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GENETIC ANALYSIS OF INHERITANCE AND MUTUAL RELATIONSHIPS AMONG YIELD COMPONENTS, MORPHOLOGICAL-BIOLOGICAL TRAITS AND YIELD OF GREEN MASS OF RED CLOVER (*TRIFOLIUM PRATENSE* L.)

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Diallel crosses involving five divergent red clover genotypes (two varieties, Junior and Diana, and three populations, Vlaška, BL-3 and M-11) were made at the Experiment Field of the Forage Crops Department of the Institute of Field and Vegetable Crops in Novi Sad. During the 2001-2002 period, progenies obtained in the crosses were tested for stem length, stem thickness, length of central lamina, yield of green mass per plant, start of flowering, persistence and growth habit. The analysis of phenotypic variance showed that the additive gene action played the dominant role in the inheritance of stem length, yield of green mass per plant and growth habit. Partial dominance was the most frequent mode of inheritance. A larger contribution of dominant variance

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to total genotypic variance and low values of narrow sense heritability (h_n^2) were obtained for stem thickness (16.67% and 23.07%, respectively). In both study years (2001-2002), using path coefficient analysis, we observed significant direct effects of stem length on green mass yield per plant (9.09*, and 6.23*, respectively).

Key words: red clover, (*Trifolium pratense* L.), diallel cross, mode of inheritance, combining ability, dominant and additive gene effects, heritability, path coefficient analysis

INTRODUCTION

Previous research on red clover has shown that the most important methods used in its breeding are mass selection, recurrent phenotypic selection and the polycross method, which limits the collection of useful genetic information such as heritability and genetic mapping (TAYLOR and QUESENBERRY, 1996). It is therefore especially important to perform detailed genetic analysis of major quantitative agronomic traits of this forage legume using controlled crosses. Diallel crossing is a very commonly used method for testing red clover progenies in order to obtain more comprehensive information on the combining abilities of parents, mode of inheritance and heritability. It is also particularly important to determine the interdependence of traits breeding is performed for.

MATERIAL AND METHODS

Based on previous studies, five divergent red clover genotypes were chosen (two cultivars, Junior and Diana, and three populations, Vlaška, BL-3 and M-11). The experimental part of our trial was carried out at the experiment field of the Forage Crops Department of the Institute of Field and Vegetable Crops in Novi Sad. First, complete diallel crosses were made during 1999-2000 among the selected red clover cultivars and populations under field conditions. Then, in 2001-2002, the progeny thus obtained was tested for stem length, stem thickness, central lamina length, green mass yield, start of flowering (UPOV, 2001), persistence (assessment of the number of surviving plants in the third year on a scale of 1 to 9), and growth habit (UPOV, 2001). The trial was set up using a randomized block design with three replicates. Each of the combinations studied was represented by a sample consisting of 30 plants. The row-to-row spacing was 80 x 80. The mode of inheritance of the traits studied was determined first based on parental and progeny means. The GCA and SCA were calculated according to GRIFFING (1956), Method 2, Mathematical Model 2, with no reciprocal crosses included (Table 1).

Tab 1. - Analysis of variance for general and specific combining abilities

Source of variation	Degrees of freedom	Sum of squares	Mean square	Expected mean squares
GCA	n-1	S_g	M_g	$V_e + V_s + (n + 2) V_g$
SCA	$\frac{1}{2}$ (n-1) n	S_s	M_s	$V_e + V_s$
E	M	S_e	M_e	V_e

The components of phenotypic variance were then calculated. (Table 1, Model II), FISHER, 1918; COCKERHAM, 1954. where:

$$V_g = \frac{1}{n+2}(M_g - M_s) - \text{GCA variance}$$

$V_s = M_s - M_e$ - SCA variance

$V_A = 4V_g$ - additive variance (for F = 0)

$V_D = 2V_s$ - dominant deviation variance (for F = 0)

$V_G = V_A + V_D$ - genotypic variance

V_E = environmental variance

$V_F = V_G + V_E$ - phenotypic variance

An estimate of heritability (h^2) in the broad ($h_b^2 = \frac{V_G}{V_F}$) and narrow senses ($h_n^2 = \frac{V_A}{V_F}$) was made.

The direct, indirect and total effects of the traits on green mass yield were determined by path coefficient analysis.

