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Testing of uniformity of seven *Lathyrus* species using Bennett's and Miller's methods

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Abstract In this paper we focused on uniformity of seven *Lathyrus* species: *Lathyrus aphaca*, *L. cicero*, *L. clymenum*, *L. hirsutus*, *L. ochrus*, *L. sativus* and *L. tingitanus*. Two statistical methods, the Bennett's and the Miller's were compared. In both methods, coefficient of variation for evaluation of the uniformity of crop varieties was used. Four quantitative traits were analyzed: length of the pod, width of the pod, weight of seeds from the pod and 100 seed weight. The results of the study demonstrated that, the Bennett's method is less restrictive (lesser species were found to be uniform in analyzed traits) compared to the Miller's one.

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Introduction

Humanity relies on a diverse range of cultivated species; at least six thousand such species are used for a variety of purposes. It is often stated that only a few staple crops produce the majority of the food supply and many minors species should not be underestimated. Basic as well as agricultural research has traditionally focused on these staples, while relatively little attention has been given to minor (or underutilized or neglected) crops very good adapted to various marginal growing conditions as: highlands, arid area, salt-affected soils, etc. (Campbell 1997). Among others for such belong some species of *Lathyrus* genus. Apart from high protein content in seeds *Lathyrus* species have a considerable potential in crop rotation, improving soil physical conditions; reducing the amount of disease and weed population, with the overall reducing of producing costs (Vaz Patto et al. 2006). The genus *Lathyrus* is large with 200 species and subspecies that are found in both the Old World and the New World. They are primarily native to temperate regions of the world, with approximately 52 species originating in Europe, 30 in North America, 78 in Asia, 24 in tropical East Africa, and 24 in temperate

South America (Asmussen and Liston 1998). However, only, one species—grass pea (*Lathyrus sativus* L.) is widely cultivated as a food and was already in use in Neolithic times. Presently is considered as a model crop for sustainable agriculture (Vaz Patto et al. 2006). The other species are cultivated to a lesser extent for both food and forage, include *L. cicera*, *L. clymenum*, and *L. ochrus* for grain but mainly for forage production and *L. tingitanus* as forage species. Another species as *L. hirsutus* and particularly *L. aphaca* are only occasionally grown for human consumption and other purposes. The accessions of all above mentioned species constituted the initial material for presented investigations.

The listed above species belong to the same plant type. It can be interesting the extent to which these species are similar in terms of uniformity of the studied measurable characteristics. If given species proves to be uniform in similar degree then it can be the good material to create new, uniform, varieties. It is important since new varieties must be uniform no worse than the registered varieties. On the other hands, it is easier to get a new variety of the more varied the starting material.

In this paper the Bennett's (1976) and Miller and Feltz (1997) methods were proposed to test uniformity all studied species. In these methods the hypothesis concerning equality of several coefficient of variation was tested.

Materials and methods

Data

Material for investigations constituted seven followed species of *Lathyrus* genus: *Lathyrus aphaca* (1), *L. cicero* (2), *L. clymenum* (3), *L. hirsutus* (4), *L. ochrus* (5), *L. sativus* (6) and *L. tingitanus* (7). The seeds were obtained from Gene Bank in Gatersleben (Germany), except *L. sativus*—derived from collection of grain legumes located in Institute of Plant Genetics Polish Academy of Sciences in Poznań. In 2010 the seeds of all species were sown in the plots (5.2 m^2) situated in Experimental Field of Institute of Plant Genetics in Cerekwica (52°31′16″N, 16°41′30″E). Field trial was established with use of random blocks method in fifteen replications. On the plots the seeds were sown in the rows with 70 cm of width and 25 cm of distance among single seeds placed in rows. In the phase of full maturity 15 pods from main stem of random chosen plants and each plot were gathered. Four quantitative traits were analyzed: length of the pod [cm], width of the pod [cm], weight of seeds from the pod [g] and 100 seed weight [g].

Statistical method

A one-way analysis of variance (ANOVA) was carried out to determine the effects of species on the variability of length of the pod, width of the pod, weight of seeds from the pod and 100 seed weight. Honestly significant differences (HSDs) for each trait were calculated. Homogeneous groups for the analyzed traits were determined on the basis of honestly significant differences.

