INSTITUTUM AGRONOMICUM "Dr.PETRU GROZA" CLUJ-NAPOCA (ROMANIA)
NOTULAE BOTANICAE HORTI AGROBOTANICI 1987, XVII

V-LEAF MARKING IN A Trifolium ambiguum Bieb. GERMPLASM COLLECTION

A.T.SZABÓ Doina CHIOREAN

Abstract:

SZABÓ T.A., CHIOREAN D., 1987, V-leaf marking in a Trifolium ambiguum Bieb.germplasm collection. Not.bot.hort.agrobot., Cluj., XVII.,77 - 84. Frequency values have been registred in the neomorph multiple allele series of V-leaf marking in a germplasm collection of Caucasian (Kura) clover - Trifolium ambiguum Bieb. This collection, obtained from the Northwest Reg.Plant Intr.Stn., Geneva N.Y., USA, comprises 6o accessions from USSR, Australia, Iran and Turky. Allele frequencies have been registered by phenotype analysis of leaflet samples collected with modified double meter method (PUIA et al.1978). Neomorph alleles have been noted following BREWBAKER. The V^h type neomorph was the most common (48 % in average for the whole collection), followed by V^o (35,8 %) V^H (7,7 %), V^b and V^B (4,5 and o,2 %). Some of the accessions differed markedly in V-leaf mark frequencies: V^o was completly lacking in two accessions (0 %)(Table 1) but was common in other two (75 %). Clinal variation, along altitunial gradient, was also detectable. The majority of accessions were oligomorph, with just 2-3 V-leaf mark phenotypes per accession.

Key words: Trifolium ambiguum, Caucasian (Kura) clover,V-leaf marking, allele frequency, germplasm.

Address: Germplasm Resource Lab., Agronomy Institute, 3400 Cluj-Napoca, Str.Mănăştur 3, R.S.Romania.

Received: 1.IX.1986.

The V-leaf marking in true clovers is determined by a series of neomorph alleles. Such neomorphs commonly display their allelic product in diploid heterozygots (3,17,2o). In polyploids, such as

Trifolium repens L. and T.ambiguum Bieb. this clear simultaneous qualitative effect of neomorphs is furtherly complicated by oligo- or poliallelic situations, seasonal phenotypic plasticity, leaf form diversity etc.(25).

The V-leaf marking is characteristic only for a group of true clovers, but to this group belong the most important cultivated species (T.pratense, T.repens, T.subterraneaum, T.medium, and the recently domesticated T.ambiguum, too). Presumably the allelic series is not equally rich in all these species, but no comparative study has been found in the available literature so far. In white clover more than 1o neomorphs have been described and interpopulational differences detected (2,3,5,7).

The biological significance of V-leaf marking is still not clear. It was regarded as caused by "ghost genes" without evolutionary significance. There was some evidence for differential grazing of different phenotypes by rumen fistulated sheep: animals showed strong bias for particular marks, selecting plants with these marks with preference to others. Due to this apostatic selection - the grazing animal uses the leaf mark as search image - the commonest phenotypes are preferred(5). Grazing birds may also contribute to the maintainance of leaf mark polymorphism (11). V-leaf mark allele frequencies seems to be correlated, to some degree, with unidentified ecological factors along altitudinal and/or latitudinal gradients, somewhat similarly as DADAY found for cyanogenic loci (8). A clover canopy with fully marked leaves is perhaps more attractive for clover pollinators than one with no leaf marks at all. D.CHIOREAN noticed in polycross populations, a better tolerance for extreme climatic conditions in the case of V0 phenotypes.

Leaf-marking differences may be useful in cultivar identification, but clover breader tend to neglect this character (26). These alleles can be used also for the study of gene flow and the evolutionary dynamics of the genetic diversity (16,21).

Caucasian or Kura clover (T.ambiguum Bieb.) reached the western part of its areal perhaps in Transylvania (13, 14, 18,22). This species was cultivated first in USSR (1); it is regarded as a good meliferous, soil conservation and forage plant suited for adverse ecological conditions, drought, low temperatures, high altitude, grazing pressure etc. (9,1o,23,28). Germplasm research and evaluation have been carried out

in USSR, USA, Australia, New Zeeland, Czechoslovakia, Romania etc., mostly phenotypic diversity of agronomically important traits, chemical composition, phosphorus nutrition, germination, palatability, legume-grass mixtures, embryoculture and interspecific hybridation (especially with white clover), polyploidy, genotype evaluation, phenotypic diversity etc. (12,15,24,26: review in 4,9,1o,23).

