Theoretical and Applied Genetics 43, 351–358 (1973)

Cytogenetic Studies in *Trifolium* Spp. Related to Berseem

I. Intra- and Interspecific Hybrid Seed Formation *

E. Putiyevsky and **J. Katznelson**

Division of Pasture and Forage Crops, Agricultural Research Organization, Newe Ya'ar Experiment Station, P.O. Haifa (Israel)

Summary. Seed formation by large-scale hybridization within and between *Trifolium* species related to *T. alexandrinum* L., was studied. The twelve species studied were: a. *T. alexandrinum* L., b. *T. berytheum* Boiss., c. *T. salmoneum* Mout., d. *T. apertum* Bobr., e. *T. meironense* Zoh. et Lern., f. *T. echinatum* M.B., g. *T. latinum* Seb., h. *T. carmeli* Boiss., i. *T. scutatum* Boiss., j. *T. plebeium* Boiss., k. *T. vavilovi* Eig. and l. *T. constantinopolitanum* Ser. Hybridization was done either by emasculation and fertilization by hand, or in nature, by utilizing the existence of natural stands and to serve as pollen source the self-incompatibility of some of the species concerned. Results of the two methods were highly comparable although seedset was much higher when crossing was done manually.

Crossability, as estimated by seedset, varied in specific cross combinations and ranged between 0 and 70%. Based on the pattern of crossability, five crossability groups were identified with > 20% seedset in interspecific—intra-group cross combinations, and usually less than 5% in inter-group cross combinations. Species a, b, c, d and e form the first crossability group; f, g and h form the second one; h, i, j belong to the third crossability group, while k and l appear as unispecific fourth and fifth crossability groups. The high level of seedset in interspecific hybridization is discussed.

Introduction

The genus *Trifolium* is one of the largest in the plant kingdom (Good, 1953), and has contributed more to the list of world cultivated plants than any other genus. Taxonomically, the genus is somewhat complicated. Several past and present authors (Presl, 1832; Bobrov, 1967; Hossain, 1961) raised various superspecific taxa to generic or subgeneric levels, while others (Gibelli and Belli, 1889; 1890 to 1893; Hermann, 1938; Bobrov, 1947; Zohary, 1969) grouped clover species into sections or subsections, with conflicting interpretations as to the nature and composition of these taxa. In addition, many of the species are ill-defined, leading often to erroneous conclusions in definition and nomenclature.

The prevalence of differing viewpoints regarding *Trifolium* taxonomy can also be attributed to the lack of biosystematical studies. Such studies have been confined mainly to the two cultivated species, *T. repens* (Chen and Gibson, 1970; Brewbaker and Keim, 1953, Pandey, 1957; Keim, 1953; Evans, 1962a) and *T. pratense* (Newton *et al.*, 1970; Taylor, 1959; Taylor *et al.*, 1963). These two species have been crossed by numerous other clover species, often belonging to remote sections, or even to another subgenus.

Berseem (*T. alexandrinum*) was included in some of the above mentioned studies (Evans, 1962a; Müller, 1960). Crosses with *T. repens* did not produce any viable seeds (Evans, 1962). Trimble and Hovin (1960) obtained 52 seeds out of 100 florets of *T. repens* × *T. alexandrinum*, but as all seedlings had died, they doubted their hybrid origin. Abdul Tawab (1968) crossed *T. alexandrinum* with *T. campestre* Schreb. and with *T. pratense* L., without obtaining any seed.

To our knowledge, no other plant species related to berseem (Oppenheimer, 1959) have ever been included in any biosystematic study. These related wild clovers, most of them E. Mediterranean, are a possible source of germ plasm for the improvement of berseem, as has already been suggested by Oppenheimer (1961). Many of these species have been regarded as synonyms of *T. alexandrinum*, whilst in others, morphological distinction between species can be made only after flowering.

It was felt that a better knowledge of the cytogenetics of this group of related species may solve some taxonomical and evolutionary problems and would facilitate their utilization in agriculture.

This paper deals with crossability between the twelve species studied and is the first of a series of publications intended to clarify some of the biosystematic relationships between the species.

