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INFLUENCE OF SOME RECESSIVE GENES ON PLANT AND INFLORESCENCE CHARACTERISTICS IN ALFALFA

ABSTRACT

The investigations of the effects of incorporation of some recessive genes into cv. Radius of alfalfa on seed productivity were performed in greenhouse and in field experiments. BC₂ progenies were produced and compared to the standard cultivar Radius. Introduction of recessive genes lp (long raceme), tf (top flowering) and br (branched raceme) affected type of inflorescence: 1) the lp trait significantly increased flower and pod number per raceme, seed number per raceme and seed weight per sq. m., 2) the tf trait increased significantly number of raceme per stem and seed yield per sq. m., 3) the branched racemes trait governed by a single recessive gene br enhanced 6 - 10 times floret number per raceme.

Key words: Medicago sativa L., alfalfa, long raceme -lp, top flowering -tf, branched raceme -br, inflorescence morphology, inheritance, seed setting.

INTRODUCTION

Improvement of the size of vegetative organs has been the aim in alfalfa breeding for long years, because the main task of breeders was to maximise dry matter and protein yield. Many valuable cultivars were released contributing to the progress in productivity, quality and resistance. The FNAMS (Fédération Nationale des Agriculteurs Multiplicateurs de Semences), France, reported on a lack of progress in seed yield capacity until 1988, but in the last decade some improvement of seed yield potentials has been reported in France and Poland. Several cultivars were bred producing 20-30% gain in seed yield compared to former standards (Staszewski, Bodzon 1987).

The change of plant and flower morphology seemed necessary to obtain a marked progress in seed yield. Accordingly three mutations useful for improvement of seed yield were selected and their inheritance was studied.

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Long inflorescence trait was found to be controlled by a single recessive gene *lp*. Plants homozygous for this gene (*lplplplp*) have racemes 2-3 times longer than standard short inflorescence forms (Fig. 1), (Staszewski 1986, Bodzon 1998).



Fig. 1 The stems and racemes of a lp-phenotype plant



Fig. 2 The stem top of a tf-phenotype plant

In the top flowering mutants (*tf tf tf tf*) flower buds grow on the stem top, thus leading to termination of the stem growth (Fig. 2 and Fig. 3), (Staszewski *et al.* 1992).

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Fig. 3 The stems of a *tf*-phenotype plant at full maturity stage



Fig. 4 The inflorescence and stem of a br-phenotype plant

Branched raceme mutation makes the flowers grow on numerous branches of a raceme, resembling a panicle (Fig. 4). Branched raceme trait was reported by Dudley and Wilsie (1957) to be controlled by four dominant genes. Another mutant of this type was reported by Childers (1966) to be controlled by a single recessive gene br.

The usefulness of single genes in tetraploid alfalfa breeding is commonly known. Numerous publications in this respect are summarised by Barnes and Hanson (1967), and Bodzon (1998).

Our study was aimed at demonstration of the effect of long raceme and top flowering on plant and inflorescence morphology. Besides, morphology and inheritance of branched raceme type of inflorescence has been described.

MATERIAL AND METHODS.

The experiment was carried out in the field to compare morphological and generative characters of BC_2 progenies and the standards.

The following plant materials were included:

1. The bulk progeny of spontaneous mutant lp (long raceme)-2002/1

- 2. The BC₂ progeny obtained by the following crossing: Radius clone × mutant 2002/1 = F₁ → F₂(1*lp*+35 normal type) (BC₁) *lp* type - F₂(*lp*) × Radius clone = F₁ → F₂(1*lp*+35 normal type) (BC₂) *lp* type - BC₁ × Radius clone = F₁ → F₂(1*lp*+35 normal type). Five basic clones of cv. Radius were used as pollinators. Bulk seeds of each clone progeny including *lp* type BC₂ plants were used in the trial to compare with the standards.
- 3. The BC_2 progeny obtained by the following crossing:

Radius clone × mutant $tf = F_1 \longrightarrow F_2$ (1tf +35 normal type),

(BC₁) tf type - $F_2(lp) \times \text{Radius} = F_1 \longrightarrow F_2(1tf + 35 \text{ normal type}),$

(BC₂) tf type - BC₁ x Radius = $F_1 \rightarrow F_2$ (1tf +35 normal type).