Material and method

The accessions of the studied T.ambiguum germplasm collection have been collected mostly by W.H.SKRDLA, 1967 (Ames, Iowa) and D.R. DEWEY et A.T.PLUMER,1977 (Utah Univ.). A duplicate series of this collection was kindly forwarded to us in 1985 by Northeast Regional Plant Introduction Station, Geneva, NY.

The original collection sites of the accessions have been localized and the collection data identified using the volumes of USDA Plant Inventories (12,29). Samples have been collected mostly from USSR with some accessions from Australia (cultivars), Iran, and Turkey. The USSR collection covers Yalta, Crimea, Krasnodar, Stavropol district, Caucasus Mountains etc. The altitudinal range of identified accessions vary between 2oo m s.m. (Isabliiuc, Stavropol distr.) and 25oo m s.m. (Teberda, Mt.Caucasus).

Samples have been sown on June 6, 1986 in the experimental field Sapca Verde (Cluj-Napoca) in rows of 2 m long, with 6o cm between the rows, using about 5(seeds/meter. Seedlings were heavily selected by summer drought.

During the first evaluation year germination and seedling vigour, seedling density, plant hight, coverage, V-leaf marking, flowering time, disease, green mars production was noted.

The V-leaf marking was registered according to BREWBAKER;this notation fitted well with our field files. Frequency values have been sampled according to a modified version of the double-meter method (PUIA et al.1978): 2o touch-points have been analyzed for each accession and sampling was repeated after a period. The percentage value of a given V-leaf mark type - considered as the frequency of the corresponding neomorph - has been calculated according to:

$$v^x = \frac{x \cdot 1oo}{n}$$

where V^x = % value of the leaf-mark type, x = the number of leaflets with this type found in the sample, n = the total number of leaflets in the given sample.

The coverage values for the accessions have also been calculated: 2 x 2o = 4o leaflets per sample have been considered as loo %.

Results

The results of the study are included in Table 1. Plant coverage in different accessions varied between 25 % and loo %, with a mean value for the 6o accesions of 79,1 % (9o days after sowing).

The accessions differed markedly in V-leaf mark allele frequencies. In two accessions - one from Caucausus, and one from Stavropol district -- all leaflets from the sample were marked. In 13 accessions 5o % or less leaflets were without V-leaf marks; the average value for the V^0-leaf mark allele calculated for all the accessions, was 35,8 %. Continuous low marks (V^h) were most frequent (48,5 % in average), but differences between the individual accessions were great (15 % - 84 %). The third most common mark was that of continuous high (V^H) with an average value of 7,7 %. The interrupted low, interrupted high, full low, median etc. phenotypes were rare.

Correlation was detectable between the frequency of no mark ($V^?$) phenotypes and the altitude of original collection in the case of about 2? accessions (fig.1), somewhat similarly as in the case of white clover or in the case of cyanogenic loci (DADAY, 1954; SZABÓ, unpubl.). In a group of 12 accessions low correlation was found, due perhaps to very different causes such as overlapping altitude - latitude - habitat effects, sampling, conservation and replication procedures in gene banks, data management etc.

In the first group (a) the correlation coefficient between V^0 allele frequency and altitude is positive and high (r = +0,72) and the value of altitudinal effects on this character is 51,8 %. In the second group (m) the correlation is much weaker (r=+0,42). Due to the heterogenity of the studied collection caused perhaps by evolutionary differentiation, but also by collection and sampling, the correlation was low for the whole collection (r = +0,11) and only 10,7 % of allele frequency variations may be attributed to altitudinal effects.

V-LEAF MARKING IN A TRIFOLIUM AMBIGUUM BIEB. GERMPLASM RESOURCE COLLECTION
Cluj-Napoca, Sapca Verde, 1986 (year I.)