Materials and Methods

a. *Parent Plants*

Our concept of taxonomy and nomenclature generally accords with that of Zohary (1969), except for the two species *T. carmeli* and *T. latinum*. As regards the former,

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* Publication of the Agricultural Research Organization No. 2195-E. The work reported here was financed by Grant FG-IS-222 from the U.S. Department of Agriculture under P.L. 480.

we felt that *T. carmeli* deserved a specific rank. The identity of *T. latinum* was not sufficiently clear, as none of the plant material that we studied fully agreed with Zohary's description; each plant possessed only one or two of the three main characteristics which distinguish this species from *T. echinatum*.

Altogether, 46 populations from twelve species of *Trifolium* were used for hybridization (Table 1). Except

Table 1. *Origin of the Trifolium species incorporated in the hybridization programme*

Species	Abbreviation	NYT number	No. of populations	Country of origin
T. alexandrinum L.	T. ale	2:		Israel (wild)
		8: 11: 16: 18:	5	Israel+Portugal (cultivars)
T. apertum Bobr.	T. apr	1471: 1772:	2	Turkey (Kurdistan)
T. berytheum Boiss.	T. ber	205: 206: 245: 252: 529:	5	Israel (North)
T. carmeli Boiss.	T. car	210: 211: 251: 266:	4	Israel (North)
T. constantinopolitanum Ser.	T. con	263	1	Israel (North)
T. echinatum M.B.	T. ech	218: 219: 220		Yugoslavia (South)
		222: 225: 226: 228:		Greece (North)
		1401: 1407: 1412: 1425: 1428:		Turkey (Kurdistan)
		1435: 1440: 1441:		Turkey (Thrace)
		1468: 1469:	17	Turkey (South-West)
T. latinum Seb.	T. lat	1451: 1497:	2	Turkey (Kurdistan)
T. meironensis Zoh. et Lern.	T. mei	230: 1485:	2	Turkey (South)
T. plebeium Boiss.	T. ple	235: 239: 270: 271:	4	Israel (North)
T. salmoneum Mout.	T. sal	260:	1	Israel (North)
T. scutatum Boiss.	T. scu	237:	1	Israel (North)
T. vavilovi Eig.	T. vav	254:	1	Israel (North)

Table 2. *Hand and Natural Hybridization according to species*. The number of flowers*

Species		T. ale	T. ber	T. sal	T. apr	T. mei	T. ech
T. ale	H	238(99+0)	146(5+3)	100(5+5)	126(8+2)	154(34+1)	143(3+1)
	N						
T. ber	H	296(81+0)	369(69+1)	316(132+2)	216(70+1)	93(20+2)	135(2+3)
	N						
T. sal	H	78(44+0)	129(73+2)	45(15+0)	81(48+1)	79(26+0)	116(33+5)
	N						
T. apr	H	130(24+3)	240(40+8)	81(35+0)	66(20+0)	119(22+5)	179(0+2)
	N						
T. mei	H	122(24+2)	99(33+1)	72(7+3)	243(26+14)	64(14+8)	131(5+3)
	N						
T. ech	H	212(2+2)	134(1+2)	83(1+1)	156(0+4)	86(2+1)	2763(204+117)
	N		3176(0+0)				
T. lat	H	130(0+0)	149(3+2)	81(1+0)	126(9+2)	97(0+0)	253(72+10)
	N						
T. car	H	401(2+0)	729(0+0)	146(0+0)	140(3+0)	169(0+0)	198(31+11)
	N		3250(112+0)				
T. ple	H	117(8+3)	136(4+3)	65(0+0)	105(0+8)	148(4+3)	127(6+1)
	N						
T. scu	H	313(6+0)	211(8+3)	58(5+1)	65(0+2)	201(10+9)	95(26+2)
	N						
T. vav	H	74(1+7)	148(2+18)	83(9+6)	80(0+12)	143(0+48)	90(0+1)
	N						
T. con	H	152(0+0)	101(0+0)	100(0+0)	48(0+0)	157(0+0)	70(0+0)
	N	571(0+0)	340(0+0)				
Total	H	2263(291+17)	2591(238+44)	1230(210+18)	1452(184+46)	1510(132+77)	4300(382+156)
	N	571(0+0)	6766(112+0)				

* H — Hand Hybridization — N — Natural Hybridization

** The figures represent the number of flowers emasculated and pollinated in the specific combination. In parentheses are for *T. alexandrinum*, all parent plants were raised from original seed collected in Yugoslavia, Greece, Turkey and Israel from natural stands. To avoid specific cases of self-incompatibility (Pandey, 1957) several plants from each population were used.

