

EFFECT OF THE MORPHOLOGICAL TRAITS ON SEED YIELD OF LUCERNE BREEDING POPULATIONS IN LITHUANIA

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Manuscript received: September 12, 2008; Reviewed: November 4, 2009; Accepted for publication: November 10, 2009

ABSTRACT

Phenotypic correlation coefficients between seed yield and morphological traits of lucerne (*Medicago sativa* L.) breeding populations were determined. Ranking of populations by seed yield and subsequent calculating of correlations showed that as higher differences in seed yield among populations was as higher correlations were obtained. Among the characters examined seed yield was positively correlated with the length of raceme, number of flowers, pods and seeds per raceme, number of seeds per pod ($r=0.31^*$, 0.61^* , 0.42^* , 0.57^* , 0.53^* , respectively). Negative relationships were determined between spring regrowth height, alfalfa mosaic, flowering period, plant regrowth height after harvest, 1000 seed weight and seed yield ($r= -0.42^*$, -0.81^{**} , -0.64^* , -0.71^* , -0.41^* , respectively).

Key words: lucerne, seed yield, morphological traits

INTRODUCTION

Lucerne (*Medicago sativa* L.) is widely grown as a forage crop due to its good quality and high herbage yield. The seed yield of lucerne cultivars is considered as secondary important characteristic. However, the ability of cultivars to give a high and stable seed yield facilitates its spread and effective distribution to farmers due to competitive selling price [15]. Seed yield also determines the economic viability of seed producers. The mean seed yield in Lithuania in many years is very low 50-100 kg ha⁻¹ in view of its large potential for phytomass production and large number of flowers. Situation is even more complicated due to relatively short productive life of lucerne crop stand for seed production in Lithuania. However, seed yield can be as high as 1000 kg ha⁻¹ when flowering period is characterized by low relative humidity and moderate to high temperatures and pod development period is characterized by moderate humidity and temperatures [23]. These climatic conditions favor long periods for pollinator activity and later seed development [12].

Genetic diversity for seed yield and its components in lucerne was described between and within populations [9, 10]. Similarly, genetic variation was identified for pollen fertility [25], ovule fertility [21], and eases tripping [19] as for traits influencing seed yield. However, the actual lucerne productivity improvement achieved in breeding has been limited [26]. These characters of plants are not easy to measure on large numbers of plants and are not the only limited factors of seed yield in dense canopies [2].

Lucerne seed yield also depends to a great extent upon environment conditions and agronomic practices. Abu-Shakra et al (1977) and Iannucci et al (2002) showed that seed yield was significantly affected by the number of forage harvests prior to seed production, and this effect was mainly associated with variation in the number of fertile stems per plant and pods per raceme. Bodzon (2004) showed that seed yield per plant was positively associated with raceme length and flower number per raceme. Askarian et al (1995), Bekovič (2007), Zhang et al (2008) reported similar effects as a consequence of different row spacing. Irrigations applied at critical stages of pods development in dry environments were beneficial to seed yield, as it resulted in more pods per raceme [17, 24].

Evaluation of initial breeding material of lucerne for high seed yield is complicated. It is due to need to screen plenty of populations in several years until year with complex of favourable conditions for seeds setting and later development occurred. In breeding for improved seed yield it is particularly important to determine the effect of generative and morphological characters on

seed yield. The presented results concern phenotypic correlation of the characters responsible for seed yield of breeding numbers of lucerne developed in Lithuania.

