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CANONICAL CORRELATIONS IN STUDYING GRAIN YIELD AND PROTEIN CONTENT AS AFFECTED BY YIELD COMPONENTS: AN ONTOGENETIC APPROACH

ABSTRACT

In the paper, we have proposed an approach to studying the relationship between two sets of variables, when one of the sets comprises plant traits that develop sequentially. The approach joins two statistical methods, namely canonical correlations and sequential yield analysis (Mądry *et al.*, 2005, J. New Seeds 7(1), pp. 85-107). Using the approach, grain yield and protein content in grain of two spring barley cultivars—Rasbet (with hulled grains) and Rastik (with hulless grains)—were studied as the effect of yield components that develop sequentially (number of spikes per m^2, number of kernels per spike, and kernel weight). Grain yield of both cultivars was determined mostly by number of spikes per m^2; the effect of two other components on yield was noticeably weaker. Yield components of cultivar Rastik did not influence protein content in grain, whereas two yield components of cultivar Rasbet, number of spikes per m^2 and kernel weight, affected protein content in grain, although the relationship was rather weak.

Key words: multivariate methods, sequential yield analysis, spring barley.

INTRODUCTION

Studying cereal grain yield formation has been an objective of many investigations. One of important aspects of this complex biological process is the effect of yield components on yield. Many researchers have attempted to assess the importance of yield components in the process of grain yield formation. Results of their studies on barley indicate that number of spikes per m^2 is the most important yield component in obtaining high grain yield; two other yield components, number of kernels per spike and kernel weight, are far less important (e.g., Conry, 1998; García and García, 1995). Number of spikes per m^2 can be modified by agronomical factors, e.g., nitrogen fertilization or date of sowing (Cantero-Martinez *et al.*, 2003); this yield component depends on number of tillers at the end of

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tillering phase (Abeledo *et al.*, 2004). A relation between number of spikes per unit area and grain yield is weaker in a case of cultivars that produce large number of kernels per spike than in a case of cultivars with small value of this component (García *et al.*, 2003). If number of spikes per m^2 is large, there is a strong competition between spikes, and thus the environmental conditions become unfavorable; therefore, the positive effect of number of spikes per m^2 on grain yield quantity may be not recorded (Jackson et. al., 1994; Dofing and Knight, 1994). Effect of number of kernels per spike and kernel weight on grain yield depends on both barley genotype and environmental conditions from flowering to kernels maturity. During less favourable weather, number of kernels per spike and kernel weight are components that have weaker influence on grain yield quantity (Voltas *et al.*, 1999a,b; Tambussi *et al.*, 2005). The mutual compensation among yield components often occurs; it consists especially in decreasing number of kernels per spike by large number of spikes per m^2 (Baethgen *et al.*, 1995).

Most investigations on yield components have been limited to studying the effect of yield components on quantity aspect of yield; formation of grain yield quality traits (e.g., protein content in grain) in these terms is less recognized. Huang *et al.* (2005) found significant negative correlation between grain yield and protein content in grain, and between protein content and number of kernels per spike; the correlation between protein content and kernel weight was insignificant in their study. In the research on different spring barley cultivars in conditions of Northern Europe, Bertholdson (1999) found that the cultivars with genetically larger tiller number and larger number of kernels per spike were characterized by lower protein content in grain.

In this paper, we study quantity and quality aspect of barley grain yield as the effect of yield components. If one deals with multivariate observations, a common statistical practice is to use a multivariate statistical approach to analyze the data. In our study, we consider two sets of variables. The first one is response variables' set (which is called in the paper the "response set"), which comprises grain yield (the quantity aspect of yield) and protein content in grain (the quality aspect of yield). The second set is grain components' set (the "component set"), which comprises number of spikes per m^2, number of kernels per spike, and kernel weight.

Suppose that our objective is to assess the relationship between the response and component sets. It could be done by two independent analyses regarding relationships: (1) grain yield versus components, and (2) protein content versus components. Nevertheless, a multivariate approach would be more efficient and would provide more thorough and overall view of the influence of components on yield formation (as yield, we understand its both aspects, i.e., quantity and quality). Two univariate analyses would provide just partial information on the importance of components in determining yield (see, e.g., Rencher 1998, Preface). In particular, in our investigation, the multivariate multiple regression (Rencher, 1998, sec. 7) or canonical correlations (Rencher, 1998, sec. 8) could be employed. We have applied the latter as a more common method. Although it is quite a well known method, its applications in agricultural studies are still rare, even though some papers employing canonical correlations in various agricultural applications and with various aims have been published (e.g., Milczak *et al.*, 1995; Flores *et al.*, 1998; or Franco and Crossa, 2002).

