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Genome-analytic studies on *Brassica trilocularis* and *B. tournefortii**

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The group of ***Brassica*** species with 10 pairs of chromosomes includes, like the cole group, a large number of cultivated species, and the most species are composed of genome ***a***, excepting only a few species (Morinaga 1934, Sikka 1940). According to the opinion of Olsson (1954), ***B. tournefortii*** will be considered to be a separate species, while all the other species with 10 pairs of chromosomes, viz., ***B. pekinensis, B. chinensis, B. narinosa, B. nipposinica, B. campestris*** and ***B. trilocularis***, are to be subspecific forms of ***B. campestris*** s. lat.

The authors' investigation on the genetic differentiation in the group of ***Brassica*** species with 10 pairs of chromosomes was started with the examination of ***B. tournefortii*** and ***B. trilocularis*** to elucidate the cytogenetical relationship established between these two forms and several other species in ***Brassica.***

Materials and method

The materials used in the present crossing experiment are as follows: ***Brassica campestris*** var. ***oleifera*** (hort. var., Kanzaki-hanana), ***B. tournefortii, B. trilocularis, B. nigra*** (hort. var., California brown) and ***B. alboglabra.*** All these forms used as materials showed exclusively normal meiotic division processes and could produce ca. 95 or still higher percentage of the well-stainable pollen-grains.

Crossings were effected by the bud-pollination technique. Cytological observations were made with the preparations made by the smear method with acetic-orcein.

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Results

1. The result of crossings and certain characteristics in the F_1 hybrids.

As shown in Table 1, ***B. tournefortii*** crossed with ***B. nigra*** or with ***B. alboglabra*** failed to yield any one true F_1 seed, while 3 other forms with 10 pairs of chromosomes, i. e., ***B. campestris***, ***B. trilocularis*** and ***B. tournefortii***, when crossed among each others, could raise certain true F_1 seeds. This table shows, in advance, that ***B. tournefortii*** was highly cross-incompatible with ***Brassica*** forms composed of ***a***, ***b or c*** genome and also that ***B. trilocularis*** was, in contrast, highly cross-compatible with ***a*** genome species. Certain outstanding characteristics appearing in these parents and their F_1 hybrids are shown in Table 2.

2. The meiotic divisions in PMCs and the fertility of F_1 hybrids.

Table 1. Results of crossing experiments.

Cross combination	Number of flowers pollinated	Number of pods developed	Number of seeds obtained	Number of seeds sown	Number of plants grown	
					true hybrid	false hybrid
B. trilocularis						
× ***B. tournefortii***	36	10	3	3	0	3
× ***B. campestris***	25	21	112	40	40	0
B. tournefortii						
× ***B. campestris***	56	26	9	9	1	3
× ***B. trilocularis***	28	21	11	11	5	2
× ***B. alboglabra***	36	18	24	24	0	18
× ***B. nigra***	25	20	14	14	0	8

Table 2. Certain characteristics as compared among ***B. campestris***, ***B. trilocularis***, ***B. tournefortii*** and their respective F_1 hybrids.

	Basal leaf	Upper leaf	Petal	Anther	Pod	Seed
B. campestris	light green, pubescent	oblong, clasping	wide, yellow	extrosed	large, cylindrical	brown
B. trilocularis	dark green, globrous	oblong, clasping	wide, yellow	introsed	large, flat	yellow
B. tournefortii	green, pubescent	narrow, not clasping	narrow, pale yellow	introsed	small, compressed	brown
(***B. camp.-tri.***) F_1	green, pubescent	intermediate, clasping	intermediate, yellow	extrosed	intermediate	——
(***B. camp.-tour.***) F_1	light green, pubescent	intermediate, not clasping	intermediate, pale yellow	extrosed	——	——
(***B. tri.-tour.***) F_1	green, pubescent	intermediate, not clasping	intermediate, pale yellow	introsed	——	——

Table 3. Chromosome pairings at meiotic metaphase-I in PMCs of the F_1 hybrids.

