RESEARCH REGARDING THE GENETIC CONTROL OF ALFALFA INTERFERTILITY

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ABSTRACT

Seven inbred lines (C_2) were included into a genetic diallel system of p(p-1) type. The 7 lines were selected from 36 inbred lines from Romanian and foreign varieties (cultivars) depending on some useful agronomic traits. The research works were performed at R.I.C.I.C. in 1997-1998. The traits used for interfertility estimation were: fructification coefficient and average number of seeds/pod. The genetic analysis was done in accordance with the statistic pattern "fix" corresponding to the diallel system p(p-1) type carried out by Cãbulea (1983). The study pointed out that the genetic determinism of seed number/pod is a complex phenomenon conditioned by both genetic – physiological actions and interactions less influenced by environmental conditions. At the same time, it was pointed out the maternal implication in the increase of alfalfa seed yield. The results of this study will be used in the strategy of alfalfa breeding for creating high productive seed cultivars.

Key words: alfalfa, combining ability, diallel system

INTRODUCTION

The increase of alfalfa seed yield represents an important concern of the Fodder Plant Breeding Laboratory from R.I.C.I.C.-Fundulea. This goal can be genetically achieved by creating varieties with a superior fructification potential. The choice of varietal components for the proposed yield traits is a main stage in the process of breeding. Consequently the value of a certain variety or line, as a breeding material is appreciated considering its capacity of transmitting the features to offsprings.

Griffing (1956) presented a mathematical pattern for estimating the value of genitors, considering their general and specific combining ability. Based on such information the genetic material can be used more judiciously.

For alfalfa, Theurer and Elling (1964) and Wilcox and Wilsie (1964) reported data regarding the transmission of physiological and yield traits by diallel crosses. Rotili (1977) analysed by the same method the transmission to alfalfa with various levels of inbreeding the traits: seed yield, fooder production and resistance to unfavourable conditions, obtaining useful results for breeding considering the analysed material.

In 1981, Varga and Gumaniuc, analysing by a genetic diallel system the traits dry matter yield and crude protein production, concluded that though the effect of additive and non-additive genes had a very important part in expressing the analysed traits, still the environment greatly influenced the two characters.

This study aims at clarifying some problems regarding the phenomenon of alfalfa interfertility and namely, its heredity, emphasizing on cytoplasmic genic effects.

MATERIALS AND METHODS

The study regarding the genetic control of alfalfa interfertility was performed during 1998-1997. The biological material utilized included 7 inbred lines (C_2) deliberately chosen from 36 inbred lines considering useful morphological traits and the tendency for selfpollination.

Some characteristics concerning the inbred lines are presented in table 1. The 7 lines are included in a diallel p(p-1) genetic system.

Crosses were carried out under isolation, in the field, in 1997 and 1998.

Pollination was done manually without emasculation, considering the principle of pollen self-incompatibility and allopollen preference (Demarly, 1963).

Direct and reciprocal crosses were carried out. Each raceme was marked with a coloured string, its colour indicating the inbred line used as a male parent and the number of pollinated flowers was written on a parchment paper attached to the raceme.

The indicators used for interfertility estimation were: fructification coefficient obtained from the ratio between the number of pods and the number of flowers and the ave-rage number of seeds per pod or the ratio between the num-

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ber of seeds and the number of pods. Grey et al. (1969) showed that the precision of the two indicators allowed their use in both the interfertility and self-fertility study.

The genetic analysis of the mentioned above two indicators was done in accordance with the “fix” statistic pattern corresponding to the diallel system p(p-1) carried out by Cãbulea (1983).

This pattern represents a modified and improved variant of Hayman’s corresponding patterns (1954) for genetic variance calculations and Griffing’s (1956) for the calculation of genetic effects corresponding to the general combining ability (additive level), specific combining ability (non-additive level) and reciprocal actions (level of cytoplasm – nucleus interaction). The pattern obtained by Cãbulea (1983) presents the advantage of analysis at cytoplasmic level too, where plasmogenic systems are localized.

RESULTS AND DISCUSSIONS

The statistic analysis of results (Ceapoiu, 1968) shows that the differences among combinations were significant.

