CYTOGENETIC STUDIES ON *Mentha arvensis* VAR. *piperascens* MALINV. AND ITS DERIVATIVE HYBRID WITH SPECIAL REFERENCE TO THE UTILIZATION OF POLYPLOIDY IN THE BREEDING OF JAPANESE MINT

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Content

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I. Introduction

Up to the present, workers in breeding projects with Japanese mint have concentrated on selection or hybridization within species, (CHAMURA 1954, KASANO 1953, 1954) and some superior varieties[1] have been released. Although most of them were resistant to the noxious rust disease, the rust fungus has become adapted to these varieties through new races, and at present they are severely damaged by this disease. Accordingly, the improvement of rust resistance is one of the most important objectives in Japanese mint breeding. This situation has prompted the breeders to attempt a transference of disease resistance from other species to Japanese mint through interspecific hybridization[2].

In order to carry out such breeding work successfully, detailed cytogenetic information on mint plants should be accumulated. Cytological and cytogenetic studies on mint plants have been conducted by several investigators such as SCHÜRHOFF (1927, 1929), LIETZ (1930), RUTTLE (1931), NAGAO (1941), IKEDA and UDO (1954 a, b, 1955 a, b, 1956, 1958, 1960), and MORTON (1956). At present, however, no decisive proposition has been made on cytogenetic relationship between Japanese mint and other species in genus *Mentha*. This is

1) Technical terms of cytogenetics whithout footnote were cited form the Nomenclature of Breeding (Jap. Jour. Breed. 9 : 194-204).

2) Cited from the Handbook of Genetics, Gihodoh 1956.

mainly due to the fact that extensive hybridization or the systematic induction of amphiploids[1] has not been attempted.

For many years, the present author has also been engaged in cytogenetic work of mint plants and has found a few species in which chromosome behaviors had the characteristics of autopolyploidy with the basic chromosome number of 6 (TSUDA 1954, 1956 a, b).

It is the first aim of the present report to obtain more detailed knowledge of polyploidy in mint plants. The author observed the chromosome behavior in a cultivated variety of Japanese mint and a variety of *Mentha longifolia* L. The observations also covered an F_1 hybrid of these two plants, its artificial amphiploid and first and second backcrossed progenies to Japanese mints. In addition to the above work, these plants were evaluated agronomically for characters such as resistance to rust disease, content of essential oil, and the content of menthol which is the main product from Japanese mint. Through these experiments, the author attempted to explore the possibility whether a variety of *M. longifolia* L. mentioned above can be used as a non-recurrent parent in the backcross method with Japanese mint.

Though this work consititutes a greater part of the present paper, some experiments and considerations were made on two artificially induced polyploid Japanese mints from a practical point of view.

The work was conducted at the Hokkaido University in Japan since 1949. Before going further, the author wishes to express his gratitude to Prof. S. NAGAO for his very helpful suggestions and invaluable critisism in regard to the present work. Likewise the author is indebted to Dr. M. KIKUCHI for his encouragement at the beginning of this work. The author also wishes to express his thanks to his former chiefs Dr. T. SHIBUYA and the late Dr. G. MISONOO who generously gave approval to the author to undertake this work. The author must acknowledge the kindness of Dr. S. HOSOKAWA and Dr. M. TAKAHASHI who encouraged him to carry out this work and rendered many helpful suggestions. He also wishes to thank a number of persons, too numerous to mention, but in particular Dr. H. SATO and the late Mr. I. MAKINO who were enrolled at the Industrial Crops Laboratory of the said University. They assisted the author on many occasions.

The author wishes to thank Dr. RAY NELSON and Dr. JOHN L. LOCKWOOD, Michigan State University in U. S. A. for their kind help in preparing this report.

1) Cited from POEHLMAN (1956).

II. Cytogenetic Studies

1. Materials

Materials which were used in this experiment are described in the Experimental Results.

2. Experimental Methods

a. Microscopic Observations

Somatic chromosome numbers were examined in root tip cells. Root tips were cooled in running tap water (ca. +10°C) for one hour before fixing so that chromosomes would be scattered and shortened. Afterwards they were fixed with FLEMMING's weaker solution or NAVASHIN's fluid for 24 hours. The paraffin sections were cut to about 15 microns in thickness, and were stained with HEIDENHEIN's Iron Alum Hämatoxylin. All drawings were made with the aide of an ABBE's drawing apparatus using a LEITZ oil-immersion objective 100×(1/12) with a Yashima K 20 ocular at a magnification of about 3000 times at table level.

Observations of chromosome behavior at meiosis were carried out by smear or squash method with SUTO's iron aceto-carmine staining. The young flower buds were cooled in running tap water for about one hour before fixing. All figures were drawn with the aid of an ABBE's drawing apparatus using a LEITZ oil-immersion objective 100×(1/12) with a LEITZ periplan OK 25 or a Yashima K 20 ocular at a magnification of about 3000 times at table level or 2500 times at the level of the stage of microscope.

Photomicrographs were taken with the aid of the same objective and an Olympus P 15 X ocular, and reproduced at the same magnification as the drawings mentioned above.

Pollen fertility was estimated by counting the pollen grains stained with SUTO's iron aceto-carmine.

b. Crossing

On the day before anthesis, the petal-attached anthers were plucked, or anthers were rolled out with pointed forceps. After the stigma unfolded, the dehisced anthers of pollen parent were smeared on the stigma. The pollination was continued for about one week on the same flower branch, and during this period, as fertilized flowers were readily detected by the fact that the pistil is separable, the procedure was repeated with possible unfertilized flowers.

3. Experimental Results

a. Japanese Mint (Fig. 14, A. and B.)

The Japanese mint belongs to *Mentha arvensis* var. *piperascens* MALINV.. The verticils appear in the axils of foliage leaves on the upper portion of the stem without a distinct inflorescence. Leaves are lanceolate and petiolate with margins sharply toothed, being more or less pubescent. The flower is pale

TABLE 1. Type of Secondary Association of Chromosomes in Japanese Mint

Type of Secondary Association			Frequency	
(3)	(2)	(1)	First Metaphase	Second Metaphase
8	11	2		1
8	10	4		2
8	8	8		2
8	7	10		1
8	5	14		1
7	11	5		1
7	10	7		1
6	12	6		1
6	10	10		1
6	9	12	1	
6	8	14		1
5	14	5		2
5	13	7		1
5	9	15	1	
4	16	4		1
4	15	6		1
4	13	10	1	
4	12	12		1
4	11	14	1	1
4	9	18	1	
3	14	11		1
3	12	15		3
3	10	19		1
2	15	12	1	
2	4	34	1	
1	13	19	1	
1	12	21	1	
1	5	35	1	
0	5	38	2	
Total			12	24

violet and protandrous. The flowering time ranges from the middle of August to the beginning of October.

The somatic chromosome number is 96 in the variety Hokushin (TSUDA 1952) and it is common in all the cultivated varieties in Hokkaido. The

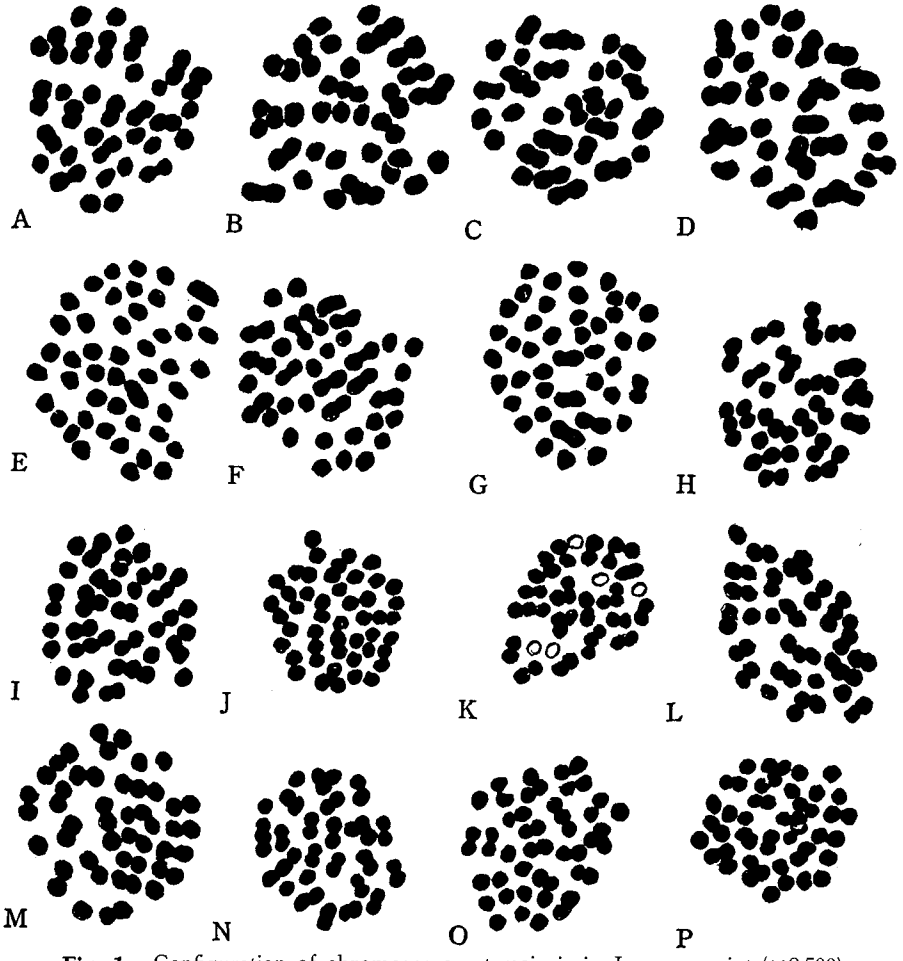


Fig. 1. Configuration of chromosomes at meiosis in Japanese mint (×2,500)

A–G. Metaphase I.

A. 6(3)+ 9(2)+12(1)	B. 5(3)+ 9(2)+15(1)	C. 4(3)+13(2)+10(1)
D. 4(3)+11(2)+14(1)	E. 2(3)+ 4(2)+34(1)	F. 1(3)+12(2)+21(1)
G. 1(3)+ 5(2)+35(1)		

H–P. Metaphase II.

H. 8(3)+11(2)+ 2(1)	I. 8(3)+ 7(2)+10(1)	J. 8(3)+ 5(2)+14(1)
K. 7(3)+11(2)+ 5(1)	L. 6(3)+12(2)+ 6(1)	M. 5(3)+14(2)+ 5(1)
N. 4(3)+16(2)+ 4(1)	O. 4(3)+12(2)+12(1)	P. 3(3)+12(2)+15(1)

meiotic chromosome behavior was observed on the variety Hokushin in 1952. Chromosomes in the early meiotic stages showed a pronounced tendency to clump, which made accurate analysis practically impossible. However, 48 bivalent chromosomes were scattered along the nuclear membrane, and no multivalent chromosomes were observed at diakinesis except for a few PMCs. A few chromosomes were observed adhering to the nucleolus. At MI, 48 bivalent chromosomes were counted and the secondary associations consisting of 2–3 bivalent chromosomes were observed as shown in Fig. 1 (A–G). As a tendency for the chromosomes to be sticky and clumped was apparent in this material, strictly objective analysis of the secondary associations was difficult. However, some types of secondary associations which were observed clearly may be shown in Table 1. At M II, secondary associations were also observed and their frequency seemed to be higher than those at M I as shown in Fig. 1 (H–P) and Table 1. During meiosis, lagging chromosomes[1] were observed in only 4 out of 57 PMCs except in materials at later flowering time. Accordingly none of pollen tetrads having one or more extra cells[2] was observed in 201 pollen tetrads. Consequently, the meiosis of the variety Hokushin is very regular.

