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Analysis of effects of nitrogen and magnesium fertilization combinations of phenotypic traits of two maize (*Zea mays* L.) cultivars using multivariate methods

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Abstract: The paper presents results of multivariate analyses assessing variation in quantitative traits after the application of nitrogen and magnesium rates in cultivation of two types of maize (*Zea mays* L.) cultivars, i.e. ES Palazzo and the stay-green ES Paroli. The purpose of this study was to assess the multivariate phenotypic variation of 16 objects which are a combination of four nitrogen doses, two doses of magnesium and two varieties characterized by varying the rate of aging. Observations were conducted for eight traits: plant height, ear height, ear length, ear diameter, ear volume, the number of plants after emergence, the number of plants before harvest, plant loss rates in the vegetation period, observed in the course of three years (2009-2011). Statistical analysis of results was performed using the multivariate methods. Analysis of canonical variables proved to be a reliable tool providing a comprehensive assessment of variation in the effect of urea and magnesium fertilisation combinations on many traits simultaneously. The most variable treatments were A2B2C2 (50 kg N ha^{-1}, 25 kg MgO ha^{-1} for ES Paroli SG) and A4B2C1 (150 kg N ha^{-1}, 25 kg MgO ha^{-1} for ES Palazzo). The most similar treatments (in terms of eight traits treated jointly) included A2B2C2 (50 kg N ha^{-1}, 25 kg MgO ha^{-1} for ES Paroli SG) and A3B2C2 (100 kg N ha^{-1}, 25 kg MgO ha^{-1} for ES Paroli SG). Mahalanobis distances between individual treatments in individual years of observations were positive and correlated statistically significantly.

Keywords: maize; stay-green; nitrogen; magnesium; multivariate analysis

INTRODUCTION

Maize (*Zea mays* L.) is a crop both supplying high grain yields and producing considerable vegetative biomass. As such it requires adequately high levels of mineral fertilisation and organic fertilisation supplemented with mineral fertilisation (Szulc et al., 2012a, b). While nutrient requirements of maize as a species have been reliably specified, information concerning appropriate nutrition of stay-green maize plants is still insufficient. The stay-green trait makes it possible for plants to maintain green leaves until full maturity (Thomas and Howarth 2000, Bekavac et al., 2002). This guarantees better health of plants and limits their lodging. Maize plants in the type of “stay-green” are healthier later during the growing season (Chen et al. 2010, Borrell and Hammer, 2000) and are more tolerant to drought stress after flowering (Rosenow and Clark 1981). This provides greater resistance of such plants to fungal diseases, resulting in lower mycotoxin contents in grain (Szulc et al., 2012c). Thus identification of response to varied mineral fertilisation of maize cultivars characterised by delayed senescence will make it possible to utilise their yielding potential. For several years now such studies have been conducted at the Department of Agronomy, the Poznań University of Life Sciences. One of the many research problems is connected with analyses of morphological traits of maize plants having different genetic profiles depending on cultivation factors, i.e. varied mineral fertilization. It seems to be of interest to analyse variation of two maize cultivars in terms of all the eight observed traits treated jointly under the influence of combined fertilisation with nitrogen and magnesium. Thus, next to statistical univariate methods assessing experimental results it is advisable to apply multivariate methods taking into consideration correlations of investigated traits (Seidler-Łożykowska and Bocianowski 2012). The features examined in this paper are very important because, from a practical point of view they determine the size of the yield. Correct plant density per unit area is one of the most important agronomic factors in the cultivation of maize for grain. According to current guidelines, plant density of maize in grain technology should be between 7–8 pcs/m^2. Failure to provide the optimum plant density at the very beginning of their vegetation does not allow full use of the full production potential of maize carried in the seed, which on the basis of the photo-

