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Cytogenetic Studies of *Trifolium* Spp. Related to Berseem

IV. The Relationships in the *Alexandrinum* and *Vavilovi* Crossability Groups, and the Origin of the Cultivated Berseem †

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Summary. The interspecific cytogenetic relationships among *T. alexandrinum* L., *T. berytheum* Boiss., *T. salmoneum* Mout., *T. apertum* Bobr., *T. meironense* Zoh. et Lern. and *T. vavilovi* Eig. were studied in several hundreds of intraspecific (WS) and interspecific (BS) F_1 hybrids in all possible combinations, and in many of their progenies.

Seed germination and development of F_1 BS hybrids were normal, except in crosses involving *T. vavilovi*. Their pollen fertility and seed set was subnormal, with somewhat lower values in *T. vavilovi* progenies. No multivalents were observed in any F_1 BS hybrid, but in some plants, four and also six chromosomes did not pair to form bivalents. B chromosomes were common in hybrids involving *T. berytheum* or *T. salmoneum*.

Some BS F_1 hybrids set ample seeds when selfed, and most of them originated from either one of two self-incompatible *T. berytheum* plants.

The six taxa studied form three groups: (i) *T. vavilovi*, which is quite remote from the other five; (ii) *T. meironense* and *T. apertum*; and (iii) *T. alexandrinum*, *T. berytheum* and *T. salmoneum*. The two last species, especially *T. salmoneum*, seem to be the progenitors of the cultivated berseem.

Introduction

In studies of various cytogenetic aspects of several *Trifolium* species related to berseem (*T. alexandrinum* L.) published earlier, we described the pattern of crossability of 12 clover species (Putiyevsky and Katznelson, 1973). These species could be divided into five crossability groups, and the relationships within and between the second and the third crossability groups have also been described (Katznelson and Putiyevsky, 1974; Putiyevsky and Katznelson, 1974). Two other crossability groups are more intimately associated with the cultivated berseem; the first one (Group I) includes, besides the cultivated berseem, four wild East Mediterranean taxa, *T. berytheum* Boiss., *T. salmoneum* Mout., *T. apertum* Bobr. and *T. meironense* Zoh. et Lerner. These five species intercross quite readily (Putiyevsky and Katznelson, 1973). Another species, *T. vavilovi* Eig., is morphologically somewhat similar to the five taxa mentioned above, and was recently located with them in subsection *Alexandrina* by Zohary (1972). Hybridization between *T. vavilovi* and the other species, however, failed almost completely and it comprises Crossability Group IV.

The six species, or taxa, concerned here impose several taxonomic problems, and they are often discussed in connection with aspects of the origin of berseem. Although the East Mediterranean region was exploited botanically quite extensively in the 19th Century, most of the species related to berseem have been described only recently (*T. vavilovi* Eig. in 1934, *T. apertum* Bobr. in 1941, *T. salmoneum* Mout. in 1953, and *T. meironense* Zohary and Lerner in 1972), in contrast to other clover sections and groups from the same region. They were, and still are, often regarded as intraspecific entities of berseem. Even *T. berytheum* Boiss., which was described in 1856 by Boissier, was named as a variety of *T. echinatum*, *T. constantinopolitanum* or *T. alexandrinum*, and intermediates between cultivated berseem and *T. berytheum* have been found in nature (Oppenheimer, 1959; Zohary, 1972). Oppenheimer's interpretation of *T. alexandrinum* var. *berytheum* as wild *T. alexandrinum* definitely focuses the search for the wild progenitor of berseem on *T. berytheum*. Bobrov (1947), on the other hand, claims that his *T. apertum* is the true progenitor of berseem.

Cytogenetic studies may clarify some of the problems mentioned above, but up to now all the interspecific studies with berseem involved crossing it with remote species such as *T. repens* L. (Evans, 1962), *T. resupinatum* (Müller, 1960), and *T. pratense* L. and *T. campestre* Schreb. (Abdel-Tawab, 1968).

In the present study large-scale hybridization was carried out with all the species related to berseem and the results obtained illuminate some of the cytogenetic problems mentioned, including the origin of the cultivated berseem.

