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Analysis of variation and interdependence of phenotypic traits in inflorescence mutants of lucerne (*Medicago sativa* L. sl.)

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Abstract. The paper presents the analysis of single- and multi-dimensional collection of sixteen genetic forms of lucerne (*Medicago sativa* L. sl.) taking into account fifteen morphological characteristics. Material for the study consisted of mutants of lucerne inflorescence: paniculate, determinate, and elongate racemose. In order to find relationships between the studied characteristics, correlation analysis was used. There were significant correlations between many phenotypic traits. Multivariate statistical analysis was carried out: an analysis of variance with entries and years as factors, followed by canonical variate analysis. A large genetic diversity among the studied lucerne forms was found. Regression analysis revealed that in different weather conditions in the years of experiment other phenotypic features had an significant impact on the mass of seeds of plants. Analysis of variance was performed taking into account the type of mutation and a statistically significant effect of mutations on the values of all fifteen characteristics was observed in the experiment. When analysing average values of individual traits for different types of mutations, it was found that the highest seed weight occurred among long-raceme mutants.

key words: lucerne, canonical variate analysis, seed yield, inflorescence mutants

INTRODUCTION

Lucerne, which has a large production capacity, is a feed rich in protein and other valuable nutrients (Hanson et al., 1988). In recent years, there has been a gradual increase in interest in growing this species, resulting from the fact that animal protein in feeds for livestock has been gradually replaced by protein from the plants (Bodzon, 2005). Due to its beneficial effects on soil fertility, on the humus balance and the structure of the soil, lucerne occupies an important place in crop production on organic farms. One of the goals of lucerne breeding is to increase the productivity of the seed (Bolanos-Aguilar et al., 2002). The possibility of obtaining high seed yield in the agroclimate of Poland determines the agronomic suitability and economics of production. Therefore, mutational lines with unusual inflorescence morphology likely to favourably affect the amount of lucerne seed yield were analysed (Broda et al., 2005).

The aim of this study was to test the morphological differentiation of the studied mutants in the context of their use for further breeding of the forms of lucerne with an increased seed setting potential, and to indicate the characteristics which determine seed yield of lucerne.

MATERIALS AND METHODS

The plant material were spontaneous inflorescence mutants of lucerne selected from a stand of plants with normal inflorescence and then self-pollinated (S1) or selected as sibs (Sib1) and varieties: Ulstar and Radius. The tested lines and varieties were developed by prof. Zygmunt Staszewski at the Plant Breeding and Acclimatization Institute in Radzików.

Five paniculate lines with gene *br* (*branched raceme*) were studied. This mutation causes the formation of branching of the second and subsequent orders in the inflorescence which results in an increase in the number of flowers in a single raceme in relation to the number of flowers in the racemes of short-raceme plants. These lines were marked with numbers 1–5 . In addition, eight determinate lines with the gene *tf* (*top flowering*), were analysed. They are characterized by shorter and more uniform period of flowering and a greater number of racemes on flower shoots as compared to conventional varieties of lucerne. Determinate lines were marked with numbers 6–13. One line and one variety of long-raceme plants with gene *lp*

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(*long peduncle*) were studied. Long-raceme mutants possess an elongated raceme and a larger number of flowers in a single inflorescence. The line with *lp* gene was marked with number 14. A long-raceme variety Ulstar was marked with number 15. A short-raceme variety Radius with inflorescence defined as normal, was marked with number 16 and constituted control for the experiment.

The field experiment was established at the Agricultural Experimental Farm of Department of Genetics and Plant Breeding of University of Life Sciences in Dłoń in 2004. The observations were conducted in 2005 and 2006, so in the second and third year of vegetation. The experiment was set up by the method of randomized blocks in three replications. Observations of all the phenotypic and cytological characteristics were performed on 480 plants.

