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EVALUATION OF THE GENETIC VARIABILITY OF WINTER PEA VARIETIES (*Pisum sativum* L.) FROM THE COLLECTION OF IPGR - SADOVO

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Abstract

Subjects of the present research paper are 48 pea specimens - winter varieties taken from the collection of the Institute for Plant Genetic Resources - Sadovo. The study aims at establishing the rate of genetic similarity and genetic remoteness of the specimens kept in the national gen-bank. The specimens are mainly of French and Bulgarian origin. A mathematical approach was used for their group formation - a cluster, correlation and factor analysis using the following indicators - total nitrogen, crude fibres, crude ash, total sugars and tannins. There were established strong negative correlations between: total nitrogen and crude fibres (r = - 0.896); total nitrogen and crude ash (r = -0.853), and total nitrogen and total sugars (r = - 0.886). The group formation of winter pea varieties allows higher objectivity in evaluation, as well as more possibilities for use of pea collection.

Key words: *peas (Pisum sativum L.), total nitrogen, crude fibres, crude ash, total sugars and tannins, genetic similarity and remoteness, correlation, cluster analysis, factor analysis.*

INTRODUCTION

Climate changes call for the search of new alternative solutions in the structure of crops and their varietal composition. The complex evaluation and analysis of plant genetic resources /PGR/ and their main indicators as part of the national and some international collections, as well as the grouping of plant materials by significant qualitative features, is a necessary condition for their preservation, management and use (Angelova & Sabeva, 2018; Kalpakchieva, 2004; Mikic et al., 2021; Sabeva, 2019; Stanek et al., 2004).

Cereals and legumes, including peas, have indisputable contribution for solving the protein problem worldwide. Peas` high nutritive value is determined by the quality and quantity of plant protein, which contains the necessary amino-acids.

Peas is rich in starch - 45-50%; crude protein - 25-30%; fibres - 6-15%; ash - 2-5%; microelements and lysine from 1.4 to 1.8%. Taking into account the nutrients and the calories, mature pea seeds exceed meat almost 3 times; fish - 4 times; rye and wheat bread - 1.5 times; potatoes - 3.5 times; and cabbage - almost 6 times. Peas` chemical composition, as well as its ecological plasticity and adaptability, determine to a great extent the wide area of distribution and make it an irreplaceable source of protein (Sabeva et al., 2014; Sabeva, 2019).

The crude protein content is a varietal feature, which is influenced to a great extent by soil and climate - the conditions necessary for grain formation (Mikic et al., 2012; Vasileva & Kosev, 2021).

Variation of crude protein influenced by external factors is most highly expressed in the high-protein varieties, reaching up to 10%. Taking into account the protein content at deviation of 1,5%, the variety is considered stable regarding this indicator. The bigger the variation amplitude is, the more interesting the studied material is for the selection. (Ali Khan et al., 1995; Cervenski et al., 2017; Sabeva, 2019)

In a study of ours, ranging over 15 years, we have established that the content of *crude* protein in winter pea varieties is influenced by the year and the variety, as well as their interaction - *year x variety* (Sabeva, 2019; Sabeva & Angelova, 2022). It has been mathematically proven. The year has the strongest influence - 61.2%, followed by the variety - 32.4%, and the weakest influence has the interaction year x variety - 6.4%.

A number of authors, based on their studies, have reported that crude protein content varies depending on seed origin and soil-climatic conditions - from 20.35% to 31.35%. (Ali Khan et al., 1995*)* have proved that crude protein content varies from 21.1% to 28.3%, according to the region of cultivation in Canada. Within the frames of one and the same variety, variation is from 24.0% to 26.3%, and with relation to the year, it is from 25.8% to 27.4%.

With relation to the soil-climatic conditions, there have been established fluctuations in the following: crude fibres - from 3.0 to 6.0%, mineral salts - from 2.0 to 3.1%, crude fats - from 1.3% to 1.5%.

Seed size is a quality feature, which varies within wide limits depending on the soil-climatic conditions and the agro-machinery. Studying the dependence between seed size and crude protein content, it has been established the correlation coefficient - r= 0.42, which shows that it is difficult to combine high protein content and large grain (Gueguen & Barbot, 1988; Srivastava et al., 2009)

A number of studies have reported that pea genotypes possessing seeds with smooth and wrinkled surface and cultivated in the same environment have diversity between varieties with relation to the crude protein content (Gueguen & Barbot, 1988; Odoardi et al., 2004). Some researchers suggest that varieties with wrinkled seeds have higher contents of sugar than these with smooth seed surface. Some Canadian authors have proven in their studies that there is negative correlation dependence between the raw protein and starch content. They claim that specimens with high starch level have low content of raw protein and raw fibres (Gueguen & Barbot, 1988; Sabeva, 2019).

