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Analysis of uniformity of new oilseed rape lines (*Brassica napus* L. subsp. *oleifera*) using coefficient of variation

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The aim of this study is to compare the suitability of Bennett's and Miller's statistical methods, respectively, for analysis of oilseed rape varietal uniformity. In both methods, coefficient of variation for evaluation of the uniformity of crop varieties was used. In order to test the suitability of the above mentioned statistical methods for analyzing the varietal uniformity in spring oilseed rape, the quantitative trait (plant height) was used. The same plant feature is also used as the obligatory plant characteristic during the official oilseed Distinctness, Uniformity, Stability (DUS) testing before variety registration in the Research Centre for Cultivar Testing (COBORU). The results of the study demonstrated that, the Miller's method is more restrictive (less varieties were found to be uniform in plant height) compared to the Bennett's method.

Key words: Bennett's methods, coefficient of variation, oilseed rape, Miller's methods, uniformity.

INTRODUCTION

Oilseed rape (rapeseed) - (*Brassica napus* L. subsp. *oleifera*) is one of the most important source of vegetable oil for human consumption in the moderate climate zone (Bocianowski at al., 2012). Rapeseed is also grown for the production of animal feed and biodiesel. Rapeseed oil contains both omega-6 and omega-3 fatty acids in 2:1 ratio although flax as well as Chia (*Salvia hispanica*) oils are richer in omega-3 fatty acid. Breeding new varieties of oilseed rape requires many years and it involves a very expensive process (Bocianowski et al., 2011; Kozak et al., 2011). Newly bred varieties apart from having an adequate value for cultivation and use (VCU) should also fulfill obligatory Distinctness, Uniformity, Stability (DUS) requirements, before being officially registered. For this reason the breeders should continuously control both whether a new variety differs from already registered varieties within a given species and whether it is sufficiently uniform and stable.

In this study only uniformity of the varieties was taken into account and analyzed using variation coefficients described in the two statistical methods. The newly bred variety must be sufficiently uniform in terms of all the officially tested traits described in the DUS testing protocols valid for a given species. In the DUS tests, the uniformity of cross fertilized varieties and measurable traits are analysed by comparing the standard deviation

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of the new genotype with a corrected (by means of moving average) standard deviation calculated for all known varieties grown in the same DUS test (Kristensen and Roberts, 2009). First, the uniformity of the annual experiments is determined and next, it is tested for series of experiments (two or three years) eliminating variability between years. These methods are computationally complicated, therefore, it was suggested that, the two simpler methods of comparing coefficient of variation in the annual experiments should be use. One of them, called Bennett's method (Bennett, 1976) has been recently suggested to be used in the DUS tests. The results of comparison of these method with other methods based on standard deviation was published by Zawieja et al. (2010, 2012). The results obtained using these methods in most of cases did not differ from each other. In this study, an additional method discovered by Miller (1991) was proposed to compare several coefficient of variation (Kozak et al., 2013). The Bennett's and Miller's (Bennett's and Miller's) methods were used to test uniformity of the new genotypes of spring oilseed rape. One trait, namely variability of plant height was calculated and can serve as an example to illustrate the possible applicability of our approach in the DUS testing work.

MATERIALS AND METHODS

The statistical analyses described in this study were performed based on the data originating from the field experiment with new genotypes of spring oilseed rape carried out in 2002/2003. The experiment was set up in the balanced design with incomplete blocks where 64 genotypes were randomized where eight were known genotypes and 56 new genotypes-inbred lines (Cochran and Cox, 1957). The experiment was set up in four replications. The calculations were made taking into consideration only one trait: plant height measured on 10 plants in each of the 256 one-row plots, 1 m long and with 0.5 m between-row distance. All genotypes were sowed at the field of the experimental station of the Poznań University of Life Sciences in Dłoń.

The DUS Test Protocol used by variety registration offices including Research Centre for Cultivar Testing (COBORU) in the official testing of oilseed rape varieties covers 14 plant quantitative characters (including plant height) and a lot of qualitative traits. In our study, one obligatory character (plant height) in the DUS testing of spring oilseed lines was analyzed in the context of varietal uniformity.

The new variety is deemed to be uniform if its coefficient of variation for each of the analyzed traits is not different from the coefficients of variation of the registered varieties used as the control varieties in the experiment. The null hypothesis takes the form:

$$\mathrm{H}_0: \quad \zeta_1 = \ldots = \zeta_v, \qquad (1)$$

where $\zeta_i = \sigma_i / \mu_i$ is the true coefficient of variation, $\mu_i > 0$, is the true mean, σ_i - is the true standard deviation of i^{th} treatment, v is the number of compared treatments. Assume that $z_i = s_i / \bar{x}_i$ is the empirical coefficient of variation for the i^{th} treatment (calculated from the sample), s_i^2 is the sample variance and $\bar{x}_i$ is the mean value of all measurements of i^{th} treatment, n_i is the number of observations for i^{th} treatment and $n = \sum_{i=1}^{v} n_i$. For small ζ_i ,that is, less than 1/3, McKay (1932) shows that, $\frac{(n_i-1)z_i^2/(1+(n_i-1)z_i^2/n_i)}{\zeta_i^2/\left(1+\zeta_i^2\right)}$ is approximately χ^2 distributed with n-1 degrees of freedom (Forkman and Verrill, 2007). The Bennett's (1976) derive approximate test for homogeneity of coefficient of variations (this test is proposed to use in genetic researches by Czajka and Kaczmarek, 2003). Next Shafer and Sullivan (1986) note that, Bennett by mistake uses a variance with devisor n-1 where McKay (1932) uses a variance with devisor n. Corrected statistical test for this method is as follows:

$$2Z = (n-v)\log\left(\frac{\sum_i y_i}{n-v}\right) - \sum_i (n_i-1)\log\left(\frac{y_i}{n_i-1}\right).$$

Miller (1991) elaborated another test of hypothesis (1), but statistics for k population was not invariant under choice of the order of the population. Feltz and Miller (1996) and Miller and Feltz (1997) presented a modified version of the Miller test with the invariance property was presented. In this method the following statistics is calculated:

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

Because ζ is not known, one must estimate it. Miller and Feltz (1997) proposed the following estimate:

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

The Statistic D distributes as a central χ^2 random variable with v-1 degree of freedom. This approach was also recommended by Forkman (2006), apart from the two over approximate methods F-tests and Bennett tests. However, it is important that, these approximate tests are adequate only for small coefficients of variation.

Only the new genotypes which coefficient of variation (CV) is not different from coefficients of variation of control varieties (there are no bases to reject null hypothesis) are deemed to be uniform. Obviously, all the objects which CV is lower than the minimal CV of control varieties are considered to be uniform as well. The new object can be recognized as uniform only when it is uniform in terms of all the tested features. The uniformity is estimated separately for every new variety testing the equality of coefficients of variation hypothesis of control varieties and one new object.

In this study four uniform varieties were chosen in terms of the CV of control varieties (k4, k49, k62). The testing was made at the α = 0.002 significance level (this level of significance is commonly used in the official tests of the uniformity). Besides, in the DUS testing, the uniformity of varieties is estimated separately for each year and each experiment series (mostly two year's tests).

Table 1. Uniformity of selected spring rape genotypes (uniform genotypes are not included) estimated by the two methods.

S/N	Acc. Bennett's method (1976)				Acc. Miller's method (1997)			
	2002	2003	Number of years in which the genotype is uniform	Uniformity in two-year series	2002	2003	Number of years in which the genotype is uniform	Uniformity in two-year series
11	*	*	2	*	*	*	2	-
16	*	-	1	*	*	*	2	-
23	*	*	2	-	*	*	2	-
2	*	*	2	*	-	*	1	*
27	-	*	1	*	-	*	1	*
29	*	-	1	-	*	-	1	-
30	*	*	2	*	-	*	1	*
31	-	*	1	*	-	*	1	*
34	*	*	2	*	-	*	1	*
35	*	*	2	*	*	*	2	-
38	*	*	2	*	-	*	1	*
40	*	*	2	*	*	*	2	-
42	*	*	2	*	-	*	1	*
43	*	*	2	*	-	*	1	-
44	*	*	2	*	–	*	1	*
56	*	*	2	*	*	*	2	-

*, Uniformity; -, non uniformity.

The results of these experiments were treated as one connected data set. This approach enables an adequate estimation of genotypes uniformity in series of DUS testing in which genotypes are compared separately in each single year and together throughout the years.

RESULTS

The uniformity of the objects was first assessed separately using two different analyses approaches. According to Bennett's method, 54 varieties from 56 tested in the experiment in 2002 were determined as uniform in plant height whereas, 47 genotypes were defined as uniform in this character while using Miller's method. In the 2003 experiment, 54 varieties were uniform in plant height using Bennett's method for statistical analysis and 55 genotypes were uniform when Miller's method was applied. The uniformity was next compared by estimating how many times the same genotype was simultaneously uniform throughout the years. In each of the two experimental years, there were 52 height-uniform varieties (Bennett's method) and 46 varieties (Miller's method). The next uniformity test was performed in two-year's experiment. 54 genotypes appeared uniform using Bennett's method and 48 varieties by using Miller's method.

The uniformity of the new tested objects was presented in Table 1 (both in single experiment and series of experiments). The uniformity was annotated with an asterisk in the Table 1.

DISCUSSION

Decisions about the variety's uniformity are made by the variety registration Offices (like COBORU in Poland) after two or three years of official DUS testing. The uniformity is estimated based on standard deviation (Pilarczyk, 1995; Kristensen and Roberts, 2009). This method is computationally complicated and for this reason using easier Bennett's method was proposed (Bennett, 1976). The method was described by Czajka and Kaczmarek (2003) to compare the population variability in terms of different features. The Miller's test was also used in their study.