Nr.	ORIGIN Country, district	US PLANT INTRODUCTION (PI) Nr.	ALT. s.m.	Gene symbols (V-leaf mark) V^A	V^a	V^h	V^b	V^c	$V^{(1)}$	V^{h-p}	F
01	Turky	206482		65	28	07					
02	Turky	206483		10	80	50		10			
03	Australia	225824		50	10	17		14			
04	Australia	225828		75	25						
05	Australia	225829		10	75	06					
06	Iran	228370		67	33						
07	Iran	229624		55	45						
08	Iran	229629		73	18		09				
09	Australia	231890		20	60	17	03				
10	Australia	231891		67	33						
11	Turky	238154		35	55	10					
12	USSR	258787		42	50		08				
13	USSR	258788		29	65	06					
14	USSR	277535		19	75	06		06			
15	Iran	277865		75	25						
16	USSR, Yalta	325484	1080	44	45	12					
17	USSR, Mt.Trebeda	325486		31	58	31					
18	USSR, Klulkchov	325487	1530	42	42	08		08			
19	USSR, Goralkov	325488	1440	21	79						
20	USSR, Yalta, hort.	325489	600	27	60	13					
21	USSR, Mihailovk.	325490		35	47	18					
22	USSR, Crimea	369329		42	42	06					
23	USSR, Krasnodar	405119		42	37	16	05				
24	USSR, "	405120		37	63						
25	USSR, Stavropol	405121	500	20	45	15					
26	USSR, "	405122		40	50	10					
27	USSR, Crimea	405123		10	68		11				
28	USSR, "	405124		22	55	23					
29	Australia, cv.SUMMIT	405934		47	47	06					
30	Australia, cv.TREELINE	405935		65	35						
31	URSS, Stavropol	440671		0	75	15	05			05	
32	URSR, Cherkisia	440672	500	29	71						
33	USSR, Borsho(Cauc.)	440673	700	50	30		20				
34	USSR, Nalchak "	440675	500	40	53	07					
35	USSR, Uryba "	440676	750	43	43	14					
36	USSR, Daut.Chal. "	440677	1900	22	35		28				
37	USSR, Obe.Mont. "	440678	2000	37	47		12				
38	USSR, Caucaz	440679	1330	05	84	05	06				
39	USSR, Mt.Elbruz "	440680	1300	20	75	05					
40	USSR, Stavropol	440681	600	43	29	07	21				
41	USSR, Maryki (Cauc.)	440682	1600	26	74						
42	USSR, Patshikova "	440683	2000	27	53	13					
43	USSR, Mt.Elbruz "	440684	1800	26	52		22				
44	USSR, Mt.Teberda	440685	1330	38	25		16				
45	USSR, Aksont "	440686	1200	27	40		07				
46	USSR, Mt. Teberda "	440687	1500	28	59		12		11		
47	USSR, " " "	440688	2200	55	45						
48	USSR, " " "	440689	2500	60	25	15					
49	USSR, Arkhish "	440690	1700	25	45	05	10				
50	USSR, " "	440691	1500	20	60		20				
51	USSR, Cherkessya "	440694	2050	35	60	05					
52	USSR, Mt.Teberda "	440695	1100	11	55	11	22				
53	USSR, Strizhamat	440696	800	0	78	17	05				
54	USSR, Stavropol	440697	510	31	55		14				
55	USSR, "	440698	550	50	35	15					
56	USSR, Svetlograd	440699	300	36	64						
57	USSR, Stv.Esenturi	440700	700	45	55	20	05				
58	USSR, Mt.Trebeda	440701	2350	35	45	20					
59	USSR, Cherkessya	440702	1700	10	85	05	10				
60	USSR, Kabardino	440703	1800	24	76	10	14				

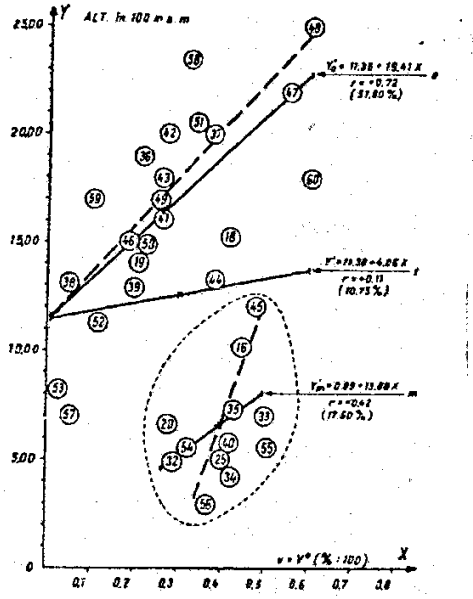

Fig. 1. The diagram of the distribution of $v = V^0$ clover leaf mark allele in the Trifolium ambiguum Bieb. collection in correlation with the original collection altitude. Linear regression, correlation coefficient (r) and the altitudinal determinism (% values in brackets) have been calculated separately for two, apparently distinct data sets (a, m) and the total (t). Dotted regression lines have been estimated by eye.