MATERIAL AND METHOD

The experiment was carried out at the Lithuanian Institute of Agriculture (LIA) during 2005-2006 in the fields of a six course rotation in forage grasses. Accessions tested were locally developed from geographically broad material. Countries of initial material origin were USA (northern states), Canada, Russia, European countries. In total, 200 populations were sown and investigated. Every breeding population was sown in two 5- meter long rows. The soil of experimental site is Endocalcari-Endohypogleyic Cambisol CMg-n-w-can (pH – 7.3, P₂O₅ – 200-270 mg kg⁻¹ and K₂O – 100-175 mg kg⁻¹, humus – 2.46 %). The lucerne nursery was sown after black fallow without a cover crop in middle of July in 2005. Sowing rate was 100 seeds (0.2 g) per 1 meter. The distance between rows of a line was 0.5 m; the distance between different lines was 1.0 m. Initial number of plants was high (up to 100 units per 1 m row), therefore, every population was evaluated for all traits as one unit. In the 2006 year crop stand was assessed for seeds yield and its components. The nursery was evaluated for winter hardiness, spring re-growth, plant height before seed harvesting and plant regrowth after it, alfalfa mosaic incidence in scores was evaluated before flowering, stem number counter before seed harvesting. Winter hardiness was evaluated after resumption of vegetation in scores 1 – 5, where 1 is the least damage by winterkill. Spring re-growth was evaluated two weeks after resumption of vegetation by score 1-3, 1 being the least value. Flowering period was evaluated in days from its start to decline. Length of racemes and flowers number per raceme was counted when plants were fully flowering. Prior to harvest, 25 well podded racemes from 25 well developed stems were sampled from each population for counting of number of pods, number of seeds and seeds per pod. Plants height was measured in cm, before harvesting and at regrowth after harvesting after two weeks. Harvested seeds were dried, cleaned and weighed. Germination of seeds, hard seed % and 1000 seed weight were evaluated after seed cleaning.

The area with fields of LIA was highly repleted with honeybee during flowering of lucerne from closely dislocated apiaries. The sowing year was favourable for establishment of plants. The winter of 2006 was severe and winter hardiness of genotypes screened was different. The spring and summer period was dryer than usual. It negatively affected final seed yield. The initial

pod setting was very high for all populations although prolonged drought hardly decreased their number at later development stages.

The correlation analysis was used for evaluation of relationships between the seed yield and assessed traits. The obtained correlation coefficients were compared for significance level at $P < 0.05$.

RESULTS AND DISCUSSION

Seed yields of 200 populations averaged 212.2 kg ha^{-1} (Figure 1), which is higher than the average commercial seeds yields in Lithuania [4] but lower than seed yields achieved in areas well suited for seed production [1, 11]. Only 1.5 % of populations were highly yielding as their seed yield exceeded 400 kg ha^{-1} . Populations characterized by seed yields from 100 to 200 and 200 to 300 kg ha^{-1} dominated and accounted for 42 and 40 %, respectively. Seed yields lower than 100 kg ha^{-1} was characteristic for 5.5 % of populations. High variability for seed yield of individual plants as well as of populations was found in other researchers, too [3, 10, 11].

Correlation analysis between seed yield and phenotypic traits is presented in Table 1. It consisted of three sets of ranked populations. Primarily, correlation coefficients were counted for all 200 populations. The second ranking involved 50 populations with lowest and 50 populations with highest seed yield. The third ranking involved 10 populations with lowest and 10 populations with highest seed yield. Rankings showed increased correlations between seed yield and the other traits when number

of the populations decreased. The main reason was that the populations tested as breeding material possessed high genetical variability inside of populations and consecutively lower variability among populations. Correlation was not found between seed yield and overwintering, stem number, plant height at harvesting, germination of seeds and hard seed percent. Populations were different by overwintering but until flowering they recovered canopy density due to rest plants, therefore this trait did not correlated with seed yield. Stem number did not affected seed yield as crop stand was young and without considerable rarefaction. Plant height at harvesting also did not influence seed yield as due to drought

plants were not logged. Seed yield correlated with spring regrowth scores, length of racemes, number of flowers per raceme, number of pod per raceme, number of seeds per pod weakly or medium ($r = -0.31^* - 0.61^*$) only in case of the third ranking. Only flowering period ($r = -0.39^*$, -0.47^* , -0.64^{**}) and plant regrowth height after harvest ($r = -0.37^*$, -0.50^* , -0.71^*) correlated with seed yield in all three rankings. Spring regrowth height, alfalfa mosaic, number of seeds per raceme and 1000 seed weight correlated weakly with seed yield in case of the second ($r = -0.41^*$, -0.35^* , 0.38 , -0.34^* , respectively) and variously in the third rankings (-0.42^* , -0.81^{**} , 0.57^* , -0.41^* , respectively).