The research material was analysed for fifteen quantitative traits: number of flowers in a raceme, length of inflorescence peduncle (mm), length of flowers in a raceme (mm), length of raceme (mm), number of ovules per ovary, pollen viability (%), number of pods per seed head, number of seeds per seed head, number of seeds per pod, pod setting efficiency (%), fertility (%), weight of seeds per plant (g), number of racemes per plant, number of seed heads per plant, and weight of 1000 seeds (g). The first 11 characters were recorded for each examined plant from ten randomly sampled racemes/seed heads of main shoot. Pod setting efficiency was understood as a quotient of number of pods per seed head and number of flowers per raceme, while fertility was the quotient of number of seeds per pod and number of ovules per ovary.

The results from biometric measurements, describing the diversity of the studied initial lucerne material were analysed statistically. The correlation coefficients were calculated on the basis of the average from entries for each year independently. For statistical analysis of research material, multivariate methods were used (Caliński and Kaczmarek, 1973). A two-way multivariate analysis of variance with the entries and years as factors was conducted. A canonical variate analysis was conducted for each year separately (Seber, 1984; Rencher, 1992, 1998; Krzanowski, 2000; Krzyśko, 2000). Simple correlation coefficients were calculated between the values of the first two canonical variables and the original values of individual traits in both years of research (Rencher, 1992; Mądry, 1993). On the basis of the values of these coefficients, features with the highest discriminative power can be detected. Using the method of stepwise regression, statistically significant characteristics affecting the mass of seeds from plants in different years of research were indicated. Considering the type of mutation as a potential differentiator, an analysis of variance for all fifteen quantitative traits was conducted. The least significant difference (LSD) was calculated for each trait to allow for direct comparison of average values for the mutation. All calculations were performed using the statistical package GenStat v. 7.1 (Payne et al., 2003).

RESULTS AND DISCUSSION

One of the major goals in lucerne breeding is to increase the yield of seeds. Direct selection for that trait is not very effective due to the polygenic nature of quantitative traits, affecting seed reproduction, and dependence on environmental factors (Volenec et al., 2002). For this reason, in this experiment, a new source of variability was used – spontaneous, monogenically inherited mutations. The tested mutants are characterized by a higher yield potential due depending on the type of mutations, to an increased number of inflorescences per plant or to a higher number of flowers in inflorescence (Bodzon, 1998, Broda et al., 2005; Weigt et al., 2009).

The difficulty of selection for high-yielding genotypes in lucerne lies in the fact that the analyzed morphological characteristics are mutually correlated. In practice this means that in order to improve the level of reproduction of seeds, many features have to be taken into account at the same time (Jaranowski and Dyba, 1983). In order to examine the relationship between the observed characteristics, correlation analysis was performed and significant correlations were found in the two years of research between: the number of flowers per raceme and the length of raceme, the length of the inflorescence peduncle and the length of raceme, number of racemes per plant and the number of seed heads per plant, the length of the raceme and the number of pods per seed head, number of ovules per ovary and the number of seeds per pod, viability of pollen grains and pod setting efficiency, number of seeds per pod and fertility, the number of pods per seed head and the number of seeds per seed head, number of pods per seed head and the mass of seeds per plant, pod setting efficiency and the mass of seeds per plant (Table 1).

Studies of other authors confirm the existence of most of the correlations that were significant in the experiment conducted. Positive correlation between the length of the raceme and the number of pods in seed heads was observed by Martura (1996) and Bodzon (2004). Moreover, Bodzon (2004) found that the raceme length of long-raceme mutants was significantly associated with the number of seeds in the seed heads, seed mass in seed heads, and seed yield. The relationship between these characteristics was also found by Guy et al. (1973) who examined different varieties of short-raceme lucerne. In addition, they observed that plants with longer racemes had more seeds per pod and a higher thousand seed weight. The positive correlation of the number of pods per seed head and the seed mass per plant is confirmed by research conducted by Pomogajbo (1981), and Vachunkowa et al. (1981). Bodzon (2004), who analysed long-raceme mutants of lucerne, also showed a relationship between these characteristics – the correlation coefficient for these traits was $r = 0.74$. Užik and Polák (1997), however, argue that the number of seeds

Table 1. The correlation coefficients for mean values of object in the first year of study (above diagonal) and year of observation (below diagonal).