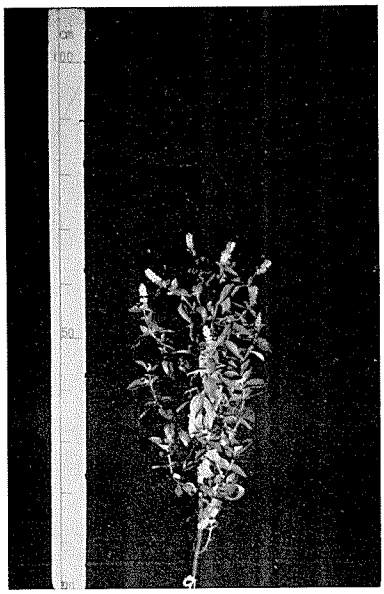


Fig. 2. A variety *Mentha longifolia* L. (D. M. A.)

b. Doitsu-Mishō-Akaguki (German Red-stem Variety grown from Seed) (Fig. 2)

The author compared this variety (designated as D. M. A.) with the specimens of *M. longifolia* L. at Bailey Hortorium, Cornell University, Ithaca, New York, U. S. A. in 1960. On the basis of the following

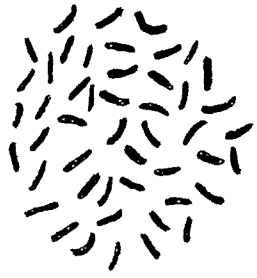


Fig. 3. Somatic Chromosomes of D. M. A. (2n=48) (×3000)

1) Cited from the Terminology of Genetics (Handbook of Genetics, Gihodoh, 1956).
2) Cited from POVILATITIS and BOYES (1956).

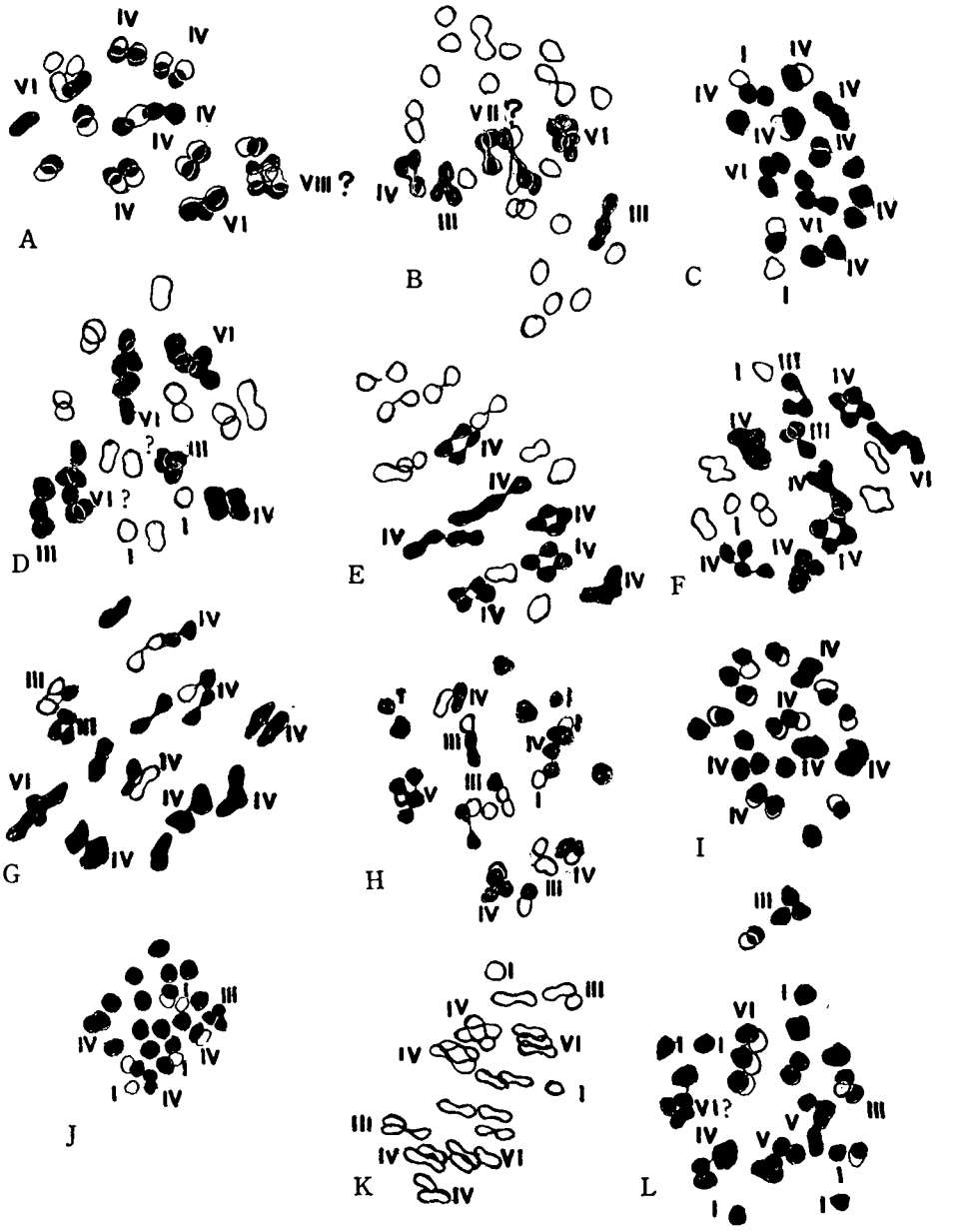


Fig. 4. Configuration of chromosomes at Metaphase I in D. M. A. (×2000)

A. $1_{VIII}+2_{VI}+5_{IV}+4_{II}$ B. $1_{VII}+1_{VI}+1_{IV}+2_{III}+5_{II}+1_{5I}$ C. $2_{VI}+7_{IV}+3_{II}+2_{I}$
D. $3_{VI}+1_{IV}+2_{III}+9_{II}+2_{I}$ E. $7_{IV}+10_{II}$ F. $1_{VI}+6_{IV}+2_{III}+5_{II}+2_{I}$
G. $1_{VI}+7_{IV}+2_{III}+4_{II}$ H. $1_{V}+4_{IV}+3_{III}+7_{II}+4_{I}$ I. $6_{IV}+12_{II}$
J. $3_{IV}+1_{III}+15_{II}+3_{I}$ K. $2_{VI}+4_{IV}+2_{III}+6_{II}+2_{I}$ L. $2_{VI}+2_{V}+1_{IV}+2_{III}+5_{II}+6_{I}$

characters, he decided that this plant belongs to a variety of *M. longifolia* L.. Leaves are ovate-lanceolate, sessile, having deep serrations. The whole plant is light green in comparison with other mints, more or less heavily pubescent. The inflorescence is rather short but not so compact. The number of flowers in a verticil is low, average 20.5. The flower is pale pink and protogamous. The flowering time ranges from the end of July to the middle of September.

The somatic chromosome number is 48 as shown in Fig. 3.

The meiotic chromosome behavior was observed in 1949 and 1960. Multivalent chromosomes ranging from tri- to octovalents were frequently observed at diakinesis and M I (Fig. 4 and Fig. 5), although high multivalent chromosomes such as octo- and heptavalent could not be clarified in a strict sense. Secondary associations consisting of four, three and two bivalent chromosomes were simultaneously observed at M I as shown in Fig. 4 and Fig. 5, A–I. If the secondary associations are included among multivalents, the configurations[1] of chromosomes at M I were as shown in Table 2. The frequency of univalent chromsomes, ranging from 1 to 16, was very high, but it seems to be greatly influenced by the temperature, since it was low in PMCs observed at the end of August. The maximum configuration observed at M I was $1_{VIII}+2_{VI}+5_{IV}+4_{II}$ (Fig. 4 A). At M II (Fig. 5 J, K and Fig. 6), the secondary associations consisting of two to four chromosomes were also observed, and its maximum configuration was 1(4)+3(3)+4(2)+3(1) as shown in Table 3. Lagging chromosomes appeared to be responsible for the formation of extra cells in the pollen tetrad which were observed in 885 out of 991 pollen tetrads (89.3 per cent). The pollen fertility, therefore, was rather low, ranging from 17.5 to 70.4 per cent, as shown in Table 4 and Fig. 7. The seed setting rate[2] is shown in Table 5, and it is considerably lower than that of male-fertile Manyo (a variety of Japanese mint) and comparable to that of male-sterile artificial autopolyploid of Manyo. Solely on the basis of these cytological observations, this plant appears to be an auto-octoploid with a basic chromosome number of 6 as in other species having a chromosome number of 48 (TSUDA 1956 a, b).

c. An Interspecific Hybrid between Japanese Mint and D. M. A.

The crossing between two species mentioned above was carried out in 1949, and D. M. A. was used as the pollen parent. Although 21 seeds were obtained from 20 crossed flowers, only one seed germinated. This F_1 seedling was designated as (H × D).

1) Cited from STEBBINS and SNYDER (1956).
2) Cited from YASUDA (1948).

TABLE 2. Chromosome Configuration at MI in D. M. A. (1960)

$1_{VIII} + 0_{VII} + 2_{VI} + 0_V + 5_{IV} + 0_{III} + 4_{II} + 0_I$	?
$1_{VIII} + 0_{VII} + 0_{VI} + 0_V + 1_{IV} + 4_{III} + 9_{II} + 6_I$	?
$1_{VII} + 1_{VI} + 0_V + 1_{IV} + 2_{III} + 5_{II} + 15_I$	?
$3_{VI} + 0_V + 1_{IV} + 2_{III} + 9_{II} + 2_I$	?
$2_{VI} + 2_V + 1_{IV} + 2_{III} + 5_{II} + 6_I$	?
$2_{VI} + 0_V + 7_{IV} + 0_{III} + 3_{II} + 2_I$	
$2_{VI} + 0_V + 4_{IV} + 2_{III} + 6_{II} + 2_I$	
$1_{VI} + 0_V + 7_{IV} + 2_{III} + 4_{II} + 0_I$	
$1_{VI} + 0_V + 6_{IV} + 2_{III} + 5_{II} + 2_I$	?
$1_{VI} + 0_V + 5_{IV} + 1_{III} + 9_{II} + 1_I$	
$1_{VI} + 0_V + 4_{IV} + 0_{III} + 11_{II} + 4_I$	
$1_{VI} + 0_V + 3_{IV} + 2_{III} + 11_{II} + 2_I$	
$1_V + 6_{IV} + 1_{III} + 8_{II} + 0_I$	
$1_V + 5_{IV} + 2_{III} + 4_{II} + 9_I$	
$1_V + 4_{IV} + 3_{III} + 7_{II} + 4_I$	
$1_V + 2_{IV} + 2_{III} + 11_{II} + 7_I$	
$1_V + 1_{IV} + 1_{III} + 11_{II} + 14_I$	
$7_{IV} + 1_{III} + 8_{II} + 1_I$	
$7_{IV} + 0_{III} + 10_{II} + 0_I$	
$6_{IV} + 1_{III} + 8_{II} + 5_I$	
$6_{IV} + 0_{III} + 12_{II} + 0_I$	
$5_{IV} + 3_{III} + 6_{II} + 7_I$	
$5_{IV} + 2_{III} + 8_{II} + 6_I$	
$4_{IV} + 2_{III} + 12_{II} + 2_I$	
$4_{IV} + 1_{III} + 12_{II} + 5_I$	
$2_{IV} + 4_{III} + 6_{II} + 16_I$	
$2_{IV} + 3_{III} + 14_{II} + 3_I$	
$2_{IV} + 2_{III} + 13_{II} + 8_I$	
$1_{IV} + 1_{III} + 19_{II} + 3_I$	

TABLE 3. Secondary Associations of Chromosomes at MII in D. M. A. (1960)

Number of Chromosomes	Number of Nuclear Plates		Type of Secondary Association			
			(4)	(3)	(2)	(1)
26	5	1	1	2	4	8
		1	1	0	6	10
		1	1	0	3	16
		1		1	6	11
		1		1	4	15
25	6	1	1	1	6	6
		1		2	8	3
		1		2	7	5
		1			9	7
		1			8	9
		1			5	15
24	13	1	1	3	4	3
		1	1	1	5	7
		1	1	1	4	9
		1	1	0	4	12
		1		2	5	8
		2		1	6	9
		3		1	5	11
		1		1	4	13
		1			6	12
		1			4	16
23	1	1		1	5	10
22	13	1	1	0	5	8
		1	1	0	4	10
		2		2	4	8
		1		2	2	12
		3		1	4	11
		2		1	2	15
		1			6	10
		1			4	14
		1			2	18
21	5	1	1	2	4	3
		1	1	1	3	8
		1		1	5	8
		2			4	13
20	4	1	1	1	1	11
		1		1	3	11
		1		1	2	13
		1			3	14
19	2	1		2	0	13
		1		1	3	10
18	1	1		1	4	7
Total	50					