According to a study conducted by Angelova and Sabeva (2013), it has been reported the presence of a weak negative correlation only between crude protein and crude fibre content. Taking into account the pea varieties and their grouping by crude protein content, authors have reported that there is no proven correlation between the morphological features of seeds, the weight of 1000 seeds and their phenotype.

Vasileva and Kosev (2021) have reported for negative correlation dependence between seed yield and phosphorus content (r = - 0.35), crude protein (r = - 0.18) and crude fibres (r = - 0.03).

With relation to the source forms for selection, more and more attention is paid not only to the morphological and productive characteristics, but to the qualitative indicators also, which determine the nutritive value of forage. There is search of genotypes combining high nutritive quality and high yield. Taking into account the choice of qualitative indicators, it is important to clarify the variability in the contents of chemical compounds, which determine the qualitative composition of forages (Burstin and Duc, 2005; Mikich et al., 2012).

IPGR keeps up a collection of over 2500 pea specimens including old varieties, forms and populations, as well as newly-selected varieties and lines.

Systematization of the information related to the evaluation of pea specimens by qualitative features, along with their chemical characteristics, give possibilities for increase of effectivity of the selection programs (Sabeva, 2019).

The present study aims at establishing the rate of genetic similarity and remoteness of a cross section including 48 winter pea specimens by some bio-chemical indicators in order to be used effectively in the selection work with peas.

MATERIALS AND METHODS

Plant material

The studied cross section of winter pea varieties (48) consisted of specimens originated mainly from Bulgaria and France. The predominant varieties were early and middle-early forms for the use of green mass and grain. The specimens had leaves of a common type. 13 of them were white-blossom, and the rest 35 - purple-blossom. Seeds were round, sometimes with an irregular shape, and had a smooth surface. Coloration was diverse and varied from light-beige to light-brown, particolored, from grey-green and dark-green to dark-brown (Angelova & Sabeva, 2013; Sabeva et al., 2014; Sabeva, 2019).

Evaluation of the specimens regarding their content of: *total nitrogen (X1)*, *crude fibres*

(X2), crude ash (X3), total sugars (X4) and concentrated tannins (X5) was conducted in the bio-chemical laboratory of IPGR - Sadovo.

The content of *total nitrogen/ crude protein* in grain was determined by Kjeldhal`s Method; *crude fibres* - by Heneverg and Shtoman; *crude ash/total sugars* - by Schoorl`s Method (A.O. A. C, 1990); *concentrated tannins* in % - by Terrill et al. (1992) with butanol-salt acid (Ilieva and Dochkova, 1999).

Soil-climatic characteristics

The specimens were cultivated in the experimental field of IPGR, on cinnamon forest soil. The town of Sadovo is situated in the Upper Thracian Plain, with an altitude - 141 m and having continental climate (42º70´58´´ N; 024´55´58´´ E.). Winter in the region is softer compared to Northern Bulgaria, and summer is hot. The average annual temperature is 12.4°C with a minimum value in January and a maximum value in July. In particular years temperatures in January and February decrease under - 18°C for 2-3 days, and in some cases – under - 20°C, which is important for the winter varieties. Precipitation sum has the highest values in May – 47.5 mm, and the lowest values in September – 14.9 mm (Boyadjieva & Stankova, 1990).

Variability of the examined indicators was determined through the use of variation analysis. The interaction between the studied indicators was evaluated and expressed through the correlation coefficient r.

The correlation analysis showed the presence of statistically significant correlations between the studied indicators, followed by a factor analysis technique (Kline, 1994) in order to reduce the number of the seven initially included indicators. The factor analysis was performed by the principle component method (PCA). The number of principle components was determined by the number of eigenvalues of the correlation matrix that were greater than 1 (Kaiser's criterion). Eigenvalues show the contribution of the eigenfactor when explaining the total dispersion in the variables.

The factor model is usually determined by the factor weights, which represent the correlation coefficients between the respective observed indicators and the factors. Thus, a smaller number of generalized factors are determined, without their own meaning, but combining the properties of several indicators.

Adequacy assessment of the factor analysis was performed by using the Kaiser - Mayer - Olkin (CMO - test) and Bartlett tests.

