P L A N T   B R E E D I N G   A N D   S E E D   S C I E N C E
Volume 60 2009

DOI: 10.2478/v10129-010-0002-x

Dorota Weigt[1], Zbigniew Broda[1], Jarosław Lira[2], Sylwia Mikołajczyk[1]

[1]Department of Genetics and Plant Breeding, Poznań University of Life Science,
[2] Department of Finance and Accounting in Agro-bussines, Poznań University of Life Science

MORPHOLOGICAL AND CYTOLOGICAL CHARACTERISTICS OF INFLORESCENCE MUTANTS IN ALFALFA (*MEDICAGO SATIVA* L. SL)

ABSTRACT

The presented study analyzed selected biometric characteristics of inflorescences, seed yield structure characters, and discussed cytological analyses concerning two spontaneous inflorescence mutants in alfalfa: top flowering (plants with the "*tf"*gene) and long peduncle mutants (plants with the "*lp*" gene). Analyses were conducted in 2005 and 2006 and the results were subjected to a one-way statistical analysis with the use of Tukey's test. It was found that mutants with gene "*lp*" exhibited the highest seed yielding potential, while plants with gene "*tf*" were characterized with the highest stability in terms of most investigated characters in the first and second year of the study; however, they set a lower number of seeds than plants of the control cultivar.

Key words: alfalfa, seed weight, long peduncle mutant, top flowering mutant

INTRODUCTION

Alfalfa is a fodder crop of high feeding and agronomic value. Its negative characteristic is low seed fertility. Direct selection towards increased seed yield is not very effective due to the polygenic character of quantitative traits affecting this character and their dependence on environmental conditions (Volenec *et al.* 2002). Improvement of seed productivity may be obtained by the introduction to native cultivars of new mutation forms characterized by a high seed yielding potential. Due to the complicated segregation ratios observed in tetraploid alfalfa, monogenic characters are considered by many authors as the most suitable for breeding of this species (Hill *et al.* 1988, Sorensen *et al.* 1988). For this reason in the presented experiment a new variation was used– spontaneous mutations, which are determined by one, recessive gene of simple inheritance (Bodzon 1998).

The aim of the study was to analyze traits affecting seed yields of alfalfa top flowering mutants (with gene *tf*) and long peduncle mutants (with gene

Communicated by Edward Arseniuk

lp) in view of the application of analyzed mutations in breeding of cultivars with a high seed yielding potential.

MATERIAL AND METHODS

Plant material

Plant material comprised spontaneous inflorescence mutants of alfalfa selected from a stand of plants with normal inflorescences, and next self-pollinated (S_1) or bred in sib-mating (Sib_1), and a synthetic cultivar Ulstar and cv. Radius. Analyzed mutants were derived by prof. Z. Staszewski at the Institute of Plant Breeding and Acclimatization at Radzików. Observations pertained to two types of inflorescence mutations: top flowering and long peduncles. Mutants with top flowering inflorescences, i.e. with gene *tf* were characterized by a shorter and uniform flowering period and a higher number of racemes on flowering branches. Long peduncle mutants, i.e. with gene *lp* have elongated receptacles and racemes as well as a higher number of flowers in the raceme. The control in the experiment consisted of a short-raceme cv. Radius, with inflorescences defined as normal.

The experiment was established in the randomized block design in 2004 (the first year of vegetation), and observations were conducted in the years 2005 and 2006 (the second and third year of vegetation).

Objects of analyses were top flowering mutants with gene *tf* – 4 inbred lines in the S_1 generation; top flowering mutant with gene *tf* – 4 progeny in the Sib_1 generation; long peduncle mutants with gene *lp* – 1 line in the S_1 generation; long peduncle mutants with gene *lp* – a synthetic cultivar Ulstar, and the control – cv. Radius.

Analyzed characters included raceme length, flower number per raceme, pollen grain viability, the number of embryos per ovary, pod number per raceme, pod setting efficiency, seed number per raceme, seed number per pod, productivity, thousand seed weight and seed weight per plant. The efficiency of pod setting referred to the percentage ratio of the pod number raceme to the flower number per raceme, while fertility defined the percentage ratio of seed number per pod to the number of embryos per ovary.