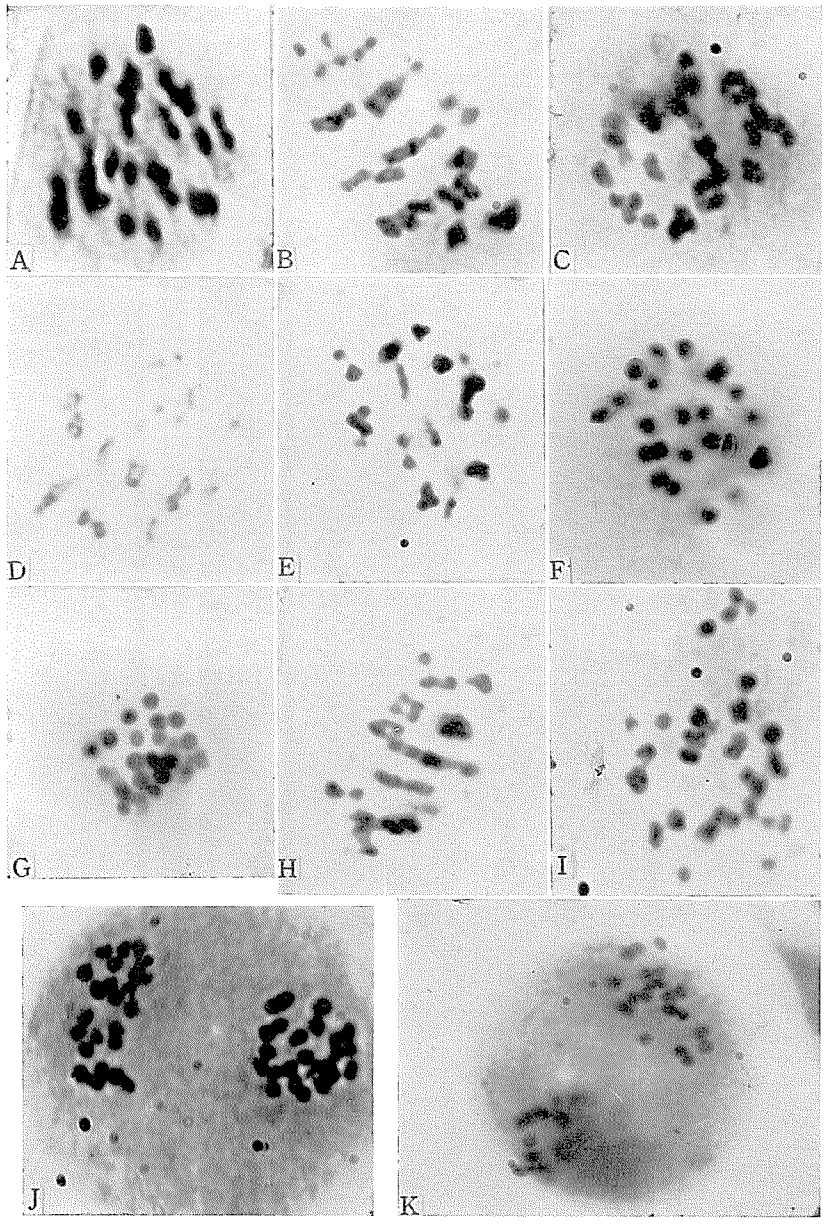


Fig. 5. Microphotograph of Metaphase in D. M. A.. A–I correspond to figures D–L in Fig. 4, and J and K correspond to Figures F and J in Fig. 6 respectively.

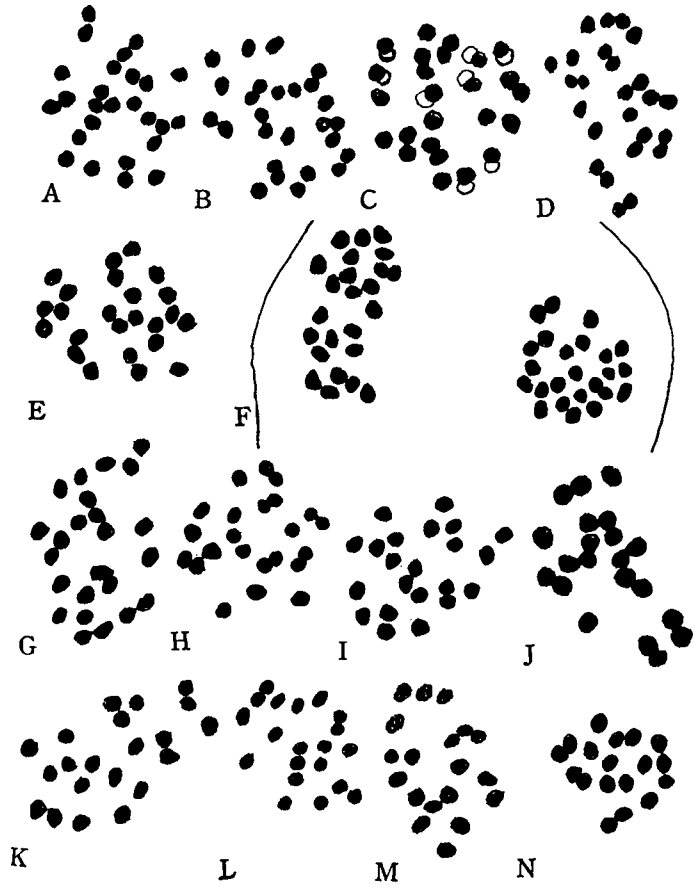


Fig. 6. Configuration of chromosomes at Metaphase II in D. M. A. (×2500)

A. 1(4)+2(3)+4(2)+ 8(1)=26
B. 1(3)+6(2)+11(1)=26
C. 5(2)+15(1)=25
D. 1(4)+1(3)+6(2)+ 6(1)=25
E. 1(4)+1(3)+5(2)+ 7(1)=24
F. 1(4)+1(3)+4(2)+ 9(1)=24
G. 1(4)+0(3)+4(2)+12(1)=24
H. 1(3)+5(2)+10(1)=23
I. 1(3)+2(2)+15(1)=22
J. 1(4)+2(3)+4(2)+ 3(1)=21
K. 4(2)+13(1)=21
L. 1(3)+2(2)+13(1)=20
M. 2(3)+0(2)+13(1)=19
N. 1(3)+4(2)+ 7(1)=18

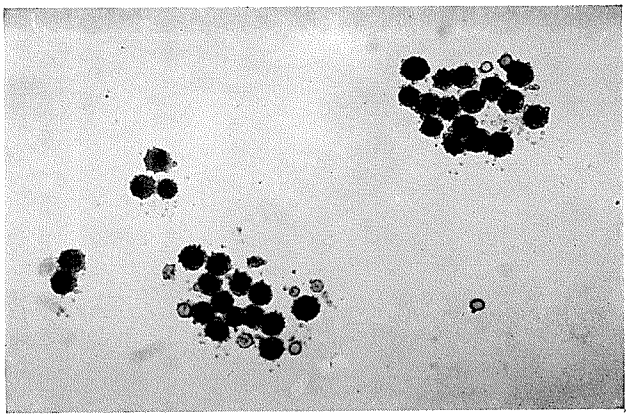


Fig. 7. Pollen grains of D. M. A. (×1000)

TABLE 4. Pollen Fertility in D. M. A. (Observed in 1958 and 1960)

Date	Number of Observed Pollens	Number of Stained Pollens	Fertility (per cent)
Aug. 21, 1958	522	273	52.3
	631	444	70.4
	1391	926	66.6
Aug. 19, 1960	515	152	29.5
Aug. 29, 1960	536	94	17.5
Sept. 24, 1960	274	169	61.7
	166	100	60.2

TABLE 5. Seed Setting Rate (Observed in1960) (Calculated on the basis of four ovules per flower)

Strain	Number of Seeds per Flower	Number of Flowers	Number of Seeds Obtained	Seed Setting Rate (per cent)
D. M. A.	0	53	0	
	1	41	41	
	2	19	38	
	3	9	27	
	4	0	0	
Total		122	106	21.7
2n Manyo	0	22	0	
	1	58	58	
	2	108	216	
	3	69	207	
	4	20	80	
Total		277	561	50.6

Strain	Number of Seeds per Flower	Number of Flowers	Number of Seeds Obtained	Seed Setting Rate (per cent)
4n Manyo	0	113	0	
	1	89	89	
	2	52	104	
	3	13	39	
	4	2	8	
Total		269	240	22.3

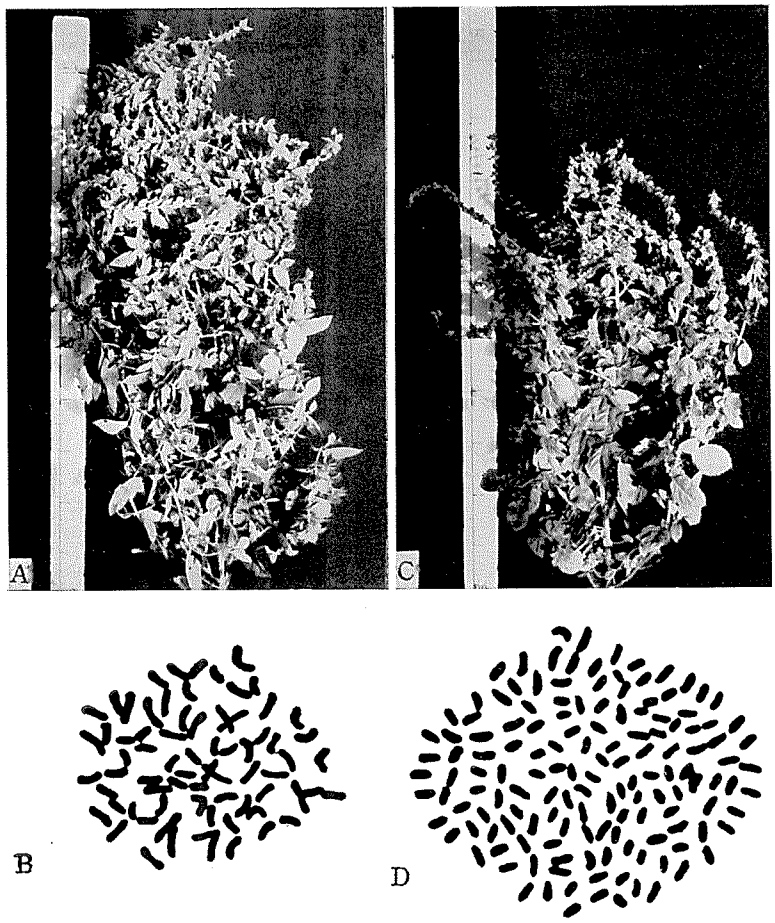


Fig. 8. A. Interspecific hybrid between Japanese mint and D. M. A.
B. Somatic chromosomes of (H×D). (2n=72) (×3000)
C. Amphiploid of the F hybrid (H×D)
D. Somatic chromosomes of 4n (H×D). (2n=144) (×3000)

As shown in Fig. 8, A, the hybrid in most its external character resembles Japanese mint, but leaves are light green, pubescent and rather ovate, resembling those of the pollen parent. The flowering time is earlier than that of the maternal parent, ranging from the beginning of August to the middle of September.

