the experiment. Phosphorus in the dose of 80 kg P_2O_5 ha^{-1} was applied in the form of triple superphosphate (46% P_2O_5), and potassium in the dose of 120 kg K_2O ha^{-1} in the form of potash salt (60% K_2O). The nitrogen, potassium and kieserite fertilizers were applied before the sowing.

Thermal and humidity conditions during the maize's vegetation period as well as the soil's richness in nutrients were described in a previous study [19].

2.2. Plant material

Specimens for analyses each year were extracted during the ear blooming stage (BBCH 67). Each experiment plot consisted of four rows. Specimens for analyses were taken from the two middle rows from each plot, with outer rows being considered outer seeding (isolation). A single specimen consisted of six plants. The following traits were analyzed: the number of leaves from one plant (pcs.), the weight of leaves from one plant (g), the weight of stems from one plant (g), the weight of ears from one plant, the weight of the plant (g), assimilation area of one plant (cm^2), specific leaf area (cm^2 g^{-1}), leaf weight fraction (%), leaf area ratio (%), stem weight fraction (%), ear weight fraction (%), leaf area index. A detailed record of the experiment can be found in a previous work [19].

The study analyzed all possible combinations of nitrogen and magnesium doses as research objects.

2.3. Statistical analysis

The multivariate analysis of variance [9] was applied to test multivariate hypotheses: about the lack of variability between objects, between years and the objects × years interaction. Based on the data for each year, as well as all three years together, the researchers were able to calculate the Mahalanobis distances [12] for analyzed objects as well as critical Mahalanobis distances D^2_{kr} (at the level of significance $\alpha = 0.05$). The canonical variable analysis [14] was applied to make it possible to visually present the multi-trait variability of the analyzed objects. It allows for a presentation of the distance based on twelve traits between the objects with the Mahalanobis distance specified. To be able to evaluate the relative share of each original trait in the multi-trait variability of the examined objects, the researchers estimated simple correlation coefficients between the values of the first two canonical variables and the values of particular original traits. All calculations in the multivariate analysis of variance as well as canonical variables analysis were conducted using the GenStat v. 15 statistical package.

3. Results and Discussion

The mean values, standard deviations, coefficients of variance as well as correlation coefficients were presented in previous works [19]. The multivariate analysis of variance (MANOVA) made it possible to discard tested hypotheses about the lack of multi-trait variability between the years of research ($P < 0.001$), the lack of multi-trait differences between the objects ($P < 0.001$) as well as the lack of interaction objects × years ($P < 0.001$). Because of that and a statistically significant influence of years on the values of particular traits analyzed separately [19], further analyses were conducted for particular years independently.

Particular traits have a different degree of significance and a different extent of contribution to the overall multi-trait variability. The multi-trait variability analysis includes identification of the most important traits in the objects' multi-trait variability. A statistical tool which helps solve this problem is canonical variable analysis. The results of the analysis for analyzed objects are presented in Table 1.

Based on the 2009 observations the researchers determined that the first two canonical variables account for 73.66% of total variability between the objects (Table 1, Fig. 1). Fig. 1 shows the variability of analyzed traits in the configuration of the first two canonical variables in the first year of research. On the graph the coordinates of the point for particular objects are values of the first and second canonical variables, respectively. The most significant linear relation with the first canonical variable was observed for: specific leaf area (positive correlation), leaf weight, assimilation area, leaf weight fraction, leaf area index and plant weight (negative correlations) (Table 1). It indicates that the six aforementioned traits were strongly correlated.

They indicate the existence of a genetic factor which to a great extent determines those six traits simultaneously. The factor may include coupled cumulative genes or pleiotropic ones. The traits with the strongest correlation with the canonical variable contributed the most to the variability of the objects. Relatively high values of the first canonical variable for analyzed objects quite often indicate high values for traits positively correlated with it and low values for traits with a negative correlation. Low values for the first canonical variable, in turn, quite often indicate low values for traits positively correlated with it and high values for traits with a negative correlation. Traits significantly correlated with the first canonical variable in 2009 indicate that the examined cultivars differed mainly in terms of leaf specific area, leaf weight, assimilation surface area, leaf weight fraction, leaf area index and plant weight (Fig. 1).