In addition, natural populations of *T. carmeli*, *T. berytheum*, *T. vavilovi*, *T. scutatum* and *T. plebeium* were used as pollen donors in the "Natural Hybridization Programme" (Katznelson, 1971).

Seeds of parents were germinated on petri dishes after scarification. Seedlings were transplanted to containers, filled with 3 kg soil and kept in a glasshouse or in a shed-house.

b. Hybridization Programme

Crossings were done within a population, between populations of the same species (intraspecific) and between species (interspecific). Those within population were performed in order to check the methods of emasculation and crossing employed throughout the study.

Hybrid seeds were obtained by two methods:

1. By a "Natural Hybridization Programme" (NHP) (Katznelson, 1971) that utilizes the existence of natural populations of several species in the north of Israel and the self-incompatibility of the wild species concerned (Putiyevsky and Katznelson, 1970). At the start of flowering in natural stands of wild clovers, single plants of various origins, grown in 3 kg pots and just starting to flower, were distributed into these stands. The pots were spaced at least 2 km apart from each other, and watered every 5—7 days. Fertilization was done by bees present in the area. On completion of flowering, the potted clovers were removed and when dead, the total number of flowers, and the number of normal and plump seeds were determined.
2. By hand hybridization (HH), i.e., emasculation and pollination by hand. The emasculation was done by removal of the ten anthers 1—2 days before their dehiscence. One or two whorls, with 7—15 flowers, were emasculated on each head; the rest of the head was untouched, but no seeds formed in the upper parts of any of the heads, except occasionally in *T. alexandrinum*. The heads were then bagged by 3 × 5 cm paper envelopes. The emasculated flowers were copiously pollinated twice, one and two days after emasculation. When several populations were available per species (Table 1) each of them served for a specific cross combination.

The study was performed at the Newe Ya'ar Experiment Station, Israel.

Results and Discussion

During 1966—71, some 162,000 flowers were crossed in NHP and 24,710 by HH, both methods yielding many thousands of hybrid seeds.

Pollen sources in NHP sometimes consisted of mixed pollen derived from 2—3 sympatric species, like T. vav + T. car, T. ber + T. car, T. vav + + T. ber or T. vav + T. car + T. ber, (see Table 1 for abbreviations). Either no hybrid seeds were formed from these pollinations, lending support to previous data, or, in case of seed formation, the findings were ambiguous. Therefore, those results of NHP involving more than one pollen species were excluded, leaving only some 142,000 florets for study.

Seedset was much higher in intraspecific combinations than in interspecific ones in both methods, and

*pollinated and the number of seeds obtained in each cross combination***

T. lat	T. car	T. ple	T. scu	T. vav	T. con	Total
198(14+11)	363(33+15)	126(6+0)	109(0+1)	63(0+0) 1716(30+100)	112(1+0)	1878(208+39) 1716(30+100)
148(16+4)	575(22+9) 77(8+2)	243(8+5)	245(5+7)	210(0+2) 4290(299+0)	162(1+1)	3008(426+37) 4367(307+2)
76(32+2)	103(18+0)	98(16+4)	96(3+15)	93(7+5) 4483(85+2)	67(0+0)	1061(315+34) 4483(85+2)
114(2+5)	149(0+0)	152(1+8)	139(3+1) 2417(0+0)	98(2+2)	106(0+0)	1573(149+34) 2417(0+0)
83(4+2)	260(5+16)	117(1+3)	63(0+1) 391(0+16)	148(17+5)	160(7+10)	1562(143+68) 391(0+16)
528(102+55)	341(62+16) 14793(594+324)	99(0+3)	93(0+1) 2033(0+0)	94(0+0)	191(4+16)	4780(378+218) 20002(594+324)
75(20+5)	397(177+28) 7243(1183+0)	126(3+7)	117(4+5)	72(0+0) 6556(7+0)	76(0+1)	1699(289+60) 13799(1190+0)
324(123+15)	512(258+12) 12004(1686+0)	218(6+1)	164(14+6)	317(1+1) 35107(56+0)	199(0+42)	3517(438+88) 50361(1854+0)
105(2+7)	191(44+14) 287(7+0)	175(76+5)	162(57+1)	101(0+0) 10535(12+10)	140(4+27)	1572(205+72) 10822(19+10)
84(8+4)	169(33+22) 3100(290+132)	99(70+0)	59(2+2)	99(0+0) 6347(17+66)	105(0+18)	1558(168+63) 9447(307+198)
56(5+4)	292(3+20) 1202(5+56)	162(1+5)	70(1+5)	33(23+2) 6201(2364+126)	85(0+0)	1316(45+128) 7403(2369+182)
59(0+0)	171(4+1) 2491(32+0)	111(0+0)	110(0+0)	90(1+0) 14411(31+0)	17(6+0)	1186(11+1) 17813(63+0)
1850(328+114)	3523(659+153) 41197(3805+451)	1726(188+41)	1427(89+45) 4841(0+16)	1418(51+17) 89646(2901+304)	1420(23+115)	24710(2775+843) 142021(6818+834)