Methods

Plants were planted in three blocks. Each block contained 16 plots. In each plot there were 10 plants growing in a 50 × 50 cm spacing. Analyses were conducted based on random samples from five plants selected from each plot. On each plant individual traits were investigated in five replications. It did not pertain to seed weight per plant – this trait was analyzed without replications by weighing all seeds collected from a single plant.

Results obtained in the field trial were subjected to the analysis of variance using the Statistica software. For this purpose the one-way analysis was applied with the use of Tukey's test and the significance of differences between values obtained for plants from individual objects was investigated. Inference was conducted at the significance level á=0.05. Results from the first and second year were elaborated separately, due to the variation in climatic factors found in the years of the study and due to the fact that the same objects were observed in the second and third year of vegetation, which could have affected characters connected with seed yielding.

Climatic conditions

The year 2005 was characterized by the periodical occurrence of semi-droughts alternating with heavy precipitation, occurring especially in May and July. Water deficit at the beginning of the vegetation period was disadvantageous for growth and development of alfalfa. Slight total precipitation in June promoted insect flights, while mean temperature in that month was higher than that in the multiyear period, which had a positive effect on flowering in alfalfa. In turn, strong precipitation in July caused new growth of young shoots and this delayed the formation and setting of seeds.

The year 2006 was characterized by low levels of precipitation. Starting from January until the end of July soil water deficit was observed, which contributed to the occurrence of alternating drought and semi-drought periods. A lack of precipitation in the period of flowering promoted insect flights, while high temperatures had an advantageous effect on flowering and pollination. Water shortage in June and July contributed to poor seed setting and pod dropping, as well as accelerated the date of harvest.

RESULTS AND DISCUSSION

In consistence with the characteristics of long peduncle mutants, plants of the inbred line with gene *lp* and the long peduncle cv. Ulstar formed the longest racemes and the highest number of flowers in the inflorescence in both years of the observations. Values of these traits for plants with gene *lp* differed significantly from the value observed in top flowering mutants and the control cv. Radius (Table 1). In the conducted experiment racemes of long peduncle mutants in the second year of vegetation were longer by over 50% than racemes of the control cv. Radius. In the third year of vegetation racemes of long peduncle plants were by 77% longer in plants of the long peduncle line in the S_1 generation and by 89% in plants of cv. Ulstar. Bodzon (2000) observed in long peduncle mutants in the S_2 generation an over three-fold elongation of the inflorescence axis in relation to cv. Radius. In turn, after the introduction, via backcrossing, of gene *lp* to cv. Radius he found that the length of racemes in hybrids was over two times bigger than that observed in plants of cv. Radius.

Table 1

Biometric traits of racemes and cytological characteristics of analyzed alfalfa inflorescence mutants

Object	Raceme length [mm]	Flower number per raceme	Number of embryos in ovary	Pollen grain viability [%]
Second year of vegetation				
"tf" S1	40.3 a	30.4 b	9.9 a	55.9 a
"tf" Sib1	41.4 a	32.9 c	9.9 a	54.3 a
"lp" S1	61.3 b	35.1 cd	9.8 a	59.8 a
"lp" -cv. Ulstar	62.8 b	37.0 d	10.0 a	84.1 c
control - cv. Radius	39.5 a	26.9 a	10.0 a	73.7 b
Third year of vegetation				
"tf" S1	38.3 b	30.9 b	9.5 a	53.9 a
"tf" Sib1	39.8 b	30.7 b	11.1 c	53.3 a
"lp" S1	60.1 c	38.1 c	10.2 b	58.8 ab
"lp" -cv. Ulstar	63.7 c	40.2 c	10.3 bc	81.2 c
control - cv. Radius	33.7 a	25.8 a	10.5 bc	63.3 b

a-d - Means denoted with identical indexes did not differ significantly (α=0.05)

As a result of introducing gene *lp* to cv. Radius Bodzon (2000), found that the first generation of hybrids was characterized by an increased number of flowers by 5%, in the raceme than in cv. Radius. In the conducted experiment the number of flowers in the raceme was observed to be by over 30% higher in long peduncle mutants in relation to plants of cv. Radius in both years of observations. A higher number of flowers in the raceme in relation to the control cultivar was recorded also in top flowering mutants (Table 1).