Phenotypic feature	Number of flowers per raceme	Length of flowers in raceme	Length of inflorescence peduncle	Length of raceme	Number of racemes per plant	Number of ovules per ovary	Viability of pollen grains	Number of seed heads per plant	Number of pods per seed head	Number of seeds per seed head	Number of seeds per pod	1000 grain weight	Weight of seeds per plant	Pod setting efficiency	Fertility
Number of flowers per raceme	1	-0.30	0.49	0.55*	0.29	0.01	-0.30	0.32	0.45	0.22	0.01	-0.69**	0.18	-0.21	0.01
Length of flowers in raceme	0.19	1	-0.03	-0.28	-0.44	-0.31	0.08	-0.46	-0.41	-0.35	-0.27	0.12	-0.39	-0.26	-0.25
Length of inflorescence peduncle	0.28	0.46	1	0.77**	0.13	-0.05	0.31	0.11	0.53*	0.46	0.31	-0.26	0.48	0.22	0.31
Length of raceme	0.81**	0.40	0.70**	1	0.34	-0.03	0.23	0.37	0.60*	0.47	0.22	-0.24	0.44	0.27	0.22
Number of racemes per plant	0.02	-0.31	-0.24	-0.04	1	0.18	-0.13	0.97**	0.29	0.15	0.07	0.10	0.38	0.12	0.06
Number of ovules per ovary	0.22	0.38	0.46	0.39	0.35	1	0.09	0.21	0.38	0.47	0.59*	0.16	0.44	0.39	0.54*
Viability of pollen grains	-0.27	-0.14	-0.07	-0.14	0.36	0.11	1	-0.05	0.48	0.67**	0.72**	0.33	0.68**	0.74**	0.73**
Number of seed heads per plant	0.07	-0.26	-0.15	0.06	0.94**	0.47	0.47	1	0.38	0.23	0.15	0.04	0.45	0.22	0.14
Number of pods per seed head	0.63**	-0.17	0.34	0.69**	0.41	0.35	0.35	0.53*	1	0.93**	0.80**	-0.37	0.89**	0.77**	0.81**
Number of seeds per seed heads	0.39	0.03	0.49	0.57*	0.34	0.67**	0.34	0.55*	0.78**	1	0.94**	-0.15	0.93**	0.85**	0.95**
Number of seeds per pod	-0.05	0.32	0.40	0.18	0.11	0.72**	0.19	0.35	0.15	0.72**	1	0.01	0.91**	0.86**	0.99**
1000 grain weight	-0.28	0.23	-0.27	-0.21	0.15	-0.07	0.04	0.13	-0.42	-0.31	0.03	1	-0.01	0.07	-0.01
Weight of seeds per plant	-0.07	-0.13	0.32	0.23	0.69**	0.5*	0.47	0.80**	0.53*	0.65**	0.49	0.04	1	0.84**	0.91**
Pod setting efficiency	-0.49	-0.53*	-0.06	-0.33	0.39	0.12	0.65**	0.48	0.30	0.34	0.19	-0.34	0.58*	1	0.87**
Fertility	-0.11	0.24	0.36	0.11	-0.04	0.45	0.19	0.21	0.08	0.64	0.94**	0.03	0.40	0.17	1

* significant at the level of P = 0.05
** significant at the level of P = 0.01

in the seed heads is the most important feature of the productivity of lucerne seeds, as it is positively correlated with the number of seeds per pod, number of pods in the seed heads and seed yield. The occurrence of correlation (r = 0.91) between the mass of seeds in the seed heads and seed yield was also shown by Bolanos-Aguilar et al. (2002).