given the number of plump seeds (left) and shrunken seeds (right).

especially in NHP. This can be seen in Table 2, showing the results of the crossing programme according to species combinations, and presenting the number of flowers pollinated and seeds obtained. On the average, seedset in intraspecific combinations, excluding *T. echinatum*, was 38% and 23% for HH and NHP respectively, while the corresponding results for interspecific hybrid combinations were 15% and 3%.

In spite of the natural pollination and fertilization in NHP, seedset was lower than that obtained by hand. This was so because plants have a lower seedset towards the end of their lives than in their prime. Whereas HH plants were not crossed late in the season, NHP plants were left in the field until their death and heads were not selected.

1. *Intraspecific Combinations*

The results in crossability within species were rather variable. There are three main reasons for this.

a) Generally, seedset was considerably higher in crosses within populations (37%) than between populations (18%). However, when *T. echinatum*, with 11% seedset in crosses between populations is excluded, the seedset in intraspecific crosses within the remaining species rises to 38%, i.e., to a level similar to that of within-populations combinations (Table 3, top part).

b) Whereas parental species and even populations differ in their ability to form seeds when emasculated, regardless of pollen source, they did not make the same proportional contribution to intra- *vs.* interspecific combinations. Four species were represented in the crossing programme by only one population, thus no intraspecific inter-population crosses could be performed on them.

c) Self-incompatibility may cause a decrease in seedset when very few plants are used. The figures for *T. scutatum* (6.8% seedset in intraspecific combination, and over 70% when crossed with *T. plebeium*) may be explained by the fact that only two *T. scutatum* plants were used for intraspecific crossing.

2. *Interspecific Combinations*

Of 20,294 flowers crossed by HH, 2,677 seeds were obtained (13%) (Tables 2, 3). By NHP, seedset was much lower — only 3,476 seeds from 124,816 flowers (2.8%) (Tables 2, 4).

Seedset varies in different combinations; sometimes it was as high as in intraspecific crossings, up to 70%, while other combinations were very low, down to zero. This variability was of two kinds: that of populations of the same species behaving differently as to crossability, and that of interrelationship between species, based on species averages. While the former may indicate some intraspecific hetero-

Table 3. *Seedset by hand hybridization*

Cross	Number of floret crosses	No. of seeds			Seedset %	
		Normal	Shrunken	Total	Total	Normal only
Intra-population	740	257	20	277	37	35
Intraspecific — total	3766	549	132	681	18	15
— without — *T. echinatum*	1022	363	23	386	38	35
— *T. echinatum*	2654	186	109	295	11	7
Interspecific — total	20294	1969	691	2677	13	10
— Group I	2920	757	54	811	28	26
— Group II	2041	567	135	702	34	28
— Group III	1003	224	44	268	27	22
— Between Groups I—V	14330	421	458	879	6	3
Total	24710	2775	843	3618	15	11

Table 4. *Summary of natural hybridization (NHP)*

Cross	Number of floret crosses	No. of seeds			Seedset %	
		Normal	Shrunken	Total	Total	Normal only
Intraspecific (different populations)	18205	4050	126	4176	22.9	22.2
Interspecific — total	124816	2768	708	3476	2.8	2.2
— Group II	22036	1777	324	2101	9.5	8.1
— Group III	3387	297	132	429	12.7	8.6
— Between Groups	99393	694	252	946	0.9	0.7
Total	142021	6818	834	7652	5.3	4.8

geneity, the latter is distinct, and may serve as one of several indices of affinity between species.