Obtained correlations showed tendency which depended on genetical variability within populations and among them. As number of populations decreased herewith variation increased among populations. The counting

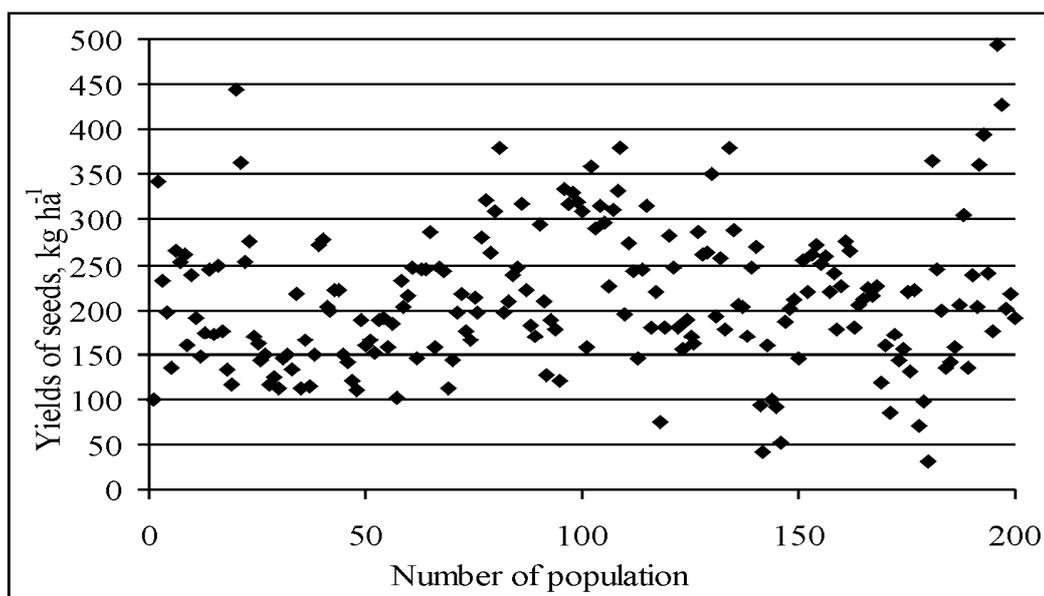


Figure 1. Distribution of lucerne breeding lines by seed yield

of correlation between seed yield and the other traits in the case of all populations did not showed considerable relations as majority of populations were characterized by similar seed yield which ranged among them in most cases up to 2 times, but more often – much less.

The strongest correlations ($r=-0.81^{**}$) were obtained between seed yield and alfalfa mosaic incidence and plant regrowth after harvest in cm ($r=-0.71^*$) in case of the third ranking. Dall et al. (1989), Jones and Nicholas (1992) researches with *Medicago* spp. species showed that alfalfa mosaic virus can decrease seed yield more than 30 %. Negative correlation of seed yield with plant regrowth after harvest can be explained by differences in root reserves. Higher seed yield negatively influenced ($r=-0.37^*$, -0.50^* , -0.71^*) plant regrowth after harvesting during all rankings of populations. It shows that plants for the higher seed yield formation root reserves exploited more intensive than low seed yielding populations. The similar tendency was obtained in researches with influence of root reserves on shoot regrowth [6, 14]. Reverse tendency though with lower correlation (-0.27^* , -0.41^* , -0.42^*) was observed in case of spring regrowth height. This relationship suggests that populations with lower plants at spring have some potential to form slightly higher seed yield.

Longer flowering period under droughty weather conditions were unfavourable for higher seed yield in the case of all three rankings ($r=-0.39^*$, -0.47^* , -0.64^{**} , respectively). Such situation could be reversed if watering could be used during flowering and later seed development. Highly positive effects of irrigations on many generative elements and seed yield were determined [16, 17]. Initial pod setting was very high and similar for all populations due to intensive activity of pollinators, mainly honeybees. Research of Ray et al. (1999) indicated that water use efficiency is heritable trait, therefore populations with highest seed yield under droughty conditions should be more drought resistant than the rest ones. Irrigation possible would be highly effective and under Lithuanian conditions. However, only few percent of fields with lucerne for seed productions could be established near water sources.