The grouping of 48-th examined specimens was performed through a hierarchical cluster analysis. The method of intergroup connection was used (Dyuran & Odelly, 1977; Desheva et al., 2016; Ward, 1963).

The mathematical and statistical processing of empirical data was performed through the statistical program SPSS Statistics 19.

RESULTS AND DISCUSSIONS

Variation and correlation analysis

A qualitative indicator showing the forage value of the pea grain is its chemical composition (Angelova & Sabeva, 2013; Sabeva et al., 2014; Sabeva, 2019).

Table 1 shows the main descriptive features of grain bio-chemical composition of the cross section of winter pea varieties for three years in average.

The variation coefficient is an important strategic indicator, which is used for establishing the homogeneity of the examined feature. The values of the variation coefficient varied in our research study within the limits from 5.54% to 49.29% (Table 1).

Data analysis showed that relatively most variable was the indicator *content of concentrated tannins* (CV= 49.29%). Tannin content in peas is related to the dark colouring of the flowers and seeds (Ilieva and Dochkova, 1999; Sabeva, 2019). With relation to our study, the variation limits of tannin content was from 0.01% to 2.71%. Winter PGR of peas in the present research included the whole number of 48 specimens, 35 of them had purple blossoms (*P. arvense*), 13 - white blossoms (*P. sativum*) and diverse coloration of seed coat. The concentration of concentrated tannins in seeds of both pea types corresponded to the results received by other authors (Cervenski et al., 2017; Kotlarz et al., 2011; Stanek et al., 2004).

Table 1. Parameter of main descriptive characteristics for grain bio-chemical composition of winter pea varieties for three years averagely

INDICATORS	Number of specimens	Min.	Max.	Mean	SE	SD	CV, %
Total nitrogen, %	48	3.74	4.65	4.19	0.03	0.23	5.54
Crude fibres, %	48	2.78	6.64	4.78	0.12	0.85	17.85
Crude ash, %	48	1.94	3.38	2.76	0.05	0.31	11.36
Total sugars, %	48	3.67	7.15	4.89	0.10	0.67	13.66
Tannins, %	48	0.01	2.71	1.63	0.12	0.80	49.29

The total nitrogen contents in grain varied within close limits, which was confirmed by the variation coefficient (CV= 5.54%). There was established a low variation coefficient for the rest of the indicators: crude fibres (CV= 17.85%); crude ash (C V= 11.36%) and total sugars (C V= 13.66%). The content of the biochemical indicators examined in the present study is comparable to the results for other pea varieties reported by other authors (Cervenski et al., 2017; Kotlarz et al., 2011; Srivastava et al., 2009; Stanek et al., 2004).

In order to establish and evaluate the relations between the studied indicators, a correlation analysis was applied (Table 2).

There were established strong negative correlations between: *total nitrogen and crude fibres* (r = - 0.896); *total nitrogen and crude ash* (r = -0.853) and *total nitrogen and total sugars* (r = - 0.886). A strong positive correlation was observed between: *crude fibers and crude ash* (r = 0.937); *crude fibres and total sugars* (r = 0.968) and *crude ash and total sugars* (r = 0.963) (Table 2).

The received results reporting for relations between the studied indicators confirm the results of numerous authors. Ali-Khan and Youngs (1973) in their study with 506 introduced plants reported that there was an insignificant negative correlational dependence between *crude protein content*, *seed yield* and *seed size*.

Other authors (Srivastava et al., 2009; Stanek et al., 2004; Vasileva and Kosev, 2021) reported for the presence of correlational relations between *seed coarseness* and *crude protein content*, as well as between *crude protein* and *starch content*.

Hierarchical cluster analysis

In order to identify the similarity and closeness of winter genotypes, it was applied a hierarchical *cluster* analysis. Evaluation of the genetic closeness between specimens was performed through a comparison of the content of: *total nitrogen, crude fibres, crude ash, total sugars and concentrated tannins.*

Table 2. A correlation matrix between the examined chemical indicators

	Total nitrogen	Crude fibres	Crude ash	Total sugars	Tannins
	x_1	x_2	x_3	x_4	x_5
x_1	1	-0.896**	-0.853**	-0.886**	0.154
x_2		1	0.937**	0.968**	0.159
x_3			1	0.963**	0.204
x_4				1	0.083
x_5					1

Cluster analysis results are presented graphically with a dendrogram (Figure 1), which shows the sequence of object collating and cluster formation. The studied winter pea varieties were grouped into six clusters and fourteen independent groups. The first cluster consisted of two subgroups - **"a"** and "**b**" and two independent groups. Genotypes forming the cluster were mainly French lines with high plants and purple blossoms. Specimens in subgroup **"a"** were close with relation to the studied indicators, only their tannin content exceeded the average value of the indicator 1.63% ± 0.12 for the group.