Due to the presence of multiple dependencies between phenotypic traits, multivariate statistical analysis of the results of the experiment was used in this study. This approach resulted in a more objective assessment of individual lines and varieties, based on an analysis of all studied traits together. Performing a two-way multivariate analysis of variance allowed to reject the hypothesis concerning the lack of differences between treatments, between years, and the hypothesis about the lack of interaction treatment × years (Table 2). Due to the significant influence of years on the phenotypic characteristics of all the studied treatments, a further analysis was conducted separately for each year.

Table 2. Values of *F* statistics in multivariable two-factor variance analysis.

Source of variation	*F* statistics for eight features together	Critical value at the level of P = 0.001
Treatment	16.47	1.33
Years	153.69	2.59
Treatments × Years	8.51	1.33

Weather conditions had a significant impact on phenotypic features in all mutation lines and varieties. In 2005, from mid-April to mid-August a large amount of rainfall was observed and temperatures differed only slightly from the long-term average. In 2006, until mid-July, there were alternating periods of drought and semi-drought and thus the period of flowering and pod ripening coincided with significant water shortages. Simultaneously, average temperatures in the months of plant growth were higher than the long-term average. Despite the high average temperatures, which favour the reproduction of the seeds of thermophilic plant, an extremely low rainfall in the second year of plant growth reduced the pod setting efficiency and fertility, and this resulted in reduced seed yield. According to Delouche (1980) and Sypniewski (1986), water shortage in the period from the beginning of flowering to harvest is associated with a significant reduction in seed yield. These observations are understandable given that the accumulation of reserve substances in legume seeds depends mainly on assimilates generated during fruiting (Bewley, 1985). Hence, a better vegetative growth of plants, and increased photosynthetic activity of the pods in favourable hydrothermal conditions lead to a substantial growth of the number of seeds per plant (Prusiński, 1997).

In the first year of research, a slightest similarity, expressed by the greatest value of the Mahalanobis distances, equal to 4.42 was observed between the determinate line marked with number 8 and the long-raceme variety Ulstar. The biggest similarity based on all the features together was found in paniculate lines 3 and 4, and the Mahalanobis distance between them was 1.34 (Table 3). In the second year of the study, the smallest Mahalanobis distance between paniculate lines 1 and 5 was observed, and the distance between them was 1.17. The smallest similarity in terms of all studied traits was found among determinate mutants of line 6 and the long-raceme Ulstar variety. The distance between them was 3.81. Correlation between Mahalanobis distance coefficients for both years was 0.45 (significant at the 0.01 level). This shows that there is similar multifeatural diversity among the studied objects in both years of the study.

Canonical variate analysis performed for the first year of the study allowed the conclusion that the first two canonical variables together explain for 55.45% of the total multifeatural variability of the studied forms of lucerne. Pollen viability, fertility, number of seeds per pod, pod setting efficiency, the number of seeds per seed head, seed weight per plant and number of pods per seed head showed a significant negative correlation with the first canonical variable. The second canonical variable was significant positively correlated with: the number of flowers per a raceme and the number of pods per seed head, while significantly negative – with a mass of 1000 seeds (Table 4). Figure 1 shows the sixteen forms of lucerne in the system of the two first canonical variables. The studied forms of lucerne formed six clusters: the first of them were mutational lines 1, 2, 3, 4, 5, 9, second – 6 and 11, third – 7, 12, 13 and 14, the fourth 8 and 10, the fifth – Ulstar variety, and the sixth – Radius variety.

In the second year of study, the first two canonical variables explained for 48.1% of the total multifeatural variability of the forms of lucerne. The first canonical variate showed a strong positive correlation with grains of pollen viability and pod setting efficiency and negative correlation with the number of flowers per raceme (Table 4). In the second canonical variable, the highest discriminative power was shown by: the number of pods per seed head, number of seeds per seed head, the number of flowers per raceme, the length of the raceme and the number of seed heads per plant (all negative correlations). Sixteen tested forms of lucerne in the system of the two first canonical variables for the second year of studies were shown in Figure 2, where we can distinguish two bigger clusters. One group are the objects 1, 9, 10, 14 and 16 and the second – 2, 8 and 13. Other forms do not constitute groups and can be only considered as eight one-object clusters.