The mean number of embryos in the ovary in the conducted experiment was similar in all analyzed lines and cultivars, ranging from 9.8 to 10.0 in the first year of vegetation and from 9.5 to 11.1 in the second year of the study (Table 1). Cebrat (1973) and Dattee (1975) stated that the number of embryos in the ovary is a relatively invariable trait and to a high degree an inheritable character. Studies carried out by the authors of this study confirm these observations.

In both years of the study viability of pollen grain was similar within individual objects. The highest viability of pollen grain was found for examined cultivars: Ulstar and Radius, while all the other objects exhibited a reduced pollen grain viability – below 60% (Table 1). Cebrat (1973) showed that populations of alfalfa are characterized by a relatively low viability of pollen grain – on average from 41% to 80%, which was confirmed also by studies conducted by Dyba *et al.* (2004) and by the authors of this study.

The pod number per raceme varied in analyzed objects. The biggest number of pods was set by mutants of the long peduncle cv. Ulstar – in the second year of vegetation it was 12% more than in plants of the control cv. Radius, while in the third year of the study it was 54% more. Bodzon (2000) stated that long peduncle forms of alfalfa are characterized by the pod number per raceme higher by approx 30% - 50% in comparison to cv. Radius. Also Guy *et al.* (1973) investigated different short raceme forms of alfalfa and they observed that plants with longer receptacles set more pods and had more seeds in the pod. Wyrzykowska and Stankiewicz (2006) studied different forms of alfalfa and selected single plants with high seed productivity, which set an average of 12.2 pods in the raceme. In this study in the second year of vegetation the mean pod number per raceme in mutants of the long peduncle cv. Ulstar was 18.2 (Table 2). Thus cv. Ulstar is characterized by a high mean number of pods in the raceme.

Table 2

Characteristics of yield structure in analyzed alfalfa inflorescence mutants

Object	Pod number per raceme	Pod seeting efficiency [%]	Seed number per raceme	Seed number per pod	Productivity [%]	Thousand seed weight [g]	Seed weight per plant [g]
Second year of vegetation							
"tf" S1	11.3 a	38.0 a	21.8 a	1.8 a	18.2 a	1.94 a	11.3 a
"tf" Sib1	12.4 b	39.0 ab	22.7 a	1.8 a	18.1 a	1.91 a	14.0 ab
"lp" S1	15.2 c	44.8 bc	38.4 b	2.3 b	23.9 b	1.93 a	20.7 bc
"lp" -cv. Ulstar	18.2 d	50.1 c	68.9 d	3.6 c	36.9 c	1.97 a	27.0 c
control - cv. Radius	16.2 c	61.2 d	42.7 bc	2.7 b	26.8 b	2.10 a	22.9 c
Third year of vegetation							
"tf" S1	11.0 a	39.1 b	19.0 a	1.7 a	17.7 a	2.01 a	8.3 a
"tf" Sib1	11.1 a	39.0 b	24.3 b	2.1 b	21.2 b	2.06 a	13.5 b
"lp" S1	12.2 a	33.2 a	29.3 bc	2.4 b	22.1 b	1.92 a	17.8 b
"lp" - cv. Ulstar	16.2 b	42.1 b	33.8 c	2.1 b	20.9 b	1.95 a	18.7 b
control - cv. Radius	10.5 a	42.4 b	22.5 b	2.2 b	20.8 b	2.03 a	14.8 b

a-d - Means denoted with identical indexes did not differ significantly (α=0.05)

Within individual mutations pod number in inbred lines was lower than in another objects which indicates the effect of inbreeding depression on this character. The same relationship was also observed by Dyba *et al.* (2004) when comparing inbred lines and synthetic populations of alfalfa. Also Broda *et al.* (2005) found the effect of inbreeding depression on a reduction of the pod number per raceme in top flowering lines.

