A Cytogenetical Study on the Origin of Twin Plants Found in the Progeny of an Aneuploid in *Brassica napus* L.

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Synopsis. Six pairs of twins were found in the progeny of an aneuploid ($2n=67$) in *Brassica napus* L. var. *oleifera* DC. The frequency of occurrence of twins was 9.09%. Five pairs had chromosomes between 60 and 68 inclusive. These were presumably of sexual origin. One pair, both members of which had 40 chromosomes, probably originated from the cleavage of a reduced unfertilized cell which developed parthenogenetically.

Introduction

Polyembryony in plants has sometimes been an object of interest, because it is frequently accompanied with the occurrence of haploid or polyploid plants (NAMIKAWA and KAWAKAMI 1934; YAMAMOTO 1936; MÜNTZING 1938; YAMASAKI 1947; MORGAN and RAPPLEYE 1954; WATANABE and MUKADE 1962). In the course of the cytological studies of polyploids and aneuploids in rape, the author obtained some twins unexpectedly. The object of this paper is to describe the morphological and cytological features of the twins and discuss their origin.

Materials and Methods

During the course of an investigation on artificial polyploids in *Brassica napus* L. var. *oleifera* DC., cultivar "Chusei-natane", an aneuploid ($2n=67$) was obtained by crossing a hypo-tetraploid ($2n=72$) ♀ with a triploid ($2n=57$) ♂. Among the open pollinated progeny of this aneuploid, six pairs of twins were found.

Eighty-four seeds obtained from the aneuploid ($2n=67$) were sown in September, 1965, after their sizes were measured with slide calipers. Seventy-one seeds germinated, of which five seedlings seemed to be hybrids with other *Brassica* species. Out of 66 genuine rape seedlings, six produced two plants each. Therefore, the occurrence frequency of twin seedlings was 9.09%. In

Received July 24, 1972

the spring of 1966, the date of the first flowering in each plant was recorded. Cytological studies were conducted on microsporocytes to determine chromosome numbers by using the aceto-orcein smear technique. Pollen viability was tested by staining with aceto-carmin, more than 2,500 pollen grains being used in each plant. Plant height was measured when the twins reached their maximum size, and the mean number of seeds per fruit was recorded in each plant.

The seeds from which the twins were derived had been numbered as "No. 6, No. 18,....., and No. 59", and one plant in each pair was denoted "a" and the other "b".

Results

1. Morphology of the twins

Any discrimination could not be made in size and shape between the twin-producing

Table 1. Plant characters of twins

Twins	Number of cotyledons	Date of first flowering	Plant height	Pollen viability	Number of seeds per capsule
No. 6-a	4	18th, Mar.	133 ^{cm}	74.2 %	3.50
-b	1	23rd, Mar.	139	59.7	3.25
No. 18-a	3	11th, Mar.	158	78.6	4.94
-b	2	—	138	57.5	5.66
No. 28-a	2	4th, Mar.	135	84.9	4.05
-b	3	14th, Mar.	156	88.8	5.12
No. 32-a	2	11th, Mar.	155	68.2	4.07
-b	2	14th, Mar.	138	70.4	3.63
No. 44-a	3	2nd, Mar.	102	Anthers did not dehisce. do.	0.03
-b	4	3rd, Mar.	98		0.01
No. 59-a	2	1st, Mar.	186	92.2	4.44
-b	2	3rd, Mar.	162	91.2	4.44

seeds and the normal ones. The major diameter of the former seeds was 4.20 mm on an average, and that of the latter was 4.04 mm. The difference between both types was not significant ($t=0.608$, $df=18$, $P=0.5-0.6$). One of the prominent characteristics observed in the twins was the high frequency of anomalous cotyledons. As shown in Table 1, half of all the twin plants had one, three, or four cotyledons. Among 60 normal sister plants of the twins, however, five were found to have three cotyledons instead of the normal two, the frequency of occurrence being 8.3%.

In each pair of the twins, there were no marked differences in vigor and growth rate between both plants. Table 1 shows the number of cotyledons, the date of first flowering, plant height, pollen viability, and seed fertility. As shown in Table 1, No. 44 twins had less difference between both constituent members in each character than any other twin pair. Moreover, both members of this pair resembled each other very closely and showed the following predominant morphological characteristics. First, they were both short in plant height, being about one meter tall, as compared with other normal or twin plants which were all above 1.2 meters. Secondly, their branching was upward spread; their stems and branches were slender, the upper portion of which was distorted and fasciated; their leaves were narrow; the intervals between flowers, hence between capsules, were short, so their flowers and capsules were aggregative (Figure 1). Thirdly, the size of flowers and thickness of petals of No. 44 twins were like those of diploids. Their flower buds were more or less rugose, and their sepals in this stage were inclined to separate. Their sepals and petals became discolored and whitish especially at the end of the flowering season. Their anthers did not dehisce, shedding no pollen. Their seed fertilities were extremely low (Table 1).