On the basis of species crossability, the 12 species dealt with here can be divided into five groups: one comprising five species, the second and third groups comprising three species each, with one species common to both groups, and the fourth and fifth groups being unispecific. Average crossability *within* groups was about 30% in HH and 10% in NHP, as compared with 6% and 0.9% for the two methods of crossing in *intergroup* combinations. This is clearly shown in the seedset percentages, summarized according to the crossability groups in Table 3 and 4, for HH and NHP respectively, and for the single-species in Fig. 1 (within-groups) and Fig. 2 (between-groups) combinations.

Group I. This group comprises *T. alexandrinum*, *T. berytheum*, *T. salmoneum*, *T. apertum* and *T. meironensis*. The seedset within this group, including reciprocals, averaged 28%, was always above 14%, and reached a maximum of 51%, as can be seen in Tables 3 and 5, and Fig. 1. This group manifests sharp reciprocal differences, which will be discussed later.

Group II comprises *T. echinatum*, *T. latinum* and *T. carmeli*. The seedset within this group ranged from 23 to 47% (Table 5), close to the seedset figures for intraspecific combinations.

Group III comprises *T. carmeli*, *T. scutatum* and *T. plebeium*. The seedset within this group was between 16% and 49%. Here again, highly significant reciprocal differences were noted.

As shown in Fig. 1, *T. carmeli* has good crossability both within Group II and Group III.

Groups IV and V comprise *T. vavilovi* and *T. constantinopolitanum*, respectively.

In a few instances certain combinations between groups resulted in a much higher seedset than the average of 5%. In some of these cases, such as *T. salmoneum* × *T. latinum*, where 34 seeds were obtained from 76 flowers, a possible error in pollination was suspected, but this possibility was ruled out later when the F_1 seeds were germinated and the plants proved to be interspecific hybrids. The females of both species seem to excel in crossability.

The crossability pattern described here is reflected also in seed quality. The percentage of shrunken seed in intraspecific combinations ranged from 2.5 to 20% (except for 36% in *T. echinatum*, suggesting the polytypic nature of this species), while the corresponding values in HH for interspecific intragroup and intergroup, combinations were 13% and 52%, respectively. Some of the shrunken seed may have been either degenerated selfers, or haploids, in which case they should not be accounted as hybrid seed at all. This would indicate a wider gap in crossability between groups, especially in the case of *T. vavilovi*, where some cross combinations produced only shrunken seeds (Table 2).