Configuration	Frequency occurrence of PMCs at metaphase-I of F_1 hybrids				
	B. trilocularis × ***B. campestris***	***B. tournefortii*** × ***B. campestris***		***B. tournefortii*** × ***B. trilocularis***	
		A	B	A	B
20_{I}	–	9	8	23	19
$1_{\mathrm{II}}+18_{\mathrm{I}}$	–	18	18	39	25
$2_{\mathrm{II}}+16_{\mathrm{I}}$	–	20	42	24	21
$3_{\mathrm{II}}+14_{\mathrm{I}}$	–	13	18	8	3
$4_{\mathrm{II}}+12_{\mathrm{I}}$	–	8	10	3	1
$5_{\mathrm{II}}+10_{\mathrm{I}}$	–	–	3	–	–
$9_{\mathrm{II}}+2_{\mathrm{I}}$	1	–	–	–	–
10_{II}	99	–	–	–	–
Total number of PMCs	100	68	99	97	69
Average number of II per cell	10	1.90	2.13	1.27	1.16
Exceptional configuration					
$1_{\mathrm{III}}+17_{\mathrm{I}}$	–	1	–	–	1
$1_{\mathrm{III}}+1_{\mathrm{II}}+15_{\mathrm{I}}$	–	–	–	1	–

N. B. A and B denote the different dates of observation with the same individuals.

(1) F_1 ***B. trilocularis*** × ***B. campestris***: There were found 10 bivalents formed at MI, of which 1 or 2 were often loosely paired (Fig. 1). Univalents rarely occurred in case of faulty pairings (Fig. 2, Table 3). The mode of chromosome distribution at AI towards the sister nuclei was 10–10 in most cells, being 9–11 in rare cases, so that the subsequent division processes could proceed quite normally, but examinations given thrice with a weekly interval revealed that pollen-fertilities, as designated by the frequency occurrence of well-stainable pollen-grains, did not exceed 80 %, i. e., 76.9, 76.8 and 79.2 %, respectively. The average seed-fertility, denoted by the percentage value of viable seeds per ovule formed, attained to 27.6 % under the open-pollination.

(2) F_1 ***B. tournefortii*** × ***B. campestris***: The configuration obtained at MI was mainly composed of loosely associated bivalents and several univalents (Figs. 3, 4), and the maximum number of bivalents per cell attained to 5. In some rare cases a trivalent appeared. The occurrence of a large number of univalents at MI resulted no doubt in the remarkable irregularity in the subsequent division processes, so that there were observed at the pollen-tetrad stage various kinds of sporads, ranging from monad to hexad, diads being fairly high in its frequency appearance. Both the pollen- and seed-fertilities became to be almost nil.

(3) F_1 ***B. tournefortii*** × ***B. trilocularis***: The configuration at MI in this

F_1, being just like that obtained in the F_1, ***B. tournefortii*** × ***B. campestris,*** was composed of a few loosely paired bivalents (0–4) and many univalents (20–12), with rare occurrence of a trivalent (Figs. 5, 6). The subsequent divisions were effected in the similar way in this F_1 as in the preceding F_1, excepting the quite frequent appearance of diads and triads. Diads showed rather more frequent occurrence as compared with the normal tetrads in some anthers examined. It appears likely that the occurrence of diads would bring about the formation of nonreductive gametes, because nearly half of pollen-grains produced in a fairly developed anther locule appeared to be quite viable in good contrast to the rest degenerated ones occurring with them.

Discussion

The 10-chromosome forms of ***Brassica,*** e. g., ***B. pekinensis, B. rapa, B. chinensis, B. japonica, B. narinosa, B. campestris*** etc., can cross easily with each other, resulting in the production of quite fertile F_1 hybrid plants, which show remarkable differences in their various characteristics (Sinskaia 1927, Morinaga 1928, Sikka 1940, Olsson 1954). Excepting the authors' F_1 hybrid plants reported herewith, the 2 F_1 hybrid plants had been obtained by Sikka (1940) through the crossing, ***B. tournefortii*** × ***B. trilocularis,*** though a number of crossings between ***B. tournefortii*** and the other ***Brassica*** forms have been tried by several workers.