The pollen parent Radius consisted of pollen mixture of 5 clones. Bulk seeds of 7 selected plants of *tf* type were included in the experiment.

4. Radius (Polish) and Europe (French) cultivars were used as standards.

Seeds were sown in pots in early March and 5-6 week old plants were space planted to avoid competition in the field properly prepared and fertilised. Plant density was 8 plants per square metre. The plots of 15 sq. m. size were repeated in three blocks.

The sample consisting of 20 stems were taken from each plot for biometric measurements in the 2^{nd} and 3^{rd} years of plant vegetation. The means were calculated from the samples: pod and seed number per raceme and seed weight per 20 racemes – 3×20 racemes, seed number per pod – 3×50 pods. The desiccated seeds were harvested from plots using HEGY-harvester. Statistical analyses were computed from 3 replications. Randomised block design was applied.

Branched raceme character of the clone no. 2 was studied in the pot experiment at spring and summer time in a greenhouse. Natural light and temperature were sufficient for plant growth under these conditions. The

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clone was selected from cv. Radius \times cv. Orca progeny. The segregating progeny of clone no. 2 were used for the following crossings:

- 1. Branched raceme plants × branched raceme plants. The seeds of four pair-crosses were produced.
- 2. Normal raceme plants × normal raceme plants. The seeds of three pairs of crosses were produced.

The seeds of the above crossings were sown to produce plants for the study of branched raceme inheritance. Plants were grown in pots of 8 kg capacity; four plants per pot. The plants reached full bloom after 85 days of vegetation. Plants having all branched racemes were denoted as branch raceme phenotype. Semi-branch type plants were classified to a normal plant group. Chi-square test served for verification of segregations.

Three samples of different inflorescence type were taken from plants being grown in the pots. Each sample consisted of 30 inflorescences.

RESULTS AND DISCUSSION.

Characteristics of generative traits of long racemes and top flowering phenotypes are presented in Table 1 and Fig. 5. Radius breeding stock was improved by introducing gene lp from the mutant 2002/1. Pod and seed number per raceme increased by over 40%. Also seed number per pod was higher by 7 per cent. Incorporation of this gene resulted in about 20 per cent gain of seed yield. It is noteworthy that cv. Radius is a population very well adapted to Polish climate conditions and possesses extremely high seed yield potential (Staszewski, Bodzon 1987). Further improvement of seed yield achieved in this cultivar confirmed high value of lp gene for breeding programs (unpublished).

Also Jakubowska and Staszewski (1994) reported on twofold increase of seed yields of male sterile alfalfa lines due to raceme elongation by introducing *lp* gene via back-crosses. The seed setting of CMS-lines with long inflorescences reached the level of standard cv. Radius in their trials.

Hopefully, the negative characters of mutant 2002/1 i. e. very low seed number per pod and low 1000 seed weight were eliminated from the Radius gene pool in the course of two back-crosses. The length of inflorescence is easy to stabilise in BC₁, since it is governed by a single gene.

Top flowering BC₂ progeny showed generative characters similar to cv. Radius, but it produced about 30 per cent higher seed yield than the standard cultivar (Table 1, Fig. 5). The extremely large number of racemes per stem was the main reason of that considerable yield gain. It is remarkable that the number of racemes per stem in BC₂ *tf* progeny surpassed by 90 per cent the check cultivar (Table 2 and Fig. 6). Inasmuch as, top flower type plants terminate growth at full bloom, so their pods ripen more evenly and stems can avoid lodging. This can be very advantageous specially when an excess of summer precipitation stimulates stem growth in common phenotypes lacking the *tf* gene.