In the third year of the experiment, characterized by a considerable water deficit throughout the entire vegetation period, in alfalfa plants mean pod numbers per raceme were lower than in the second year of vegetation. This was caused by a reduced pod setting in the third year of the study, since the expression of this trait is strongly dependent on the amount and distribution of precipitation as well as air temperature (Simon 1984, Lorenzetti 1993, Katepa-Mupondwa *et al.* 1996). In our study among analyzed lines and cultivars only progeny with the *tf* gene exhibited considerable stability in terms of the pod number in the raceme and the efficiency of pod setting. In top flowering mutants in the third year of the study the pod number per raceme decreased only slightly, while the pod setting efficiency in top flowering progeny in generation Sib_1 remained at the same level, whereas in top flowering lines in the S_1 generation it even increased (Table 2). Cebrat (1973) and Dattee (1975) were of the opinion that the pod setting efficiency is dependent on genetic factors; what is more, it is strongly modified by weather conditions. Our investigations indicate a slight effect of environmental conditions on the expression of genetic factors influencing this trait in mutants with gene *tf*.

The biggest seed number per raceme was recorded for the long peduncle cv. Ulstar – in the second year of vegetation on average 68.9, while in the third year of the experiment it was 33.8 (Table 2). A considerable reduction in the number of seeds per raceme in the third year of vegetation was also observed in plants of long peduncle lines in generation S_1 and in the control cv. Radius. This was probably caused by drought, which resulted in withering of inadequately nourished embryos (Cebrat 1973). We need to focus here on top flowering progeny in the Sib_1 generation, which in the third year of vegetation, despite long-term drought, had more seeds in the raceme than in the second year of the experiment. The seed number per raceme was also investigated by Wyrzykowska and Stankiewicz (2006). Single specimens selected by these researchers for their high seed productivity set on average 22.8 seeds in the raceme. In this study mutants with gene *lp* were characterized by a higher mean seed number per raceme in both years of vegetation.

The seed number per pod is directly connected with fertility. Both these characters are strongly modified by environmental factors. Studies conducted in Poland showed that the mean number of seeds per pod in alfalfa ranged from 2 to 5, depending on the genotype and weather conditions (Jabłoński 1973, Jelinowska and Rene 1984, Bodzon 2004, Broda *et al.* 2005). Tassei (1971) was of the opinion that from 3 to 4 seeds in the pod constitutes an adequate number of plants per area unit. In this experiment in the second year of vegetation the biggest seed number per pod was observed in the long peduncle cv. Ulstar – mean 3.6 (Table 2). In terms of this trait cv. Ulstar differed significantly from the other progeny and the control cv. Radius. In turn, the lowest seed number was found in pods of top flowering mutants – mean 1.8, which significantly distinguished these progeny from the other analyzed objects. In the third year of vegetation the seed number per pod was more uniform and only inbred lines with gene *tf* differed significantly from the other object by the lowest mean, i.e. 1.7 seeds in the pod.

In both years of the observations the most fertile plants were those of the long peduncle cv. Ulstar, which in the second year of vegetation considerably exceeded in this respect, plants of the other lines and cultivars and set seeds with mean efficiency of 36.9% (Table 2). In the third year of vegetation a strong decrease was observed in fertility of most analyzed objects. An exception in this respect was found for top flowering progeny in generation Sib_1, in which fertility increased from 22.7% in the second year of the experiment to 24.3% in the third year of vegetation. This fact confirmed the resistance of top flowering mutants from sib-mating to adverse weather conditions, observed in the third year of vegetation.

Thousand seed weight was similar in all analyzed objects (Table 2). Statistical analysis showed no significant differences in this trait. Adverse weather conditions in the third year of vegetation did not have an effect on thousand seed weight, which confirms findings by Delouche (1980), in whose opinion water deficit during generative development is connected mainly with a reduced quantity and not deterioration of seed quality.