Generative traits such as number of flowers, pods and seeds per raceme, number of seeds per pod and raceme length showed tendency for stronger positive correlations with seed yield in the case of rankings and selecting populations with marginal seed yield. Correlations were not obtained in the case of the first ranking. In the case of the second ranking only number of seed per raceme showed weak correlation ($r=0.38$). The correlations of the third ranking were weak for length of raceme and number of pods per raceme ($r=0.31^*$, 0.42^* , respectively)

and medium for number of flowers and seeds per raceme and number of seeds per pod ($r= 0.61^*$, 0.57^* , 0.53^* , respectively). Similar relationships were observed in recent studies [8, 11, 22]. Seed weight was negatively associated with seed yield in the second and the third rankings. As seed yield increased plants were forced to distribute lower assimilates per seed, therefore, seed weight decreased.

The data shown in Table 2 is information about some traits that expressed highest correlations with seed yield of populations from the third ranking. The most significant negative effect is seen for alfalfa mosaic incidence on seed yield. The group of populations with lowest seed yield was estimated in average incidence of alfalfa mosaic by 6.2 score, whereas the group with highest seed yield in average had only alfalfa mosaic score 3.2. An average number of flowers per raceme differed by 30.7 %, or 21.5 and 28.1 for low yielding and high yielding populations, respectively. Low yielding populations flowered in average longer for 6 days. The regrowth after harvesting among the population groups was in average 37.8 cm for low yielding and 27.8 cm for high yielding populations.

The ultimate purpose of developing line is high and stabile seed yield as breeders are economically interested in as possible faster distribution of the new varieties and receiving of the subsequent royalty. Seed growers have the same interest as their incomes very depend on seed yield. The greatest shortcomings of conventional methods used to determine seed- yield are labour-consuming harvesting and processing of harvested seed and chaff mass. The determination of traits which allow breeders select effectively under local conditions for high and stabile yield is highly desirable. However, climate warming results in high unpredictability of weather conditions. When seed yields of lucerne breeding populations are low and similar for all populations, it is difficult to select population's superior by seed yield. At this situation, lucerne breeders should identify the potential of populations by the traits related to high seed yield. An overall there are two types of traits which could help to indicate seed yielding ability of populations. The first type is available until seed harvesting and the second ones are traits available just before of after seed harvesting. For initial breeding the most desirable are traits which can be assessed visually without long time and labour consumes for precise measurements.

The first type traits with considerable correlation in the case third ranking were alfalfa mosaic ($r=-0.81^{**}$) and flowering period ($r=-0.64^*$). The both traits visually are easy identifying and take short time. Some information about yielding capability can supply measurement of

Table 1. Correlation of seed yield (X) with the other traits (Y) in lucerne

Trait (Y)	(X) r, n=200	(X) r, n=100	(X) r, n = 20
Overwintering, scores	-0.01	0.07	0.05
Spring regrowth, scores	-0.03	-0.08	0.32*
Spring regrowth, cm	-0.27*	-0.41*	-0.42*
Alfalfa mosaic, scores	-0.24	-0.35*	-0.81**
Length of raceme, cm	0.09	0.19	0.31*
Number of flowers per raceme	0.15	0.14	0.61*
Flowering period, days	-0.39*	-0.47*	-0.64**
Number of pods per raceme	0.26	0.29	0.42*
Number of seeds per raceme	0.21	0.38	0.57*
Number of seeds per pod	0.18	0.27	0.53*
Stem number	-0.04	-0.05	0.08
Plant height at harvesting, cm	-0.15	-0.24	-0.25*
Plant regrowth after harvest, cm	-0.37*	-0.50*	-0.71*
1000 seed weight, g	-0.24*	-0.34*	-0.41*
Germination of seeds, %	-0.06	-0.02	0.14
Hard seed, %	0.01	-0.02	-0.21