In order to compare the degree of uniformity same methods can be used. For example one of them is based on standard deviation. It is the method used in the DUS (distinctness, uniformity and stability) research, conducted in the registration research. Another method can be to compare coefficients of variations (CV) of researched objects. In this method the hypothesis H_0: $\zeta_1 = \cdots = \zeta_v (\zeta_i = \sigma_i/\mu_i$ is the population coefficient of variation of ith population, μ_i and σ_i are the mean and standard deviation of ith population, respectively) ($i = 1, 2, \ldots, v$) is tested versus the hypothesis that at least one of CV differs from the others. In the literature can be found several statistics used to test this hypothesis. In this paper two methods going to use.

Let $z_i = s_i/\bar{x}_i$ is the empirical coefficient of variation for the ith treatment (calculated from the sample), s_i^2 is the sample variance and $\bar{x}_i$ is the sample mean value of ith treatment.

The first of used method is the Bennett (1976) method. The test statistic takes the form

$$B = (n - v)\log\left(\frac{\sum_{i=1}^{v} y_i}{n - v}\right) - \sum_{i=1}^{v}(n_i - 1)\log\left(\frac{y_i}{n_i - 1}\right),$$

where $y_i = n_i z_i^2/(1 + z_i^2)$ and $y_i \neq 0$, n_i is the number of observations ith population ($i = 1, 2, \ldots, v$), v is the number of compared populations and $n = \sum_{i=1}^{v} n_i$. The B statistic is asymptotic distributed as χ^2 with $(v - 1)$ degrees of freedom.

The second method is statistic proposed by Miller (Miller and Feltz 1997):

$$D = \frac{\sum_{i=1}^{v}(n_i - 1)z_i^2 - \frac{1}{n-v}\left(\sum_{i=1}^{v}(n_i - 1)z_i\right)^2}{\zeta^2(0.5 + \zeta^2)},$$

where

$$\zeta = \frac{\sum_{i=1}^{v}(n_i - 1)z_i}{n - v}.$$

The Statistic D is also asymptotic distributed as a central χ^2 random variable with v-1 degrees of freedom. It should be noted that all CV can be smaller than 33 % in both used tests.

After testing the uniformity of all researched species, if the null hypothesis is rejected then one of the extreme CV (largest first) is omitted and then the null hypothesis is tested again. In researches concerning uniformity in the DUS the hypothesis about uniformity is conducted at significance level equal 0.002. However, it would be good check the uniformity at a higher level (the confidence interval is shorter), e.g. at $\alpha = 0.05$ too, thus both of these significance was used in this study.

The parallel coordinate plot is proposed as an efficient tool for visualization of four traits of seven *Lathyrus* species. Parallel coordinates are a two-dimensional technique for visualisation of multidimensional data sets and are an efficient tool for visualizing multivariate data (Bocianowski et al. 2015; Seidler-Łożykowska et al. 2015). The y-axes are parallel, have the same length, and start with a minimum of the corresponding trait and end with its maximum. Note thus that when a particular species is placed in the middle of a y-axis, it does not mean that its value is around the mean of the corresponding trait—it is the middle point within the trait's range. For a particular species, the points on the adjacent y-axes are joined by a line, thereby picturing a multidimensional characterization of the species.

Results

The coefficients of variation (CV) of all tested species are given in the Table 1. Three of them are larger than 33 % thus they not be taken into account in testing uniformity. There are CV received for two characteristics: weight of seeds from the pod and 100 seed weight and in the case of three species *Lathyrus clymenum*, *L. hirsutus L. sativus*.