2. Cytology of the twins

The somatic chromosome number in each plant was determined mainly from the total number of chromosomes appearing on both daughter nuclear plates at metaphase II in the same microsporocyte. The numbers of chromosomes located on the plates at this stage are shown in Table 2. The normal sister plants of the twins, *i. e.* the progeny of the aneuploid ($2n=67$), had chromosome numbers ranging between 58 and 68 with a peak of 64. The twins covered the same range, except both individuals of No. 44 having 40 chromosomes.

Fig. 1. Illustration of branches showing distortion, fasciation, and aggregative fruit-setting in No. 44 twins. (Left two, No. 44-a; right two, No. 44-b)

Table 2. Chromosome numbers located on the nuclear plates at metaphase II of microsporocytes in the twin plants

Twins	Number of chromosomes										Number of plates	Somatic chromosome number
	27	28	29	30	31	32	33	34	35	36		
No. 6-a -b			1	1	5	5	6	3			21 4	65 60±
No. 18-a -b				1	2	3					6 26	63± 63
No. 28-a -b				1		1	8	11	3		24 26	67 68
No. 32-a -b			1	2	7	8	9	3	1	1	32 22	65 65
No. 59-a -b	1 1			3	7	8	6	2	2		29 39	65 60
	Number of chromosomes											
	16	17	18	19	20	21	22	23				
No. 44-a -b	1 1		3	9	16	10	4	1			44 41	40 40

Table 3. Lagging chromosomes observed at anaphases I and II of microsporocytes in No. 44 twins

	Number of laggards	Number of microsporocytes	
		No. 44-a	No. 44-b
Anaphase I	0	10	7
	1	16	7
	2	17	2
	3	8	2
	4	3	1
	5	1	
	6	2	
	7		
Anaphase II	8		1
	0+0	7	4
	0+1	10	11
	0+2	3	1
	1+1	3	2
	0+3	2	1
	1+2	4	6
	2+2		2
	1+4	1	
	2+3	1	1
	2+4	1	

In each of No. 18, No. 32, and No. 44 pairs both constituent members had the same chromosome number as each other, but in No. 6, No. 28, and No. 59 pairs they did not.

Chromosome configurations at metaphase I were not observed in every twin, except No. 44 ones in which only a few microsporocytes were examined. One plant of No. 44 twins had one microsporocyte showing $5_{III}+12_{II}+1_I$ configuration, and another plant two sporocytes showing $6_{III}+11_{II}$ and $5_{III}+12_{II}+1_I$, respectively. In both of them, many laggards were observed at anaphases I and II as shown in Table 3.

Discussion

The frequency of occurrence of twins or triplets in crops is generally quite low: *e. g.*, 0.02~0.09% in wheat (YAMASAKI 1947; KAWAKAMI and HATAMURA 1952; WATANABE and MUKADE 1962), 0.06~0.65% in *Capsicum* (MORGAN and RAPPLEYE 1950), 0.02~0.70% in rye (MOREY and MOREY 1959), 0.37% in cotton (ENDRIZZI 1959), 0.05~0.17% in tomato (FOSKETT 1960), and 0.01~0.07% in beet (KRUSE 1960). The frequency of the twins in this study showed as high as 9.09%. Such a high

frequency has rarely been reported. KRUSE (1960) reported that in swede (*Brassica napus* var. *rapifera*) the frequencies of twins, triplets, and quadruplets varied from 0.004% to 0.057%, the average being 0.023%, according to different Danish strains. On the other hand, TASAKA (1945) found the frequencies of twins to be 0.02~9.27% in some Japanese varieties of the rape (*Brassica napus* var. *oleifera*), among which the highest frequency was shown in the variety "Tohoku 9" (quoted by WATANABE 1958). The frequency of this variety (9.27%) is nearly equal to that of the present study (9.09%). In other species of Brassiceae, however, frequencies of twins are low: *e. g.* 0.135% (double seeds) in *Brassica oleracea* (HOWARD 1939) and 0.00~0.08% in *Raphanus* (IZUKA 1960).

In many crops there are found differences in the incidence of polyembryony among varieties or strains, and such varietal differences are suggested to be hereditary (*e. g.*, YAMASAKI 1947; MOREY and MOREY 1959; McEVAN and VIZER 1966). Whether the exceedingly high frequency of the twins found in this study is hereditary or not, is uncertain. Throughout a series of experiments about polyploids and aneuploids in rape, however, there has been no occurrence of polyembryony, except in the present study, during the last eight years (TOKUMASU unpublished). If hereditary, twins would have been obtained not only in the present case but also in the successive generations.