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Materials and Methods

The origin of lines used as parent plants as well as the two methods of obtaining hybrid seed, i.e., manually and by the natural hybridization programme (NHP), have been presented elsewhere (Putiyevsky and Katznelson, 1973; Katznelson, 1971). Altogether, 245 intraspecific and 594 interspecific hybrid seeds of this group were germinated, each seed separately in a petri dish. The seedlings were planted in soil in 3-kg pots and kept in a glasshouse.

The methods employed in the cytological and pollen fertility studies were described earlier (Putiyevsky and Katznelson, 1970). In each F_1 plant, several heads were bagged and triggered for self-pollination. Other heads were back-crossed, usually to the two parental lines (of the same accession number), and the number of flowers treated and the seeds produced was counted. All other heads were collected, threshed, the seed weight was determined, and calculated as percent of total plant dry weight. Some F_2 and BC_1 seeds were germinated and their pollen fertility, cytology and seedset upon selfing and open pollination were determined, as in their parents.

Results

a. *Germination and Development*

The results of germination and development of intra- and interspecific F_1 hybrids in various combinations among the six species dealt with in this publication are presented in Table 1. These data deserve two explanations: *First,* the seeds of T. mei* intraspecific (WS) hybrids that turned to albinos resulted from two parent plants which produced many albinos in various intra- and intergroup combinations (Katznelson and Putiyevsky, in press); both plants were albino heterozygotes, probably in the same locus. *Second,* the relatively low germination and high mortality rates in T. vav intraspecific combination may stem from the fact that most of these seeds were obtained by the NHP, in which heads and seeds were not selected, and many of them were somewhat shrunken (see Putiyevsky and Katznelson, 1973). This does not exclude some cytogenetic heterogeneity *within* T. vav as another possible cause.

Except for these cases, most of the intraspecific hybrids (86—100%) reached maturity, higher than the percentage in an interspecific (BS) cross combination. Within the latter, those including T. mei as a parent usually had better germination and development, while those of T. vav were much poorer (43.6%) than hybrids within crossability group I (64.7%).

Table 1. *Germination and development of F_1 hybrids*

Species combination	Total F_1 seed planted	No. F_1 seed germinated	No. albino seedlings	No. dead before flowering (albinos excluded)	No. reached maturity	% of germinated seedlings
T. ale—T. ale	15	15		2	13	86.7
T. ber—T. ber	10	10			10	100.0
T. apr—T. apr	8	8		1	7	87.5
T. mei—T. mei	2	2	2		0	0.0
T. vav—T. vav	210	129		32	97	75.2
Total intraspecific combinations	245	164	2	35	127	77.4
T. ale—T. ber	32	32		14	18	56.2
T. ale—T. sal	28	22		9	13	59.1
T. ale—T. apr	30	28		6	22	78.6
T. ale—T. mei	27	27		4	23	85.2
T. ber—T. sal	68	65		27	38	58.5
T. ber—T. apr	75	70		35	35	50.0
T. ber—T. mei	26	26	1	2	23	88.5
T. sal—T. apr	45	39		13	26	64.5
T. sal—T. mei	26	25	4	3	18	72.0
T. apr—T. mei	35	29	1	9	19	65.5
Interspecific within crossability Group I	392	363	6	122	235	64.7
T. ale—T. vav	28	19		8	11	50.8
T. ber—T. vav	76	52		39	13	25.0
T. sal—T. vav	72	36		20	16	44.4
T. apr—T. vav	7	7	3	3	1	14.3
T. mei—T. vav	10	10		2	8	80.0
Between crossability Group I and *T. vavilovi*	193	124	3	72	49	40.0

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* Abbreviations: T. ale—*T. alexandrinum* L., T. apr—*T. apertum* Bobr., T. ber—*T. berytheum* Boiss., T. meis—*T. meironense* Zoh. et Lern., T. sal—*T. salmoneum* Mout., T. vav—*T. vavilovi* Eig.

b. *Pollen Fertility of F_1 Hybrids*

Pollen fertility was examined in 127 WS and 293 BS F_1 hybrids (Table 2). Although a few BS hybrids were sterile or semi-sterile, most of the hybrids were fertile or subfertile (70–80% fertile pollen). There was only a small difference in pollen fertility between WS and BS combinations, and hardly any difference between BS crosses within crossability group I and between them and *T. vavilovi*.