The analysis of results (Table 2) showed that, the Miller's method was more restrictive (less objects were found as uniform) than the Bennett's test (at the significance level of 0.002). A reverse situation appeared only in 2003. If an object was not uniform in one year of tests, then it was found to be uniform more frequent in the Miller's test than in the Bennett's one (Table 1). Finally more objects were found to be uniform in the Bennett's test. Generally, it can be concluded that, in the performed experiment most of the tested objects were sufficiently uniform in terms of the measureable feature considered. Therefore, there is a possibility to register some of the new obtained and tested genotypes as commercial varieties.

Zawieja et al. (2010) testing the uniformity of winter rye varieties found that more varieties were uniform by using the Bennett's method than using official DUS methods

Table 2. Number of uniform genotypes calculated using Bennett's and Miller's method. Total number of analyzed genotypes was 56.

Test	Number of uniform genotypes in 2002	Number of uniform genotypes in 2003	Number of uniform genotypes in each of the two years of experiment	Number of uniform genotypes in two years experiment
2Z	54	54	52	54
D	47	55	46	48

2Z, Bennett's test statistics; D, Miller's test statistics.

(based on standard deviations). Zawieja et al. (2010) while comparing the two methods (Bennett's and DUS) by using the example of experiments with oil seed rape, found that the Bennett's method appeared to be more restrictive. However, the differences between the numbers of uniform varieties, found by the two methods, were not significant. Therefore, one can start to recommend the use of the simpler method of Bennett in parallel to more complicated officially recognized methods used in the official DUS tests. The method presented in this study appears to be comparable to the existing official DUS testing approach in oilseed rape. Miller's method seems to be more restrictive than Bennet's, so it could be useful at early breeding stages in the light of preliminary uniformity evaluation of younger oilseed breeding materials.

REFERENCES

Bennett BM (1976). On an approximate test for homogeneity of coefficients of variation, Contributions to Applied Statistics (ed. W.I. Ziegler). Birkhäuser Verlag. pp. 169–171.

Bocianowski J, Kozak M, Liersch A, Bartkowiak-Broda I (2011). A heuristic method of searching for interesting markers in terms of quantitative traits. Euphytica 181:89-100.

Bocianowski J, Mikołajczyk K, Bartkowiak-Broda I (2012). Determination of fatty acid composition in seed oil of rapeseed (*Brassica napus* L.) by mutated alleles of the FAD3 desaturase genes. J. Appl. Genet. 53:27-30.

Cochran WG, Cox GM (1957). Experimental design. John Wiley Inc., New York.

Czajka S, Kaczmarek Z (2003). On testing the homogeneity of coefficients of variation. Biuletyn IHAR, 226/227/1:25-29 (in polish).

Feltz CJ, Miller GE (1996). An Asymptotic test for the equality of coefficients of variation from *k* population. Stat. Med. 15:647–658.

Forkman J (2006). Statistical inference for the coefficient of variation in normally distributed data. Centre of biostatistics. Report. P. 2.

Forkman J, Verrill S (2007). The distribution of McKay's approximation for the coefficient of variation. Stat. Probab. Lett. 78:10–14.

Kozak M, Bocianowski J, Liersch A, Tartanus M, Bartkowiak-Broda I, Piotto FA, Azevedo RA (2011). Genetic divergence is not the same as phenotypic divergence. Mole. Breed. 28:277-280.

Kozak M, Bocianowski J, Rybiński W (2013). Note on the use of coefficient of variation for data from agricultural factorial experiments. Bulg. J. Agric. Sci. 19(4):644-646.

Kristensen K, Roberts A (2009). Potential approaches to improving COYU. UPOV Geneva. TWC/27/15:1-8.

McKay AT (1932). Distribution of the coefficient of variation and the extended t distribution. J. R. Stat. Sec. 95:695–698.

Miller GE (1991). Asymptotic test statistics for coefficients of variation. Commun. Stat. Theory Methods 20:3351–3363.

Miller GE, Feltz CJ (1997). Asymptotic inference for coefficients of variation. Commun. Stat. Theory Methods 26:715–726.

Pilarczyk W (1995). Badanie odrębności, wyrównania i trwałości odmian II. Wykorzystanie analizy tablic kontyngencji przy opracowaniu wyników badań OWT. Colloquium Biometryczne 25:23-34.

Shafer NJ, Sullivan JA (1986). A simulation study of a test for the equality of the coefficients of variation. Commun. Stat. Simul. Comput. 15:681–695.

Zawieja B, Bocianowski J, Rybiński W (2012). Testing uniformity of mutants of the *Lathyrus sativus* L. (grasspea) using Bennet's Method. Russian J. Genet. 48(2):224-229.

Zawieja B, Pilarczyk W, Kowalczyk B (2010). Comparison of uniformity decisions based on COYU and Bennett's methods – simulated data. Colloquium Biometricum 40:53-61.