Fig. 5 Comparison of generative characters of long raceme and top flowering lines BC_2 and standards in per cent of cv. Radius

TableComparison of generative characters of long raceme (lp) and top flowering (tf) BC2 lines and standards (harvested in 1993 - 1994)							
	Dod no nor	Sood no nor	Sood no nor	1000 good	Sood weight per 20 Seed vield		

Genotypes	Pod no per raceme	Seed no per raceme	Seed no per pod	1000 seed weight [g]	Seed weight per 20 racemes [g]	Seed yield $[g \times m^{-2}]$
		Iy	ear of utilizatio	n		
			<i>lp</i> - lines			
BC ₂ 3015	23.4	100.4	4.3	2.0	4.0	41.8
BC ₂ 3179	23.5	92.1	3.9	2.0	3.6	38.5
BC ₂ 3151	25.6	109.9	4.3	2.0	4.4	43.5
BC ₂ 3052	27.3	114.6	4.2	2.0	4.5	45.6
BC ₂ 3088	26.1	110.3	4.2	2.0	4.4	43.2
BC ₂ mean	25.2	105.5	4.2	2.0	4.2	42.5
			tf - lines			
BC ₂ 96013	15.0	56.0	3.7	2.1	2.4	41.6
BC ₂ 96031	15.4	61.2	4.0	2.2	2.7	43.1
BC ₂ 96051	16.2	69.8	4.3	2.0	2.8	48.3
BC ₂ 96057	15.4	66.4	4.3	2.0	2.7	46.4
BC ₂ 96058	16.4	64.8	4.0	2.1	2.7	45.5
BC ₂ mean	15.7	63.6	4.1	2.1	2.6	45.0
Mutant 2002/1	24.9	51.6	2.1	1.8	1.9	20.8
cv. Radius	15.9	61.8	3.9	2.2	2.7	34.7
cv. Europe	15.0	54.9	3.7	2.2	2.9	31.5

Continued						
Genotypes	Pod no per raceme	Seed no per raceme	Seed no per pod	1000 seed weight [g]	Seed weight per 20 racemes [g]	Seed yield $[g \times m^{-2}]$
		II yea	ar of utilization	1		
			<i>lp</i> - lines			
BC ₂ 3015	26.4	122.7	4.7	2.0	4.9	43.2
BC ₂ 3179	24.4	102.8	4.2	2.0	4.1	40.5
BC ₂ 3151	26.2	120.2	4.6	2.1	4.8	46.6
BC ₂ 3052	28.9	132.9	4.6	2.1	5.2	47.0
BC ₂ 3088	26.7	121.4	4.5	2.1	5.0	47.1
BC ₂ mean	26.5	120.0	4.5	2.1	4.8	44.9
			<i>tf</i> - lines			
BC ₂ 96013	15.9	69.1	4.3	2.0	2.8	43.3
BC ₂ 96031	17.7	71.2	4.0	2.1	3.0	44.4
BC ₂ 96051	16.4	76.2	4.6	2.1	3.2	50.1
BC ₂ 96057	17.1	79.5	4.5	2.1	3.3	48.9
BC2 96058	17.4	75.2	4.4	2.2	3.3	47.2
BC ₂ mean	16.9	74.2	4.4	2.1	3.1	46.8
Mutant 2002/1	25.6	54.5	2.1	1.9	2.0	22.4
cv. Radius	17.2	72.0	4.2	2.2	3.2	37.8
cv. Europe	16.6	65.9	4.0	2.2	3.1	35.1
LSD _{0.05} A/B *	4.50	20.21	0.70	0.24	0.73	8.24
LSDo of B/A *	1 17	7 78	0.27	0.10	0.28	3 1 9

*A/B - to compare genotypes within a single year; B/A - to compare a genotype between two years



Fig. 6 Comparison of morphological characters of long raceme and top flowering lines BC_2 and standards in per cent of cv. Radius.