Four of the features observed in the experiment (length of flowers in a raceme, length of inflorescence peduncle, number of racemes per plant and number of ovules per

Table 3. Mahalanobis distances between lucerne mutants in the first year of the study (above diagonal) and the second year of observation (below diagonal).

Mutation lines and varieties	1 – paniculate line	2 – paniculate line	3 – paniculate line	4 – paniculate line	5 – paniculate line	6 – determinate line	7 – determinate line	8 – determinate line	9 – determinate line	10 – determinate line	11 – determinate line	12 – determinate line	13 – determinate line	14 – elongate racemose line	15 – Ulstar variety	16 – Radius variety
1 – paniculate line	0	1.62	2.09	1.71	1.89	2.92	2.15	2.36	2.11	2.45	2.81	2.08	2.12	1.86	3.32	2.97
2 – paniculate line	2.45	0	2.04	1.52	2.33	2.45	1.80	2.82	2.19	2.58	2.65	2.21	2.38	2.11	3.37	3.07
3 – paniculate line	2.23	1.92	0	1.34	1.95	3.01	1.87	2.46	1.39	2.38	2.63	2.59	2.17	2.15	3.25	2.47
4 – paniculate line	2.23	1.67	2.36	0	2.09	2.86	1.62	2.50	1.69	2.18	2.67	2.09	1.83	1.74	3.33	2.57
5 – paniculate line	1.17	2.00	2.09	1.93	0	3.13	2.21	2.24	2.38	3.17	2.49	2.36	2.17	2.18	3.54	2.84
6 – determinate line	3.36	2.97	3.37	2.24	2.68	0	1.85	2.88	2.67	2.55	1.76	2.37	2.03	2.46	3.86	3.65
7 – determinate line	2.66	2.60	2.95	2.39	2.35	2.09	0	2.17	1.95	2.15	1.66	1.53	1.24	1.51	3.20	2.48
8 – determinate line	2.64	2.57	2.11	2.33	2.27	2.94	2.90	0	2.39	2.49	2.59	1.80	1.88	2.74	4.42	3.51
9 – determinate line	1.95	2.97	2.31	2.42	2.05	2.76	2.34	2.27	0	1.67	2.91	2.43	2.17	2.34	3.71	2.77
10 – determinate line	2.21	2.81	2.46	2.22	2.25	2.83	2.35	2.08	1.32	0	2.86	1.98	2.02	2.66	4.35	3.66
11 – determinate line	2.53	2.30	2.66	1.30	2.02	1.62	2.25	2.35	2.48	2.38	0	2.11	1.72	2.20	3.66	3.44
12 – determinate line	2.52	2.55	2.93	1.75	2.44	2.17	2.01	3.00	2.39	2.54	1.46	0	1.55	2.27	4.03	3.32
13 – determinate line	2.10	1.93	1.83	1.76	1.92	2.96	2.72	2.20	2.42	2.34	1.75	1.91	0	1.52	3.58	2.92
14 – elongate racemose line	2.19	2.43	2.05	2.27	2.26	3.10	2.30	2.58	2.57	2.29	2.20	2.17	1.85	0	2.40	2.09
15 – Ulstar variety	2.49	3.40	3.07	3.32	2.85	3.81	2.42	3.56	2.76	2.90	3.23	2.93	3.19	2.21	0	1.94
16 – Radius variety	1.44	2.83	2.17	2.40	1.65	3.03	2.56	2.17	1.57	1.47	2.24	2.45	1.92	1.83	2.64	0