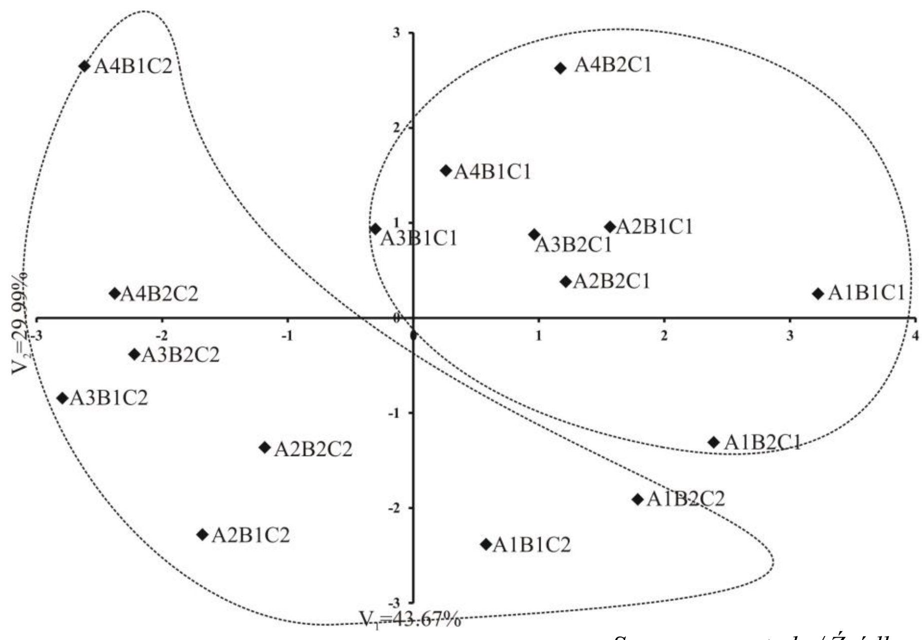
The second canonical variable was significantly positively correlated with the number of leaves, leaf weight, stem weight, ear weight and plant weight, and negatively correlated with leaf specific area, leaf weight fraction and leaf area ratio (Fig. 1).

The biggest differences with regard to all traits (measured in Mahalanobis distances) were recorded for objects A4B1C2 and A1B2C1 (the Mahalanobis distance between them was 6.65). It was a result of both the application of doses of nitrogen (A4 and A1) and magnesium (B1, B2) and the cultivars' different genetic profiles. The biggest similarities were recorded for A4B2C2 and A3B2C2 (1.50). It means that applying doses of 100 and 150 kg $N \cdot ha^{-1}$ in the case of the "stay-green" cultivar does not influence the analyzed traits.

Table 1. Correlation coefficients between the first two canonical variables and the observed traits
Tab. 1. Współczynniki korelacji pomiędzy dwoma pierwszymi zmiennymi kanonicznymi a obserwowanymi cechami

Trait	2009		2010		2011		For 3 years	
	V_1	V_2	V_1	V_2	V_1	V_2	V_1	V_2
Number of leaves	0.452	0.692 **	0.031	0.811 ***	-0.839 ***	0.107	0.752 ***	-0.256
Leaf weight	-0.823 ***	0.527 *	-0.275	-0.562 *	0.010	0.260	-0.092	0.968 ***
Stem weight	-0.486	0.833 ***	0.624 **	-0.116	-0.770 ***	-0.003	0.616 *	0.747 ***
Ear weight	-0.310	0.744 ***	0.599 *	0.083	-0.788 ***	0.110	0.728 **	0.539 *
Plant weight	-0.506 *	0.807 ***	0.609 *	-0.153	-0.758 ***	0.080	0.598 *	0.745 ***
Assimilation surface area	-0.827 ***	0.395	-0.753 ***	-0.332	0.687 **	0.115	-0.475	0.837 ***
Specific leaf area	0.621 *	-0.533 *	-0.526 *	0.330	0.375	-0.144	-0.464	-0.690 **
Leaf weight fraction	-0.654 **	-0.661 **	-0.726 **	-0.460	0.976 ***	0.060	-0.933 ***	0.300
Leaf area ratio	0.044	-0.872 ***	-0.845 ***	-0.157	0.895 ***	-0.035	-0.941 ***	-0.107
Stem weight fraction	0.035	0.287	0.180	0.011	-0.163	-0.205	0.255	-0.159
Ear weight fraction	0.303	0.069	0.356	0.310	-0.591 *	0.111	0.655 **	-0.158
Leaf area index	-0.906 ***	0.132	-0.976 ***	0.038	0.855 ***	-0.224	-0.885 ***	0.385
Percentage of elucidated multivariate variability	43.67	29.99	48.60	16.14	57.25	11.66	51.77	33.65
* $p< 0.05$; ** $p< 0.01$; $p< 0.001$								
V_1 – first canonical variable; V_2 – second canonical variable								