First the test of hypothesis H_0, concerning all tested species, was conducted, excluding those with too high CV. The lack of uniformity in the case of two characteristics was detected at 0.05 significance level, namely for length of the pod and width of the pod (see Table 2). However at $\alpha = 0.002$ only in the case of width of the pod the null hypothesis have to be rejected if the Miller's test was used. These results allow us to conclude that not all of the tested species are uniform to a similar degree as regards these characteristics. In the purpose assessment the maximum number of uniform species the CV of one or more species was removed from (takes the smallest or the largest value and, in the first, the largest CV is deleted because the small CV can be mean that researched species is uniform in the degree better than over researched species) and again test hypothesis H_0 was tested. The results are given in Table 3. In the case of length of the pod only one species was removed—*Lathyrus clymenum*. This removed species as not uniform in the satisfactory degree. CV of this species was quite large. After excluding this species from groups of tested species the there is no reason to rejection the null hypothesis. This means that the other species are uniform in the similar degree. For width of the pod the CV of two species proved to be too large to show their uniformity to a degree not worse than the other species. In the case of $\alpha = 0.002$ only *Lathyrus clymenum* is not uniform.

In Table 4 the average values of the characteristics of considering species were presented. In the case of *L. clymenum* species for both length and width of the pod the appropriate average do not different from over averages (homogeneous groups are: 1 and 4, 2 and 4 for length of the pod as well as 2, 3 and 7 for width of the pod). However for *L. aphaca* species for all four traits we observed the smallest values (Fig. 1). For width of the pod species 1, 4, 5 and 6 are one-elementary homogeneous group. *L. clymenum* had the largest CV and the same a fairly large variance. In the tests of uniformity based on standard deviation, using by the variety registration Offices, if are the numbers of researched objects are groups (most often ten) of objects which have a closest averages are compared simultaneously. However, as shown, such objects can vary in terms of CV at the same different as regards the uniformity.

Table 1 Coefficients of variation (in %) of tested *Lathyrus* species

No	Species	Length of the pod	Width of the pod	Weight of seeds from the pod	100 seed weight
1	*L. aphaca*	10.358	12.877	17.528	19.097
2	*L. cicera*	11.264	7.8211	26.218	15.773
3	*L. clymenum*	21.313	14.626	**35.165**	31.149
4	*L. hirsutus*	9.5421	6.1323	**140.61**	25.127
5	*L. ochrus*	10.301	9.3807	31.447	22.845
6	*L. sativus*	8.4628	7.4246	22.069	**40.573**
7	*L. tingitanus*	8.7371	6.348	20.546	19.922

Bold font—CV bigger than 33 %

Table 2 The p value of the test hypothesis concerning the equality of coefficients of variation of all species which CV is smaller than 33 %

Test	Trait			
	Length of the pod	Width of the pod	Weight of seeds from the pod	100 seed weight
Bennett p value	0.0043**	0.0059**	0.3953	0.3037
Miller p value	0.0038**	0.0017**	0.2570	0.1914

** $p < 0.01$

Table 3 The p value of the test hypothesis concerning the equality of coefficients of variation of some species chosen in this way that there was not reason to reject null hypothesis

Trait	Length of the pod	Width of the pod	
Omitted species	*L. clymenum*	*L. clymenum*	*L. aphaca* and *L. clymenum*
Bennett p value	0.9498	0.0713	0.6465
Miller p value	0.8970	0.0276*	0.4893

* $p < 0.05$

Discussion

The uniformity of tested objects (cultivar, genotypes) by the variety registration Offices (like COBORU in Poland) is estimated based on standard deviation (Kristensen and Roberts 2009). Decisions about the variety's uniformity are made after two or three years of official DUS testing. This method is computationally complicated and for this reason using easier Bennett's (1976) method or Miller method (Feltz and Miller 1996; Miller and Feltz 1997) was proposed (Forkman 2006; Forkman and Verrill 2007). The Bennett method was used to compare the population variability in terms of different features. In this paper these method were used to compare CV some of species belonging to the legume family.