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synthetic efficiency is amounts 32 tons of grain ha^{-1}. On the other hand, the correct plant density per unit area strongly influences the size of the surface of assimilation, which largely determines the efficiency of solar radiation use. Excessive plant density leads to reduced productivity of photosynthesis. It is the result of mutual shading of the plant and reduction of the intensity of leaf photosynthesis. The photosynthesis uses increased by nitrogen the rate of respiration and consequently, to a decrease in biomass yield. On the other hand, significantly reduced plant density despite the improvement of conditions for the growth of individual plants, does not always ensure high yields. The aim of this study was to conduct a multivariate characteristic of phenotypic variability in 16 treatments being combinations of nitrogen and magnesium fertilisation levels and two maize cultivars. The analysis of canonical variables was applied (Yeater et al., 2004), based on the model of multivariate analysis of variance (MANOVA), for observations of eight quantitative traits in an experiment established in the split-split-plot design.

MATERIAL AND METHODS

Experimental field

The field experiment was performed at the Department of Agronomy, the Poznań University of Life Sciences, in the fields of the Teaching and Experimental Station in Swadzim in the years 2009–2011. The study was conducted in the random block (split-split-plot) design with three experimental factors in four field replications. The experiment was designed to analyse the effect of four urea application rates (factor A: A1 – 0, A2 – 50, A3 – 100, A4 – 150 kg N ha^{-1}), two magnesium application rates (factor B: B1 – 0, B2 – 25 kg MgO ha^{-1} applied as kieserite) on morphological traits of plants and ears in two types of maize cultivars (factor C: C1 – ES Palazzo [FAO 230-240] and the stay-green C2 – ES Paroli [FAO 250]). A detailed description of the experience characterizing agronomic issues are presented in the earlier work (Szulc et al., 2013). The test plot consisted of four rows of maize plants (30 m^2). Observations were performed on the two middle rows of plants. Two lateral rows of the experimental plot were so called seeding rows.

Methodology of observation

Plant density per area unit was determined twice: after the full emergence of plants and before the harvest. Counting the number of plants twice during their vegetation period made it possible to determine plant losses. Measurements of the height of plants and the height of ear setting were made using a graduated strip, with an accuracy of 1 cm, to 15 consecutive plants from the plot of the first row to be harvested. Plant height was measured from the surface of the ploughland to the tip of the perch or the end of the last leaf, if it reached higher than the perch. At the same plants, the height of ear setting was also measured. The measurement was made from the surface of the ploughland to the node from which the corn rachis grow. From each plot there were 10 ears collected, which were measured within the length and diameter of the ear by the electronic caliper. Ear volume (V) was calculated using the formula $V = \pi \cdot r^2 \cdot D$, where r denotes ½ ear diameter, D – ear length.

Statistical analysis

The relationships between traits were estimated using Pearson's correlation coefficients at different levels (Kozak et al., 2010). Due to the correlation of observed traits the material was analysed using multivariate methods assuming as experimental treatments all possible combinations of hybrids, nitrogen and magnesium fertilisation rates. The multivariate analysis of variance (Chatfield and Collins 1986) was used to test multivariate hypotheses on a lack of differences in effects between treatments, between years and treatments × years interactions. The Mahalanobis distances (Mahalanobis 1936) for investigated traits between treatments and the Mahalanobis critical distance D^2_{kr} (at $\alpha = 0.05$) were calculated for the data for all observations in three years. Analysis of canonical variables (Rencher 1998) was applied in order to present graphically multivariate variation of the investigated treatments. This facilitates a graphic presentation of the distance based on eight traits between these treatments with the Mahalanobis distance metric. In order to determine the relative share of each original trait in the multivariate variation of analysed treatments Pearson's simple correlation coefficients were estimated between values of the first two canonical variables and values of individual original traits. The year-to-year averages for observed traits were then used for cluster analysis to study relationships between treatments. The Euclidean distance was used as the resemblance coefficient for cluster analysis using the unweighted pair group arithmetic means method (UPGMA). All calculations for data analysis were performed using the GenStat v. 15 statistical package.

RESULTS AND DISCUSSION

Testing of Pearson's correlation coefficients made it possible to observe several statistically significant interdependencies between observed traits of maize. Plant height

Table 1. Correlation coefficients between observed quantitative traits of maize (*Zea mays* L.).