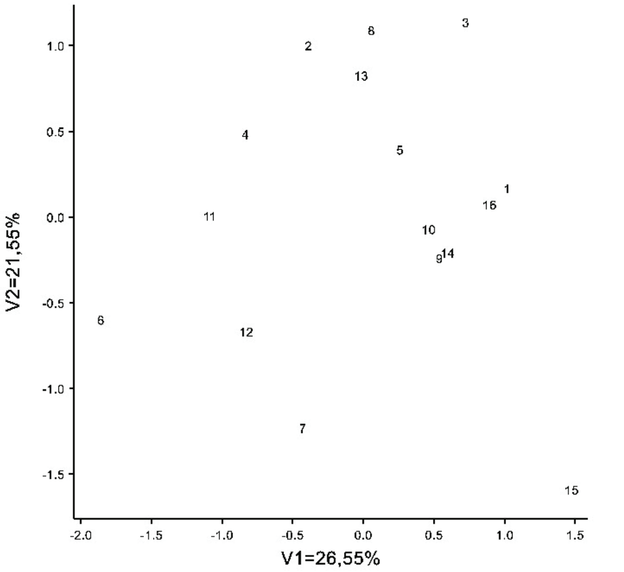
Tabela 4. Own values $\hat{\lambda}_i$ for two first canonical variables (Z_1, Z_2) and correlation coefficients with average values for every feature.

Phenotypic feature	Canonical variables			
	first year of study		second year of study	
	Z_1	Z_2	Z_1	Z_2
Number of flowers per raceme	0.14	0.84**	-0.67**	-0.57*
Length of flowers per raceme	0.01	-0.48	-0.04	0.27
Length of inflorescensce peduncle	-0.43	0.46	0.02	-0.08
Length of raceme	-0.34	0.42	-0.381	-0.52*
Number of racemes per plant	0.09	0.21	0.23	-0.38
Number of ovules per ovary	-0.20	0.21	0.057	-0.24
Viability of pollen grains	-0.96**	-0.232	0.84**	-0.48
Number of seed heads per plant	0.01	0.25	0.31	-0.52*
Number of pods per seed head	-0.67**	0.65**	-0.05	-0.93**
Number of seeds per seed head	-0.83**	0.45	0.14	-0.67**
Number of seeds per pod	-0.84**	0.29	0.31	-0.06
1000 seed weight	-0.16	-0.82**	0.21	0.38
Weight of seeds per plant	-0.81**	0.38	0.47	-0.41
Pod setting efficiency	-0.83**	0.12	0.66**	-0.34
Fertility	-0.85**	0.29	0.34	0.01
Own values	1.18	0.61	0.79	0.64
Percent of explained multi-feature variability of treatments	36.64	18.81	26.55	21.55

* significant at the level of P = 0.05
** significant at the level of P = 0.01

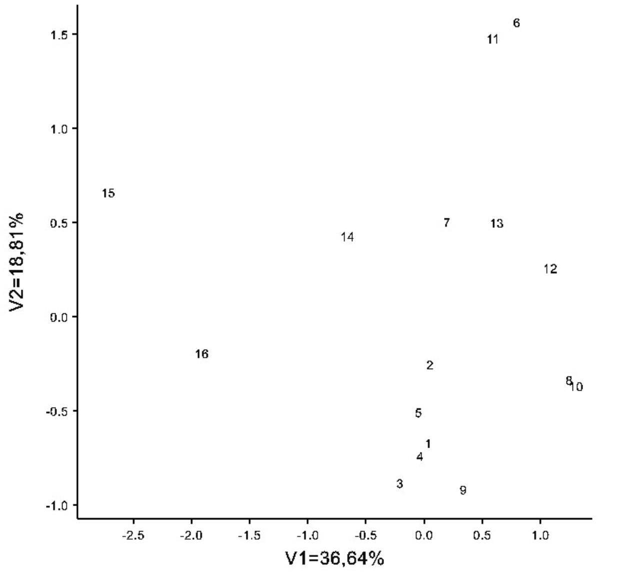
ovary) were not significantly correlated with either the first or the second canonical variable for any year of research. This means that these features do not have a high discriminative power in the experiment conducted.