References

1. BORBOV G. - 1950, Species of clover new to cultivation (In Russian), Acad.Sci.URSS, Moscow.

2. BREWBAKER J.L. - 1955, V-leaf marking of white clover. Journ. Hered., 46, 13: 115-123.

3. BREWBAKER J.L. - 1967, Angewandte Genetik. Die genetische Grundlagen der Pflanzen- und Tierzüchtung. Fischer Vg.,Stuttgart.

4. BRYANT W.G. - 1974, Caucasian Clover (Trifolium ambiguum Bieb.)- a review.Journ.Austr.Inst.Agr.Sci., 40, 11-19.

5. CAHN M.A., HARPER J.L.- 1976, The biology of leaf mark polymorphism in Trifolium repens L. 1. Distribution of phenotypes on a local scale; 2. Evidence for selection of leaf marks on white clover (Trifolium repens L.) by rumen fistulated sheep. Heredity, 37: 309-333.

6. CARNAHAM H.L., HULL H.D., HANSON A.A., BROWN K.G. - 1955, Inheritance and frequencies of leaf markings in white clover. Journ.of Heredety, 46, 3: 109-114.

7. CORKILL L.- 1971, Leaf markings in white clover. Journ.of Heredity, 62, 3: 307-310.

8. DADAY H. - 1954, Gene frequencies in wild populations of Trifolium repens L. II. Distribution by altitude. Heredity, 8, 61-78.

9. DEAR B.S., ZORIN M. - 1985, Persistence and productivity of Trifolium ambiguum M.Bieb. (Caucasian clover) in a high altitude region of south-eastern Australia. Aust.J.Exp.Agric. 25, 124-132.

10. DUKE J.A. - 1981, Handbook of Legumes of World Economic Importance. Plenum Press, New York - London.

11. HARPER J.L. - 1977, Population Biology of Plants. Acad.Press, London etc.

12. HYLAND H.L. - 1970-1978, Plant Inventory, USDA, NRARC-West, Beltsville, Maryland, No.176, 180, 183, 184.

13. JANKA V. - 1853, Linnea, 30: 563 (ap.NYÁRÁDY).

14. JORDANOV D. (Ed.) - 1976, Flora R.P.Bulgaricae, Ed.Acad.Sci.Bulg., Sofija.Vol.IV. 332-411: Trifolium L. by St.KODZUHAROV.

15. KANNENBERG L.W., ELLIOT F.C. - 1962, Ploidy in Trifolium ambiguum in relation to some morphological and physiological characters. Crop Sci., 2, 378-381.

16. MANI G.S. (Ed.) - 1984, Evolutionary Dynamics of Genetic Diversity. Springer, Berlin etc.

17. MULLER H.J. - 1932, Further studies on the nature and causes of gene mutations. Proc.6th Int.Congr.Genet. 1:213 (ap. RIEGER et al.).

18. NYÁRÁDY A. - 1957, Trifolium L. In Flora R.P.Române, V, 176.

19. PUIA I., PÁZMÁNY D., TIRNAVEANU I., SZABÓ T.A., CHIRCA E. - 1978, Utilisation de la methode "double-metre" dans l'etude des prairies de Secuieu (Monts Apuseni). Not.bot.hort. agrobot., Cluj., 9: 97-103.

20. RIEGER R., MICHAELIS A., GREEN M.M., 1976, Glossary of Genetics and Cytogenetics. Classical and Molecular. G. Fischer, Jena.

21. SLATKIN M. - 1985, Rare alleles as indicators of gene flow. Evolution, Lawrence, 39, 1:53-65.

22. SOÓ R., PRISZTER SZ. - 1966, 1985, Synopsis systematico-geobotanica florae vegetationisquae Hungariae, II, VII, Akadémiai K., Budapest.

23. SPEER G.S., ALLINSON D.W. - 1985, Kura clover (Trifolium ambiguum): legume for forage and soil conservation. Economic Botany, 39, 2: 165-176.