Trait	Plant height	Ear height	Ear length	Ear diameter
Ear height	-0.33***	1		
Ear length	-0.13	0.31***	1	
Ear diameter	0.10	0.09	0.56***	1
Ear volume	-0.03	0.24**	0.90***	0.86***

** P<0.01, *** P<0.001

was significantly negatively correlated with ear height (Table 1). Setting height of marketable ears was characterised by a statistically significant positive correlation with ear length and ear volume (Table 1). Ear length and ear diameter were statistically significantly directly proportional to ear volume (Table 1). Ear length and diameter were characterised by statistically significantly positive Pearson's correlation coefficients (Table 1). The number of plants after emergence and before harvest were characterised by a very significant interdependence (*r* = 0.91) (Table 2). Interesting dependencies were observed for plant loss rates: a positive correlation with the number of plants after emergence and a negative correlation with the number of plants before harvest (Table 2).

Table 2. Correlation coefficients between the number of plants after emergence and before harvest and plant loss rates in maize (*Zea mays* L.).

Trait	Number of plants after emergence	Number of plants before harvest	Plant loss rate
Number of plants after emergence	1		
Number of plants before harvest	0.91***	1	
Plant loss rate	0.22*	-0.21*	1

* P<0.05, *** P<0.001

The conducted multivariate analysis of variance (MANOVA) made it possible to reject tested hypotheses concerning a lack of average multivariate differences between treatments (P < 0.001) and a lack of treatments × years interactions (P < 0.001). In contrast, it was not possible to reject the zero hypothesis on a lack of average multivariate differences between years of the study (P = 0.073). In relation with the above the next analyses were performed for years jointly.

Table 3. Correlation coefficients between the first two canonical variables and original traits.

Trait	First canonical variable	Second canonical variable
Plant height	-0.21	0.82***
Ear height	-0.59*	-0.06
Ear length	-0.60*	0.79***
Ear diameter	-0.87***	0.28
Ear volume	-0.80***	0.53*
Number of plants after emergence	0.99***	0.04
Number of plants before harvest	0.98***	-0.01
Plant loss rate	0.41	0.38
Percentage of explained multivariate variability	65.48	18.30

* P<0.05, *** P<0.001

Individual traits are of different importance and have a different share in the joint multivariate variation. A study on the multivariate variation for treatments includes also identification of the most important traits in the multivariate variation of treatments. Analysis of canonical variables is a statistical tool making it possible to solve the problem of multivariate relationships (Bocianowski and Rybiński 2008). Results of the analysis of canonical variables for investigated treatments are presented in Table 3. The first two canonical variables explained jointly 83.78% total variation between treatments (Table 3, Fig. 1).

Figure 1 presents variation in traits of investigated treatments in the system of the first two canonical variables. In the graph the coordinates of a point of a given treatment are values of the first and second canonical variables, respectively. The greatest, significant linear relationship with