The interrelationship existing among ***B. campestris, B. trilocularis*** and ***B. tournefortii,*** as presumed from the manner of chromosome pairings in these F_1 hybrids, may be summarized as follows: (1) The genome of ***B. trilocularis*** will be considered to be homologous to that of ***B. campestris*** with very slight difference. A slight meiotic irregularity observable in the F_1 ***B. trilocularis*** × ***B. campestris*** could also be recognized in the F_1 ***B. rapa*** × ***B. trilocularis*** (Sikka 1940), in the F_1 ***B. campestris*** (var. ***oleifera,*** var. ***toria***) × ***B. trilocularis*** and in the F_1 ***B. chinensis*** × ***B. trilocularis*** (Olsson 1954), and in the F_1 ***B. pekinensis*** × ***B. rapa*** (Richharia 1937). Most of the interspecific F_1 hybrids in ***Brassica*** having 10 pairs of chromosomes showed rather normal meiotic behaviours and the resultant high fertility, but the remaining several hybrids showed certain meiotic irregularities and resulted in the reduced fertility, probably because of various degree of genetic differentiations which have taken place within the genome ***a.*** ***B. trilocularis*** showed, however, rather definite difference from the typical ***a*** genome species in the following characteristics; the general morphology, the self-fertile reproductiveness, and certain biochemical features of the methanol-soluble leaf extract (cf. Sinskaia 1928, Alam 1945, Fukushima and Iwasa 1965). Olsson (1954) has destined this form as a good subspecies of ***B. campestris*** in clearcut contrast to any other forms. Thus the authors would prefer to give the designation *a′* to this genome of ***B. trilocularis.*** (2) The authors' investigation has disclosed that ***B. tournefortii*** was fairly different in its genome constitution from either of ***B. campestris*** or ***B. trilocularis.*** Thus the designation ***T*** was duly given to the genome of ***B. tournefortii.***

As shown in Table 3, from the rather rare appearance of a trivalent chromosome in the PMC of F_1 hybrid it may be duly conceivable that one bivalent would be possible to occur as an autosyndetic pairing within either one of the genomes *T*, *a* or *a′*. Ramanujam (1941) found rarely in his haploid mutant of ***B. campestris*** one single bivalent which may be produced by an autosyndesis within *a* genome. If so, it would naturally follow that each two of bivalents formed in the F_1 ***B. tournefortii*** × ***B. trilocularis*** and in the F_1 ***B. tournefortii*** × ***B. campestris*** are the product of autosyndetic pairings. In consequence, granted that such two autosyndetic bivalents will occur at most the formation of two or three allosyndetic bivalents in the F_1 hybrids would be taken as the proved fact from the maximum chromosome pairings at MI. The observations described above will support no doubt that the genome *T* is partially homologous to *a* and *a′* genomes.

Summary

The authors have tried to raise interspecific crosses between ***B. trilocularis*** or ***B. tournefortii*** and several other ***Brassica*** species, and succeeded in obtaining true F_1 hybrids in the following three cross-combinations: ***B. tournefortii*** × ***B. campestris***, ***B. tournefortii*** × ***B. trilocularis*** and ***B. trilocularis*** × ***B. campestris***. ***B. tournefortii*** was highly cross-incompatible with ***Brassica*** species having *a*, *b* or *c* genome, and ***B. trilocularis*** was, in turn, highly cross-compatible with ***Brassica*** species having *a* genome. Meiotic chromosome associations obtained with those F_1 hybrids were $(0\text{–}4)_{II}+(20\text{–}12)_{I}$ in F_1 ***B. tournefortii*** × ***B. trilocularis***, $(0\text{–}5)_{II}+(20\text{–}10)_{I}$ in F_1 ***B. tournefortii*** × ***B. campestris*** and $(9\text{–}10)_{II}+(2\text{–}0)_{I}$ in F_1 ***B. trilocularis*** × ***B. campestris***.

The result of genome-analysis has made clear that ***B. tournefortii*** is composed of the genome *T*, being partially homologous with the genome *a*, and that the genome *a′* of ***B. trilocularis*** is taken as that distinctly different from the genome *a*, though rather slight in its degree.