RESULTS

In the majority of the combinations, partial dominance was the most common mode of inheritance of most of the traits under study (stem length, green mass yield in both study years, start of flowering in 2002, growth habit) (pd - Table 2), indicating that additive gene action was of greater importance in the inheritance of these characteristics (Table 4). Analysis of variance revealed highly significant GCA values for the above traits (Table 3). A highly significant PCA value and greater importance of the dominant gene effect were found for the inheritance of start of flowering in 2001.

A greater contribution of dominant variance to total genotypic variance and low values of narrow sense heritability were obtained for the following traits: stem thickness in both study years, start of flowering in 2001, and central lamina length in 2002.

Equal importance of the additive and nonadditive components of genotypic variance and low values of both narrow and broad sense heritability (h_n^2 = 22.64%, h_b^2= 41.51 %) were observed for the inheritance of persistence, indicating that environmental factors played a greater role in the inheritance of this trait (Table 4). The most frequent mode of inheritance of this character was superdominance of the parent with better persistence (h^+ - Table 2).

Using path coefficient analysis, it was determined that in both study years (2001 and 2002) stem length had a significant direct effect on green mass yield per plant (9.09* and 6.23*, respectively - Table 5). In 2001, the effect was pronouncedly negative in itself (r = -0.141), but the overall effects were positive due to the positive indirect effects of stem length (r = 0.379) and growth habit (r = 0.164).

Table 2. - Mean values and mode of inheritance of stem length, stem thickness, central lamina length, green mass yield per plant, start of flowering, persistence and growth habit in 2001-2002

Parents and F1 hybrids (♀ x ♂)	Stem length ($\bar{x}$)		Stem thickness ($\bar{x}$)		Lamina length ($\bar{x}$)		Green mass yield ($\bar{x}$)		Start of flowering ($\bar{x}$)		Persistence ($\bar{x}$)	Growth habit
♀ x ♂	2001.	2002.	2001.	2002.	2001.	2002.	2001.	2002.	2001.	2002		($\bar{x}$)
Vlaška	55,8	45,4	3,94	4.05	34,65	34,69	158,00	578,33	4.00	3,33	4,00	5,33
Vlaška x Junior	55,7 d'	48,6 pd'	3,83 d'	4.29 h'	36,60 d*	34,13 d	190.33 pd'	763,67 pd'	4.67 d*	5,33 d*	3.00 h'	5,60 h*
Vlaška x Diana	53.5 pd*	47,5 d*	3,70 pd'	4.04 d*	37.64 h*	35.27 d*	160,00 pd'	638,00 pd	4.00 d*	5.67 pd*	4.50 i	5,20 pd*
Vlaška x BL-3	49,7 pd	48,0 h	3,74 pd	4.12 h*	36.77 h*	33,99 h	133,34 pd	691,00 pd*	3,67 h	6,33 h*	4.17 d*	5,03 h
Vlaška x M-11	54.1 pd'	47,0 pd*	4,20 h*	4.27 d'	38.55 pd*	37,12 pd'	224,67 h*	805,34 pd*	2.67 h'	3,00 h'	6,67 h*	6,50 pd'
Junior	56,6	53,4	3,86	4.12	36,67	36,62	205,67	858,67	4,67	5,33	5,00	5,10
Junior x Diana	50,9 pd	49,7 pd	3,74 pd	3.93 pd	34,32 h	35,12 d	167,33 d	854,67 pd*	4,33 pd*	5.33 d	4.67 h	5,75 h*
Junior x BL-3	51,7 pd'	47,6 d'	3,56 h'	3,98 d'	37,00 h*	36,45 d'	155,34 pd'	745,00 pd'	3,00 h'	4,67 d'	5,67 h*	5,40 h*
Junior x M-11	51,9 h'	49,6 h'	3,62 h'	4.32 h'	37,70 pd'	35,19 h	206,67 d'	835,67 h'	3,67 pd'	3,67 pd'	4,83 h'	6,24 pd
Diana	49,7	47,6	3,67	3.89	36,51	35,54	167,00	752,00	3,67	6,00	5,00	4,33
Diana x BL-3	48,9 i	48,5 h*	3,87 h*	4,08 h*	38,46 h*	35,62 d	151,00 pd*	847,34 h*	3,00 h	7,00 h*	5,67 h*	5,46 h*
Diana x M-11	47,6 h	47,4 d	3,51 h	4.25 pd*	34.48 h	36,56 pd	174,34 pd	760,00 pd	2,33 h	5,33 pd*	6,83 h*	5,83 pd
BL-3	48,1	47,8	3,68	4,01	35,69	37,18	122,34	698,67	5,00	4,67	4,17	5,30
BL-3 x M-11	51,3 pd*	49,5 pd'	3,65 h'	3,90 h	34,73 h	33,92 h'	204,34 pd*	718,34 pd	2,00 h	4,67 d*	7,00 h*	5,81 pd
M-11	53,5	50,1	4,04	4,28	40,10	40,88	218,00	839,67	3,33	3,33	5,67	7,89
	3,6	2,9	0,25	0,21	2,85	2,78	48,22	146,83	1,16	1,69	2,28	0,82
	4,8	4,0	0,33	0,28	3,84	3,75	65,05	198,09	1,56	2,29	3,08	1,10