Cereal grain yield components develop sequentially during plant ontogenesis (e.g., Dofing and Knight, 1992; García del Moral *et al.*, 2003). It causes the possibility of a particular yield component to be determined by components previous to it in the plant ontogeny; this component, however, cannot influence those components that develop earlier than it does. Such biological concept of yield components should be taken into consideration in analyzing the influence of components on their putative response (in our case, two response variables—grain yield and protein content in grain). It is done by applying the so-called Gram-Schmidt orthogonalization (Winer, 1971). This linear transformation of the data results in orthogonality and hence the stochastic independence of the variables transformed. Therefore, assessing the influence of components appropriately orthogonalized (i.e., transformed subject to the ontogenetic order assumed) can be treated as assessing the relative influence of components on yield after taking into account the cause-and-effect relationships among yield components. A method to analyze such relationships, so-called sequential yield analysis, was presented in details by Mądry *et al.* (2005).

We suggest combining the two approaches mentioned, that is, canonical correlations and sequential yield analysis. The aim of the combined method is to describe a set of response variables as the effect of a set of sequentially developing traits. The method proposed, canonical correlations for sequentially developing predictors, can be treated as a novel approach and can be applied for any data being appropriate for both statistical methods.

The objective of the paper is (a) to propose an approach to studying a relationship between two sets of variables, when one of the sets comprises the sequentially developing traits, and (b), using the proposed approach, to compare influence of sequential grain yield components on grain yield and protein content in grain of two spring barley cultivars, Rasbet (with hulled grains) and Rastik (with hulless grains).

MATERIAL AND METHODS

Site and soil

The experiment was carried out in the years 1999, 2001, and 2002 at the Chylice Experimental Farm of the Warsaw Agricultural University (N 52°05’ E 20°32’, 105 m a. s. l.) located in Central Poland, 40 km west of Warsaw (Mazovia Region). The climate of this region, similarly to whole Poland, is transitional between humid oceanic climate of Western Europe and the dry continental climate of Eastern Europe (Czerwiński *et al.*, 1989). The vegetation period in Central Poland lasts 170-200 days. There is quite a long period with temperatures above 0 degrees (about 250 days) and short period with snow cover (about 35-65 days). At the experimental site, total rainfall averages 500 mm annually, with ca. 270 mm received during growing season (April-July). The course of the weather during years 1999, 2001, and 2002 is presented in Table 1.

In the years 1999 and 2001, the distribution of precipitations was favorable. During the growth period of spring barley, precipitation was close to or more than plant needs. In 2002, a shortage of precipitation during growth period occurred. Low amount of rainfall in April and June was recorded. The experiment was established on soils classified as black earth (*Mollic Gleysols*) formed of loamy sand of glacial origin.

Table 1

Monthly precipitation (mm) and average monthly temperatures (°C) in Chylice and the precipitation needs of barley; according to Dzieżyc *et al.* (1987)

Date	IV	V	VI	VII
The monthly sum of rainfall (mm)				
1999	75.1	67.4	160.8	39.0
2001	92.5	24.6	64.3	104.3
2002	11.1	66.4	71.7	43.0
The precipitation needs of barley (Dzieżyc *et al.* , 1987)				
	39	59	82	87

Treatments and design

In each year of the study (1999, 2001, and 2002), the experimental design was a randomized complete block in a split-plot arrangement with four replications within the years. Combinations of cultivar (Rasbet and Rastik) and date of sowing (early and delayed) were the main plots, whereas rates of N (0, 30, 60, and 90 kg N ha^{-1}) were the subplots within the main plots.

Two different cultivars, i.e., Rasbet (with hulled kernels) and Rastik (with hulless kernels), both bred by ZDHAR Radzików (Poland), were investigated. Their description is presented in Table 2.