The values of the fructification coefficient (arcsin $\sqrt{\%}$) were comprised between 48.8 in combination Lc-3 x Lc-1 and 80.5 in combination Lc- 5 x Lc-7; on an average, the fructification coefficient was 67.3. Other combinations with high fructification coefficient values were: Lc-1 x Lc-5; Lc-2 x Lc-5; Lc-4 x Lc-5; Lc-2 x Lc-7 (Table 2).

The average number of seeds per pod was comprised between 2.28 and 7.85, the average being 4.31. The highest values were recorded in combinations Lc-4 x Lc-5 (7.85); Lc-5 x Lc-4 (7.50); Lc-2 x Lc-5 (7.50) and the lowest values in combinations Lc-1 x Lc-2 (2.28); Lc-1 x Lc-3 (2.55); Lc-6 x Lc-3 (2.68).

Corresponding to the object of this study, the determination of the genetic control of alfalfa interfertility was obtained by the study of

Table 1. Short characterization of parental forms included into crossing system

No.	Parental lines	Inbred level	Origin	Country	Characteristics
1.	Lc-1	C_2	Multiking	USA	multi-leaflet leaves
2.	Lc-2	C_2	Topaz	Romania	disease resistance
3.	Lc-3	C_2	Erecta	Hungary	lodging resistance
4.	Lc-4	C_2	Pleven 13	Bulgaria	large number of ovules
5.	Lc-5	C_2	Sigma	Romania	somaclonal line
6.	Lc-6	C_2	Kometa	Poland	rich foliage
7.	Lc7	C_2	Vela	Denmark	long-racemes

Table 2. Fructification coefficient [(arcsin $\sqrt{\%}$)(numerator)] and average number of seeds per pod (denominator) resulted from diallel crossing of seven inbred lines
Fundulea, 1997-1998

Inbred lines	Lc-1	Lc-2	Lc-3	Lc-4	Lc-5	Lc-6	Lc-7
Lc-1	-	53.5 2.28	62.7 2.55	66.8 5.75**	77.0* 7.20***	64.2 3.35	75.2 3.28
Lc-2	69.9 3.48	-	69.5 3.43	66.0 6.28***	76.0* 7.50***	70.1 3.78	78.3* 3.98
Lc-3	48.8 2.85	56.7 3.05	-	64.7 5.30	69.2 5.23	55.6 3.23	72.3 3.65
Lc-4	56.6 3.88	69.1 3.58	60.7 3.93	-	78.4* 7.85***	61.3 3.38	77.7* 3.88
Lc-5	59.9 4.03	62.7 3.98	60.2 3.03	71.9 7.50***	-	59.8 3.30	80.5 4.03
Lc-6	64.9 3.70	69.0 3.48	62.8 2.68	67.3 5.93	67.5 6.83***	-	71.3 3.13
Lc-7	68.2 3.70	72.0 3.60	72.8 3.08	75.3 5.98	78.9** 7.40***	69.6 3.33	-

$\overline{x}$ 67.3
4.31
LSD 5% 8.26
0.83

two traits: fructification coefficient and the average number of seeds per pod. For the two traits, the variances of additive and cytoplasmic genic actions, of non-additive genic interactions and cytoplasm x nucleus interactions were calculated. At the same time, the variances of genotype interactions x years, additive genic actions x years, non-additive genic actions x years, cytoplasmic actions x years, were calculated in order to establish the influence of environment conditions upon the investigated traits.

The analysis of genetic variances regarding the fructification coefficient is presented in table 3. At the level of genotypes, a very significant variance of additive genic actions and cytoplasmic actions (maternal) could be noticed first of all. Total variance (genotypes + years) was also very significant.

At the level of interactions genotypes x years, additive x years, non-additive x years, cytoplasm x years, nonsignificant low values were recorded. The fact that the variances of these interactions are not significant may be considered as an argument in favour of the genetic factors. Consequently, the fructification coefficient depends very much on the interactions between the style and the male gametophyte and less on the environmental conditions.

The great share of additive genic actions (435.7 in comparison with non-additive actions 57.6) at the genotype level proves the real possibilities of genetic breeding of alfalfa fructification coefficient, because the additive variance represents the variance value determinated by multiple genes with cummulative or additive effects and which are transmitted to offsprings, having the possibility to be fixed by recurrent selection.