* P < 0.05; ** P < 0.01

Table 2. Detailed analysis of traits with highest correlation with seed yield

No. of Catalog	Seed yield, kg ha ⁻¹	Spring regrowth, cm	Alfalfa mosaic, score	Flowers per raceme	Flowering periods, d	Regrowth after harvest, cm	1000 seeds weight, cm
LIA2438	30.6	33.2	8	20.2	69	39.1	2.6
LIA2431	42.0	37.4	5	20.8	68	43.7	2.4
LIA2430	52.2	29.7	8	20.8	68	35.5	2.1
LIA2436	70.6	33.4	5	21.3	69	44.7	2.4
LIA14	74.8	28.7	5	22.1	62	25.9	2.3
LIA2430	84.6	35.1	5	19.8	69	35.2	2.3
LIA2434	91.4	32.8	8	22.5	68	34.5	2.4
LIA1172	92.6	31.7	5	20.1	68	40.7	2.1
LIA2437	98.0	34.4	8	28.6	69	43.3	2.1
LIA2433	99.2	30.6	5	18.7	68	35.0	2.1
Average	73.6	32.7	6.2	21.5	67.8	37.8	2.3
SD	24.4	2.6	1.5	2.7	2.1	5.7	0.2
LIA63	358.8	31.9	3	25.1	63	25.8	2.2
LIA9	361.0	35.4	3	30.8	68	33.1	2
LIA407	362.6	28.4	5	30.2	69	30.0	2.2
LIA2458	365.8	29.3	3	28.9	53	21.7	2.3
LIA2439	379.4	31.7	3	35.9	62	28.3	1.9
LIA1978	380.0	30.6	3	22.5	62	32.6	2.2
LIA80	380.4	29.5	3	25.8	63	24.2	2.3
LIA9	427.2	23.9	3	25.6	53	24.3	2.1
LIA 2	444.4	34.8	3	33.4	63	33.7	2.2
LIA 62	493.0	27.5	3	23.1	62	25.1	2.2
Average	395.3	30.3	3.2	28.1	61.8	27.9	2.2
SD	44.9	3.4	0.6	4.4	5.3	4.3	0.1

spring regrowth height ($r=-0.24^*$ to -0.42^*), however it takes some time for measurements of plants height.

The use of length of raceme and number of flowers per raceme was complicated as these traits correlated only in the case of third ranking ($r=0.31^*$, 0.61^*). Considering correlation level number of flowers per raceme could be used for selection of populations with higher seed yielding potential but adequate visual scoring should be developed. This trait could be more useful for visual evaluation of breeding material grown under single spaced plants.

The second type traits were number of pods, seeds per raceme and number of seeds per pods. However, weak to medium correlations and demand of time-consuming labour doesn't make evaluation of these traits attractable. These traits seem are more suitable for selecting of parental material until crossing.

Considering our findings we can suggest evaluation of flowers number per raceme

as positively seed yield influencing trait for selecting of highly yielding populations and alfalfa mosaic, flowering period and spring re-growth height as the least labour-consuming criteria under droughty conditions for rejecting of low yielding populations among the initial breeding material of lucerne.