The somatic chromosome number was 72 (Fig. 8, B), but because of the degeneration of stamens at an early development stage, the meiotic chromosome behavior could not be observed. Seeds have not been obtained under open pollination up to the present time (Table 6).

d. An Induced Amphiploid of the F_1 Hybrid (H × D)

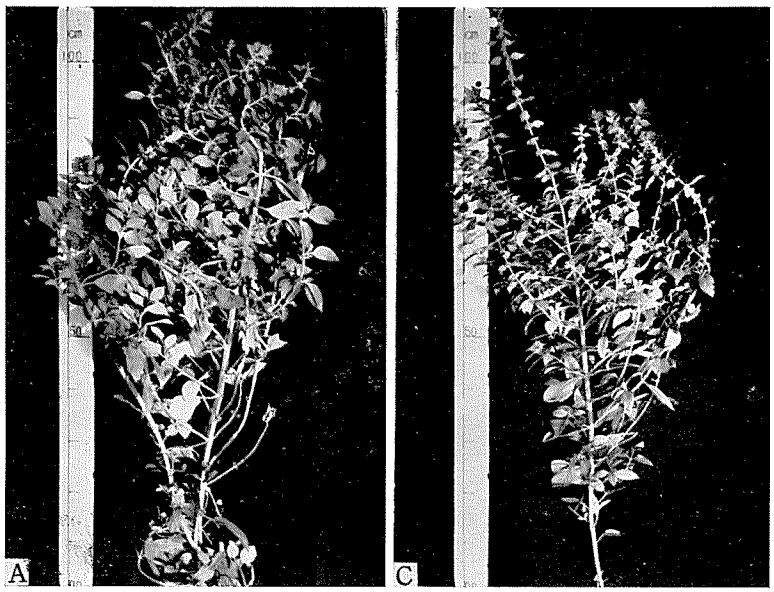
In 1956, an amphiploid of this F_1 hybrid was induced by the same method as that described in the following section, and it was designated as 4n (H × D).

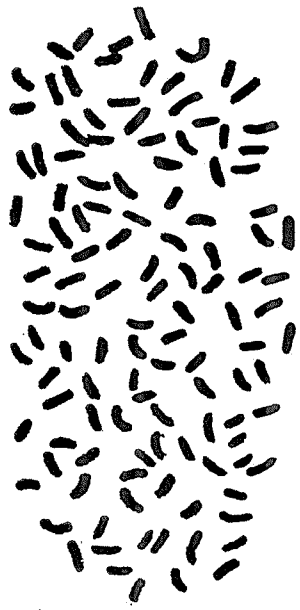
The external character of this plant is shown in Fig. 8, C. Leaves are thicker and have deeper serrations than the F_1 hybrid, and the length of internode shortened. The green leaves of 4n (H × D) was darker than those of (H × D), but still lighter in comparison with those of Japanese mint.

The somatic chromosome number was 144 as expected (Fig. 8, D), but since stamens did not develop as those in (H × D), the observation of meiotic chromosome behavior could not be carried out. The female gamete had con-

TABLE 6. Seed Setting Rate (Observed in 1960) (Calculated on the basis of four ovules per flower)

Strain	Number of Seeds per Flower	Number of Flowers	Number of Seeds Obtained	Seed Setting Rate (per cent)
(H × D)	0	262	0	
	1	0	0	
	2	0	0	
	3	0	0	
	4	0	0	
Total		262	0	0.0
4n (H × D)	0	178	0	
	1	29	29	
	2	8	16	
	3	0	0	
	4	0	0	
Total		215	45	5.2
Suzukaze	0	251	0	
	1	48	48	
	2	22	44	
	3	6	18	
	4	1	4	
Total		328	114	8.7





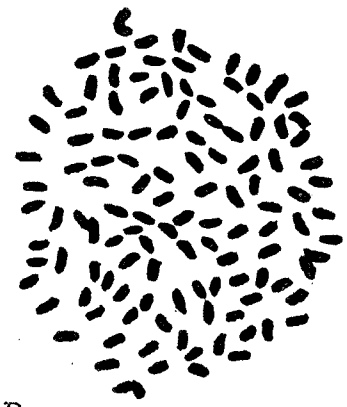


Fig. 9. Progenies of the amphiploid backcrossed to Japanese mint.
A. B_1F_1-1
B. Somatic chromosomes of B_1F_1-1. (2n=120) (×3000)
C. B_1F_1-2
D. Somatic chromosomes of B_1F_1-2. (2n=120) (×3000)

siderable fertility under open pollination as shown in Table 6. It appeared to have almost the same fertility as that of the variety Suzukaze (a variety of Japanese mint) which was male sterile in 1960.

e. The Progenies of the Amphiploid Backcrossed to Japanese Mint

In 1956, backcrosses of 4n (H × D) to a variety of Japanese mint (Manyo) were made, and 31 seeds were obtained. Harvested seeds were immediately sown in sterilized sand in a petri-dish and stored in a refrigerator. On March 28, 1957, they germinated at room temperature. Thirteen seedlings were obtained from which 2 were selected on the basis of external characters and taste and odor of leaves. These two plants were designated as B_1F_1-1[1] and B_1F_1-2 respectively.

The external character of these two backcrossed progenies was comparable

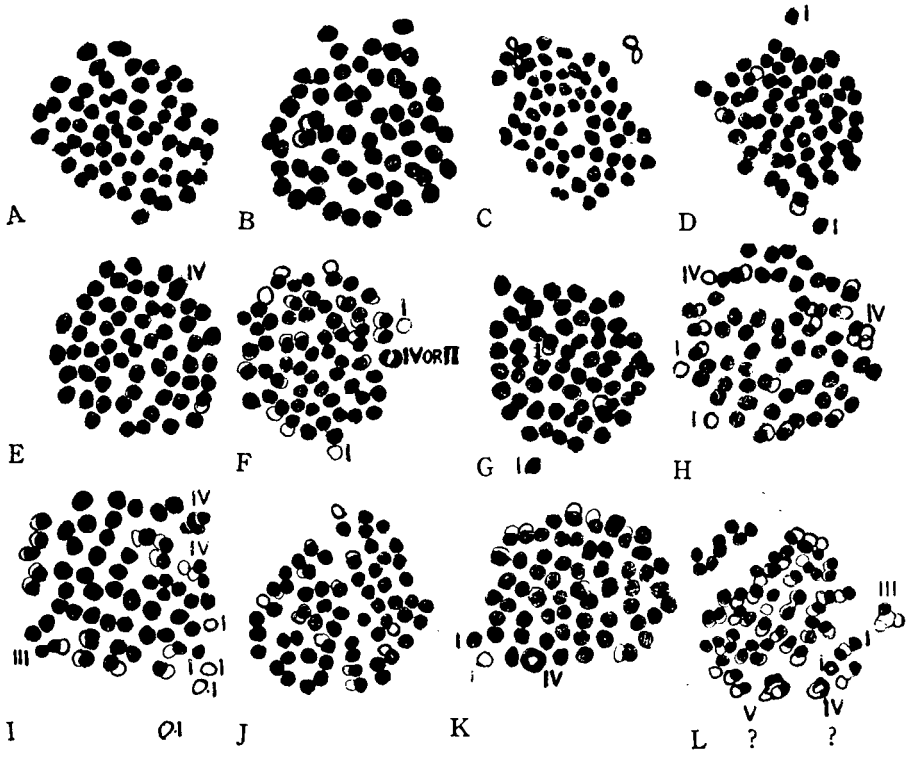


Fig. 10. Configuration of chromosomes at Metaphase I in B_1F_1-1. (×2500)

A. 60_{II} B. 60_{II} C. 60_{II} D. $50_{II}+2_I$ E. $1_{IV}+58_{II}$
F. $59_{II}+2_I$ G. $59_{II}+2_I$ H. $2_{IV}+55_{II}+2_I$ I. $2_{IV}+1_{III}+52_{II}+5_I$
J. 60_{II} K. $1_{IV}+57_{II}+2_I$ L. $1_V+1_{IV}+1_{III}+53_{II}+2_I$?

1) Cited from SAKAI (1952).

to the Japanese mint except for the light green color (Fig. 9, A and C).

Both of them have a chromosome number of 120 in somatic cells (Fig. 9, B and D) and they do not differ from each other except in minor characters.

The chromosome behavior at meiosis was observed mainly in B_1F_1-1, in

TABLE 7. Chromosome Configuration at MI in B_1F_1-1

Configuration	Number of Nuclear Plates
$1_V + 1_{IV} + 1_{III} + 52_{II} + 4_I$?	1
$2_{IV} + 1_{III} + 52_{II} + 5_I$	1
$2_{IV} + 0_{III} + 56_{II} + 0_I$	1
$2_{IV} + 0_{III} + 55_{II} + 2_I$	1
$2_{IV} + 0_{III} + 54_{II} + 4_I$	1
$1_{IV} + 0_{III} + 58_{II} + 0_I$	1
$1_{IV} + 0_{III} + 57_{II} + 2_I$	1
$60_{II} + 0_I$	6
$59_{II} + 2_I$	3
Total	16

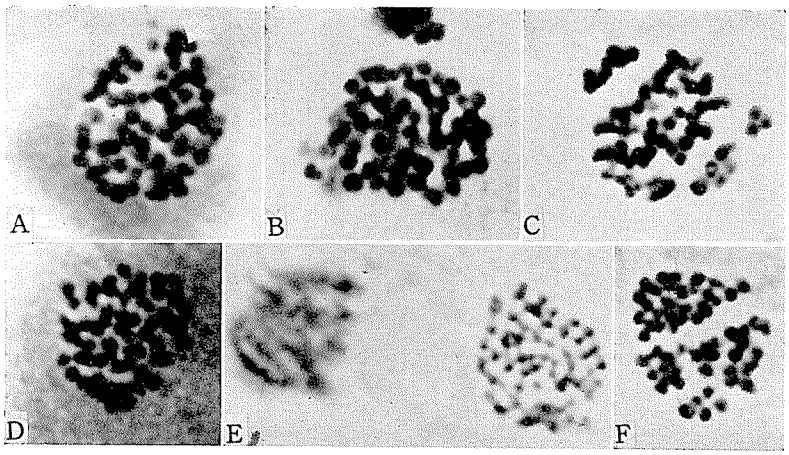


Fig. 11. Microphotograph of the meisosis in B_1F_1-1. (×2500)

A-C. Metaphase I.

A. Corresponds to J. in Fig. 10.

B. Corresponds to K. in Fig. 10.

C. Corresponds to L. in Fig. 10.

D-F. Metaphase II.

D. Corresponds D in Fig. 12.

E. Corresponds E in Fig. 12.

F. Corresponds F. in Fig. 12.

1958 and 1960. The observed configurations of chromosome pairing are shown in Table 7, Fig. 10 and Fig. 11, A–C. The chromosomes counted at M I ranged in numbers from 58 to 61. The number of bivalent chromosomes ranged from 52 to 60, and the most complete pairing 60_{II} was observed in 6 out of 16 PMCs. Univalent chromosomes ranging from 2 to 5 were observed in a half of 16 PMCs and tri- and quadrivalent chromosomes in 6 PMCs. A possible pentavalent chromosome was observed in only one PMC (Fig. 10, L and Fig. 11, C). The maximum number of multivalent chromosomes in a PMC was 3 as shown in Fig. 10, I and L and in Table 7.