The considered species of *Lathyrus* are uniform in the same degree as regards two characteristics: weight of seeds from the pod and 100 seed weight. In terms of length of the pod and width of the pod one species was nouniform. There is *Lathyrus clymenum*. It is after used the Bennett test as well as Miller method at both used significance levels. In the case the Miller's method at $\alpha = 0.05$ also the *Lathyrus aphaca* is nouniform in terms width of the pod characteristic. Generally, it can be concluded

Table 4 Mean values for *Lathyrus* species

Species	Length of the pod (cm)	Width of the pod (cm)	Weight of seeds from the pod (g)	100 seed weight (g)
Lathyrus aphaca	2.524f	0.491e	0.093d	1.587c
Lathyrus cicera	3.079e	0.745c	0.233c	6.129b
Lathyrus clymenum	4.685b	0.768c	0.395b	8.565b
Lathyrus hirsutus	2.767ef	0.547d	0.165cd	2.217c
Lathyrus ochrus	4.103c	0.867b	0.385b	8.350b
Lathyrus sativus	3.837d	1.207a	0.525a	22.379a
Lathyrus tingitanus	7.037a	0.736c	0.449ab	7.829b
$HSD_{0.05}$	0.382	0.054	0.093	2.724
ANOVA $F_{6;96}$	127.5***	2.95***	23.75***	50.49***

In columns, means followed by the same letters are not significantly different

*** $p < 0.001$

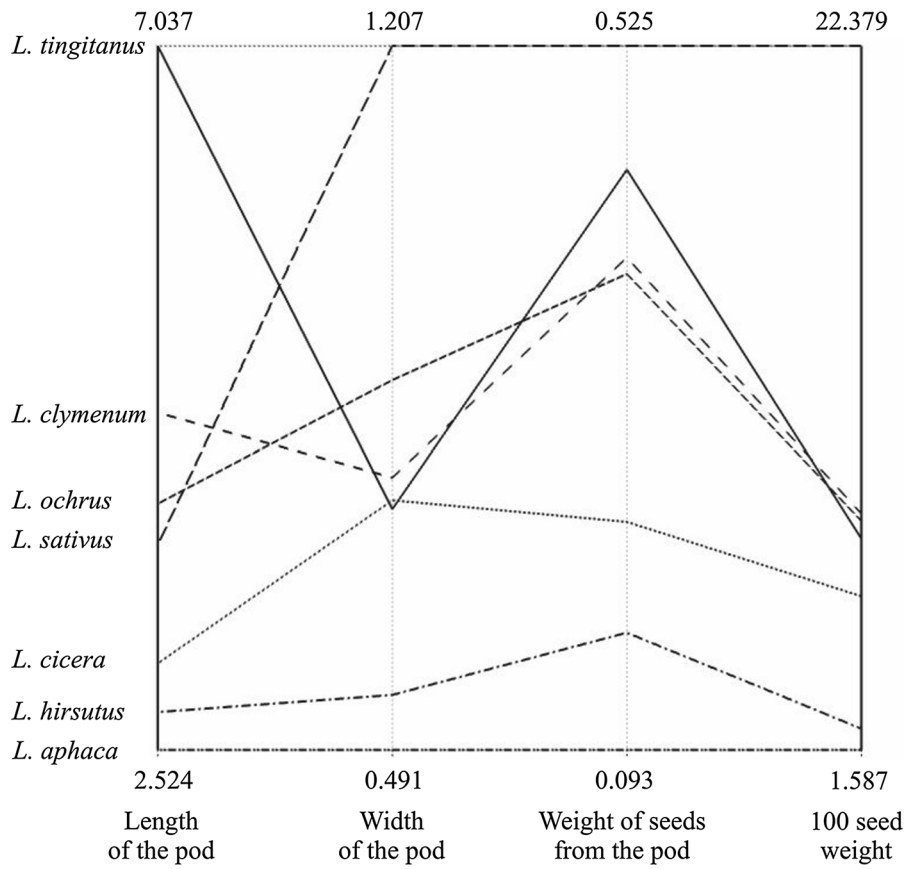

Fig. 1 Parallel coordinate plots (PCPs) for seven *Lathyrus* species and four traits

that, in the performed experiment most of the tested objects were sufficiently uniform in terms of the measureable characteristics considered. Tested species which did not differ in the terms of averages can vary in terms of CV at the same different as regards the uniformity.

More general conclusion is following the Bennett's method was less restrictive than the Miller's test (at the

significance level of 0.002). This is confirmed of results obtained in the previously research (Zawieja et al. 2012, 2013). Finally more species were found to be uniform in the Bennett's test.

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