As seen in Table 1, half of the twins showed the irregularity of cotyledons. WATANABE (1958) reported that in *Brassica napus* all of the conjoined twins found had malformed cotyledons, and some of the unattached twins had excessive or deficient numbers of cotyledons. In *Brassica napus*, therefore, the anomalous number of cotyledons seems to be closely associated with polyembryony. This anomaly might be caused by the forced development of polyembryos within a limited space in a single ovule. WATANABE (1958) further reported that polyembryos in *B. napus* were obtainable from small seeds rather than from large ones. IZUKA (1960) also found the same tendency in *Raphanus*. If such a tendency is common, smallness of twin-producing seeds may be the

result of incomplete development of embryos. In this study, however, twinning was not concerned with the size of seeds.

Embryological or histological studies may be desirable to determine the modes of origin of polyembryonic seeds. However, they are practically difficult or almost impossible due to the extreme limitation in their occurrence. Instead of the embryological studies, genetical and cytological methods are sometimes employed for this purpose. There are some examples in which the origin of twins was explained with the aid of one or more genetic markers (KAWAKAMI and HATAMURA 1952; MORGAN and RAPPEYE 1954; FOSKETT 1960; IIZUKA 1960), but few in which the origin of twins was discussed on a cytological basis. ENDRIZZI (1959) obtained some twins of cotton from crossing between, or selfing of, parents that were not only different genetically, but also different cytologically. Comparing the cytological aspects of the twins with those of their parents, he inferred the origin of the twins from the cytological and phenotypical point of view. ENDRIZZI's materials, unlike the rape in the present study, were not polyploids, but diploids which had some chromosome aberrations such as deletion, translocation, chain, or ring. The deduction of the origin of twins through polyploidy and aneuploidy as in the present study may also be referred to as a cytological method. If aneuploid twins occur in diploid population, the aneuploidy itself suggests the peculiar modes of their origin (YAMASAKI 1947 in wheat; IIZUKA 1960 in *Raphanus*). However, it does not seem strange that some of the twins occurring in tetraploid population are of aneuploidy, because tetraploids usually produce aneuploid seeds as well as euploid ones (*e. g.* TOKUMASU 1961). Such an instance was found in tetraploid barley (TSUCHIYA 1953).

WEBBER (1940) has given a detailed review of polyembryony in plants and classified them as follows: (1) sporophytic budding, (2) cleavage, *i. e.* the separation of zygote or young embryo into two or more units, (3) simple polyembryony, *i. e.* the formation of more than one egg (including egg-like synergids, or antipodals) in a single embryo sac, and (4) the development of more than one embryo sac in

an ovule. It is also a problem whether twins are of parthenogenetic or sexual origin. The following are the discussions about the origin of the twins conducted in this study on the basis of cytological and morphological evidences.

As previously stated, the majority of the plants, among which the twins were found, had chromosome numbers ranging from 58 to 68. And the chromosome numbers of the twins fell within this range, with the exception of No. 44 (Table 2). The twins as well as their sister plants were presumably of sexual origin. These plants were grown from open-pollinated seeds. But most seeds were produced possibly by selfing, as *Brassica napus* is autogamous species. The parent ($2n=67$) of these plants showed the nuclear plates having 30 to 36 chromosomes at metaphase II (TOKUMASU, unpublished). If the gametes receiving such chromosome numbers are functional, the parent could produce seeds having 60 to 72 chromosomes. Such expected chromosome numbers of seeds generally agreed with those of the twins and their sister plants. On the other hand, the possibility that No. 28-a was derived from an extra adventitious embryo arising from maternal tissue cannot be excluded, because it had the same chromosome number as the parent. No. 44 twins had much lower chromosome number ($2n=40$) as compared with the above-mentioned plants. This pair perhaps originated from a reduced unfertilized sexual cell. If more nuclear plates are counted at metaphase II of the parent, 40 chromosomes may be encountered. It seems likely that the egg cell with 40 chromosomes developed parthenogenetically into an adult.

In some of the twins (Nos. 6, 28 and 59), both constituent members of each pair were different in chromosome numbers from each other. In these cases, both members of each pair probably arose from two separate sexual cells fertilized with male nuclei. But it is uncertain whether two fertilized cells were within one or two embryo sacs, or whether they were eggs or synergids or antipodal cells. Among the twins both members of which had the same chromosome numbers (Nos. 18, 32 and 44), No. 44 twins showed striking resemblances in every respect, morphologically and cytologically, between each other. It seems