At M II, chromosomes ranging in numbers from 54 to 63 were observed as

TABLE 8. Number of Chromosomes at MII in B_1F_1-1

Number of Chromosomes	54	55	56	57	58	59	60	61	62	63	Total
Number of Nuclear Plates	1	2	2	4	2	1	10	3	2	1	28

Average = 58.9

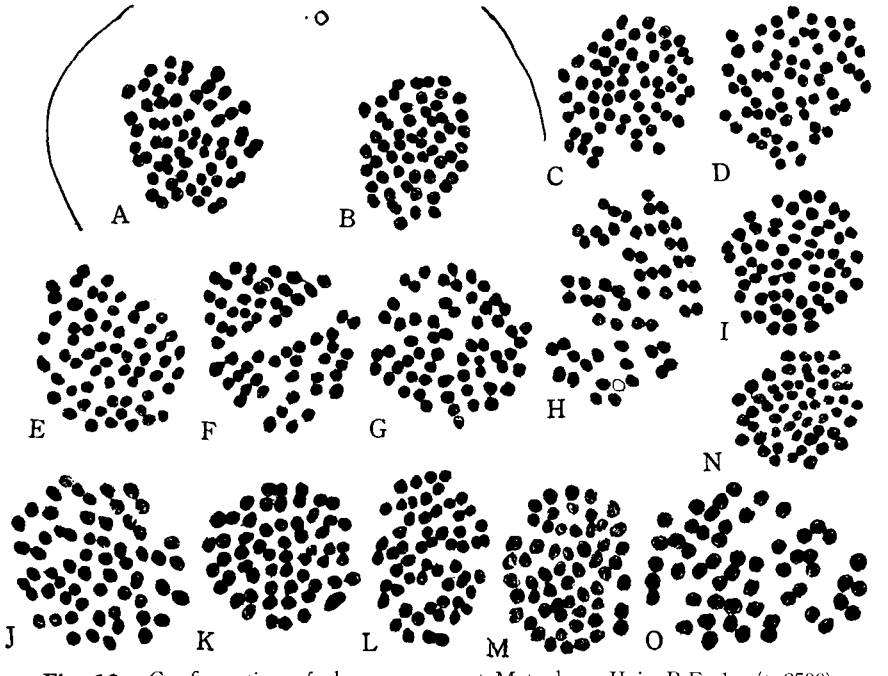


Fig. 12. Configuration of chromosomes at Metaphase II in B_1F_1-1. (×2500)

A. 63 + B. 56 and a lagging chromosomes C. 62
D. 61 E. 61 F. 60 G. 60 H. 60 I. 60
J. 60 K. 59 L. 58 M. 57 N. 55 O. 54

shown in Table 8 and Fig. 12 and Fig. 11, D–F. However, 60 chromosomes were counted in about a third of 28 nuclear plates.

Lagging chromosomes were observed in 21 out of 42 PMCs (50 per cent) and 71 out of 553 pollen tetrads (ca. 13 per cent) had one or more extra cells.

As shown in Table 9 and Fig. 13, both B_1F_1s had a high pollen fertility in comparison with that of Manyo, even though the difference in environmental factors was taken into account.

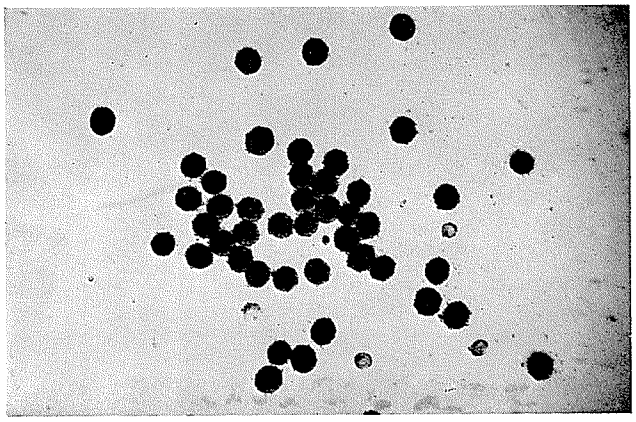


Fig. 13. Pollen grains of B_1F_1–1.

TABLE 9. Pollen Fertility of B_1F_1s and Manyo
(Observed in 1958 and 1960)

Strain	Date	Number of Observed Pollens	Number of Stained Pollens	Fertility (Per cent)
B_1F_1–1	Aug. 16, '58	1634	1586	97.1
		1637	1600	97.7
		800	774	96.8
B_1F_1–2	Aug. 12, '58	923	653	70.7
		801	529	66.0
		910	646	71.0
Manyo	Sept. 22, '60	1029	896	87.1
		979	829	84.7

The seed setting rate is shown in Table 10. Both B_1F_1s have a high seed fertility in comparison with that of D. M. A. shown in Table 5, and B_1F_1–2 has almost the same fertility as that of the variety Manyo shown in Table 5. (All the strains were male fertile)

TABLE 10. Seed Setting Rate of B_1F_1s (Observed in 1960) (Calculated on the basis of four ovules per flower)

Strain	Number of Seeds per Flower	Number of Flowers	Number of Seeds Obtained	Seed Setting Rate (per cent)
B_1F_1-1	0	45	0	
	1	82	82	
	2	80	160	
	3	53	159	
	4	12	48	
Total		272	449	41.3
B_1F_1-2	0	53	0	
	1	78	78	
	2	120	240	
	3	88	264	
	4	26	104	
Total		365	686	47.0

f. The Progenies Successively Backcrossed to Japanese Mint

Since the expectation of high fertility in B_1F_1s was fulfilled, the author made repeated backcrosses in 1958. As the male gamete of Japanese mints was completely sterile except for a few plants of diploid Manyo, B_1F_1s were used as pollen parents and the following crosses were made.

Combination	Designation
2n[1] Manyo × B_1F_1-1	B_2F_1-5
2n Suzukaze × B_1F_1-1	B_2F_1-3
4n[2] Manyo × B_1F_1-1	B_2F_1-4
4n Suzukaze × B_1F_1-1	B_2F_1-1
2n Manyo × B_1F_1-2	B_2F_1-2
B_1F_1-1 Self Pollination	B_1F_2-1
B_1F_1-2 Self Pollination	B_1F_2-2

The seed setting rate in these crosses are shown in Table 11.

After harvest at the beginning of October, seeds were wrapped in moistened filter paper and polyethylene film, and stored in a refrigerator until February, 1959.

On Feb. 3, 1959. part of the seeds were germinated in a growth cabinet

1), 2) Cited from POEHLMAN (1959).

TABLE 11. Seed Setting Rate in Second Backcrosses (1958) (Calculated on the basis of 4 ovules per flower)

Combination	Number of Crossed Flowers	Number of Seeds Obtained	Seed Setting Rate (per cent)
2n Manyo × B_1F_1-1	9	13	36.1
2n Suzukaze × B_1F_1-1	21	47	56.0
4n Manyo × B_1F_1-1	19	43	56.6
4n Suzukaze × B_1F_1-1	21	52	61.9
2n Manyo × B_1F_1-2	18	27	37.5
B_1F_1-1 Self.	10	17	42.5
B_1F_1-2 Self.	20	40	50.0

which was regulated so that the temperature was 25°C with illumination for 18 hours, followed by 10°C for 6 hours in darkness. The germinating percentage[1] in this case is shown in Table 12. Other seeds were taken to U. S. A. by the author and sown in unglazed pots in the greenhouse of the Department of Botany and Plant Pathology, Michigan State University. The germinating percentage in this case is also shown in Table 12. These results revealed that the difference of environment did not affect the germination of these hybrids except for B_2F_1-4.

TABLE 12. Germinating Percentage of B_2F_1s

Strain	Localities	Number of Seeds Sown	Number of Seeds Germinated	Germinating Percentage
B_2F_1-5	Japan U. S. A.	20 12	12 7	60.0 58.3
B_2F_1-3	Japan U. S. A.	47 48	28 24	59.6 50.0
B_2F_1-4	Japan U. S. A.	43 23	37 9	86.0 39.1
B_2F_1-1	Japan U. S. A.	18 55	15 45	83.3 81.8
B_2F_1-2	Japan U. S. A.	19 8	13 5	68.4 62.5
B_1F_2-1	Japan	26	22	84.6
B_1F_2-2	Japan	9	5	55.6

1) Cited from YASUDA (1948).

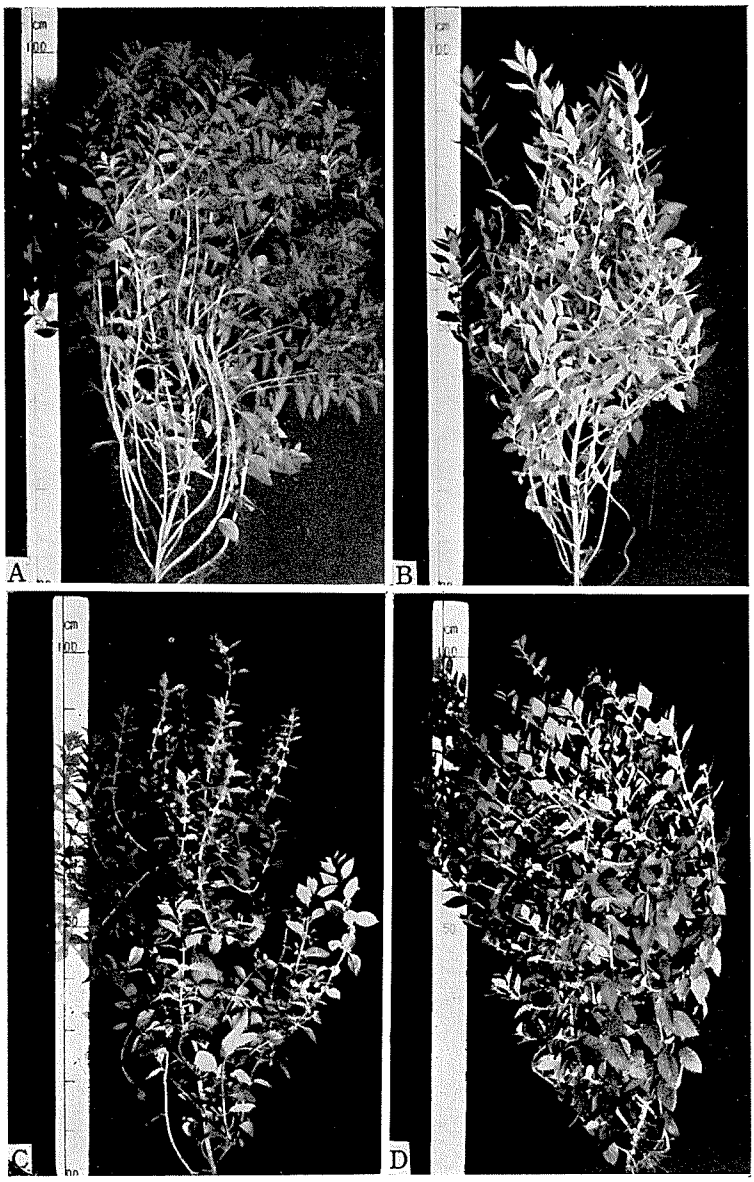


Fig. 14. A. A variety of Japanese mint (Manyo).
B. A variety of Japanese mint (Suzukaze).
C. A progeny successively backcrossed to Japanese mint (Suzukaze), B_2F_1-3-12.
D. A progeny successively backcrossed to 4n Japanese mint (4n Suzukaze), B_2F_1-1-7.

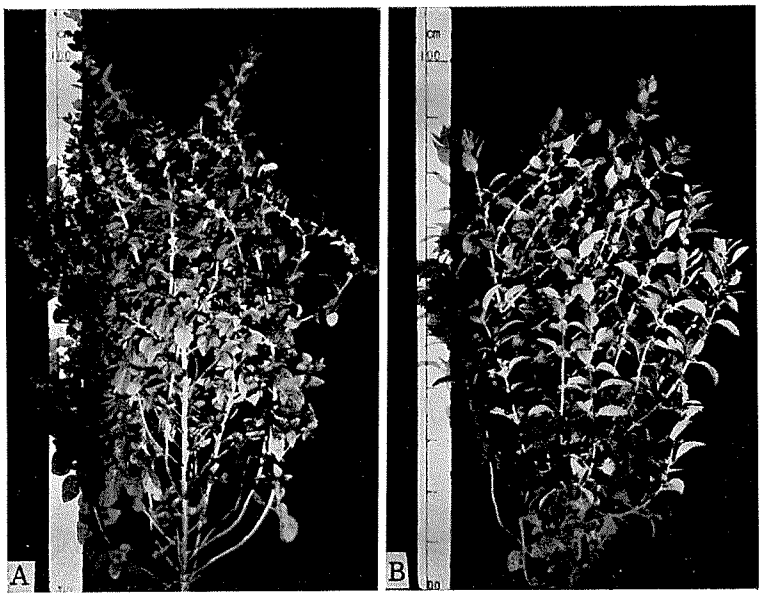


Fig. 15. A. A progeny successively backcrossed to Japanese mint (Manyo), B_2F_1-5-2.
B. A progeny successively backcrossed to 4n Japanese mint (4n Manyo), B_2F_1-4-7.