Table 3. Analysis of variance of combining abilities for stem length, stem thickness, central lamina length, green mass yield per plant, start of flowering, persistence and growth habit in five parental genotypes of red clover

Source of variation	F experimental												F tabular	
	Stem length		Stem thickness		Lamina length		Green mass yield		Start of flowering		Persistence	Growth habit	0,05	0,01
	2001.	2002.	2001.	2002.	2001.	2002.	2001.	2002.	2001.	2002.				
GCA	14,72**	7,66**	6,35**	7,44**	1,43	4,65**	9,73**	6,49**	7,15**	8,82**	3.57*	23,31**	2,72	4,08
SCA	1,64	1,31	3,68**	2,91*	3,50**	2,81*	0,96	1,25	3,96**	2,08	1,48	2,09	2,19	3,04
GCA/SCA	8,97	5,85	1,72	2,56	0,40	1,65	9,76	5,19	1,80	4,24	2,41	11,15		

Table 4. Components of phenotypic variability and heritability for stem length, stem thickness, central lamina length, green mass yield per plant, start of flowering, persistence and growth habit

Components of variance	Stem length		Stem thickness		Lamina length		Green mass yield		Start of flowering		Persistence	Growth habit
	2001.	2002.	2001.	2002.	2001.	2002.	2001.	2002.	2001.	2002.		
V_A	11,36	3,88	0,012	0,012	-	0,96	1389,36	7692,40	0,28	1.32	0.72	0.96
V_D	1,96*	0,64	0,04	0,02	4,8	3,34	-	1294,38	0,94	0,74	0,60	0,18
V_G	13,32	4,52	0,052	0,032	4,8	4,3	1389,36	8986,78	1,22	2,06	1,32	1,14
V_E	4,57	3,20	0.02	0,02	2,90	2,77	831,45	7710,04	0,48	1,03	1,86	0,24
V_F	17,89	7,72	0,072	0,052	7,7	7,07	2220,81	16696,82	1,7	3,09	3,18	1,38
h_b^2	74,45 %	58,55 %	72.22 %	61,54 %	62,34 %	60,82 %	62,56 %	53,82 %	71,76 %	66,67 %	41,51 %	82,61 %
h_n^2	63,50 %	50,26 %	16,67 %	23,07 %	-	13,58 %	62,56 %	46,07 %	16,47 %	42,72 %	22,64 %	69,56 %

Table 5. Direct, indirect and total effects of the traits studied on green mass yield of red clover cultivars during 2001- 2002

Components of variance	Stem length		Stem thickness		Lamina length		Start of flowering		Persistence		Growth habit	
	2001.	2002.	2001.	2002.	2001.	2002.	2001.	2002.	2001.	2002.	2001.	2002.
VIA: Stem length	0,676	0.559	0.379	0,057	0,144	0,116	0,206	0,069	-0,201	0,034	0,125	0,102
VIA: Stem thickness	-0,079	0,021	-0,141	0,204	-0,073	0,068	-0,009	-0,076	0,001	-0,004	-0,064	0,122
VIA: Lamina length	0,014	0,023	0.034	0,037	0,066	0,111	-0.005	-0,046	0,005	0,035	0,032	0,073
VIA: Start of flowering	0,036	0.035	0.008	-0.105	-0,009	-0,118	0,118	0,282	-0.103	-0,050	-0.036	-0,181
VIA:Persist.	-0,176	0,012	-0,004	-0,004	0,044	0,064	-0,516	-0,036	0,591	0,203	0,212	0,073
VIA: Growth habit	0,066	0.039	0.164	0,130	0,175	0,142	-0.111	-0.139	0,129	0,078	0.360	0,217
Total effect	0,538	0,690	0,440	0,319	0,347	0,383	-0,318	0,054	0,422	0,296	0,628	0,405
F value	9,09*	6,23*	0,32	0,56	0,09	0,16	0,11	0,98	2,57	0,74	2,58	0,29