24. STEWART A.V. - 1979, Genotypic evaluation of Trifolium ambiguum M.Ag.Sc.Thesis, Univ.Cantebury, Christchurch (ap.DEAR et ZORIN).

25. SZABÓ T.A. - 198o, Phenotypical variability of Trifolium repens populations collected in Romania. Bul.Inst.Agr.Cluj., Ser.Agr., 34: 35-42.

26. TAYLOR N.L. (Ed.) - 1985, Clover Science and Technology.ASA,Madison.

27. TOWNSEND C.E. - 197o, Phenotypic diversity for agronomic characters and frequency of self compatible plants in Trifolium ambiguum. Canad.Journ.Plant.Sci., 5o, 331-338.

28. VACEK V,, ROD J. - 1957, Perennial clover Trifolium ambiguum Bieb. Herb.Abstr.nr.1385 (ap.SPEER et al,).

29. WHITE G.A. - 1983, Plant Inventory No.188, Part.II., Plant Material Introduced July 1 to December 31, 198o (Nos 443o14 - to 452o71). USDA, ARS, Beltsville, ARC, Maryland.

Rezumat

SZABÓ T.A., CHIOREAN D.,1987,Marcajul folier într-o colecţie de germoplasmă de Trifolium ambiguum Bieb. Not. bot. hort. agrobot.,XVII,77-84.

A fost studiat marcajul foliar V la Trifolium ambiguum Bieb. într-o colecţie obţinută de la Northwest Reg. Plant Int. Station Geneva, USA şi cultivată la Sapca Verde, Cluj-Napoca. Nomenclatura folosită pentru alele din seria neomorfă multiplă a fost cea adoptată pentru specia înrudită, T. repens L. Probele au fost recoltate prin metoda dublului metru. Fenotipul cel mai frecvent a fost cea determinată de alela V^H (48 %), urmată de fenotipuri fără marcaje foliare, determinate de v =V^o (35,8%). Intr-un grup distinct de proveniența (Fig. 1, a) s-a putut evidenţia o corelaţie pozitivă relativ ridicată între frecvenţa alelelor v şi altitudinea (r=+0,72). Pentru întreaga colecţie însă corelaţia a fost mult mai slabă (r=+0,11) frecvenţa alelelor v fiind influenţată evident de alţi factori cum ar fi diferenţiere evolutivă, nişe ecologice specifice, metode de colectare şi manipularea datelor de germoplasmă metodologia folosită pentru luarea probelor etc.

INSTITUTUM AGRONOMICUM " Dr. PETRU GROZA " CLUJ-NAPOCA(ROMANIA)
NOTULAE BOTANICAE HORTI AGROBOTANICI 1987, XVII

PLANTES-HOTES POUR DES INSECTES SEMINIPHAGES IV. (Brassicaceae, Campanulaceae, Caryophyllaceae, Euphorbiaceae, Geraniaceae, Resedaceae et Scrophulariaceae)

T. PERJU, I. MOLDOVAN

Abstract:

PERJU T., MOLDOVAN I., 1987, Host-plants for seminiphagous insects.IV. (Brassicaceae, Campanulaceae, Caryophyllaceae, Euphorbiaceae, Geraniaceae, Resedaceae and Scrophulariacea). Not. bot. hort. agrobot. Cluj., XVII, 85-91.

During the period 197o-1984 inflorescences with fruits and seeds have been collected, from 14 host-plants of several botanical families and the species of insectes fee - ding on their flowers, fruits and seeds have been identified.

The species of seminiphagous have been for the first time obteined in our country by the method of pure cultures in laboratory conditions.

The following species of seminiphagous have been identified in the fruits and seeds of these cultivated or spon - taneous species of host-plants: Gymnetron campanullae L. Sibinia pellucens Scop., Ceuthorrynchus assimilis Payk., Gymnetron anthyrrhinii Payk., G. netum L., Systole euphor - biae Zer.

Among the identified seminiphagous species, Gymnetron campanullae L., Systole euphorbiae Zer., (Eurytomidae) are new in the fauna of our contry.

Key words: seminiphagous species.

Address: Agricultural Institut of Cluj-Napoca, Departement of Entomology, Str. Mănăştur 3 , Cluj-Napoca. R.S.ROMANIA

Received: 6.V.1986