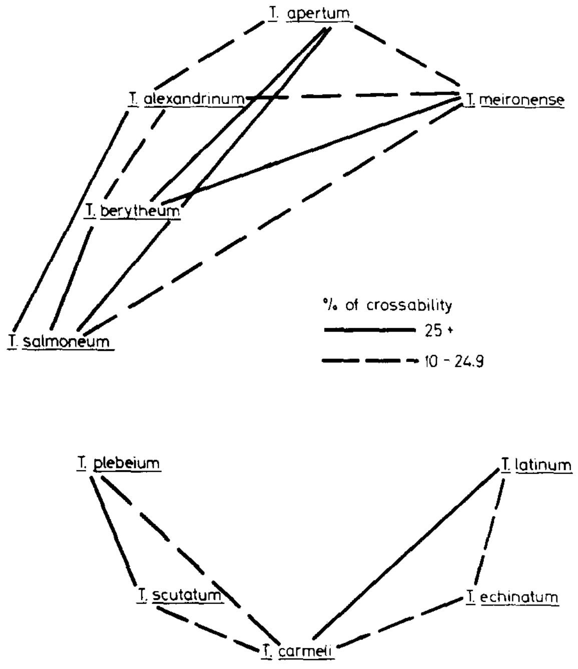

Fig. 1. Seedset (crossability) in intragroup cross combination

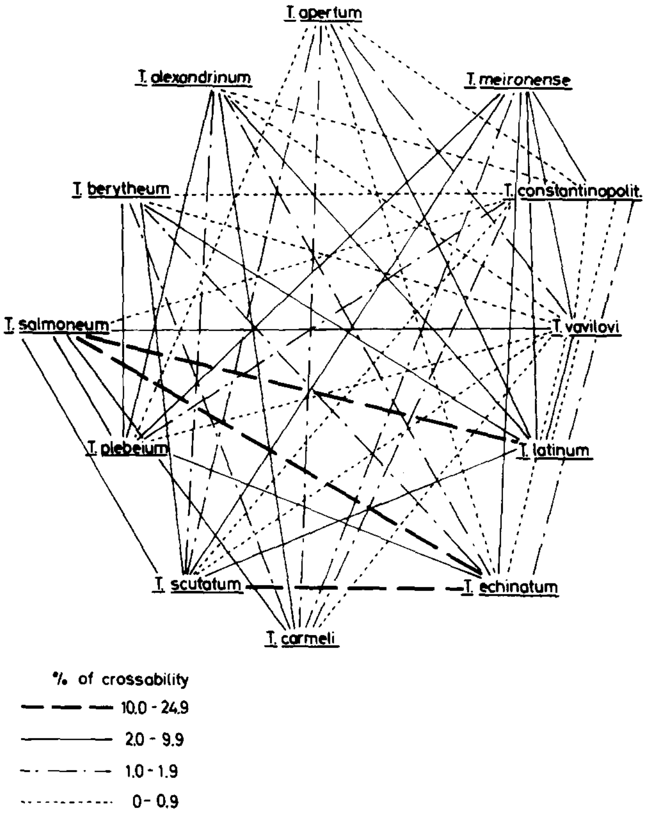

Fig. 2. Seedset (crossability) in intergroup cross combination

Table 5. *Reciprocal differences in crossability between hand-hybridized Trifolium species (% seedset of plump seeds only)*

cross ♀ × ♂	*T. ale*	*T. ber*	*T. sal*	*T. ape*	*T. mei*	*T. ech*	*T. lat*	*T. car*	*T. ple*	*T. scu*	*T. vav*	*T. con*
T. ale × *T.*	41.6											
T. × *T. ale*												
T. ber × *T.*	27.4	18.7										
T. × *T. ber*	3.4***											
T. sal × *T.*	56.4	58.1	33.3									
T. × *T. sal*	5.0***	41.8*										
T. ape × *T.*	18.5	16.7	43.2	30.3								
T. × *T. ape*	6.3***	32.4***	59.3*									
T. mei × *T.*	19.7	33.3	9.7	10.7	21.9							
T. × *T. mei*	22.1	21.5*	32.9	18.5								
T. ech × *T.*	0.9	0.7	1.2	0	2.3	7.4						
T. × *T. ech*	2.1	1.5	28.4**	0	3.8							
T. lat × *T.*	0	2.0	1.2	7.1	0	28.5	26.7					
T. × *T. lat*	7.1**	10.8***	42.1***	1.7**	4.8	19.3						
T. car × *T.*	0.5	0	0	2.1	0	15.7	38.0	50.4				
T. × *T. car*	9.1***	3.8**	17.5***	0	1.9	18.2	44.6					
T. ple × *T.*	6.8	2.9	0	0	2.7	4.7	1.9	23.0	43.4			
T. × *T. ple*	4.8	3.2	16.3***	0.7	0.8	0	2.4	2.7***				
T. scu × *T.*	1.9	3.8	8.6	0	5.0	27.4	9.5	19.5	70.7	3.4		
T. × *T. scu*	0	2.0	3.1	2.2	0**	0***	3.4*	8.5*	35.2***			
T. vav × *T.*	1.3	1.3	10.8	0	0	0	8.9	0.1	0.6	1.4	69.7	
T. × *T. vav*	0	0	7.5	2.0*	11.5**	0	0 *	0.3	0	0		
T. con × *T.*	0	0	0	0	0	0	0	2.3	0	0	1.1	35.3
T. × *T. con*	0.9	0.6	0	0	4.4	2.1	0	0	2.9	0	0	

* Reciprocal difference significant at 5% level.
** Reciprocal difference significant at 1% level.
*** Reciprocal difference significant at 0.1% level.

3. Reciprocal Differences in Crossability

Reciprocal differences in crossability were found in many cross combinations, especially when *T. alexandrinum*, *T. berytheum*, *T. salmoneum* from Group I and *T. carmeli* from Group III were used as parents, but also in many instances of intergroup pairs. This is illustrated in Table 5, which summarizes the results of crossability by HH in reciprocal pairs (plump seeds only are given). The Table also indicates statistical significance, as calculated by the χ^2 method for each pair. Some of the fairly sharp differences are not significant, owing to low crossability and/or small sample sizes.