Table 2

Characteristics of cultivars (source: IHAR, 1999; COBORU, 2002)

Parameters	Rasbet	Rastik
Year of registration	1998	1999
Parent's forms	Rudzik × Alexis	Grosso × (Bielik × H 2238)
Grain	hulled	hulles
Grain usage	brewery	feed, food
Yield of grain (COBORU experiments)	94-102% of standard	81-84% of standard
Content of grain (COBORU experiments)	11.0%	13.7%
Spikes	2-row	2-row

The early sowing date of spring barley, depending on the weather conditions, was at the end of March or the beginning of April. The first sowing dates were (according to the weather conditions in the years): early sowing—07.04 (1999), 04.04

(2001), and 28.03 (2002); delayed sowing—28.03 (1999), 30.04 (2001), and 18.04 (2002).

Nitrogen was applied in the ammonium sulphate form. The rates of 30 and 60 kg N ha^{-1} were applied once before sowing, whereas the rate of 90 kg N ha^{-1} was divided into 60 kg N ha^{-1} before sowing and 30 kg N ha^{-1} at stem elongation (DC 32; Zadoks *et al.*, 1974).

Root plants cultivated with manuring were the forecrop for spring barley: sugar beets in 1999 and 2002, and potatoes in 2001. The plot area was 30 m^2; seeds were sown at a density of 350 seeds per m^2 for Rasbet and 400 per m^2 for Rastik (because of a worse field emergency ability of the latter cultivar).

Plant sampling

Based on the samples taken from 1m^2, grain yield and number of spikes per m^2 were measured during harvesting. Second yield component, average kernel weight (thousand kernel weight), was determined by measuring two independent samples of 500 kernels. Third yield component, average number of kernels per spike, was calculated indirectly on the basis of grain yield, number of spikes per m^2, and kernel weight. Protein content in dry mass of grain was evaluated after harvesting on the basis of nitrogen content in grain (6.25% × N content) measured with the Kjeldahl method (Concon and Soltess, 1973).

Statistical analysis

Two multivariate sets were considered: the set of response (dependent) variables (yield set dealing with quantity and quality aspect of yield) $Y = (Y_1, Y_2)^T$, where Y_1 is grain yield and Y_2 is protein content in grain; and the set of grain yield components (component set) $X = (X_1, X_2, X_3)^T$, where *X*s are sequentially developing grain yield components, that is, number of spikes per m^2, number of kernels per spike, and kernel weight.

For the detailed description of sequential yield analysis, see Mądry *et al.* (2005). Canonical correlations were applied using the methodology presented by Rencher (1998). Namely, canonical correlations were calculated on the standardized data. Testing of the general hypotheses of canonical correlations (both canonical correlations are zero) was performed using the exact Wilks Lambda, Lawley-Hotellting Trace, and Pillai's tests (Rencher, 1998). If the general hypothesis was rejected, the hypothesis on the second canonical correlation (equal to zero) was tested via the likelihood ratio test (Johnson and Wichern, 1999). Following suggestions of Rencher (1998), besides standardization, no other tools for interpreting canonical variates, as the rotation of canonical variate coefficients or interpreting correlation between each variable and the corresponding canonical variate, were employed. Redundancy analysis was not applied, either, since it does not enrich the analysis (Rencher, 1998, p. 329). The validation of the basic assumptions for canonical correlation analysis (not included in the paper) was done by ordinary plotting the points of canonical variates (Rencher, 1998, sec. 8.5).

The computation in the approach proposed consists of (a) transforming the original data with the Gram-Schmidt othogonalization (subject to assumed ontogenetic order of the predictors), and (b) applying canonical correlations for two sets, Y (response set) and Z (orthogonal component set).

For each cultivar, the analysis was carried out for all the observations from plots and years, which were treated as a representative sample from the population generated by agronomical (treatments studied) and environmental (years) conditions. Therefore, there were 96 observations for each cultivar. Interpretation based on data pooled from various conditions relates to the causal relationships in the population mentioned.

The Gram-Schmidt orthogonalization was performed using the R language and environment (R Development Core Team, 2005), whereas canonical correlations using SAS (SAS Institute, 2004).

RESULTS

The correlation matrices of traits studied for two cultivars of spring barley (Rasbet and Rastik) are presented in Table 3. Grain yield of both cultivars was significantly positively correlated with all its components. In both cases, the strongest correlation was detected between grain yield and number of spikes per m^2. The weak negative correlation between the two response variables, grain yield and protein content, was detected, although for cultivar Rastik the coefficient was significant only at $P \leq 0.05$. In the case of cultivar Rastik, protein content was weakly and negatively correlated with number of spikes per m^2 (significant at $P \leq 0.05$), and in the case of cultivar Rasbet with kernel weight. In the case of cultivar Rastik, the only significant correlation (negative) detected among components was between number of kernels per spike and kernel weight. In the case of cultivar Rasbet, weak positive correlation between number of spikes per m^2 and kernel weight, and weak negative correlation between number of kernels per spike and kernel weight was observed.