Table 2. *Frequency distribution of pollen fertility of intraspecific* (*W.S.*) *and interspecific* (*B.S.*) *F_1 hybrids*

Hybrids combination	Total plants examined	No. of plants with the following of pollen fertility percentage										Average pollen fertility %
		0–10	10–20	20–30	30–40	40–50	50–60	60–70	70–80	80–90	90–100	
T. ale–T. ale	13								1	3	9	90.8
T. ber–T. ber	10							2		3	5	85.6
T. apr–T. apr	7									2	5	91.4
T. vav–T. vav	97									2	95	94.8
Total intraspecific combinations	127							2	1	10	114	93.6
T. ale–T. ber	18						1			5	12	90.2
T. ale–T. sal	13								3	3	7	87.5
T. ale–T. apr	22	1						1	4	9	7	80.5
T. ale–T. mei	23							2	3	8	10	84.4
T. ber–T. sal	38	1				2	3	1	3	8	20	81.7
T. ber–T. apr	35				1	1	2	2	4	17	8	80.3
T. ber–T. mei	23								1	8	14	86.1
T. sal–T. apr	26					2		1	4	11	8	81.5
T. sal–T. mei	18							1	7	8	2	80.0
T. apr–T. mei	19							1	1	10	7	87.0
Interspecific within crossability Group I	235	2			1	5	6	9	30	87	95	84.5
T. ale–T. vav	11					1	3	4		2	1	63.6
T. ber–T. vav	13						1	2	3	5	2	78.6
T. sal–T. vav	16							6	2		8	79.0
T. mei–T. vav	8							1	2	4	1	81.4
T. apr–T. vav	1									1		81.0
Between crossability Group I and *T. vavilovi*	49					1	4	13	7	12	12	78.0

Table 3. *Chromosome configurations in meiosis of hybrids* (*2n = 16*)

Hybrids combination	F_1					F_2				
	No. plants examined	No. cells examined	Cells with		% Cells with univalents	No. plants examined	No. cells examined	Cells with		% Cells with univalents
			7II + 2I	8II				7II + 2I	8II	
T. ale × T. ber	4	110	9	101	8.1	5	84	1	83	1.2
T. ale × T. sal	1	30	—	30	0	3	45	—	45	0
T. ale × T. apr	5	131	11	120	8.4	6	99	5	94	5.0
T. ale × T. mei	5	92	5	87	5.4	8	127	8	119	6.3
T. sal × T. ber	7	192	4	188	2.0	6	101	—	101	0
T. ber × T. apr	10	236	3	233	1.3	5	77	1	76	1.3
T. ber × T. mei	3	55	5	50	9.0	4	68	—	68	0
T. sal × T. apr	5	124	2	122	1.6	2	24	—	24	0
T. sal × T. mei	4	114	1	113	0.7	3	48	—	48	0
T. apr × T. mei	6	164	2	162	1.2	10	172	3	169	1.1
T. ale × T. vav	1	30	4	26	13.3	1	18	1	17	5.5
T. ber × T. vav	3	90	1	89	1.1	2	30	—	30	0
T. sal × T. vav	4	88	9	79	10.2	—	—	—	—	—
T. mei × T. vav	1	30	1	29	3.3	2	28	—	28	0
T. apr × T. vav	1	21	1	20	4.8	—	—	—	—	—

Table 4. *Seed set upon selfing, backcrosses and open pollination of F_1 hybrids*