Source: own study / Źródło: opracowanie własne



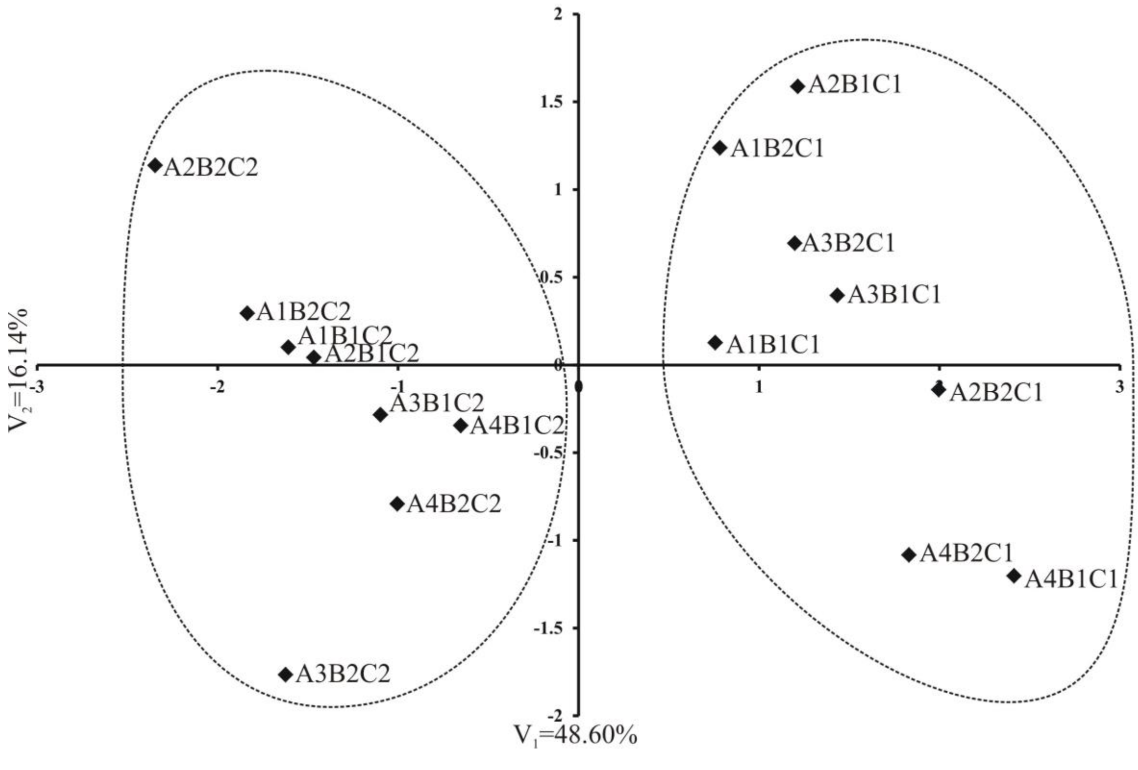
Source: own study / Źródło: opracowanie własne

Fig. 1. Distribution of treatments in the space of two first canonical variables in 2009
Rys. 1. Rozmieszczenie kombinacji traktowań u układzie dwóch pierwszych zmiennych kanonicznych w 2009 r.

Analyzing the data from a previous study conducted in 2010 the researchers determined that the first two canonical variables accounted for 64.74% of total variability (Table 1, Fig. 2). The position of the objects in the configuration of the first two canonical variables is presented in Fig. 2. A positively significant correlation was noted for stem weight, ear weight and plant weight, while a negative correlation, for assimilation surface area, leaf specific area, leaf weight fraction, leaf area ratio and leaf area index (Table 1). It means that the objects were characterized by relatively high stalk weight, high ear weight and high plant weight, but small leaf assimilation area, small specific leaf area, small leaf weight fraction, low leaf area ratio and small leaf area index. As can be noted in Fig. 1, the first canonical variable significantly varied the cultivars. The second canonical variable was positively correlated with number of leaves, and negatively correlated with leaf weight (Table 1). The smallest multi-trait variability was recorded for A4B2C2 and A3B1C2 (1.27), which indicates a lack of variability for 100 and 150 kg N·ha^{-1} and magnesium doses with regard to the trait values for the "stay-green" type. The biggest variability, in turn, was noted for A4B1C1 and A2B2C2 – the Mahalanobis distance between them was 5.37. It is a result of significant differences between the cultivars (Fig. 2).