The choice of the adequate breeding methods for a superior utilization of additive genic actions might lead to the creation of some synthetic types with a high potential of producing alfalfa seeds. The results presented in table 3, also confirm the possibility to exploit the cytoplasmic actions (s^2 = 123.0) demonstrating that the sense of crossing is important for alfalfa interfertility.

The analysis of genetic variances for the average number of seeds per pod is presented in table 3. Very significant variances for additive genic actions, cytoplasmic ones, non-additive genic interactions and nucleus-cytoplasm genic actions are evident. The data presented point out that the heredity of ave-rage number of seeds per pod is a very complex physiologic-genetic phenomenon produced by the genetic structure of parteners, the non-additive genic actions and the nucleus-cytoplasm interactions. As in the case of fructification coefficient, the action of environment is less significant. The weight of additive genic actions (s^2 – 19.0) and cytoplasmic actions (s^2 = 14.4) in comparison with non-additive interactions (s^2 – 0.59) should be noticed.

The priority exploitation of additive genic and cytoplasmic actions allows the selection of the most important lines with genetic structure favourable to the proposed purpose, in our case the increase of seed number per pod.

The phenotype expressiveness of interfertility is determined by the genetic structure of

Table 3. The genetic variances s^2 involved in alfalfa interfertility control

Source of variation	DF	Fructification coefficient				Average no of seeds/pod			
		SS	s^2	F values	Signif.	SS	s^2	F values	Signif.
Total (genotypes + years)	123	5403.65	43.93	2.15	***	200.48	1.62	18.39	***
Genotypes (6)	41	4694.33	114.49	5.62	***	199.74	4.87	54.98	***
Additive actions (A)	6	21614.21	435.70	21.39	***	114.00	19.00	214.45	***
Non-additive actions (NA)	14	807.21	57.65	2.83	**	8.33	0.59	62.72	***
Cytoplasmic actions (C)	6	758.22	123.03	6.04	***	86.73	14.45	163.15	***
Nucleus-cytoplasm interactions (NC)	15	534.68	35.64	1.75	*	8.48	0.57	7.12	***
Genotype x year interactions	82	709.31	8.65	0.42	NS	0.74	0.01	0.10	NS
A x years	12	111.55	9.29	0.45	NS	0.15	0.01	0.15	NS
NA x years	28	279.11	9.96	0.48	NS	0.31	0.01	0.12	NS
C x years	12	125.01	10.41	0.51	NS	0.19	0.01	0.18	NS
NC x years	30	193.63	6.45	0.31	NS	0.07	0.00	0.02	NS
Genetic error	82	1669.96	20.36			7.26	0.08		

*, **, *** – significant for: p<0.05, p<0.01 and p<0.001
NS – Non-significant

genotypes. For the genetic analysis of interfertility expressed by the fructification coefficient and the average number of seeds per pod, the mathematic pattern worked out by Cãbulea (1983) which was used for estimating the experimental results allows the quantification of the genic effects of parental forms regarding the weight of genic contribution to the achievement of interfertility and the clarification of the type of genic actions proper for the determinism of the characteristics involved in the process of interfertility.

The estimation of additive genic effects gives the possibility to appreciate the genetic value of inbred lines (parental forms) considering the general combining ability.The inbred lines with high general combining ability are highly important in alfalfa breeding activities, having the capacity to transmit uniformly the traits involved in the determination of interfertility. The values of additive genic effects of the 7 inbred lines and for the analysed traits are present in table 4.

The calculation of the genic effects determined by the additive genic actions offers the possibility to select the most valuable inbred lines for breeding the traits in the desired direction.

Regarding the fructification coefficient, the inbred line Lc-7 has has been especially remarked by its very high positive values of additive genic effects.

As for the average number of seeds per pod, the lines Lc-5 and Lc-4, with high values of additive genic effects (table 4), have been very well noted.

Considering the values of additive genic effects for the two analysed traits, the lines Lc-7, Lc-5 and Lc-4 are the most valuable parental forms of the diallel crossing system. These lines may lead to the development of new performing synthetic alfalfa lines, in hybrid combinations in which they take part.