Hybrid combination	Total No. plants examined	Seed set upon: Selfing			Backcross to ♀ parent			Backcross to ♂ parent			Open pollination	
		No. of flowers	No. of seeds	% seedset	No. of flowers	No. of seeds	% seedset	No. of flowers	No. of seeds	% seedset	Seed weight, g	$\frac{\text{seed weight}}{\text{plant weight}}$ %
Intraspecific												
T. ale—T. ale	13	2015	464	23.1	—	—	—	—	—	—	5.92	1.73
T. ber—T. ber	10	1659	45	2.1	—	—	—	—	—	—	7.96	7.19
T. apr—T. apr	7	781	0	0	—	—	—	—	—	—	0.76	1.69
Interspecific												
T. ale—T. ber	18	5688	1089	19.1	243	70	28.8	282	119	42.2	17.11	3.70
T. ale—T. sal	13	3886	1090	28.0	89	33	37.0	78	35	44.9	16.85	3.50
T. ale—T. apr	21	5728	227	4.1	318	38	11.9	172	39	22.7	11.82	1.96
T. ale—T. mei	23	5755	788	13.8	252	63	25.0	230	82	35.6	22.15	1.57
T. ber—T. sal	37	10712	1192	11.1	206	100	48.5	231	106	45.9	26.45	2.74
T. ber—T. apr	34	9136	490	5.4	273	75	27.5	337	79	23.4	17.22	1.27
T. ber—T. mei	23	4398	255	5.1	284	43	15.1	175	50	28.6	12.20	1.79
T. sal—T. apr	25	5846	5	0.1	117	49	41.9	115	33	28.7	15.59	2.21
T. sal—T. mei	18	5201	3	0.1	220	59	26.8	213	54	25.3	6.06	0.38
T. apr—T. mei	19	6401	67	1.0	260	28	10.8	297	47	15.8	12.37	0.75
T. ale—T. vav	11	1247	48	3.8	—	—	—	—	—	—	9.35	1.91
T. ber—T. vav	13	4000	1	0.02	25	7	28.0	21	0	0.0	4.35	1.61
T. sal—T. vav	16	3441	53	1.6	111	17	15.3	45	12	20.8	1.74	0.46
T. apr—T. vav	1	230	0	0	25	0	0.0	25	0	0.0	0	0
T. mei—T. vav	8	1369	0	0	25	11	44.0	—	—	—	2.05	2.50

c. Cytology of F_1 Hybrids

Cytology in meiosis was examined in 60 interspecific F_1 hybrids and appeared to be quite regular (Table 3). No multivalents were discerned in 1498 cells that were examined, but quite a few of the cells had two univalents instead of a bivalent. These were mainly in T. ale × T. vav, T. sal × T. vav and T. ber × T. mei cross combinations. The more or less normal meiosis corresponds to the normal or subnormal pollen stainability.

An interesting feature found in the majority of F_1 hybrids within crossability group I (but *not* between them and T. vav) is the appearance of B chromosomes. This topic will be reported and discussed elsewhere.

d. Seed Set of F_1 Hybrids

The data on seed set in F_1 hybrids upon selfing, backcross to both parental populations and on exposure to a large variety of pollen sources, are given in Table 4.

i) *Selfing.* Seeds were formed in many cross combinations. Ample seeds (up to 28% seed set) were produced when T. ale was one of the parents. Good seed set upon selfing, though lower than the T. ale combinations, was obtained in progenies of T. ber — expecially T. ber × T. sal, T. ber × T. mei, and reciprocals. However, a closer look at the results of the individual plants traces the good seed set upon selfing to two individual T. ber plants, NYT 206/1 and NYT 206/2. This can be seen in Table 5.

T. ber × T. sal hybrids: Of 37 F_1 plants, only 12 set any seed when selfed, and 11 of them were progenies of NYT 206/1.

In the *T. ber × T. apr hybrids*, seed set upon selfing may be similarly traced to Plant NYT 206/2. However, one T. apr × T. ber F_1 hybrid, a progeny of NYT 206/3, a third plant of NYT 206, did not set any seed. It should be stressed here that seed set upon selfing of the two parental plants, 206/1 and

Table 5. *Seed set upon selfing in some F_1 hybrids, progenies of T. ber (206, 252, 529) crossed with T. sal (260) and T. apr (1471, 1772)*

Parents		Number of plants	Hybrid	
♀	♂		florets	seeds
206/1	260	6	2015	313
252	260	6	1506	6 (all from one plant)
529/1	260	11	3192	0
260/1	206/1	5	1902	873
260/1	252	6	1130	0
260/1	529	3	967	0
206/2	1471	6	1021	241
206/2	1772	3	1298	200
529/3	1772	3	803	0
529/3	1471	8	2039	0
252	1772	4	1107	0
but 1471	206/3	1	240	0

206/2, was 0.51% and 0.0%, respectively (Putiyevsky and Katznelson, 1970).