By analyzing objects in 2011 we determined that the first two canonical variables account for 68.91% of total variability (Table 1, Fig. 3). Fig. 3 shows examined objects in the configuration of the first two canonical variables in the third year of research (2011). The objects were grouped mainly by cultivar type. The first canonical variable was significantly positively correlated with the assimilation surface area, leaf weight fraction, leaf area ratio and leaf area index and negatively correlated with number leaves, stem weight, ear weight, plant weight and ear weight fraction (Table 1). It means that the "stay-green" type in 2011 was characterized by bigger assimilation area, leaf weight fraction, leaf area ratio and leaf area index, but number leaves, stem weight, ear weight, plant weight and ear weight fraction (Fig. 3). The second canonical variable was not significantly correlated with any of the traits (Table 1). The biggest variability with regard to all 12 analyzed traits was observed for objects A4B2C1 and A1B2C2 (the Mahalanobis distance between them was 4.70), which indicates large differences between the examined cultivars with regard to analyzed traits. The smallest differences were noted for objects A2B2C2 and A2B1C2 (1.05), which indicates lack of influence of a 50 kg N·ha^{-1} magnesium dose in the case of the "stay-green" type.

Analysis of all data from the three years of research made it possible to determine that the first two canonical variables account for 85.42% of total variability (Table 1, Fig. 4). The position of the objects in the configuration of the first two canonical variables is presented in Fig. 4. A positively significant correlation with the first canonical variable was noted for leaf count, stem weight, ear weight, plant weight and ear weight fraction, and negative correlation for leaf weight fraction, leaf area ratio and leaf area index (Table 1). It indicates that objects of relatively high leaf count, stalk, ear and plant weight and high ear weight fraction have relatively low leaf weight fraction, low leaf area ratio and leaf area index.



Source: own study / Źródło: opracowanie własne

Fig. 2. Distribution of treatments in the space of two first canonical variables in 2010
Rys. 2. Rozmieszczenie kombinacji traktowań w układzie dwóch pierwszych zmiennych kanonicznych w 2010 r.