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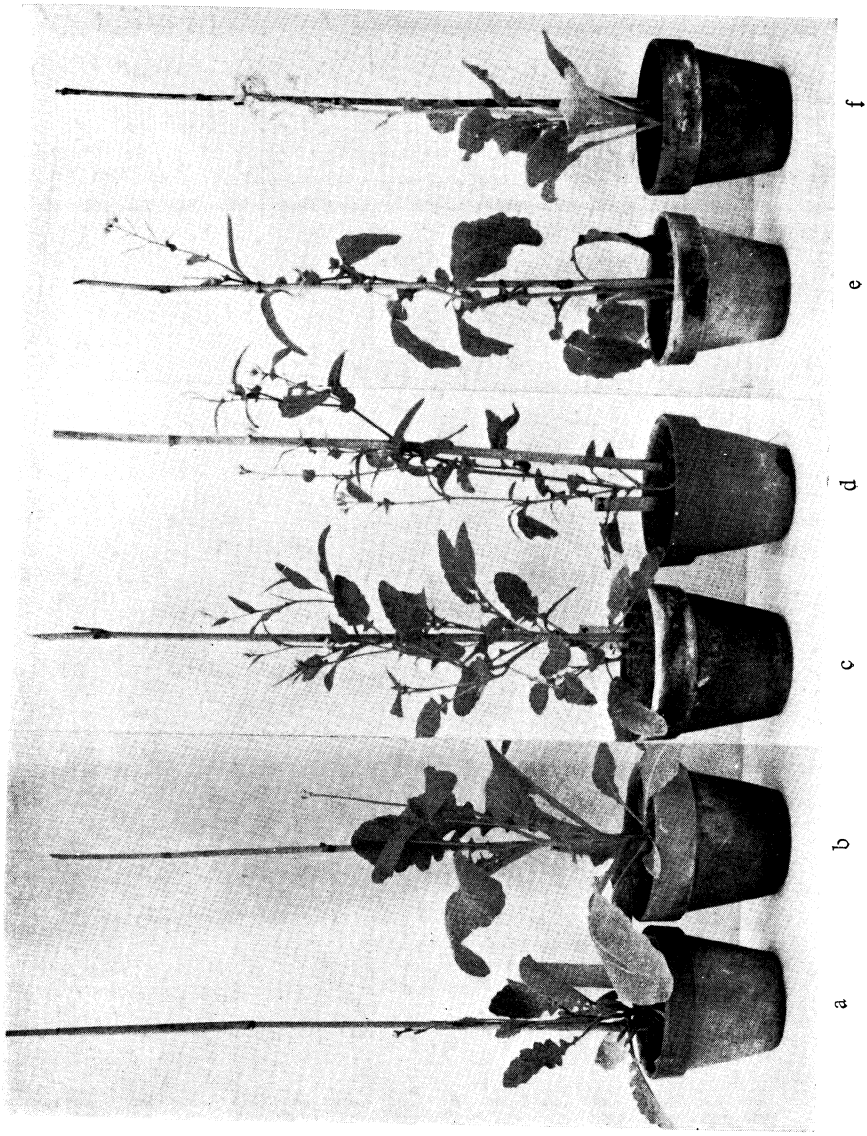
Explanation of Plate 14

Flowering plants of F_1 hybrids and their parents.
a, ***B. tournefortii***; b, F_1 hybrid of ***B. tournefortii*** × ***B. campestris***; c, F_1 hybrid of ***B. tournefortii*** × ***B. trilocularis***; d, ***B. trilocularis***; e, F_1 hybrid of ***B. trilocularis*** × ***B. campestris***; f, ***B. campestris.***