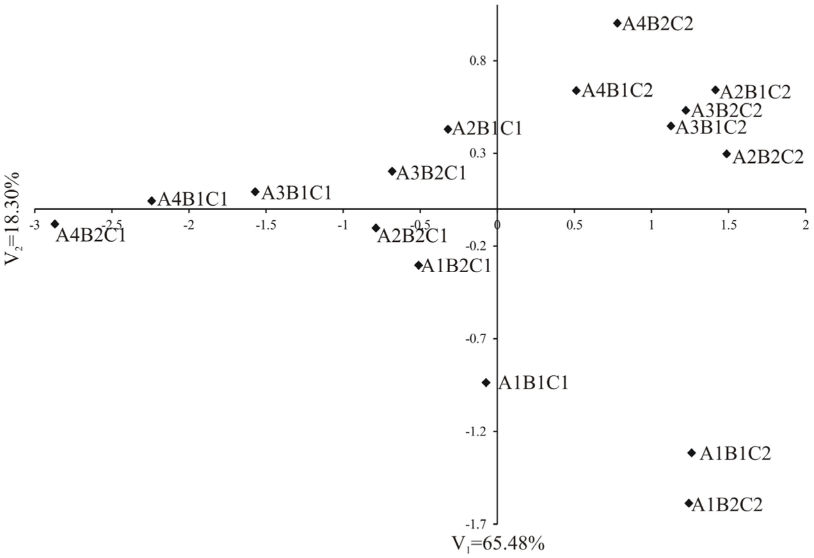

Fig. 1. Location of maize (*Zea mays* L.) treatments in the system of the first two canonical variables (A1 – 0, A2 – 50, A3 – 100, A4 – 150 kg N ha^{-1}; B1 – 0, B2 – 25 kg MgO ha^{-1}; C1 – ES Palazzo, C2 – ES Paroli SG)

Table 4. Mahalanobis distances between analysed treatments of maize (*Zea mays* L.)

	A1B1C1	A1B1C2	A1B2C1	A1B2C2	A2B1C1	A2B1C2	A2B2C1	A2B2C2	A3B1C1	A3B1C2	A3B2C1	A3B2C2	A4B1C1	A4B1C2	A4B2C1
A1B1C1	0														
A1B1C2	1.499	0													
A1B2C1	1.035	2.169	0												
A1B2C2	1.608	0.741	2.264	0											
A2B1C1	1.555	2.511	1.155	2.698	0										
A2B1C2	2.263	2.053	2.243	2.309	1.809	0									
A2B2C1	2.020	2.717	1.583	3.127	2.072	2.910	0								
A2B2C2	2.111	1.713	2.343	2.123	2.138	1.047	2.680	0							
A3B1C1	1.951	3.259	1.587	3.361	1.478	3.097	2.184	3.188	0						
A3B1C2	1.975	1.843	1.994	2.143	1.627	0.676	2.536	0.755	2.797	0					
A3B2C1	1.446	2.520	0.858	2.736	0.902	2.261	1.421	2.289	1.335	1.897	0				
A3B2C2	2.114	1.998	2.205	2.254	1.958	1.087	2.701	0.644	2.978	0.683	2.066	0			
A4B1C1	2.540	3.803	1.971	3.905	2.305	3.804	2.058	3.794	1.134	3.453	1.790	3.571	0		
A4B1C2	1.781	2.193	1.573	2.366	1.349	1.170	2.337	1.311	2.287	1.005	1.529	0.994	2.876	0	
A4B2C1	2.969	4.365	2.515	4.403	2.751	4.411	2.811	4.454	1.518	4.099	2.312	4.185	1.095	3.489	0
A4B2C2	2.299	2.530	1.955	2.773	1.802	1.252	2.502	1.596	2.869	1.424	1.970	1.437	3.341	0.923	3.922

$D^2_{kr} = 3.501$

A1 – 0, A2 – 50, A3 – 100, A4 – 150 kg N ha^{-1}; B1 – 0, B2 – 25 kg MgO ha^{-1}; C1 – ES Palazzo, C2 – ES Paroli SG