Of 23 *T. ber* × *T. mei* F_1 *hybrids* only two set any seed. Their common parent was T. ber NYT 252/1, which had quite good seed set when selfed.

Other cross combinations set hardly any seeds, and those which did form were often shrunken.

ii) *Backcrosses*. Seed set upon backcrossing, though varying considerably in individual cross combinations, was quite high on average, and exceeded 40% in some combinations. There seems to be a positive correlation between seed set in backcrosses to the two parental populations, the only serious deviation being crosses involving T. vav.

Also, seed set was higher in the combinations between T. ale, T. ber and T. sal (41%) than in combinations between any of the three taxa and T. mei—T. apr (22.6%), or between any of all five taxa and T. vav (16.9%). Reciprocal differences of the magnitude found in the crossability between species (Putiyevsky and Katznelson, 1973) were quite common.

iii) *Open pollination*. The seed set in WS and BS F_1s, given in grams seeds and as percent of plant weight, is also presented in Table 4. Outstandingly high amounts of seed were produced in T. ber × T. ber combinations, followed by those of T. ale—T. ber—T. sal. This is in accordance with the success in backcrossing. However, unexpected high seed set was found in the T. mei × T. vav cross combination, both in backcrossing and in open pollination. The hybrid nature of these plants was ascertained both by morphology and pollen data, and we cannot explain this.

e. F_2 Plants

As in the case of the second and third crossability groups (Katznelson and Putiyevsky, 1974; Putiyevsky and Katznelson, 1974), germination of F_2 seeds was excellent: 367 out of 381 seeds, which was much better than that of parents or F_1 hybrid seed. One of the seedlings, T. ber × T. mei, was albino, and 92 plants died before flowering.

Pollen stainability was estimated in the remaining 274 plants; 57 F_2 plants, of relatively low pollen stainability, were studied cytologically.

The results on pollen fertility of F_2 and BC_1 hybrids appear in Table 6. Comparison of these results with those of their parents (Table 2) shows that pollen fertilities of F_2 were no higher, and often lower, than those of F_1s. This is especially obvious in the hybrids involving T. vav, which fact helped us to ascertain the hybrid nature of their highly fertile F_1 parents.

Table 6. *Pollen fertility of F_2 + $B.C._1$ interspecific hybrids*

Combination		No. of plants with the following pollen fertility percentage										Total no. plants examined	Ave. pollen fertility %
♀ = F_1	♂	0—10	10—20	20—30	30—40	40—50	50—60	60—70	70—80	80—90	90—100		
T. ale—T. ber	B.C.	1						2	1	7	12	23	85.6
T. ale—T. sal	B.C.							1	1	5	5	12	87.0
T. ale—T. apr	B.C.						4	3	4	8	4	23	80.6
T. ale—T. mei	B.C.					1	3	5	4	4	2	19	71.9
T. ber—T. sal	B.C.								5	9	16	30	88.9
T. ber—T. apr	B.C.						1	1	2	15	9	28	85.6
T. ber—T. mei	B.C.								2	3	3	8	86.2
T. sal—T. apr	B.C.					1			2	6	2	11	82.6
T. sal—T. mei	B.C.								2	2	3	7	86.4
T. apr—T. mei	B.C.								3	6	12	21	89.1
T. ale—T. ber	Selfing						1	1	1	8	4	15	82.9
T. ale—T. sal	Selfing									3	5	8	90.0
T. ale—T. apr	Selfing							1	1	6	8	16	84.8
T. ale—T. mei	Selfing			1	1	2	1	2	2	7	6	22	75.0
T. ber—T. sal	Selfing	1							2	4		7	70.0
T. ber—T. apr	Selfing								1	2		3	84.0
T. ber—T. mei	Selfing					1				2	2	5	81.0
T. sal—T. apr	Selfing									2		2	85.5
T. apr—T. mei	Selfing								1	3	3	7	87.9
Within crossability Group I		2		1	1	5	10	16	34	102	96	267	83.2
T. mei—T. vav	B.C.					2						2	44.5
T. ale—T. vav	Selfing						1	1	1	1		4	66.0
T. ber—T. vav	Selfing						1					1	50.0
Between crossability Group I and *T. vavilovi*						2	2	1	1	1		7	60.7