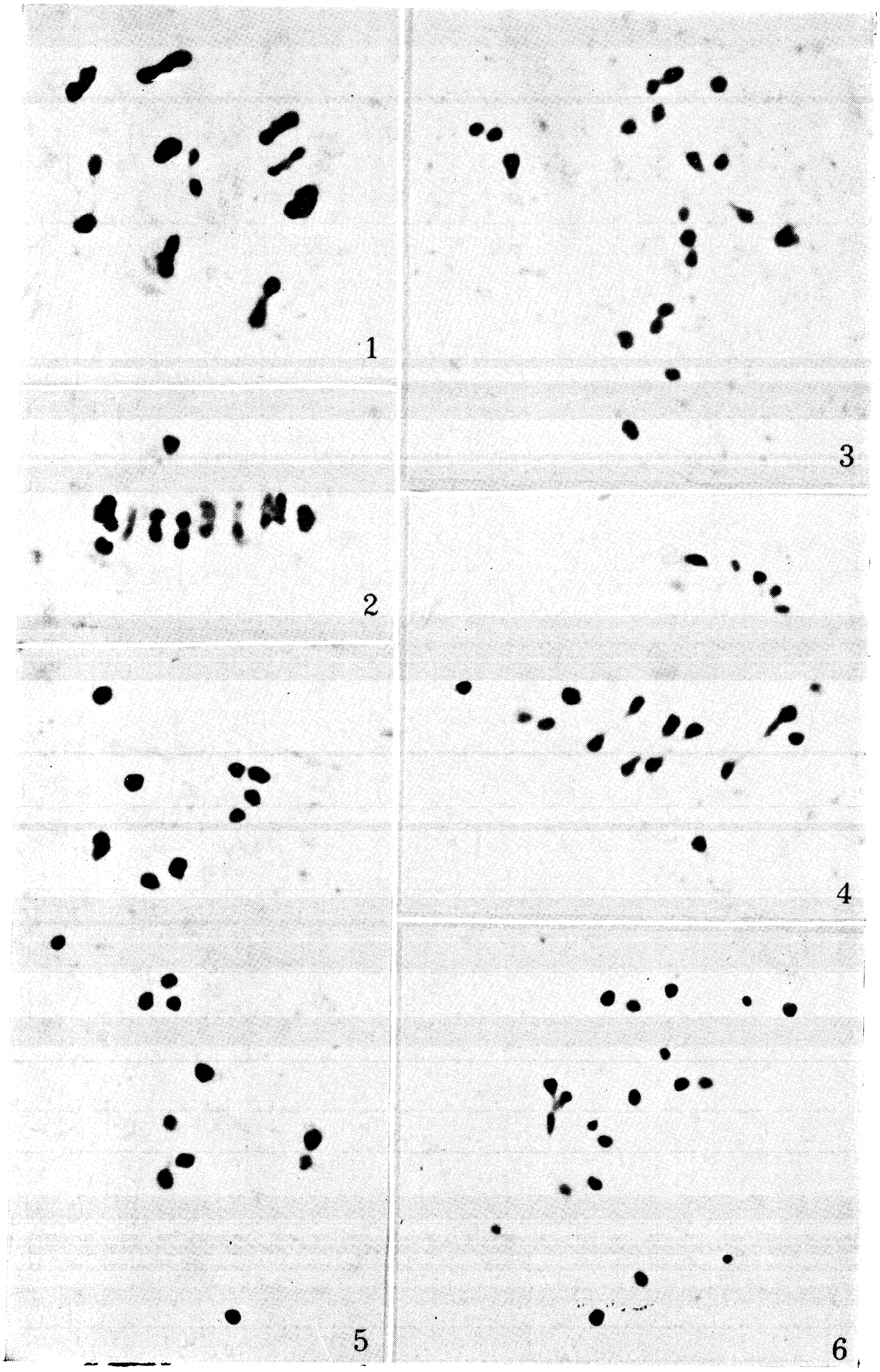


Genome-analytic studies on *Brassica trilocularis* and *B. tournefortii*

Explanation of Plate 15

Microphotographs of metaphase-I in PMCs of F_1 hybrids, ***B. trilocularis*** × ***B. campestris*** (Figs. 1 and 2), ***B. tournefortii*** × ***B. trilocularis*** (Figs 3 and 4), and ***B. tournefortii*** × ***B. trilocularis*** (Figs 5 and 6).

Fig. 1. 10_{II}, containing two loose bivalents. Fig. 2. $9_{\mathrm{II}}+2_{\mathrm{I}}$. Fig. 3. $\mathrm{I}_{\mathrm{II}}+18_{\mathrm{I}}$. Fig. 4. $4_{\mathrm{II}}+12_{\mathrm{I}}$. Fig. 5. 20_{II}. Fig. 6. $1_{\mathrm{III}}+17_{\mathrm{I}}$.



Genome-analytic studies on *Brassica trilocularis* and *B. tournefortii*