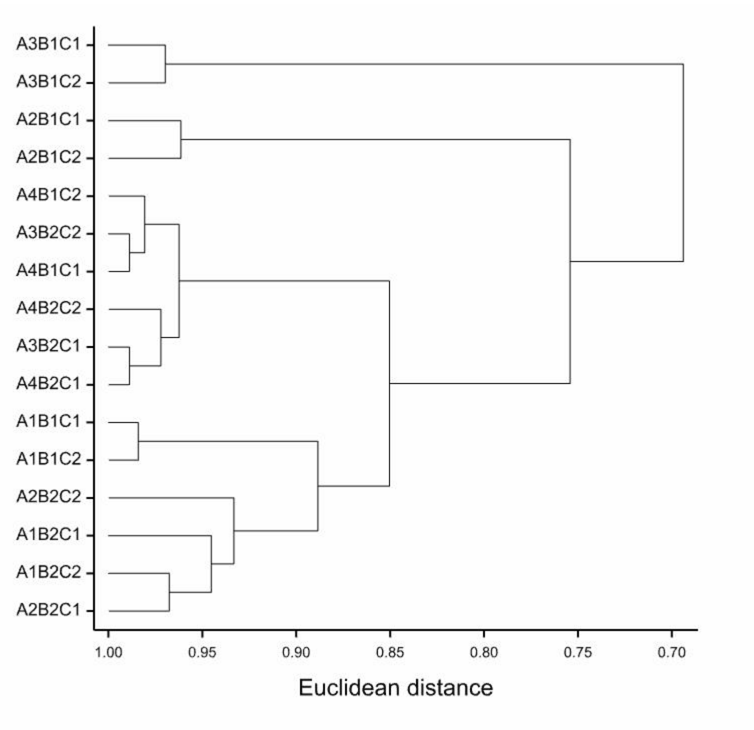

Fig. 2. Dendrogram based on means of eight qualitative traits of 16 treatments of maize (*Zea mays* L.) (A1 – 0, A2 – 50, A3 – 100, A4 – 150 kg N ha^{-1}; B1 – 0, B2 – 25 kg MgO ha^{-1}; C1 – ES Palazzo, C2 – ES Paroli SG)

the first canonical variables was found for the number of plants after emergence, the number of plants before harvest (positive dependencies), ear diameter, ear volume, ear length and ear height (negative dependencies) (Table 3). The second canonical variable was significantly positively correlated with plant height, ear length and ear volume (Table 3). The greatest variation in terms of all the traits jointly (measured Mahalanobis distances) was found for treatments denoted with symbols A2B2C2 (50 kg N ha^{-1}, 25 kg MgO ha^{-1} for ES Paroli SG) and A4B2C1 (150 kg N ha^{-1}, 25 kg MgO ha^{-1} for ES Palazzo) (the Mahalanobis distance between them amounted to 4.454). The greatest similarity was found for treatments A2B2C2 (50 kg N ha^{-1}, 25 kg MgO ha^{-1} for ES Paroli SG) and A3B2C2 (100 kg N ha^{-1}, 25 kg MgO ha^{-1} for ES Paroli SG) (0.644). Values of Mahalanobis distances for all pairs of treatments are presented in Table 4.

Clustering of investigated treatments on the basis of Euclidean distances for values of means produced three clusters at the similarity level of 0.85. One cluster comprised treatments denoted with symbols A3B1C1 and A3B1C2, the second - treatments A2B1C1 and A2B1C2, with the third cluster being composed of all the other treatments (Fig. 2). In the third cluster we may distinguish two subclusters. One subcluster contained treatments at fertilisation with 100 and 150 kg N ha^{-1} (denoted with symbols A3 and A4), while the other comprised those with fertilisation levels of 0 and 50 kg N ha^{-1} (Fig. 2). Similar results for hop obtained Skomra et al. (2013).

The presented multivariate characteristic of the behaviour of analysed treatments is a convincing illustration of this aspect. In this way efficiency of the analysis of canonical variables was shown. This results from the fact that these variables explained a considerable part of total variation (83.78%). Thus this is a reliable method, which may be confirmed by its extensive application by breeders and geneticists (Shamsuddin 1985, Seidler-Łożykowska et al., 2013, Nowosad et al. 2016).

CONCLUSIONS

1. The most variable treatments included A2B2C2 (50 kg N ha^{-1}, 25 kg MgO ha^{-1} for ES Paroli SG) and A4B2C1 (150 kg N ha^{-1}, 25 kg MgO ha^{-1} for ES Palazzo). The most similar treatments (in terms of the eight traits treated jointly) included A2B2C2 (50 kg N ha^{-1}, 25 kg MgO ha^{-1} for ES Paroli SG) and A3B2C2 (100 kg N ha^{-1}, 25 kg MgO ha^{-1} for ES Paroli SG).
2. Results obtained using the analysis of canonical variables confirm applicability of this method for an effective assessment of multivariate similarity and multivariate characteristic of behaviour in the investigated treatments.
3. Recorded results indicate a great variation in the tested maize cultivars, irrespective of the applied nitrogen and magnesium fertilisation rates.

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