Table 7. *Seedset in various progenies of interspecific F_2 hybrids*

Combinations			No. of plants examined	Seedset upon selfing			Seedset upon open pollination	
♀ = F_1		♂		No. of flowers	No. of seeds	% Seedset	Seed weight, g	$\frac{\text{seed weight}}{\text{plant weight}}$ %
T. ale	T. ber	B.C.$_1$	23	4929	322	6.5	4.38	0.55
		Selfing	15	2159	97	4.5	5.26	1.44
T. ale	T. sal	B.C.$_1$	12	2455	250	10.2	10.20	1.71
		Selfing	8	903	11	1.2	3.12	1.92
T. ale	T. apr	B.C.$_1$	23	3556	151	4.3	5.33	0.44
		Selfing	16	2997	184	6.1	4.44	1.15
T. ale	T. mei	B.C.$_1$	19	4010	29	0.7	1.50	0.19
		Selfing	22	4830	12	0.2	0.12	0.02
T. ber	T. sal	B.C.$_1$	30	5915	408	6.9	5.17	0.46
		Selfing	7	1435	14	1.0	0.45	0.34
T. ber	T. apr	B.C.$_1$	28	4773	304	6.4	20.97	1.90
		Selfing	3	630	5	0.8	0.20	0.28
T. ber	T. mei	B.C.$_1$	8	1318	47	3.6	2.07	0.83
		Selfing	15	1140	110	0.1	0.12	0.13
T. sal	T. apr	B.C.$_1$	11	1812	111	6.1	6.72	2.28
		Selfing	2	300	62	20.7	1.18	3.76
T. sal	T. mei	B.C.$_1$	7	1000	0	0	0.07	0.04
T. apr	T. mei	B.C.$_1$	21	4120	0	0	0.07	0.01
		Selfing	7	1600	0	0	0.07	0.06
T. ale	T. vav	Selfing	4	462	47	10.2	1.80	2.40
T. ber	T. vav	Selfing	1	618	213	34.5	1.82	1.74
T. mei	T. vav	B.C.$_1$	2	270	0	0	0.03	0.01

Cytologically, the F_2 were similar to their F_1 parents in the absence of multivalents in meiosis and in the appearance of 7II and 2I cells (Table 3).

Seed set upon selfing of F_2 hybrids varied between 0--34.5% (Table 7), and some seed was obtained in most combinations. Quite obviously, plants that originated from selfed F_1 produced many seeds when selfed. Thus, the single F_2 T. ber × T. vav seed that was obtained from selfing some 4000 florets (Table 4) produced a highly self compatible plant. It is also obvious that selfed flowers formed seeds in many BC plants, in combinations whose F_1 plants were almost completely self incompatible. A good correlation found between seed set upon selfing and seed set by open pollination may suggest that many of the seeds obtained in open pollination may result from selfing.

Discussion

An obvious conclusion from the data presented is that the six taxa of subsection *Alexandrina* Zoh. (Zohary, 1972) represent a heterogeneous group. First, *T. vavilovi*, though superficially somewhat similar morphologically to the five other taxa, is quite isolated genetically from them, both in terms of crossability (Putiyevsky and Katznelson, 1973) and of lower seed germination and development of F_1 seedlings, much smaller plants with somewhat lower pollen fertility and only few seeds. The effect of isolation through even lower pollen fertility and seed formation is obvious also in F_2 and BC plants. Thus, *T. vavilovi* is a clear-cut species, well isolated from the five other taxa.

It may be added that there is some evidence that *T. vavilovi* is not homogeneous cytogenetically, as some hybrids between geographically remote populations were sterile and did not set seeds (Katznelson, 1969).

The five other taxa are hardly isolated genetically. Crossability, germination, development, pollen fertility and seed set (BS F_1s) are similar to WS F_1s (normal) or only somewhat lower (subnormal).