All the plants except for a few plants grown in Japan were lost during the author's absence from Japan. Consequently, the observations were conducted on the plants which were grown in U. S. A. and brought back to Japan in 1960. Thus, the author was compelled to omit the B_1F_2 plants from the observations.

The external characters of B_2F_1s were influenced by the maternal parents and it was difficult to distinguish them from Japanese mint without cytological observations (Fig. 14 and Fig. 15). The B_2F_1s between 4n Suzukaze and B_1F_1-1 showed this tendency very prominently.

Cytological observations were carried out mainly in 1959 at Michigan State University, and some of them in 1960 in Japan. The somatic chromosome numbers of all these plants could not be observed, because the high number and the small size of chromosomes prevented their exact distinction. From the chromosome behavior in B_1F_1s and chromosome number of maternal parents, the following somatic chromosome numbers were expected in B_2F_1s.

2n Manyo × B_1F_1 ····· ±108
2n Suzukaze × B_1F_1 ····· ±108
4n Manyo × B_1F_1 ····· ±156
4n Suzukaze × B_1F_1 ····· ±156

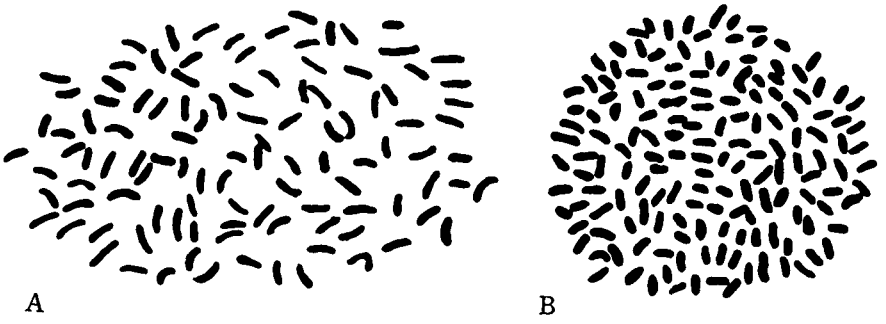


Fig. 16. A. Somatic chromosomes of B_2F_1-3-12 (2n=108) (×3000)
B. Somatic chromosomes of B_2F_1-1-7 (2n=156) (×3000)

The results of the observations of two plants met this expectation (Fig. 16, A and B).

B_2F_1s showed a great deviation in the initiation of flowering, ranging from the middle of August to the beginning of October. Generally, however, the strains backcrossed to tetraploid Japanese mint were late flowering. Some of them did not have flower until the beginning of October.

In 1959, observations of meiotic chromosome behavior were made on only two individuals in the strain 2n Suzukaze × B_1F_1-1 (B_2F_1-3), because the male gamete of these backcrossed progenies developed poorly in the greenhouse of Michigan State University. These two individuals were designated as B_2F_1-3-8 and B_2F_1-3-12 respectively and their somatic chromosome number was 108 as shown in Fig. 16, A. Since these two plants did not always develop stamens, a sufficient number of PMCs could not be obtained for detailed cytogenetic analysis in 1959. In 1960, the author concentrated on cytogenetic observations of B_2F_1-3-12.

The results of cytogenetic studies on B_2F_1-3-12 were as follows: As shown in Table 13, the chromosome number at M I ranged from 54 to 60, and averaged 57.1. The most complete pairing, 54_{II} was observed in 6 out of 65 PMCs (9.2 per cent) (Fig. 17, A and B and Fig. 18, A), with the most

TABLE 13. Observed Chromosome Number at MI in B_2F_1-3-12

Number of Chromosomes		54	55	56	57	58	59	60	Total
Number of Observed PMCs	1959	4	5	6	3	7	4	3	32
	1960	2	3	5	8	6	5	4	33
	Total	6	8	11	11	13	9	7	65

Average=57.1

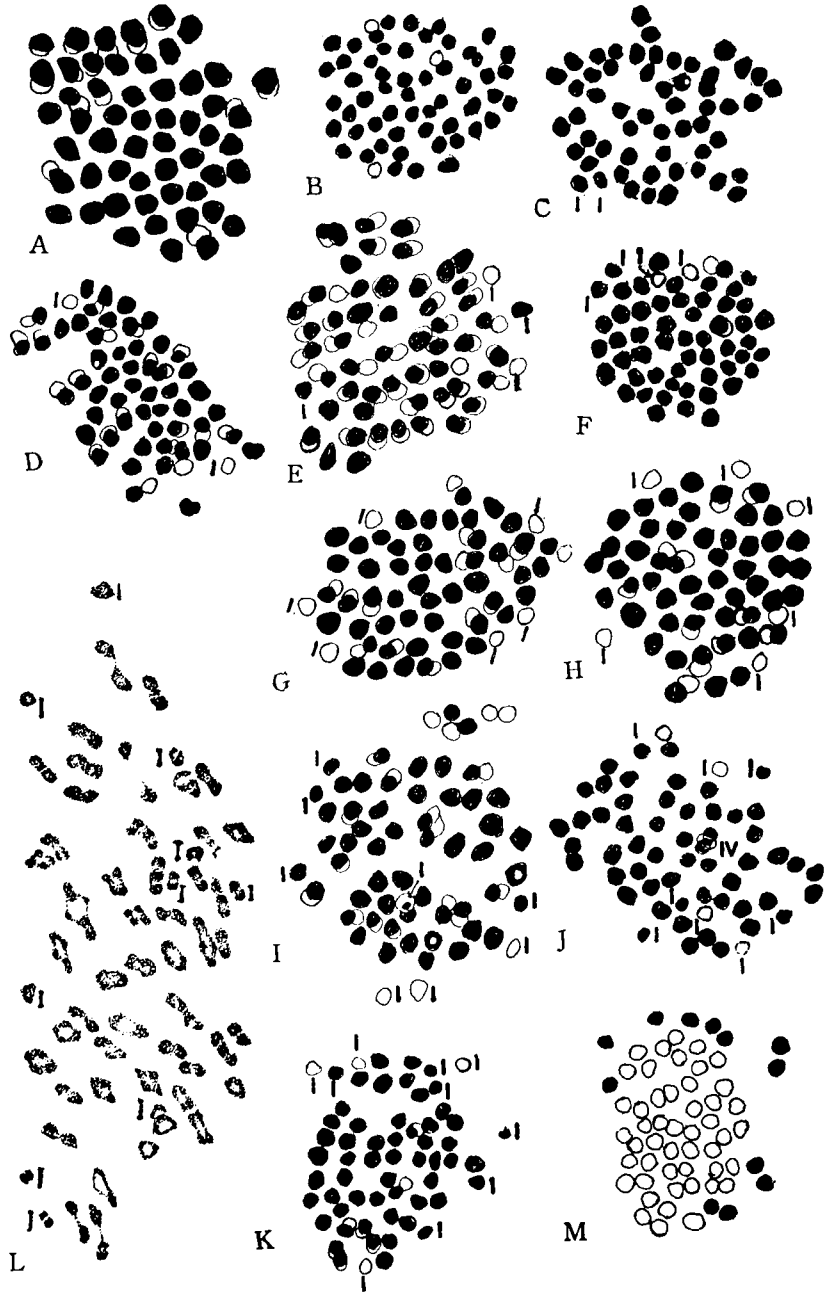


Fig. 17. Configuration of chromosomes at Metaphase I in B_2F_1-3-12. (×2500 except for A. ×3000).

A. 54_I B. 54_{II} C. $53_{II}+2_I$ D. $53_{II}+2_I$
E. $52_{II}+4_I$ F. $52_{II}+4_I$ G. $51_{II}+6_I$ H. $51_{II}+6_I$
I. $50_{II}+8_I$ J. $1_{IV}+48_{II}+8_I$ K. $49_{II}+10_I$
L. $49_{II}+10_I$ M. $48_{II}+12_I$

incomplete at $48_{II}+12_{I}$, which was observed in 7 out of 65PMCs (10.8 per cent) (Fig. 17, M and Fig. 18, E).

The number of univalent chromosomes ranged from 0 to 12 as shown in Table 14, and averaged 6.3. The univalent chromosomes tended to be closely adjacent to each other in a secondary association of two univalents as shown in Fig. 17, C, F, I, K, M and Fig. 18, D, E). The multivalent chromosomes

TABLE 14. Frequency of Univalent Chromosomes Observed at MI in the B_2F_1-3-12

Number of Univalents	0	2	4	6	8	10	11	12	Total
Number of PMCs	6	8	11	10	14	8	1	7	65

Average=6.3

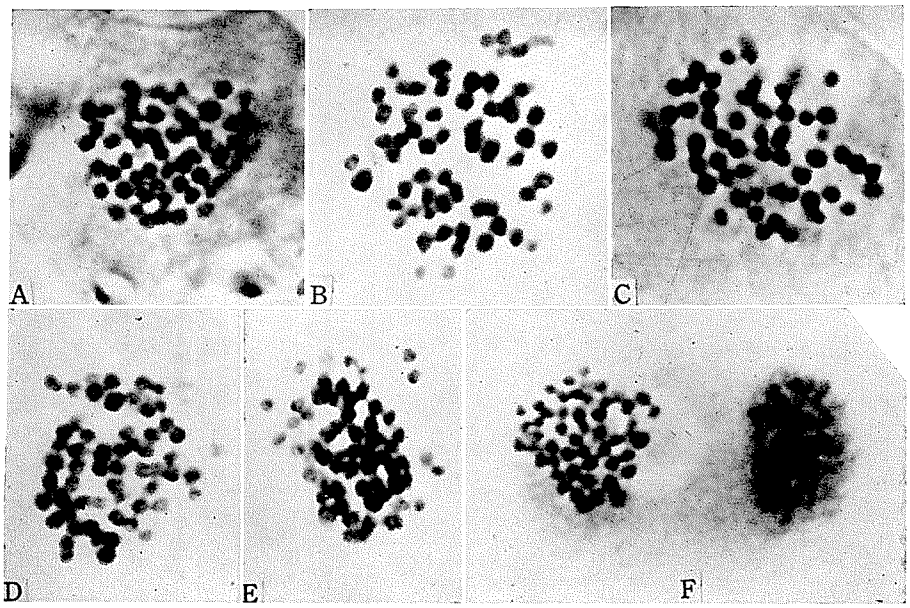


Fig. 18. Microphotograph of the meiosis in B_2F_1-3-12 (×1500)

A-E. Metaphase I.
A. Corresponds to B. in Fig. 17.
B. Corresponds to I. in Fig. 17.
C. Corresponds to J. in Fig. 17.
D. Correspods to K. in Fig. 17.
E. Corresponds to M. in Fig. 17.
F. Metaphase II.
Corresponds to C in Fig. 19.

(a quadri- and a trivalent) were observed in 2 out of 65 PMCs (Fig. 17, J.).

The number of chromosomes at M II ranged from 50 to 59 as shown in Table 15 and Fig. 19 and averaged 54.0 in 1959. The 54 chromosomes were counted in 2 out of 8 nuclear plates (25 per cent). It was rather difficult to find out the second metaphase in this plant.

TABLE 15. Number of Chromosomes at M II in B_2F_1-3-12.