Having high general combining ability, these lines increase the value of varieties, as seed yield is concerned.

Cytoplasms with significant genetic value are important in alfalfa breeding. These lines can be used mainly as maternal forms in creating hybrids with good behaviour regarding the increase of seed production.

Regarding the fructification coefficient, only the line Lc-1 is considered to have a high value of cytoplasmic effects, and for that reason is recommended to be used as a maternal form.

For the average number of seeds per pod, five out of the seven analysed lines present very high cytoplasmic genic effects (Table 4).

Lines Lc-2, Lc-6 and Lc-7 are especially remarkable and they are recommended to be used as maternal forms in hybrid combinations. Lines Lc-4 and Lc-5 having low cytoplasmic effects are recommended to be used only as paternal forms.

Consequently, the maternal effect is preponderant for seed production, a logic result because the maternal parent has a direct effect for the whole period of pod maturation unlike pollen which intervene only in the moment of fecundation. Cytoplasmic and additive genic effects allow the selection of the most valuable lines for the analysed traits and the most favou-

Table 4. Additive and cytoplasmic genic effects of parental forms for both fructification coefficient and average number of seeds per pod
Fundulea, 1997-1998

Parental lines	Additive genic effects		Cytoplasmic genic effects	
	Fructification coefficient	Average number of seeds per pod	Fructification coefficient	Average number of seeds per pod
Lc-1	-4.21^{ooo}	-0.58^{ooo}	3.19*	0.23***
Lc-2	-1.54	-0.33^{ooo}	1.82	0.71***
Lc-3	-4.65^{ooo}	-0.98^{ooo}	-1.75	0.38***
Lc-4	1.35	1.14***	-0.71	-0.85^{ooo}
Lc-5	2.08	1.60***	-2.82^{o}	-1.38^{ooo}
Lc-6	-1.91	-0.57^{ooo}	1.83	0.45***
Lc-7	8.98***	-0.28^{ooo}	1.55	0.44***
LSD 5%	2.65	0.08	2.65	0.08

rable position in hybrids can be established for each line used in the diallel system.

The non-additive genic effects reflect the specific combining ability of inbred lines determined by intraallelic interactions. The non-additive genic actions allow the assessment of the relationship degree between the two lines, the positive values showing an emphasized genetic differentiation and the negative values demonstrate that between the two parental forms there is a genetic similitude. It is the case of the combination between lines the Lc-2 and Lc-5, lines developed from Romanian cultivars Topaz and Sigma with low values of non-additive genic effects (-7.24). In a positive way, the combination between the lines Lc-2 and Lc-6 with significant positive value of the specific combining ability was remarked (Table 5).

The values of non-additive genic effects regarding the average number of seeds per pod emphasize few combinations with high positive values of specific combining ability: Lc-4 x Lc-5, Lc-1 x Lc-6, Lc-3 x Lc-7 (Table 5). Therefore, the number of seeds from a crossing depends on the female and male parent and also on a specific couple effect.

The values of cytoplasmic-nucleus genic interaction effects for the analysed traits are presented in table 5. As regards the fructification coefficient, low values were found and no combination was remarked. The values of cytoplasmic-nucleus interaction effects regar-ding the average number of seeds per pod emphasize a positive effect only in the case of combinations Lc-3 x Lc-7; Lc-3 x Lc-6.

The differences between direct and reciprocal hybrids could be attributed to cytoplasmic-nucleus interactions. The significant negative values and the non-significant ones being in a majority (Table 5), the result is that it is not the case to produce direct and reciprocal hybridization regarding analysed traits. It results that in the case of alfalfa, direct and reciprocal hybridization are not necessary.