The mechanism isolating T. ale—T. ber—T. sal from T. mei—T. apr is geographical and ecological. T. apr grows in the Caucasus and Turkey in shrubby habitats (Bobrov, 1950), and the Type Specimen of T. mei was collected in a small clearing within a dense maquis on Mt. Meiron, Israel, *i.e.*, both taxa grow in regions and conditions where T. ale is not grown, and where T. sal and T. ber do not occur naturally. The natural habitats of T. sal and T. ber are wet places, roadsides and waterlogged soils, etc., often border areas sown to berseem. Hybrid swarms between T. ale and T. ber under these conditions are quite common, and they are often regarded as subspontaneous berseem. T. ale-like plants are eliminated by natural selection in a process — and due to causes — similar to *Hordeum vulgare* — *H. spontaneum* hybrid swarms (Zohary, 1971). Moreover, in a study of the domestication process in T. ber we found a clear-cut gene flow from T. ale to T. ber, originating from some 2—3% interspecific hybridization, and the F_1 plants were easily discerned morphologically. By this slow process, lines intermediate between T. ale and T. ber in many traits are being formed (Katznelson, in preparation).

Thus, T. ber and/or T. sal are, or are closest to, the wild progenitor of berseem, the cultivated T. ale. This contrasts with Bobrov's opinion that *T. apertum* is the wild progenitor of berseem. He concluded this on the basis of the open calyx tube of *T. apertum*, without knowing *T. berytheum*, and especially *T. salmoneum*, whose calyx throat is not hairy. The two latter species are much more similar morphologically to berseem than either T. apr or T. mei. In fact, the five species of crossability group I can be separated into two species complexes that differ morphologically: T. mei—T. apr are more delicate, have smaller heads and leaves, and the fruiting head, especially in T. mei, is more cylindrical than in the T. ale—T. ber—T. sal group.

It should be stressed here that as the five taxa are very homogeneous cytogenetically, our conclusion on the origin of berseem is based mainly on morphology, but we have studied a wealth of material at all growth stages. While some taxonomic considerations will be discussed elsewhere, the following are worth discussing here. The three taxa, T. ale, T. sal and T. ber, show morphological intergradation in many characteristics. However, some characteristics of T. ale were not found in the two wild taxa, and vice versa.

a) A very important agronomic trait in the cultivars of the Musgawi group is the very low basal branching of erect branches, which is the basis for the multiharvest nature of these cultivars. T. sal and some populations of T. ber are erect, but they never branch (and rebranch) at their bases. (This trait was seen in a few plants of T. apr, continuously grazed, from Sinop). On the other hand, the branching pattern of all cultivars of the Fahli and Saidi groups of T. ale is similar, if not identical, to that of some wild types of T. sal and T. ber.

b) The calyx tube in T. ale is completely open, while it is closed by a ring of hairs in T. ber, and by a callus ring without hairs in T. sal. This character affects the easy threshability of the cultivated berseem and much stronger seed retention in the wild taxa. However, the callus ring in T. sal is sometimes hardly seen.

In other morphological characters the three taxa show more or less continuous variation.

c) We found that many populations of T. ber—T. sal have B chromosomes, in almost every plant examined. We, and many other scientists, have never found them in T. ale (Mehta et al., 1963; Almeida and Carvalho, 1962; Putiyevsky and Katznelson, 1970).

d) While the wild T. ber and T. sal are practically self-incompatible, T. ale varies in its genetic system from selfers to self-incompatibles, even within one population (Putiyevsky and Katznelson, 1970). This change in the genetic system is quite common in many other cases of domestication, such as tomato, barley, cotton and, to some extent, wheat. This may lead to the conclusion that berseem is a newly domesticated plant, but this is erroneous, as berseem was probably the earliest forage crop to be sown from the first Egyptian dynasty, i.e., 3500—3800 B.C.

One may connect the last topic to the breakdown of self-incompatibility in crosses involving T. ber. Such breakdown in interspecific hybrids has already been reported by Pandey (1957) in the F_1 of *T. uniflorum* × *T. repens*. Pandey concluded that although the two quite remote species have the same system of incompatibility, they differ in the location of the S gene in the chromosomal complement.

Our case, however, is quite different. Intrapopulation differences (NYT 206/1 and 206/2 versus NYT 206/3) show that we deal here not with species but with individuals. This apparently rules out differences in *location* of the S locus, and suggests that the breakdown of the incompatibility system is genetic. Genetical analysis in the future may elucidate this subject.

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