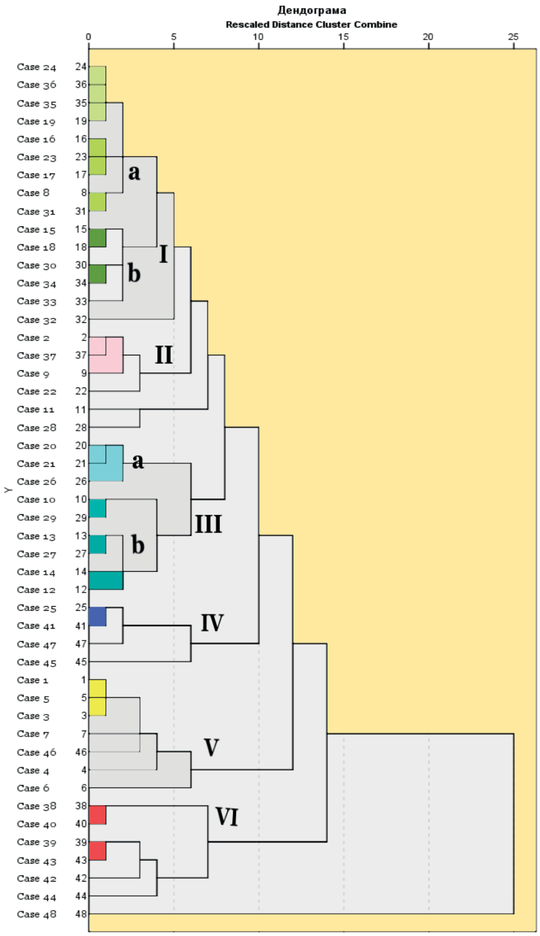

Figure 1. A dendrogram for the similarity of winter pea varieties

Subgroup "**b**"consisted of genotypes with higher content of total nitrogen and crude fibres than the average values of the group indicators as follows: 4.19% ± 0.03; 4.78% ± 0.12. The second cluster was formed by one subgroup and three independent groups. This cluster consisted of Mir variety and the populations 12 AB and П- 1 of Bulgarian origin, as well as three French lines. All, except the selection line 12 AB, were with purple blossoms. Specimens in this cluster had close values of the examined indicators, which were lower than the average reveal of the indicators. The third cluster also consisted of two subgroups - **"a"**and **"b",** and two independent groups. Varieties included in this cluster were characterized with close average values of the studied indicators, but with higher content of crude fibers and crude ash from the average reveal of the relevant indicators. They were different by phenotype, purple-blossom plants were predominant, only two of them – Pleven 10 and П-13 were white-blossom. The prevailing lines were those of French origin. Specimens in the fourth cluster were characterized with high content of total sugars and crude ash (with relation to the average value of the relevant indicator). Varieties in the fifth cluster were close by all studied indicators and did not contain tannins. Specimens in the sixth cluster had higher content of total nitrogen compared to the former group, and the rest of the studied indicators were under the average reveal of the indicators.

Dendrogram analysis showed that genotype formation into 6 groups was mainly due to the differences in the content of total nitrogen, crude fibres and tannins.

Most of the studied genotypes had good indicators and could be used as initial materials. The selection process could be directed towards the improvement of seed quality. Differences found in the present research study could be useful for the selection of potential parent couples for cross-breeding.

Analysis of the main components - PCA

Results from PCA application are presented in Table 3. Analysis showed that there were two own values, which determined the choice of *two* main components (Table 3).

The first component explained 35,09% of the total variation, and the second - 24,31%. The first component was related to the indicators - *crude fibres and total sugars*. *Total nitrogen, crude ash and tannins* formed the second component (Table 3).

Table 3. A factor matrix obtained by the method of the component principle

Factors	Components	
	PC 1	PC 2
Total nitrogen		0.660
Crude fibres	0.900	
Crude ash		0.415
Total sugars	0.846	
Tannins		0.762
% of the variance	35.09%	24.31%

Figure 2 shows the spatial visualization of the studied features and the relations between them, and Figure 3 presents the position of winter pea varieties in the component plane.