REFERENCES

- [1] Abu-Shakra S., Bhatti M.L., Ahmed H., Effect of forage harvest frequency on subsequent seed production and pollen quality, *Agron. J.* (1977) 69: 428-431.
- [2] Annicchiarico P., Prediction of indirect selection for seed and forage yield of lucerne based on evaluation under spaced planting, *Plant Breeding* (2006) 125: 641-643.
- [3] Anonymous, Descriptor: Alfalfa seed productivity (SEEDPROD), <http://www.ars-grin.gov/cgi-bin/npgs/html/desc.pl?68001>.
- [4] Anonymous, Statistic department of Government of Lithuanian Republic <http://www.stat.gov.lt>.
- [5] Askarian M., Hampton J.G., Hill M.J., Effect of row spacing and sowing rate on seed production of lucerne (*Medicago sativa* L.) cv. Grasslands Oranga. *New Zeal. J. Agr. Res.* (1995) 38: 289-295.
- [6] Avice J.C., Lemaire G., Ourry A., Boucaud J., Effects of the previous shoot removal frequency on subsequent shoot regrowth in two *Medicago sativa* L. cultivars, *Plant Soil* (1997) 188: 189-198.
- [7] Bekovič D., The effect of inter- row distance and environmental conditions on seed yield of alfalfa (*Medicago sativa* L.), *Plant Breeding Seed Sci.* (2007) 13: 25-29.
- [8] Bodzon Z., Correlations and heritability of characters determining the seed yield of the long-raceme alfalfa (*Medicago sativa* L.), *J. App. Genet.* (2004) 45: 49-59.
- [9] Bolanos-Aguilar E.D., Huyghe C., Julier B., Ecalle C., Genetic variation for seed yield and its components in alfalfa (*Medicago sativa* L.) populations, *Agronomie* (2000) 20: 333-345.
- [10] Bolanos-Aguilar E.D., Huyghe C., Djukic D., Julier B., Genetic control of alfalfa seed yield and its components, *Plant Breeding* (2001) 120: 67-72.
- [11] Bolanos-Aguilar E.D., Huyghe C., Ecalle C., Hacquet J., Julier B., Effect of cultivar and environment on seed yield in alfalfa, *Crop Sci.* (2002) 42: 45-50.
- [12] Cane H.J., Pollinating bees (Hymenoptera: apiformes) of U.S. alfalfa compared for rates of pod and seed set, *J. of Econ. Entomol.* (2002) 95: 22-27.
- [13] Dall D.J., Randles J.W., Francki R.I. B., The effect of alfalfa mosaic virus on productivity of annual barrel medic, *Medicago trunculata*, *Aust. J. Agr. Res.* (1989) 40: 807-815.
- [14] Dhont C., Castonguay Y., Nadeau P., Belanger G., Chalifour F.P., Alfalfa root nitrogen reserves and regrowth potential response to fall harvests, *Crop Sci.* (2003) 43: 191-194.
- [15] Facinelli M., Temperate forage seed production: conventional and potential breeding strategies, *International Herbage Seed Production Research Group Newsletter* (2000) 3: 7-15.
- [16] Guoi Z.G., Liu H.X., Wanh Y.R., Hu Y.K., Yang I N., Irrigating at podding and regrowth stages increases seed yield and improves pod distribution in lucerne grown in the Hexi corridor in China, *New Zeal. J. Agr. Res.* (2007) 50: 285-290.
- [17] Iannucci A., Fonzo N.D., Martiniello P., Alfalfa (*Medicago sativa* L.) seed yield and quality under different forage treatment systems and irrigation treatments in a Mediterranean environment, *Field Crop. Res.* (2002) 78: 65-74.
- [18] Jones R.A.C., Nicholas D.A., Studies on alfalfa mosaic virus infection of burr medic (*Medicago polymorpha*) swards: seed-borne infection, persistence, spread and effects on productivity, *Aust. J. Agr. Res.* (1992) 43: 697-715.
- [19] Knapp E.E., Teuber L.R., Selection progress for ease of floret tripping in alfalfa, *Crop Sci.* (1994) 34: 323-326.
- [20] Ray I.M., Townsend M.S., Muncy C.M., Henning

J.A., Heritabilities of

water-use efficiency traits and correlations with agronomic traits in water stressed alfalfa, *Crop Sci.* (1999) 39: 494-498.

[21] Rosellini D., Lorenzetti F., Bingham E.T., Quantitative ovule sterility in *Medicago sativa*, *Theor. Appl. Genet.* (1998) 97: 1289-1295.

[22] Sengul S., Using path analysis to determine lucerne (*Medicago sativa* L.) seed yield and its components, *New Zeal. J. Agr. Res.* (2006) 49: 107-115.

[23] Šlepetyš J., Effect of the cutting date on the first crop of lucerne (*Medicago sativa* L.) on the seed yield and maturation in the aftermath, *Agriculture.* (2004) 87: 157-172.

[24] Taylor A.J., Marble V.L., Lucerne irrigation and

soil water use during bloom and seed set on a red-brown earth in south-eastern Australia, *Aust. J. Exp. Agr.* (1986) 26: 577 – 581.

[25] Viands D.R., Sun P., Barnes D.L., Pollination control: mechanical and sterility. In Hanson A.A., Barnes D.K., Hill R.R. (Eds.) *Alfalfa and Alfalfa improvement*, Madison, MI, 1988, pp. 931-960.

[26] Volenec J.J., Cunningham S.M., Haagenson D.M., Berg W.K., Joern B.C., Wiersma D.W., Physiological genetics of alfalfa improvement: past failures, future prospects, *Field Crop. Res.* (2002) 73: 97-110.

[27] Zhang T., Wang X., Han J., Wang Y., Mao P., Majerus M., Effects of between-row and within-row spacing on alfalfa seed yields, *Crop Sci.* (2008) 48: 794-803.