In the conducted experiment the highest seed weight per plant in both years of vegetation was found for the long peduncle cv. Ulstar – in the first year of the study 27.0 g, while in the second year of the experiment 18.7 g (Table 2). High seed productivity of long peduncle forms of alfalfa was confirmed in studies by Užik and Polak (1997) as well as Bodzon (1998). In turn, Wyrzykowska and Stankiewicz (2006) stated that not always the long peduncle character had a positive effect on seed productivity, since most of their analyzed long peduncle lines had infructescences with a low pod number, which in turn resulted in a reduced seed weight per plant.

As it is the case with most of the investigated traits, a reduced seed weight was observed in the third year of the study in all analyzed plots. The highest decrease in the value of this trait was found in the long peduncle cv. Ulstar and in the control cv. Radius, which could have been caused by their high sensitivity to low precipitation. The least reduced seed weight per plant in the third year of vegetation in response to adverse weather conditions was recorded in top flowering progeny in the Sib_1 generation. A decrease was observed in seed weight in all inbred lines. This was probably caused by a decrease in values of many traits affecting seed yield, connected with inbreeding depression. A similar trend was also found by Broda *et al.* (2005).

Despite high mean temperatures, which promote reproduction of seeds in this thermophilous plant, the extremely low amount of precipitation caused a reduction in the efficiency of pod setting and fertility, and as a result also seed yield. In the opinion of Delouche (1980) and Sypniewski (1986) water deficit in the period from the beginning of flowering to harvest is connected with a considerable reduction of seed yield. These observations are understandable, in view of the fact that the accumulation of reserve substances in seeds of legumes depends mainly on assimilates formed during fruiting (Bewley 1985). Thus the better vegetative development of plants and increased photosynthetic activity of pods under advantageous hydrothermal conditions, contribute to a significant increment in the number of seeds per plant (Prusiński 1997).

Despite the high sensitivity of long peduncle mutants to hydrothermal conditions they were characterized by a higher seed reproduction than top flowering mutants and exceeded top flowering lines and the control cultivar in terms of most analyzed traits. In turn, mutants with gene *tf*, despite the highest stability of analyzed characters, gave the lowest seed weight per plant among all the analyzed objects.

CONCLUSIONS

The mutant with the *lp* gene had a high seed yielding potential, manifested in the elongation of racemes by 31% in the second year of vegetation and 77% in the third year of vegetation, as well as the formation of an over 50% higher number of flowers per raceme in both years of the study in relation to the control cv. Radius.

A high potential in terms of seed productivity in the long peduncle mutant of cv. Ulstar, making it possible to increase pod number per raceme in relation to the control cv. Radius, as well as increase the seed number per raceme, led to an increase in seed weight per plant by 18% in the second year of vegetation and by 26% in the third year of the experiment.

The top flowering progeny in the Sib_1 generation exhibited the highest stability of analyzed traits of seed yield structure under diverse hydrothermal conditions found in the first and second years of vegetation and it showed the least decrease in seed weight per plant in the second year of vegetation, characterized by a very small level of precipitation.

The top flowering mutants characterized by a higher number of flowers per raceme in relation to the control cv. Radius; however, they set the lowest seed number among all the analyzed objects.

Inbred lines in the S_1 generation of the top flowering and the long peduncle mutants were characterized by inbreeding depression, manifested in the following traits: the length of raceme, the pod number per raceme, the seed number per raceme and seed weight per plant.

ACKNOWLEDGEMENTS.

The authors are grateful to Prof. dr hab. Zygmunt Staszewski from Plant Breeding and Acclimatization Institute for his kind providing mutants of alfalfa inflorescences for morphological and developmental analyses.

REFERENCES

Bewley J. D. 1985. Seeds physiology and germination. Plenum Pres. New York and London.

Bodzon Z. 1998. Inheritance of spontaneous long peduncle mutation in alfalfa (*Medicago sativa* L.) Plant Breeding Seed Sci. 42/1: 3-9.