Reciprocal differences may have several causes.

a) Differential response of species, populations and plants to mechanical manipulation during emasculation and pollination; this is unrelated and in addition to the intrinsic variation in seedset, present when plants are naturally pollinated. These differences may be reflected in the variation in seedset of intraspecific crosses (Table 5, the diagonal line), or in differences in seedset of various populations within species. Such variation was observed in *T. carmeli* where the average seedset in NYT 266 was 16.6% (2257 crosses), and 23.9% in NYT 251 (1543 crosses).

This kind of information may be used for laying down the order of taxa in seedset, from best parent to poorest one; it may also explain cases where Species B is poor in a reciprocal pair with Species A, but superior to Species C in a reciprocal pair with A. Thus, *T. vavilovi* seems to be the best female plant, having the highest seedset in intraspecific hybridization, and highest seedset as female in 7 out of ten pairs of reciprocal interspecific combinations. The percentage of shrunken seeds, (not included in Table 5) supports this *T. vavilovi* superiority in this respect.

b) Sharp deviations from the general pattern, as described under *a*), probably indicate pair-specific compatibility. The performance of female *T. alexandrinum* for example, is poor when crossed with *T. berytheum*, *T. salmoneum* and *T. apertum*, but better than the reciprocal when crossed with *T. meironensis* and *T. carmeli*. Its crossability relations with *T. vavilovi* and *T. latinum* can be predicted on the basis of the general crossability of these two species, as described in *a*).

There are two obvious factors that may induce specific species-pair deviations. These are the self-fertility of *T. alexandrinum*, and the system of B chromosomes in *T. salmoneum* and *T. berytheum* (Putiyevsky and Katznelson, 1970). But other, less conspicuous factors may be more important. The effect of the B chromosomes on reciprocity becomes evident by the consistently higher seedset in plants possessing them when they are used as females.

The seedset in interspecific, intragroup cross combinations was found to be very high. In many of these combinations, even when parental species exhibit striking morphological differences, such as *T. carmeli* and *T. scutatum*, crossability is close to that found in intraspecific combinations. This was an unexpected finding, as the general crossability level among *Trifolium* species (Evans, 1962a, 1962b; Newton *et al.*, 1970; Keim, 1953) is very low or nil. It may be explained either by our better technique or by the nature of crossability as an isolation mechanism, i.e. weak in intragroup combinations, but very decisive and almost complete in other species such as *T. repens* (Evans, 1962b) *T. pratense* (Newton *et al.*, 1970) and *T. subterraneum* (Katznelson, 1967). The second possibility seems more likely, as evidence of breakdown of hybridization was shown by Evans (1962 b), Müller (1960) and Pandey (1957). Polyploidization and the use of graft and embryoculture techniques, facilitated somewhat the postponement of embryo breakdown and even gave rise to interspecific hybrids (Evans, 1962 a; Hovin, 1962; Chen and Gibson, 1970).

Even the intergroup combinations manifest better seedset performance than was shown by other studies in *Trifolium*; seeds have formed in most of the combinations, without resorting to special devices.

Although low crossability is only one out of several isolation mechanisms, (Stebbins, 1958) the results obtained in this study show that the twelve clover species, as a group, show much closer kinship than other studied cases in *Trifolium* — an indication that they probably share a recent, common origin. Basically, we are dealing here with two levels of kinship; that of between groups, were seedset in inter-hybridization is usually between 0.5—5%, and that of within groups, where the corresponding value is 20—25%. The ease of crossability in the latter case raises some doubts about the validity of the species as a taxonomical unit.

The fact that *T. carmeli* belongs to two crossability groups — *T. plebeium*—*T. scutatum* and *T. echinatum*—*T. latinum* — suggests that it is relatively closer to the common origin than other species.

The conclusions presented here are backed by studies of the other components of the isolation mechanism that will be discussed in subsequent parts of this series of publications.

Acknowledgement

We are indebted to Prof. D. Zohary for his encouragement.

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Received October 10, 1972

Communicated by H. Stubbe

E. Putiyevsky
Dr. J. Katznelson
Division of Pasture and Forage Crops
Agricultural Research Organization
Newe Ya'ar Experiment Station
P. O. Haifa (Israel)