very likely that No. 44 twins were derived from the cleavage of a parthenogenetic embryo. As to the origin of this pair, another possibility remains that both members developed from two separate reduced cells within one embryo sac. MORGAN and RAPPLEYE (1954) stated that stimulation of the synergids, followed by disintegration of the sexual embryo, could account for the development of haploid-haploid twins. With regard to No. 18 twins, both members seemed to be genetically dissimilar as there were found considerable differences in pollen fertility and plant height. And it is not necessarily determinate that they had the same chromosome numbers (Table 2). Therefore, the origin of No. 18 twins was probably two different embryos. FOSKETT (1960) demonstrated in tomato that even in identical twins frequently one member of a pair was larger than the other, indicating differences in growth due to factors other than genetic. As indicated in Table 1, No. 32 twins were similar to each other in various characters except plant height. If plant height is a character susceptible to environmental factors, No. 32 twins were likely to be genetically identical. Their chromosome numbers being considered, they probably originated from the cleavage of one zygote. Another possibility of the origin of the identical twins is that two sexual cells within one embryo sac unite with two male nuclei from the same pollen tube. This possibility, however, seems to be small because one of the two male nuclei usually unites with an egg and the other with polar nuclei.

Summary

(1) Among the open pollination progeny of an aneuploid ($2n=67$) in *Brassica napus* L. var. *oleifera* DC., six pairs of twins were found in 1966. The occurrence frequency of the twins was 9.09%. There were no differences in size and shape between the twin-producing seeds and the normal ones.

(2) Half of the twins had anomalous cotyledons. In each pair of the twins, there were no marked differences in vigor and growth rate between both constituent members. But some pairs showed considerable differences in the date of first flowering, plant height, pollen

viability, and seed fertility. In one pair (No. 44), both members resembled each other very closely and showed predominant morphological characteristics.

(3) Somatic chromosome numbers of six pairs of the twins were as follows: 65-60; 63-63; 67-68; 65-65; 65-60; and 40-40. In No. 44 twins ($2n=40$), one plant showed $5_{III}+12_{II}+1_I$ chromosome configuration, and another one showed $5_{III}+12_{II}+1_I$ and $6_{III}+11_{II}$ configurations. In other twins, however, the first metaphasic figures were not observed.

(4) The modes of origin of the twins were determined cytologically and morphologically as follows. All the twins, except No. 44, were presumably of sexual origin. In most of them each pair arose from two separate sexual cells, which were fertilized with male nuclei, within one or two embryo sacs. While, No. 32 twins ($2n=65$) were possibly derived from the cleavage of one zygote. No. 44 twins ($2n=40$) perhaps originated from the cleavage of a reduced unfertilized cell which developed parthenogenetically.

Acknowledgement

The author wishes to thank Prof. K. MATSUMOTO of Ehime University for reading the manuscript.

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ナタネの異数体の次代にあらわれた双生子植物の 起源に関する細胞遺伝学的研究

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ナタネ *Brassica napus* var. *oleifera* の低四倍体 ($2n=72$) と三倍体 ($2n=57$) との交配より生じた異数体 ($2n=67$) の次代より 6 対の双生子植物を得た。その出現率は 9.1% であった。双生子を生じた種子と正常の種子との間には形態的にも、また大きさの点においても差異を認めなかつた。双生子植物の半数は子葉数の異常を示したが、その後の生育には各対を構成している植物体間に著しい差異は見られなかつた。

一方、開花始期・草丈・花粉稔性・種子稔性についての調査では、双生子対によつて若干の差異を示すものと、何らの差異をも示さないものがあつた。特に No. 44 は両植物とも特異な形態を示し、両者はきわめて類似していた。6 対の双生子の体細胞染色体数は MII における両娘核板上の染色体数の観察から、65-60, 63-63, 67-68, 65-65, 65-60, および 40-40 と決定された。MI の観察は 40 染色体数を持つ一対 (No. 44) のほかには行なわなかつた。No. 44 では、一個体が $5_{III}+12_{II}+1_I$ および $6_{III}+11_{II}$ の対合型を示し、他の一個体は 5_{III}

$+12_{II}+1_I$ を示した。本実験で得られた双生子の出現頻度は他作物のそれにくらべきわめて高く、また子葉数の異常を伴うことも特徴的である。この高出現頻度が遺伝的なものかどうか明確にし得ないが、その後代に再び双生子の出現が見られず、非遺伝的である可能性が強い。双生子の起源について細胞学的ならびに形態学的観点から考察すると、60 ないし 68 染色体数を有する双生子対の大部分の個体は 2 個の別々の生殖細胞 (卵細胞あるいは助細胞) が、それぞれ雄核と結合して生じたものと思われる。その際 2 個の生殖細胞は同一の胚嚢のものか別々の胚嚢のものは不明である。No. 32 は、その構成個体が共に同一染色体数 ($2n=65$) を有し、形質も差異がなく恐らく一つの接合体から分離して生じた一卵性双生子であろう。No. 44 はその染色体数 ($2n=40$) から減数卵が単為生殖的に発達したと推定され、またその構成個体の形態的、細胞学的類似性から一卵性双生子と考えられる。