Critical values of F-distributions at 1 and 7 degrees of freedom and 5 and 1% probabilities are 5.59 and 12.25

DISCUSSION

Results obtained in this paper on the inheritance of green mass yield, start of flowering, and persistence are in agreement with those reported by several other authors (ANDERSON, 1960; ANDERSON, *et al.* 1974; CORNELIUS, *et al.* 1977; CORNELIUS and TAYLOR, 1980). Concerning the inheritance of growth habit, particularly prominent in the present paper was partial dominance, which is in agreement with the findings of MIRZAIE-NODOUSHAN *et al.* (1999). Studying the inheritance of red clover stem thickness at the third internode below the terminal inflorescence, MIRZAIE-NODOUSHAN *et al.* (1999) found that slenderer stems were partially to completely dominant over thicker ones. In the present paper, in agreement with the above, partial dominance or dominance of the parent with the slenderer stem was manifested as the mode of inheritance of stem thickness in four combinations in 2001 and two in 2002 (Table 2). It is interesting to note that in the first year of the study superdominance of the slenderer stem was particularly prominent.

In our study, in agreement with the data reported by CECCARELLI (1968), genotypic variance for red clover stem length was found to have been determined primarily by additive gene action.

A number of researchers (WIONCEK *et al.*, 1976; KUHBANCH and VOIGTLANDER, 1981) have found significant correlations betwen green forage yield and stem length in red clover, which suggests that selection for a longer stem may lead to increased green mass yields. Data obtained in the present paper support this conclusion, because our path coefficient analysis showed there were significant direct effects of stem length on green mass yield in both study years.

CONCLUSION

Our variance analysis of diallel crosses for GCA revealed years highly significant values of stem length, stem thickness, green mass yield per plant, start of flowering and all the morphological/biological traits in both study years. At the same time, highly significant SCA values were recorded for stem thickness, central lamina length and start of flowering in 2001.

Analysis of phenotypic variance showed additive gene action played the dominant role in the inheritance of stem length and green mass yield in both years of study. Relative to the morphological/biological characteristics studied, a dominant contribution of the additive component of genotypic variance was found in the inheritance of growth habit and start of flowering in 2002. In this connection, partial dominance was the most common mode of inheritance in most of the combinations. In the inheritance of persistence, the additive and nonadditive components of genotypic variance were both equally important. The larger contribution of dominant variance to total genotypic variance and low values of narrow sense heritability (h_n^2) were obtained for stem length in both years (16.67% and 23.07%), start of flowering in 2001 (16.47%), and central lamina length in 2002 (2.73 %). In both study years, path coefficient analysis revealed a significant direct effect of stem length on green mass yield per plant (9.09* and 6.23*, respectively).

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GENETIČKA ANALIZA NASLEĐIVANJA I MEĐUZAVISNOST KOMPONENTI PRINOSA, MORFOLOŠKO-BIOLOŠKIH KARAKTERISTIKA I PRINOSA ZELENE MASE CRVENE DETELINE (*TRIFOLIUM PRATENSE* L.)

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Izvod

U radu je korišćeno pet divergentnih genotipova crvene deteline (dve sorte: Junior, Diana i tri populacije: Vlaška, BL-3, M-11) koje su dialelno ukrštene. Eksperimentalni deo ogleda je bio urađen na oglednom polju Zavoda za krmno bilje Naučnog Instituta za ratarstvo i povrtarstvo u Novom Sadu. Tokom 2001 i 2002 godine izvršeno je testiranje dobijenog potomstva u odnosu na ispitivane osobine (dužina stabljike, debljina stabljike, dužina centralne liske, prinos zelene mase po biljci, vreme početka cvetanja, perzistentnost i forma rasta). Analizom fenotipske varijanse može se zaključiti da je aditivan način delovanja gena imao dominantnu ulogu u nasleđivanju: dužine stabljike, prinosa zelene mase po biljci, forme rasta, a kao najčešći način nasleđivanja ispoljila se parcijalna dominacija. Veće učešće dominantne varijanse u ukupnoj genotipskoj varijansi, kao i niske vrednosti heritabilnosti u užem smislu (h_n^2) su dobijene za: debljinu stabljike (16,67 % i 23,07 %). Tokom obe godine (2001-2002) analizom path koeficijenta utvrđen je značajan direktan efekat dužine stabljike na prinos zelene mase po biljci crvene deteline (9,09*, odnosno 6,23*).

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