Table 3

Correlation matrices for grain yield, protein content, and yield components of spring barley cultivars Rastik and Rasbet

Cultivar	Trait	Y_1	Y_2	X_1	X_2
Rastik	Y_2	-0.22*	1		
	X_1	0.82**	-0.25*	1	
	X_2	0.20*	0.10	-0.16	1
	X_3	0.30**	-0.04	0.02	-0.33**
Rasbet	Y_2	-0.35**	1		
	X_1	0.81**	-0.11	1	
	X_2	0.40**	-0.14	-0.04	1
	X_3	0.41**	-0.44**	0.20*	-0.27**

*, ** significant at $P \leq 0.05$ and $P \leq 0.01$, respectively

The correlation matrices for the data after orthogonalization of components are presented in Table 4. Of course, there were no correlations among orthogonal components (Zs). For both cultivars, the correlation between grain yield and orthogonal components were quite similar to the corresponding correlations before transformation, besides the noticeably larger correlations between grain yield and orthogonal number of kernels per spike and between grain yield and orthogonal kernel weight.

Table 4

Correlation matrices of grain yield, protein content in grain, and orthogonal yield components of spring barley cultivars Rastik and Rasbet

Cultivar	Trait	Y_1	Y_2	Z_1	Z_2
Rastik	Z_1	0.82**	-0.25*	1	
	Z_2	0.34**	0.06	0.00	1
	Z_3	0.42**	-0.01	0.00	0.00
Rasbet	Z_1	0.81**	-0.11	1	
	Z_2	0.43**	-0.15	0.00	1
	Z_3	0.38**	-0.48**	0.00	0.00

Results for cultivar Rasbet

The results of canonical correlation analysis for spring barley cultivars studied are presented in Table 5. For cultivar Rasbet, both canonical correlations were significant at $P \leq 0.01$, but the first one was noticeably more important than the second one (Table 5). Because the eigenvalue (which is the square of the corresponding canonical correlation; see Rencher, 1988) for the first pair of canonical variates was very large (0.98; Table 6), we conclude that the relationship between both sets (Y and Z) was very strong.

Table 5

Importance of squared canonical correlations of spring barley cv. Rasbet and Rastik

Cultivar	No. canon. variate	Importance (%)
Rasbet	1	85.1
	2	14.9
Rastik	1	96.7
	2	3.3

Table 6

Canonical correlations of spring barley cv. Rasbet and Rastik grain yield and protein content (set Y) versus set of orthogonal components (Z)

Cultivar	No. canon. variate	Canon. Correlation	Eigenvalue
Rasbet	1	0.991**	0.982
	2	0.415**	0.172
Rastik	1	0.986**	0.972
	2	0.181	0.033

Table 7

Standardized coefficients of canonical variates for the first set (grain yield and protein content) of spring barley cv. Rasbet and Rastik

Cultivar	Variable	1^{st} canon. variate	2^{nd} canon. variate
Rasbet	Grain yield	1.00	0.36
	Protein content	0.02	1.07
Rastik	Grain yield	1.00	0.20
	Protein content	0.02	1.02

The first canonical variate was definitely the grain yield variate (Table 7); the contribution of protein content to this variate was negligible. All orthogonal components, but especially number of spikes per m^2, contributed to the first canonical variate (Table 5). Hence, the strong relationship between these two sets resulted especially from the strong relationship between grain yield and all orthogonal components, but especially number of spikes per m^2. Because the coefficients for all orthogonal components and for grain yield influenced the corresponding variates positively, we conclude that all components influenced grain yield positively.

The second canonical correlation was rather weak (Tables 5 and 6). Among the response variables, especially protein content contributed to this variate, but also grain yield did (Table 7). This canonical variate was determined especially by orthogonal kernel weight (negatively) and, to lower extent, number of spikes per m^2 (positively); see Table 8. Number of kernels per spike had no significant contribution to this canonical variate.