Taking into account the 48-th winter pea plants, there could be distinguished the following varieties as sources of total nitrogen variation: the French lines - 95202083, 302, 306 and 335; Pleven 10 Bulgarian variety and the populations - 35 [A], 37 [A], as well as Austria winter variety.

The rest of the specimens, which were closer to the centre of the component plane, could be unified into a core collection with relation to the studied features. This group of winter pea plants could be used for the creation of new varieties with appropriate qualitative features, as well as for their direct use in different production fields.

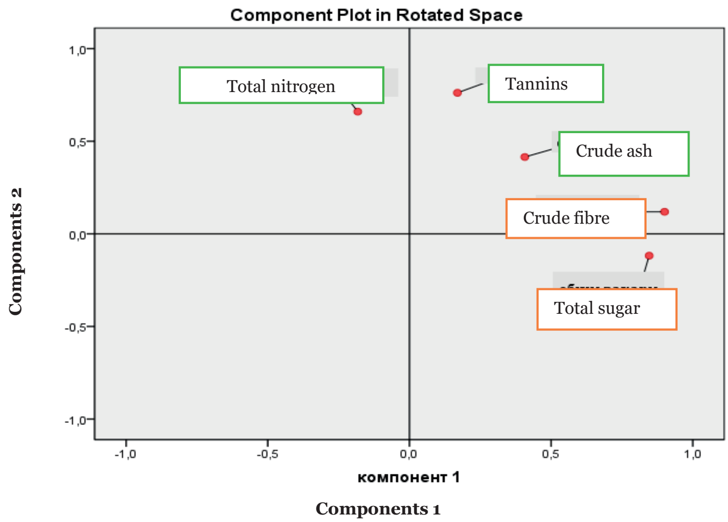

Figure 2. A projection of the examined features in the factor plane

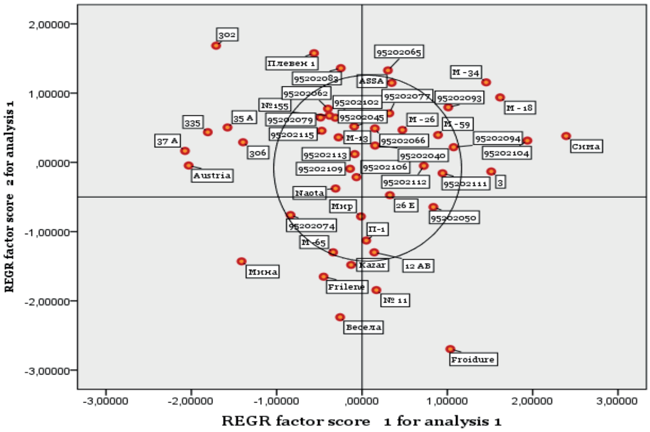

Figure 3. A projection of the examined specimens in the factor plane

CONCLUSIONS

In order to group genotypes by genetic similarity or remoteness, there could be successfully applied cluster and principle-component analyses. Their combined application allows the reception of a clearer concept for the influence of the studied indicators used for the differentiation of the examined genotypes.

There were established strong negative and positive correlations between: *total nitrogen and crude fibres* (r = - 0.896); *total nitrogen and crude ash (*r = -0.853), *and total nitrogen and total sugars* (r = - 0.886); *crude fibres and crude ash* (r = 0.937); *crude fibres and total sugars* (r = 0.968) and between *crude ash and total sugars* (r = 0.963).

Some winter pea plant genetic resources were determined as appropriate to be unified into core collections according to the examined indicators, as well as the possibilities to be included in different directions for application.

Database created according to the available connections between the studied indicators can be used in the selection and improvement work with peas for the search of relevant features.

The relationship " Yield - total ET " is best represented by Davidov's two-power formula. The same is expressed graphically through the S-curve, with a high correlation coefficient (R = 0.986) and value of powers n = 2.3 and m = 9.1.

The relationship Yield-evapotranspiration” by phases is the best presented by two-power formula with R = 0.921. The power’s value for entire vegetation period is N = 1.3. The power values by phases are as follows: I period - m_1 = 0.05, II period - m_2 = 0.79, III period - m_3 = 0.49, IV period - m_4 = 0.28. this means that the sensitivity of the first period is the smallest, followed by the period including the time between the end of grain filling and ripening. The second and third periods show the highest sensitivity, with the advantage being on the side of the budding - flowering period.

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