Differences in the values of correlation coefficients of the analysed characteristics of the first and second canonical variable, and differences in the distribution of the studied forms of lucerne in the system the two first variables Z_1 and Z_2 in the first and second year of studies are a proof of a strong influence of environmental factors on the studied traits. It was found that the stronger is the influence of external factors on the phenotype, the lower is the degree of heritability of a given feature. Also, the share of the total genetic variation in the variability of the organism in terms of this trait is lower. The results of analysis of the canonical variables indicate a low degree of heritability of characteristics, for which significantly opposing values of the variables Z_1 and Z_2 in the subsequent years of obser-



1–5 – branched raceme lines; 6–13 – top flowering lines; 14 – long raceme line, 15 – Ulstar variety, 16 – Radius variety

Fig. 1. Layout of the tested lucerne mutation lines and varieties in the system of two first canonical variables in the first year of study.



1–16 – see Fig. 1.

Fig. 2. Layout of the tested lucerne mutation lines and varieties in the system of two first canonical variables in the second year of study.

Table 5. The analysis of the regression of particular features per weight of seeds from the plant in the first year of study.

Variance analyses		
Source of variation	d.f.	Mean square
Model	5	2557.19**
Error	234	50.22
Total	239	102.67
Evaluation of parameters		
Variable	d.f.	Evaluation of parameter
Regression constant	1	-24.40**
Number of ovules per ovary	1	1.25**
Viability of pollen grains	1	0.09**
Number of seed heads per plant	1	0.02**
Number of seeds per seed head	1	0.17**
1000 seed weight	1	0.01*
Percent of explained variability: 51.1		

* significant at the level of P = 0.05
** significant at the level of P = 0.01

Table 6. The analysis of regression of particular features per weight of seed from the plant in the second year of study.

Variance analyses		
Source of variation	d.f.	Mean square
Model	7	1077.67**
Error	232	62.65
Total	239	92.38
Evaluation of parameters		
Variable	d.f.	Evaluation of parameter
Regression constant	1	3.53*
Number of flowers per raceme	1	-0.13*
Length of inflorescence peduncle	1	0.17**
Number of racemes per plant	1	-0.01**
Number of seed heads per plant	1	0.01**
Number of pods per seed head	1	0.29*
Number of seeds per pod	1	3.17*
Fertility	1	-0.29*
Percent of explained variability: 32.2		

* significant at the level of P = 0.05
** significant at the level of P = 0.01

vation were recorded. These characteristics are: number of flowers per raceme, viability of pollen grains, number of pods per seed head and pod setting efficiency. Therefore, drawing genetic and breeding conclusions requires a continuation of research in subsequent years as well and using other locations in order to find highly hereditable features which would ensure desired breeding gain from selection. The distribution of individual objects in the system of the two first variables indicates a large genetic diversity between the examined forms of lucerne, even within the same mutation. Due to this, the analyzed lines, particularly those characterized by the greatest genetic diversity, can constitute a valuable starting material for the crosses.

Table 7. Mean squares from variance analysis for particular features. when the type of mutation was a differentiating factor.

Source of variation	Mutation	Year	Mutation × Year
Number of degrees of freedom	2	1	2
Number of flowers per raceme	2024.87**	511.70*	236.75
Length of flowers in raceme	220.91**	179.24**	8.63*
Length of inflorescence peduncle	6435.34**	8805.05**	44.17
Length of raceme	13111.01**	1911.81**	225.85
Number of racemes per plant	3098.40**	16349.40**	766.93*
Number of ovules per ovary	30.83**	57.30**	32.82**
Viability of pollen grains	47098.04**	13579.73**	6880.24**
Number of seed heads per plant	5662.77**	87260.19**	2151.43**
Number of pods per seed head	3759.89**	841.48**	245.48**
Number of seeds per seed head	62632.02**	5373.11**	15628.86**
Number of seeds per pod	62.44**	9.90**	25.72**
1000 seed weight	1924.09**	3501.86**	490.30**
Weight of seeds per plant	9407.14**	1482.42**	384.11*
Pod setting efficiency	51558.79**	514.14	1754.24*
Fertility	5082.36**	375.33	2958.50**

* significant at the level of P = 0.05
** significant at the level of P = 0.01

Table 8. Average values of particular features for different types of mutations.