Number of Chromosomes	50	51	52	53	54	55	56	57	58	59	Total
Number of Nuclear Plates	1	1	1	0	2	0	2	0	0	1	8

Average = 54.0

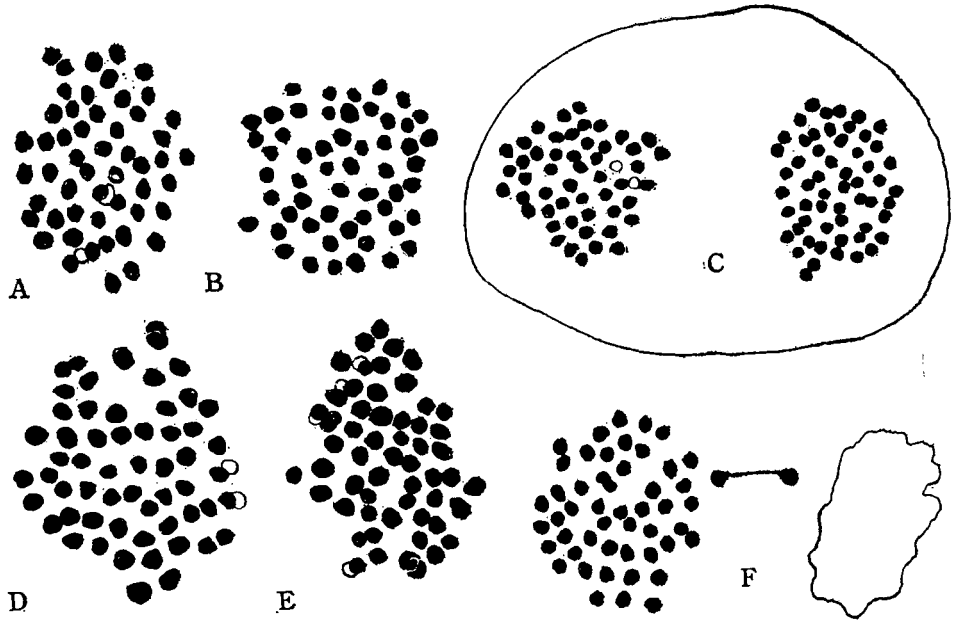


Fig. 19. Configuration of chromosome at Metaphase II in B_2F_1-3-12. (A. B. D. E. × 3000, C. F. ×2000)

A. 54 B. 54 C. 52+56 D. 58 E. 56
F. 59+chromosome bridge

The pollen tetrads and pollen fertility were observed in the greenhouse of Michigan State University. Ninety nine out of 311 pollen tetrads had one or more extra cells (31.8 per cent). The pollen fertility is estimated from data in Table 16.

The seed setting rate observed in 1959 was 3.1 per cent (30 seeds in 958

TABLE 16. Pollen Fertility in B_2F_1-3-12 (Aug. 27, 1959)

Number of Observed Pollens	Number of Stained Pollens	Fertility (per cent)
1083	537	49.6
556	170	30.6
868	359	41.4
414	151	36.5
Total 2921	1217	41.7

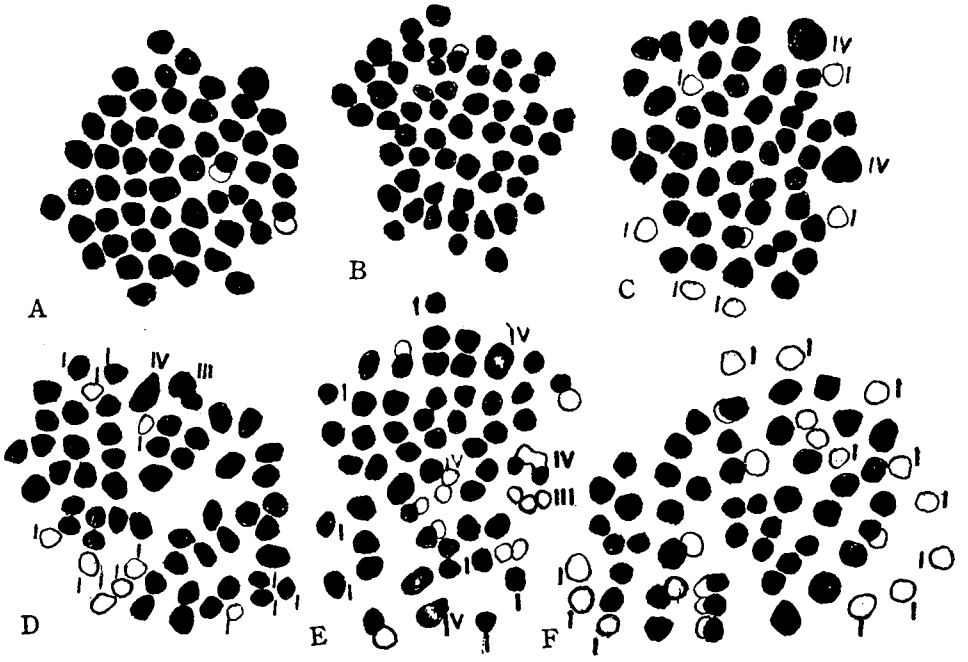


Fig. 20. Configuration of cromosomes at Metaphase I in B_2F_1-3-8. (×3000)
A. 54_{II} B. 54_{II} C. $2_{IV}+47_{II}+6_I$ D. $1_{IV}+1_{III}+44_{II}+13_I$
E. $4_{IV}+1_{III}+41_{II}+7_I$ F. $48_{II}+12_I$

TABLE 17. Observed Chromosome Number at MI in B_2F_1-3-8

Number of Chromosomes	52	53	54	55	56	57	58	59	60	Total
Number of Observed PMCs	2	4	3	3	0	1	1	0	1	15

Average = 54.5

ovules) under open pollination. The observation in 1960 will be described later collectively with those of other B_2F_1s.

In B_2F_1-3-8, the chromosome behavior at meiosis was somewhat different

TABLE 18. Seed Setting Rate in B_2F_1s (1960)

Progenitors (male-fertile)	Seed Setting Rate	Progenitors (male-sterile)	Seed Setting Rate
D. M. A.	21.7	Suzukaze	7.5
Manyo	50.6	(H×D)	0.0
B_1F_1-1	41.3	4n (H×D)	5.2
B_1F_1-2	47.0		

Strain	Number of Seeds per Flower	Number of Flowers	Number of Seeds Obtained	Seed Setting Rate (per cent)
B_2F_1-5-6	0	345	0	
	1	15	15	
	2	0	0	
	3	0	0	
	4	0	0	
Total		360	15	1.0
B_2F_1-4-1	0	321	0	
	1	1	1	
	2	0	0	
	3	0	0	
	4	0	0	
Total		322	1	0.1
B_2F_1-3-12	0	272	0	
	1	105	105	
	2	14	28	
	3	1	3	
	4	1	4	
Total		393	140	8.9
B_2F_1-3-15	0	278	0	
	1	12	12	
	2	0	0	
	3	0	0	
	4	0	0	
Total		290	12	1.0
B_2F_1-1-1	0	262	0	
	1	0	0	
	2	0	0	
	3	0	0	
	4	0	0	
Total		262	0	0.0

from that in B_2F_1-3-12. As shown in Table 17, the chromosome number observed at M I ranged from 52 to 60 and averaged 54.5. The mean number of bivalent chromosomes was 47.3, and the most complete chromosome pairing, 54_{II} was observed in 2 out of 15 PMCs (about 13 per cent). The number of univalent chromosomes ranging from 2 to 14 was observed, and quadri- and trivalent chromosomes were frequently observed as shown in Fig. 20, C. D. E.. Fig. 20, E shows the most complicated configuration: $4_{IV}+1_{III}+41_{II}+7_{I}$.

A detailed observation at M II was not conducted, because the number of samples was not sufficient. But 53 chromosomes were observed in a nuclear plate.

Including the B_2F_1-3-12, the seed setting rate in B_2F_1s was observed in plants selected at random from each strain under open pollination in 1960. A comparison with results of progenitors mentioned previously, is shown in Table 18. The strains in which maternal parents were teraploids were almost completely sterile. On the other hand, the strains in which maternal parents were diploids had considerable seed fertility. The variation in seed fertility within the same strain appeared to be partially due to the distance from a pollinator; in 1960, all the B_2F_1s were male sterile and, consequently, only four strains (2n Manyo, D. M. A., B_1F_1-1 and-2) furnished the pollens.

4. Discussion and Conclusion

The purpose of the present cytogenetic observation was to obtain detailed information on the polyploidy of Japanese mint (a variety of *M. arvensis* L.) and D. M. A. inferred as a variety of *M. longifolia* L. (*M. silvestris*).

In regard to the polyploidy of Japanese mint, IKEDA and UDO (1954, 1959) have already reported that it is an allopolyploid at high level on the basis of the meiotic chromosome behaviors in 2n and 3n plants. The author's observation on the meiosis in the variety Hokushin supported their assertion.

In D. M. A., the occurrence of multivalent and univalent chromosomes was distinctive. Environmental conditions such as temperature appeared to be partially responsible for such chromosome configuration. The later the observation was made in the flowering season, the more regular the chromosome behavior became, because of the decrease in the number of univalent chromosomes. In any event, solely on the basis of the occurrence of the multivalent chromosomes ranging from tri- to octovalent, D. M. A. may be inferred to be an autooctoploid with a basic chromosome number of 6.

It is regretable that the male gamete of F_1 hybrid ($H \times D$) degenerated at an early development stage and that the chromosome behavior could not be observed. However, since the observation on the seed setting rate for many

years under open pollination indicated that the female gamete was completely sterile, it is considered that the meiosis in (H × D) is extremely irregular. The restoration of fertility in the amphiploid of this F_1 hybrid (4n (H × D)) suggested a rather regular meiosis in the female gamete in this plant.

The chromosome behavior at meiosis in B_1F_1-1 was remarkably regular and the author could not find any evidence that the two orignial parents, the variety Hokushin and D. M. A. had similar genomes: None of the multivalent chromosomes higher than quadrivalent was found except in one out of 16 PMCs (6.3 per cent) in this generation, and the frequency of multivalent chromosomes was rather low. In other words, if there are isogenomes[1] in these plants, the occurrence of many trivalents and univalent chromosomes in B_1F_1 can be expected on the condition that both D. M. A. and the Japanese mint are clear allopolyploids. However, because of the occurrence of multivalent chromosomes in D. M. A., if they had contained isogenomes, there would have been multivalent chromosomes higher than quadrivalent at meiosis in B_1F_1-1.

Based on the facts that regular chromosome configuration without univalent chromosomes was observed in half of the PMCs and the frequency of univalent chromosomes was below 5 per PMC, it is reasonable to estimate that the 24 chromosomes derived from D. M. A. frequently form 12 bivalent chromosomes through autosyndesis, and 48 chromosomes derived from the variety Hokushin form 48 bivalent chromosomes with those of the variety Manyo. Furthermore, it may be possible to estimate that the multivalent chromosomes (tri- and quadrivalent) were formed between chromosome sets derived from D. M. A. through autosyndesis.

In any event, such rather regular meiotic behavior of chromosomes in B_1F_1 indicates that chromosomes derived from D. M. A. consist of two highly homologous chromosome sets. Furthermore, the occurrence of multivalent chromosomes suggests the existence of homologous chromosomes within each chromosome set, mentioned above.

The most prominent distinction of chromosome behavior at meiosis between B_1F_1-1 and B_2F_1-3 was detected in the frequency of univalent chromosomes as shown in Table 19. The frequency of univalent chromosomes in B_1F_1-1 appears to follows a Poisson distribution, whereas a normal distribution was possibly seen in B_2F_1-3-12. Furthermore, the mean number of univalent chromosomes was higher in B_2F_1-3-12 than in B_1F_1-1. As the lowest bivalent chromosome number in B_2F_1-3-12 was 48, univalent chromo- somes are considered to be derived from D. M. A.. Rare occurrence of mul- tivalent chromosomes in this plant and its rather high frequency in B_1F_1-3-8 can not

1) Cited from the Iwanami Dictionary of Biology (1960).