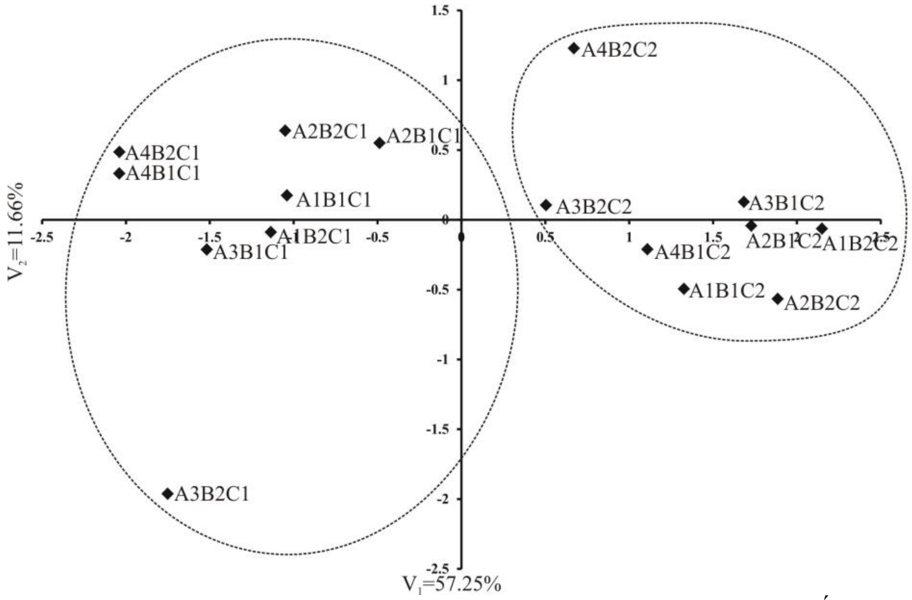


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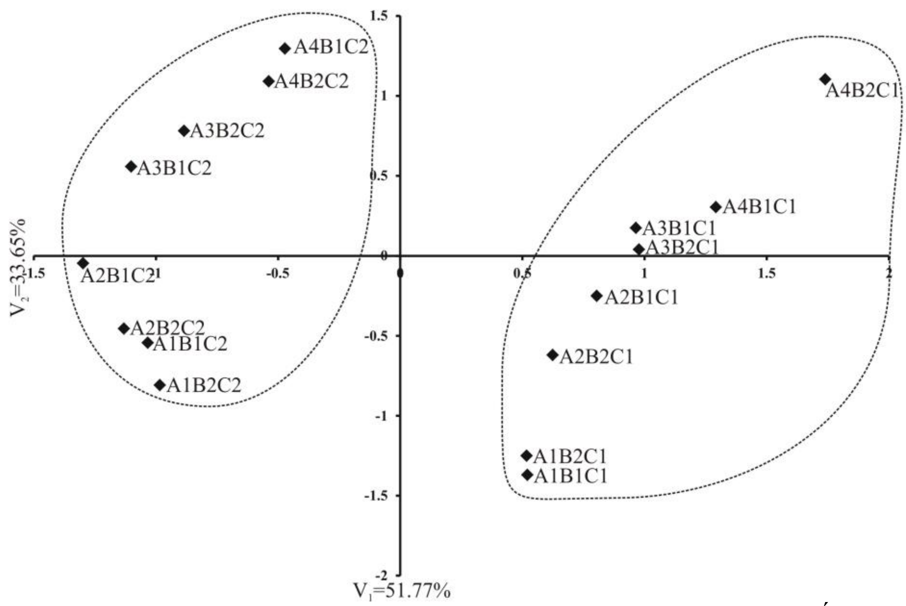
Fig. 3. Distribution of treatments in the space of two first canonical variables in 2011
Rys. 3. Rozmieszczenie kombinacji traktowań w układzie dwóch pierwszych zmiennych kanonicznych w 2011 r.

The three years of research saw significant differences in the objects with regard to cultivar type (Fig. 4). The second canonical variable was positively correlated with leaf weight, stem weight, ear weight, plant weight and assimilation surface area, and significantly negatively correlated with specific leaf area (Table 1). The smallest multi-trait differences were noted for A4B2C2 and A3B2C2 (0.59), which indicates lack of variability for doses of 100 and 150 kg N·ha^{-1} with regard to the trait values for the "stay-green" type. The biggest differences were recorded for A4B2C1 and A1B2C2 – the Mahalanobis distance between them was 3.38.

The presented multi-variable characteristics of the analyzed objects is a convincing illustration. It proves that the canonical variable analysis method is effective. It stems from the fact that those variables accounted for most of total variability (73.66%, 64.74%, 68.91% and 85.42% for experiments conducted in 2009, 2010, 2011, respectively, as well as for all three years together). Thus the method is reliable, which is why it has been widely used by cultivators and geneticists [2-6, 15-18, 20].

We calculated and tested correlation coefficients between the Mahalanobis distances for particular object pairs in particular years and for all three years together. All coefficients were positive and statistically significant. The range of Mahalanobis distances was smaller with each year of research. Throughout all three years of research the range of those values was smaller than in particular years, which indicates that conducting such analyses for all years together is valid and necessary.



Source: own study / Źródło: opracowanie własne

Fig. 4. Distribution of treatments in the space of two first canonical variables in 2009-2011 years
Rys. 4. Rozmieszczenie kombinacji traktowań w układzie dwóch pierwszych zmiennych kanonicznych dla średniej z lat 2009-2011

4. Conclusions

1. Results obtained using the canonical variable analysis method confirm that the method is useful for effective evaluation of multi-trait similarity and multi-trait analysis of the objects.
2. The most varied objects were: A4B1C2 and A1B2C1 (in 2009), A4B1C1 and A2B2C2 (in 2010), A4B2C1 and A1B2C2 (in 2011) and A4B2C1 and A1B2C2 (for all three years). The most similar objects (with regards to the 12 traits analyzed together) were: A4B2C2 and A3B2C2 (in 2009), A4B2C2 and A3B1C2 (in 2010), A2B2C2 and A2B1C2 (in 2011) and A4B2C2 and A3B2C2 (for all three years). Thus, increasing the dose of nitrogen from 100 to 150 kg $N \cdot ha^{-1}$ does not influence the manifestation of traits for the "stay-green" type.
3. The differences between the objects are determined mainly by cultivar type. The results indicate significant differences between the analyzed maize cultivars, regardless of the dose of nitrogen and magnesium used.

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