Table 1

Comparison of morphological characters (Table 2) demonstrates that only raceme length and flower number per raceme of the BC₂ long racemes progenies significantly exceeded these characters of cv. Radius. Other traits, such as plant height, stem number per plant, node number per stem, raceme number per stem were similar in cv. Radius and BC₂ lp progenies.

The above information could suggest that lp gene does not negatively influence dry matter yields. Pomogajbo (1982), who experimented with several alfalfa cultivars, stated that plants with elongated inflorescences were characterised by higher seed and forage productivity. This relationship was confirmed also by Užik (1996). The correlation between the inflorescence length and seed yields and between the seed yield and insect pollination was reported by Guy *et al.* (1973) and Dattee (1974). But it should be taken in consideration that mentioned above authors studied populations with normal (short) racemes governed by a single dominant gene. Bodzon (1998) compared both populations, with a common-short raceme and that with long raceme. The inflorescence length in the first gene pool ranged from 30 to 70 mm whereas in the long raceme gene pool it was 80 to 160 mm.

Table 2

Comparison of morphological characters of long raceme (lp) an	nd top flowering (tf) E	C ₂ lines and stan-
dards (harvested in 1993 - 1	1994)	

Genotypes	Raceme length	Flower no. per raceme	Plant height [cm]	Main stem no per plant	Node no per main stem	Raceme no per stem
		I year	of utilization			
			<i>lp</i> - lines			
BC 2 3015	12.2	46.5	87.8	24.2	13.2	21.8
BC 2 3179	10.8	38.2	95.4	23.2	14.9	22.8
BC 2 3151	11.0	40.6	96.2	24.0	14.0	23.3
BC 2 3052	13.3	47.1	98.1	22.6	13.8	25.2
BC 2 3088	13.4	46.1	99.3	24.0	15.8	23.6
BC 2 mean	12.6	44.6	97.9	23.5	14.5	22.8
			<i>tf</i> - lines			
BC 2 96013	5.7	18.7	87.7	20.9	13.8	40.1
BC 2 96031	6.0	19.1	85.1	22.4	14.3	43.9
BC 2 96051	6.6	26.2	97.2	22.1	15.4	47.1
BC 2 96057	6.4	24.6	88.6	21.3	14.1	42.4
BC 2 96058	6.2	21.4	92.1	22.8	15.0	46.1
BC 2 mean	6.2	22.0	90.1	21.9	14.6	43.9
Mutant 2002/1	14.0	48.6	102.5	23.9	17.3	20.8
cv. Radius	5.9	21.1	90.2	22.6	13.4	23.1
cv. Europe	6.0	21.0	86.1	21.7	13.5	22.2

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Genotypes	Raceme length [cm]	Flower no per raceme	Plant height [cm]	Main stem no per plant	Node no per main stem	Raceme no. per stem
		II year	r of utilization			
			p - lines			
BC 2 3015	12.8	47.6	91.7	21.2	13.8	25.0
BC 2 3179	13.1	40.5	100.7	21.4	14.5	23.4
BC 2 3151	10.6	42.2	100.3	22.2	14.5	25.2
BC 2 3052	14.5	49.9	103.4	21.8	14.9	25.9
BC 2 3088	11.4	48.8	105.1	21.9	16.1	23.9
BC 2 mean	12.5	45.8	100.2	21.7	14.8	24.7
			tf - lines			
BC 2 96013	6.1	19.6	92.3	19.4	14.6	42.7
BC 2 96031	6.3	21.2	90.6	20.4	15.2	45.1
BC 2 96051	6.8	26.6	102.8	21.6	17.0	51.6
BC 2 96057	6.6	26.2	91.2	22.1	16.2	44.8
BC 2 96058	6.5	24.3	95.6	23.5	16.4	48.4
BC 2 mean	6.5	23.6	94.5	21.4	15.9	46.5
Mutant 2002/1	14.7	51.2	104.4	23.3	18.9	21.4
cv. Radius	6.2	22.7	94.6	22.0	14.6	24.5
cv. Europe	6.0	21.2	90.7	21.1	13.9	23.6
LSD _{0.05} A/B *	2.07	6.59	14.41	4.23	2.13	3.98
LSD _{0.05} B/A *	0.79	2.56	5.50	1.64	0.82	1.54