Table 8

Standardized coefficients of canonical variates for the second set (orthogonal components) of spring barley cv. Rasbet and Rastik

Cultivar	Trait	1st canon. variate	2nd canon. variate
Rasbet	Z_1	0.82	0.42
	Z_2	0.44	0.00
	Z_3	0.37	-0.91
Rastik	Z_1	0.83	-0.53
	Z_2	0.35	0.75
	Z_3	0.43	0.41

In summary, the relationship between the two studied sets of variables for cultivar Rasbet was very strong. The set of the response variables, grain yield and protein content, was determined by all grain yield components, but especially by number of spikes per m^2 and kernel weight, the former influencing both dependent variables and the latter influencing protein content.

Results for cultivar Rastik

For cultivar Rastik, only first canonical correlation was significant (Table 6); hence, its importance was very large (Table 5). Therefore, we will not use the second pair of canonical variates in interpretation. As in the case of cultivar Rasbet, grain yield determined the first canonical variate, whereas contribution of protein content was negligible. The eigenvalue for the first pair of canonical variates was very large (0.97; Table 6). Because protein content did not contribute to the first canonical variate, we conclude that this trait was not significantly influenced by any grain component. Grain yield was influenced positively by all components, but especially by number of spikes per m^2.

The comparison of the results obtained for both cultivars leads to the conclusion that pattern of influence of grain components on two aspects of grain yield (its quantity and quality) was slightly different. Components of cultivar Rasbet grain yield affected grain yield and protein content (the latter was affected by first and third component); in the case of cultivar Rastik, only grain yield was affected by its components—protein content in grain appeared to be independent from yield components. For both cultivars, number of spikes per m^2 was the most important grain yield component in affecting the response set.

DISCUSSION

The approach to analyze the relationship between two sets of variables, i.e., the response variables' set and orthogonal predictors' set, is proposed in the paper. The example of the application of the method is presented for the two-dimensional response variables' set Y (which comprised two response variables—grain yield and protein content in grain) and the three-dimensional orthogonal predictor variables' set Z (which comprised orthogonal yield components). If a number of variables investigated is small and a correlation matrix R_y of Y is diagonal (i.e., correlation coefficients between response variables are nil), the inference based on the multivariate approach would be, in fact, similar to the inference based on the set of univariate analyses. In the case of the near-diagonal matrix R_y, the gain of applying multivariate approach might be negligible as well, although it would depend on particular data considered. Nevertheless, when a number of response variables is large and off-diagonal components of R_y are not nil or near-nil, the gain would be large. This is because the multivariate statistical methods take into account correlations among dependent and independent variables, the information on which is lost when one applies a set of univariate analyses.

The statistical analysis via the approach proposed confirmed the large influence of first component, number of spikes per m^2, on grain yield of both spring barley cultivars studied, as former investigations proved (e.g., Conry, 1998; García and García, 1995). The importance of two other components in yield formation, i.e., number of kernels per spike and kernel weight, was far smaller. Then, cultivars of high yielding potential are likely to produce a large number of productive shoots, even in unfavorable environment conditions. Previous investigations have proven negative relation between yield components and protein content in grain (Bertholdson, 1999; Huang *et al.*, 2005). In the case of cultivar Rasbet, we recorded the significant relation between protein content in grain and two yield components, number of spikes per m^2 and kernel weight. In the case of cultivar Rastik, protein content in grain was not significantly determined by yield components. It can indicate that the lower level of protein content in grain of a particular cultivar, the greater the influence of yield components on protein content in grain, as well as that the higher level of protein content in grain, the weaker (or even none) the relationship. Nevertheless, generalization of this conclusion would require more research that would comprise multiplicity of spring barley cultivars.

CONCLUSIONS

We have proven the usefulness of the proposed approach to studying two aspects of grain yield, i.e., its quality and quantity, as the effects of grain yield components that develop sequentially during plant ontogenesis. Moreover, we have also shown that canonical correlation analysis, rarely employed in agricultural studies even though commonly known, is a convenient and efficient statistical method in spite of its theoretical complexity. In the example presented, in which grain yield and protein content in grain of two spring barley cultivars were studied as the effects of grain yield components, it has been shown that grain yield of both cultivars was determined especially by number of spikes per m^2. Number of kernels per spike and kernel weight influenced grain yield to noticeably smaller extent. Protein content in grain of cultivar Rastik was not influenced by grain yield components, whereas in the case of cultivar Rasbet, the weak influence of number of spikes per m^2 and kernel weight on this yield quality trait was observed.

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