Phenotypic feature	Mutation			LSD(0.001)
	paniculate	determi-nate	elongate racemose	
Number of flowers per raceme	28.68	31.57	29.62	2.94
Length of flowers in raceme	12.33	11.45	11.20	0.44
Length of inflorescence peduncle	35.11	31.52	38.07	2.29
Length of raceme	51.11	46.79	56.68	4.30
Number of racemes per plant	821.7	889.6	988.5	118.67
Number of ovules per ovary	9.89	9.811	10.32	0.38
Viability of pollen grains	59.5	53.41	72.97	4.05
Number of seed heads per plant	675	748	904	131.90
Number of pods per seed head	10.13	11.43	16.02	1.47
Number of seeds per seed head	19.3	21.95	42.6	5.29
Number of seeds per pod	1.86	1.83	2.53	0.30
1000 seed weight	2.058	1.981	1.944	0.069
Weight of seeds per plant	11.76	11.78	20.28	2.50
Pot setting efficiency	37.46	38.95	56.14	5.22
Fertility	18.82	18.8	25.06	3.10

The observations conducted in the first year of the study showed that characteristics which significantly determine the mass of seeds per plant were: the number of ovules per ovary, pollen viability, number of seed heads per plant, number of seeds per seed heads and 1000 seed weight. In the second year of the study, statistically significant characteristics were: length of inflorescence peduncles, the number of seed heads per plant, number of pods per seed head and the number of seeds per pod. Results of regression analysis of individual characteristics on the mass of seeds from the plants were shown in Table 5 and 6. Observations of the same genotypes for seed yield traits showed their variable response to environmental conditions. Lack of rainfall during the spring and summer, in the second year of research, caused the underdevelopment of inflorescences. Water-deficient flowers withered and some of the ovules died. This explains the decrease in seed yield in the second year of the study and is the cause of the impact of other phenotypic traits in seed weight per plant than in the first year of the experiment.

The results of the analysis of variance taking into account the type of mutations showed a statistically significant impact of this differentiating factor on the values of all fifteen characteristics observed in the experiment (Table 7). The average values for different traits by type of mutation are shown in Table 8. Long-raceme mutants had higher fertility potential of seeds in relation to the other mutants and control variety Radius. They yielded almost twice as high the seed weight – an average of 20.28 g of the plant, and were also higher than the other mutants in terms of: number of ovules per ovary, pollen viability, number of seed heads per plant, number of pods per seed head, number of seeds per seed head, number of seeds per pod, pod setting efficiency and fertility.

The higher potential seed yield of the long-raceme plants was also observed by other authors. The study of Broda et al. (2005) showed that the average seed yield of long-raceme plants mutants was higher than that in other mutation lines or in control variety Radius. Bodzon (2004) found that long-raceme plants set a greater number of pods in seed heads and more seeds in the raceme, and thus seed yield of these lines is higher. Wyrzykowska and Stankiewicz (2006) found that the long-raceme feature does not always have a positive effect on productivity of lucerne seed, as most of the analysed long-raceme lines had seed heads poor in pods, which caused a decrease in reproduction of seeds of the studied lines.

CONCLUSIONS

1. Canonical variate analysis showed a large phenotypic variation among the studied mutants, which can be used in cross-breeding of lucerne.

2. Different weather conditions during the test had a modifying influence on phenotypic features of lucerne mutants, determining the mass of seeds of plants.

3. Long-raceme plants were characterized by the highest seed yield potential of lucerne mutants giving the highest average seed weight per plant.

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