Broda Z., Weigt D., Hegenbarth R. 2005. Charakterystyka morfologiczna i cytologiczna oraz ocena struktury plonu nasion w liniach wsobnych mutantów kwiatostanu lucerny (*Medicago sativa* L. sl.). Prace z Zakresu Nauk Rolniczych PTPN, Tom 98/99: 155-165.

Cebrat J. 1973. Cytoembriologiczne badania nad przyczynami niskiej płodności lucerny mieszańcowej (*Medicago media* Pers.) Zesz. Probl. Post. Nauk Roln. 131: 99-110.

Dattee Y. 1975. Facteurs genetiques lies a l'auto et i'interfeftilite chez luzerne. Ann. Amelior. Plant. 30: 4-11. Delouche J. C. 1980. Environmental effects on seed development and seed quality. Hort Sc. 15: 775-780.

Dyba S., Wojciechowski A. 1979. Współzależność między kiełkowaniem ziarn pyłku i wzrostem łagiewek pyłkowych a plonem nasion u lucerny. HRAiN, Tom 23, Zeszyt 6: 371-378.

Dyba S., Broda Z., Łuczkiewicz T., Nawracała J., Boberska K. 2004. „Effect of the pollination method on fertility of inbred lines and synthetic populations of alfalfa (*Medicago sativa* L. sl.). PTPN, Tom 97-2004: 93-102.

Guy P., Ecalle C., Genier G. 1973. Influence de la morphologie d'une inflorescence sur la production de graines. Zesz. Probl. Post. Nauk Roln. 131: 25-32.

Hill R. R. jr., Shenk J. S., Barnes R. F. 1988. Breeding for yield and quality. Monog. 29: Alfalfa and alfalfa improvement. Am. Soc. Agron.: 809-825.

Jabłoński B. 1973. Badania biologii kwitnienia i zapylenia lucerny mieszańcowej. Pszczel. Zesz. Probl. 14: 1-7.

Jelinowska A., Rene B. 1984. Variability de la floraison et de la formation des graines par la luzerne. Proc. of M. sativa Group Eucarpia. Brno.

Katepa-Mupondwa F. M., Barnes D. K. Smith JR S. R. 1996. Influence of parent and temperature during pollination on alfalfa seed weight and number of seeds per pod. Can. J. Plant Sci. 76(2): 259-262.

Lorenzetti F.1993. Achieving potential herbage seed yield in species of temperate regions. Proc. of XVIIth Greassland Congress: 1621-1628.

Pedersen M. W., Barnes D. K. 1973. Alfalfa seed size as an indicator of hybridity. Crop Sci. Vol. 13: 72-75.

Simon U. 1984. Variation of seed setting in a lucerne polycross. Proc. of the VIth Eucarpia Meeting of Group Medicago. Brono: 239-244.

Sorensen E. L., Byers R. A., Horber E. K. 1988. Breeding for insect resistance. Monog. 29 Alfalfa and alfalfa improvement. Am. Soc. Agron.: 859-902.

Sypniewski J. 1986. Problemy uprawy roślin strączkowych w Polsce. Fragm. Agron. 1: 29-26.

Tassei A. 1971. Relative efficiencies of four self-pollinations techniques in alfalfa. Crop Sci. 11: 175-192.

Užik M., Polák E.1997. Selection of alfalfa for fodder and seed yield. Proc. of the XIIth Eucarpia Meeting of the Group Medicago, Brno: 67-70.

Volenec J. J., Cunningham S. M., Haagenson D. M., Berg W. K., Joern B. C., Wiersma D. W. 2002. Physiological genetics of alfalfa improvement: past failures, future prospects. Field Crops Research 75: 97-110.

Wyrzykowska M., Stankiewicz C. 2006. Wielowymiarowa analiza zmienności cech rolniczych różnych form lucerny mieszańcowej (*Medicaga sativa ssp. media*). Biul. IHAR 242: 243-251.