* A/B - to compare genotypes within a single year; B/A - to compare a genotype between two years

Simple inheritance of the long raceme and top flowering characters enables utilisation of this mutations for breeding plants with better seed yield potential. It opens also a possibility of preliminary selection for improved seed yield in alfalfa as for a qualitative, rather than quantitative character.

The segregation of the branched raceme trait is presented in Table 3. In F_2 progenies of sib-crosses of branched raceme plants segregations were 1:1 and 3:1 in two groups. The first group can be assumed to be simplex × nulliplex crosses, while in the second group probably simplex × simplex were crossed. Sib-crosses of normal (unbranched) raceme plants gave segregation typical for intercrossing of duplexes: 35:1. The presented results confirm that the branched raceme trait in clone no. 2 is transmitted by a single recessive gene *br*.

Dudley and Wilsie (1967) studied the branched raceme character controlled by four genes, namely A, B, Ra and Ra'. The genes A and B were inherited independently and tetrasomically, while Ra and Ra' were duplicated genes inherited disomically. The authors concluded that this character brings no advantage because many florets were deformed.

Table 2

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Cara a in a comitant	Number of	Observed	segregation	Expected s	Expected segregation		Chi - square	
	plants	Normal	Branched	Normal	Branched	value	р	
Clone no 2	31	28	3					
(open pollination)								
			$br \times br *$:					
2-29 × 2-1	11	6	5					
2-1 × 2-31	105	58	47					
Total:	116	64	52	58	58	1.24	0.26	
2-31 × 2-31	48	35	13					
2-29 × 2-29	75	52	23					
Total:	123	87	36	92.25	30.75	1.17	0.28	
			$N \times N *$:					
2-26 × 2-12	133	130	3					
2-28 × 2-3	90	85	5					
2-72 × 2-12	79	78	1					
Total:	302	293	9	293.6	8.4	0.041	0.83	

Segregation of br - phenotypes in F₂

* *br* - branched raceme, N - normal (unbranched) raceme

Childers (1966) was of the opinion that a branched raceme trait is transmitted by a single recessive gene *br*. Our observations are consistent with this statement. It would be interesting to know whether all *br* genes are present at the same locus.

From the data presented in Table 4 it can be seen, how great advantage brings the incorporation of br gene. The number of 180 florets in inflores-cence is impressing.

Comparison of various affaita inflorescences								
Types of inflorescence	Inflorescence length [cm]	Number of branches	Flower no per branch	Flower no per raceme				
Normal type	5.9	1	21.0	21.0				
<i>lp</i> type	12.6	1	44.6	44.6				
br type	13.6	15	12.0	180.0				

Similarly to the reports of the above mentioned authors, a part of branched raceme plants produced deformed florets. Deformed, vestigial florets were not able to produce seeds. The inflorescence with deformed florets resembles a cauliflower. Heterozygous plants possessing both, normal and vestigial florets, occurred in a small number. Such plants produced selfed progeny segregating for normal and deformed types. According to our unpublished results, vestigial florets trait is transmitted by a separate gene. This observation is in agreement with the results

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Table 3

Table 4

of Mariani et al. (1976) who pointed out that vestigial, cauliflower inflorescence character is controlled by an independent recessive gene. The possibility to select branched raceme population free of vestigial florets seems realistic.

It has been demonstrated in this study that the lp and tf genes are useful for improvement of alfalfa seed yield. We were able to study only the effect of back-crosses, but selection and heterosis effects can increase seed yield still more. Perhaps, in future a breeder would develop a cultivar improved by interactions of all three genes: lp, tf and br.

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