CONCLUSIONS

The analysis of genetic variances for some features which contribute to obtain seeds yield (average number of seeds/pod, fructification coefficient emphasized that their genetic deter-

Table 5. Non-additive genic (sij) and nucleus – cytoplasm interaction effects (rij) for the both fructification coefficient and the average number of seeds per pod

Combination	Additive genic effects		Cytoplasmic genic effects	
	Fructification coefficient	Average number of seeds per pod	Fructification coefficient	Average number of seeds per pod
Lc-1 x Lc-2	-2.98	-0.52^{ooo}	-6.15	-0.12
Lc-1 x Lc-3	-2.27	-0.05	2.04	0.00
Lc-1 x Lc-4	-2.36	0.07	1.19	-0.14
Lc-1 x Lc-5	3.70	0.25**	2.58	0.00
Lc-1 x Lc-6	3.79	0.35***	-1.62	0.04
Lc-1 x Lc-7	0.08	0.03	-1.22	0.00
Lc-2 x Lc-3	2.34	0.24**	2.82	-0.14^{o}
Lc-2 x Lc-4	0.85	-0.19	-4.05	-0.21^{oo}
Lc-2 x Lc-5	-7.24^{oo}	0.16	-7.10^{oo}	-0.29^{ooo}
Lc-2 x Lc-6	6.11*	0.22	0.54	-0.11
Lc-2 x Lc-7	0.88	0.19*	-0.17	-0.09
Lc-3 x Lc-4	0.67	0.14	3.25	-0.55^{ooo}
Lc-3 x Lc-5	0.34	-0.82^{ooo}	3.44	-0.62^{ooo}
Lc-3 x Lc-6	-1.10	0.18*	-0.02	0.34***
Lc-3 x Lc-7	1.35	0.29**	-0.07	0.31***
Lc-4 x Lc-5	4.78	0.61***	1.14	0.31***
Lc-4 x Lc-6	-1.93	-0.24^{oo}	-0.38	0.03
Lc-4 x Lc-7	-0.65	-0.25^{oo}	0.34	0.24
Lc-5 x Lc-6	-3.39	-0.28^{oo}	0.81	0.03
Lc-5 x Lc-7	1.81	0.08	2.06	0.09
Lc-6 x Lc-7	-3.47	-0.24^{oo}	-2.51	-0.11
LSD 5%	5.31	0.17	4.60	0.14

minism is very complex.

The variances of cytoplasmic additive genic actions and of non-additive and nucleus - cytoplasm interactions were very high for the average number of seeds / pod.

For the fructification coefficient only the variances of cytoplasmic and additive genic actions recorded very significant values.

No significant values were recorded at the level of interactions for genotypes x years, additive x years, non-additive x years, cytoplasm x years.

Significant cytoplasmic genic effects were emphasized in five out of the seven studied lines, higher values being recorded in Lc 2 and Lc 7. Being used as maternal forms, these lines can contribute positively to the increase of alfalfa seeds yield.

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Table 1. Influence of aluminum ions, in reaction mixture, on the level of saccharasic activity in a reddish-brown soil fertilized with compost with different quantities (glucose+fructose-mg/100 g soil dw/24 hours)

A- Factor	B – Factor – COMPOST (t/ha)								Average (A)	
	b1-0	%	b2-0	%	b3-0	%	b4-0	%		%
a1–without Al^{3+}	b 3287	100	b 4028	100	b 2579	100	b 3472	100	b 3341	100
a2- with Al^{3+}	a 4228	129	a 5019	125	a 3472	135	a 4528	130	a 4312	129
Average (B)	3757 c		4523 a		3025 d		4000 b			

LD P	5%	1%	0,1%
A	291	673*	2143
B	101	142	201*
AB	302	628*	1799
BA	144*	201	284

Table 2. Influence of aluminum ions, in reaction mixture, on the level of saccharasic activity in a chernozem mineral fertilized or manured with farmyard compost (glucose+fructose-mg/100 g soil dw/24 hours)

A- Factor	B – Factor – COMPOST (t/ha)										Average (A)	
	b1-0	%	b2-$N_{32}P_{32}$	%	b3-$N_{94}P_{96}$	%	b4-$N_{128}P_{128}$	%	b5 compost	%		%
a1–without Al^{3+}	b 1564	100	b 1496	100	b 1459	100	b 1401	100	b 1732	100	b 1530	100
a2- with Al^{3+}	a 1686	108	a 1581	106	a 1684	115	a 1589	113	a 1864	108	a 1681	110
Average (B)	1625 b		1538 d		1571 c		1495 e		1798 a			

LD P	5%	1%	0,1%
A	7	17	54*
B	14	20	27*
AB	19	28	45*
BA	20*	28	39