TABLE 19. Frequency of Univalent Chromosomes in B_1F_1-1 and B_2F_1-3-12

Number of Univalent Chromosomes	0	2	4	5	6	8	10	11	12	Total of Observed PMCs	Average
B_1F_2-1	8	5	2	1						16	1.4
B_2F_1-3-12	6	8	11	0	10	14	8	1	7	65	6.3

TABLE 20. Somatic Chromosome Number of Genus *Mentha*

Species	Somatic Chromosome Number	Investigator
aquatica	36	SCHÜRHOFF, (1929), TSUDA (unpublished)
	96	RUTTLE (1931), MORTON (1956)
arvensis	12	LÖVE and LÖVE (1942)
	54	WOLF (1929)
	60	LÖVE and LÖVE (1952)
	64	NAGAO (1941)
	72	RUTTLE (1931), LÖVE and LÖVE (1952) MORTON (1956)
	92	NAGAO (1941)
	96	TSUDA (1950), IKEDA (1954)
cardiaca	60	MORTON (1956)
citrata	84	MORTON (1956)
	120	MORTON (1956)
crispa	96	MORTON (1956)
gentilis	84	MORTON (1956)
	96	IKEDA (1954), MORTON (1956)
	108	MORTON (1956)
	120	MORTON (1956)
gracilis	54	MORTON (1956)
japonica	48	NAGAO (1941)
	49	IKEDA (1954)
longifolia (*silvestris*)	18	HEIMANS (1938)
	24	RUTTLE (1931), JUNELL, L. & L. (1942), MORTON (1956)
longifolia (*silvestris*)	36	MORTON (1956)
	48	NAGAO (1941), SUZUKA (1949), MORTON (1956), TSUDA (1961)

Species	Somatic Chromosome Number	Investigator
muelleriana	60	MORTON (1956)
niliaca	24	RUTTLE (1931), MORTON (1956)
	56	RUTTLE (1931)
piperita	36	GLOTOV (1940), TSUDA (unpublished)
	64	GLOTOV (1940)
	66	RUTTLE (1931), MORTON (1956)
	68	RUTTLE (1931), NAGAO (1941)
	70	RUTTLE (1931)
	72	NAGAO (1941), IKEDA (1954), MORTON (1956)
	84	NAGAO (1941)
	128	GLOTOV (1940)
pulegium	10	MORTON (1956)
	20	RUTTLE (1931), MORTON (1956)
	30	MORTON (1956)
	40	RUTTLE (1931), MORTON (1956)
	48	IKEDA (1954)
requieni	18	RUTTLE (1931)
rotundifolia	18	HEIMANS (1938)
	24	NAGAO (1941), MORTON (1956), IKEDA (1954)
	36	MORTON (1956), TSUDA (unpublished)
	48	MORTON (1956)
	54	SCHÜRHOFF (1929)
smithiana	120	MORTON (1956)
spicata (*viridis*)	36	SCHÜRHOFF (1929), NAGAO (1941), LÖVE and LÖVE (1952), IKEDA (1954)
	48	NAGAO (1941), LÖVE and LÖVE (1952), IKEDA (1954)
	54	IKEDA (1954)
	84	NAGAO (1941)
verticillata	42	MORTON (1956)
	84	MORTON (1956)
	120	MORTON (1956)
	132	MORTON (1956)

1) The table arranged by DARLINGTON was supplemented by the results of MORTON, IKEDA and UDO, and the present author.

be explained clearly, even though we may assume the structural differentiation such as translocation. Based on the fact that complete pairing of 54_{II} was observed in about 10 per cent of observed PMCs, it is reasonable to estimate that the 12 chromosomes derived from D. M. A. occasionally form 6_{II} through the autosyndesis. Nevertheless, the high frequency of univalent chromosomes indicates that the homology between these two chromosome sets is imcomplete.

From a thorough examination of this inference, as far as this experiment is concerned, it may be concluded that D. M. A. consists of 8 chromosome sets (genomes), and that they have more or less a homology to each other. Depending upon the proposal by STEBBINS (1947), therefore, this plant may possibly be named a segmental allooctoploid.

IKEDA and UDO (1955 a, b, 1956, 1960) explain the chromosome behaviors at meiosis in this genus with the assertion that the basic chromosome number of this genus is 12. With this basic chromosome number, however, any suitable expllanation of the chromosome behavior mentioned above could not be obtained. Furthermore, based on results in Table 20, it may be more reasonable to infer that one of the basic chromosome numbers in this genus is 6.

SHIMOTOMAI (1935) found many species in genus *Chrysanthemum* to be autopolyploids. STEBBINS (1947) also enumerated 7 intervarietal autopolyploids. It is very interesting that these plants are propagated through vegetative organs as are mint plants.

III. Investigations on Some Available Characters in Interspecific Hybrids Backcrossed to Japanese Mint

As mentioned in the previous section, the author used a segmental allooctoploid as a non-recurrent parent, and obtained numerous backcrossed progenies of an artificial amphiploid of the interspecific hybrid Hokushin × D. M. A.. He examined the following characters of these backcrossed progenies from an agronomical point of view, namely such characters as the resistance to rust disease, the content of essential oil and the content of menthol in essential oil. The yield test of herb was not conducted because of insufficient materials.

1. Observations on External Characters and Selection

In 1957, two vigourous B_1F_1s were selected, based on external characters and taste and odour of leaves. In 1959, 42 plants were selected from 90 B_2F_1s at Michigan State University, based on external characters.

In 1960, all of the plants including check plants (Manyo, Suzukaze, D.M.A., F_1, 4n F_1 and two B_1F_1s) and B_2F_1s grown in Japan were planted in the Experi-

mental Field of the Hokkaido University. Strains were spaced 1 meter apart so that competition between strains was prevented. Each stand consisted of three plants. The quantity of base fertilizers per 1/10 ha was as follows:

Ammonium Sulphate	18.75 kg.
Calcium Superphosphate	30.00 kg.
Potassium Sulphate	9.40 kg.

Ammonium Sulphate at 5 kg per 1/10 ha was side-dressed at the end of June. The following general judgement of the external characters could be made. F_1 (H × D) showed considerable vigor in regards to external characters such as tall and thick stems, many branches and large leaves (Fig. 8, A). As mentioned previously, in 4n (H × D) shorter but thicker stems, smaller but thicker leaves were observed (Fig. 8, C). In B_1F_1s (Fig. 9, A and C), the vigor appeared to be still maintained in tall and thick stems, and very large leaves. In B_2F_1s, the vigor changed to some extent, and generally speaking, B_2F_1–2s and B_2F_1–5s in which the maternal parent was the variety 2n Manyo, were inferior in vigor to other strains. B_2F_1–3s and B_2F_1–1s showed almost the same vigor as that of the variety Manyo, and were superior to the variety Suzukaze (Fig. 14).

2. Resistance to Rust Disease

a. Experimental Method

A test of disease resistance of all plants used in this experiment was made in 1960. One seedling of each strain was planted in a unglazed pot (20 cm diameter) and placed in a greenhouse at the beginning of May. In late August, about 20 plants of the variety Manyo infected[1] with rust disease were shipped from the north-eastern part of Hokkaido (Engaru and Kun-neppu district). The urediospores borne on these plants were collected with a brush into 800 cc of sterilized distilled water and filtered through four layers of sterile gauze. The inoculation was made in a growth cabinet. The moisture in this cabinet was maintained at 100 per cent by filling a metal container with water, placed at the bottom and covering the walls and ceiling with moistened cloth. The temperature was regulated from 18° to 25°C. The sample plants placed in the growth cabinet were sprayed with the urediospore suspension by a hand sprayer on August 25, 1960. After 24 hrs, the innoculated plants were transferred to the greenhouse, and on Sept. 9, 15 days after inoculation, the evaluation of rust resistance was made.

1) Technical terms in this section were cited from BAXTOR (1953).

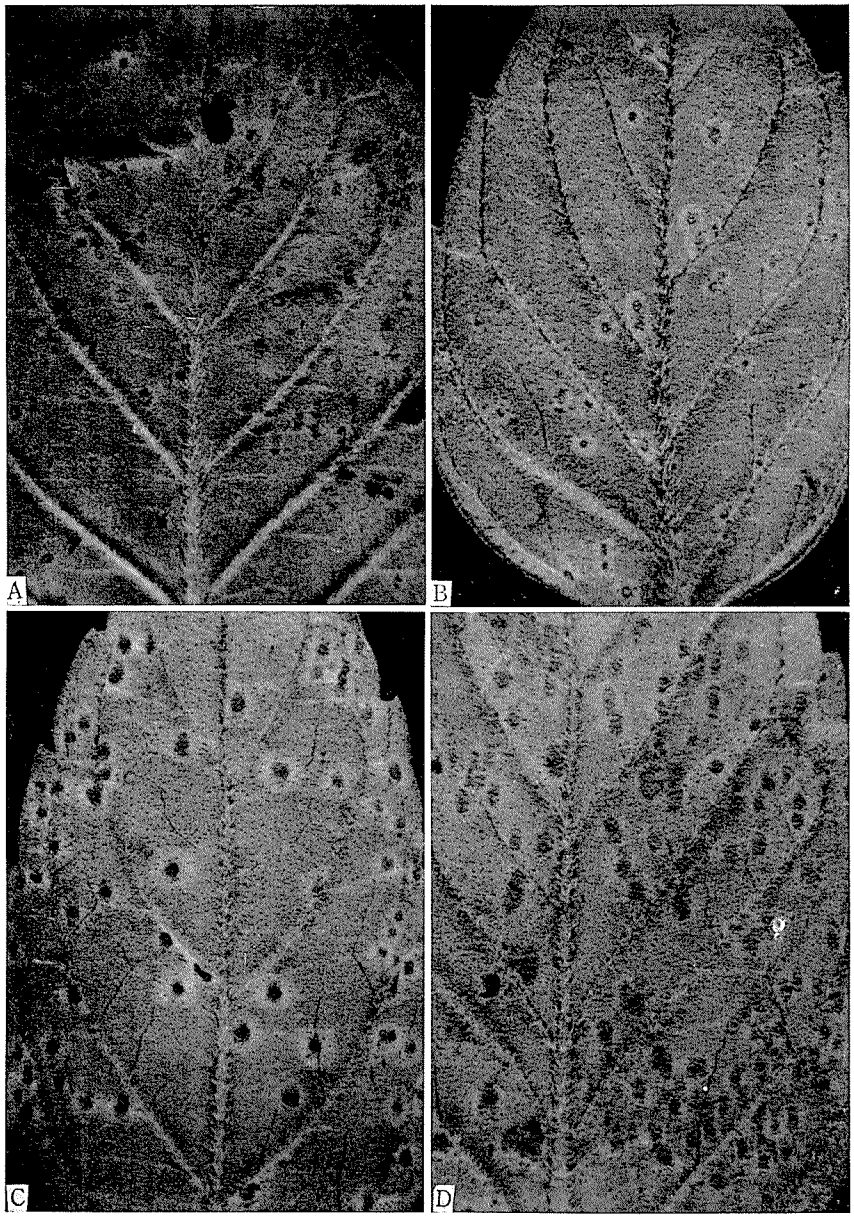


Fig. 21. Infection type to rust disease. (ca×3)

A. Type 1. (B_2F_1-3-4)
B. Type 2. (B_2F_1-3-8)
C. Type 3. (the variety Manyo)
D. Type 4. (B_2F_1-1-22)

b. Experimental Results

The intensity of infection was highly variable. Thus, the author modified the evaluation standards proposed by BAXTOR (1953), and the intensity of infection was classified into the following 5 types.

Infection Type	Resistance	Symptoms
0	Immune	Flecking or small necrotic spots, no uredia formed.
1	Highly Resistant	Uredia were few and smallest with flecking or small necrotic spots. (Fig. 21, A)
2	Moderately Resistant	Uredia were few, with a moderate size in chlorotic spots. (Fig. 21, B)
3	Moderately Susceptible	Uredia abundant, but smaller than Type 4 with chlorosis. (Fig. 21, C)
4	Highly Susceptible	Uredia abundant and large, no necrosis or chlorosis. (Fig. 21, D)

The results of the evaluation are shown in Table 20. D. M. A., (H × D) and 4n (H × D) showed infection type 0, and were found to be immune to rust disease. All the cultivated varieties were evaluated as moderately susceptible, based on the abundant uredia in rather large chlorotic spots as shown in Fig. 21, C. The B_1F_1s were infected, but they were evaluated as type 1

Fig. 22. Mint plants suffered from rust disease. Plants on the left hand side are B_1F_1s and plants on the right hand side are 4n Japanese mints (Manyo).

and highly resistant. This high resistance to rust disease can be seen in Fig. 22 taken at the experimental field in 1958. In this picture, 4n Manyo suffered severely from rust disease and all the leaves except for young and small leaves had fallen, whereas B_1F_1 showed no symptoms of disease at first sight, though the leaves had many necrotic spots.

In B_2F_1s, the infection type ranged from type 1 to type 4. From Table 22, it is shown that, of 45 B_2F_1s, 6 were highly resistant, 10 moderately resistant, 10 moderately susceptible and 19 highly susceptible. It appeared that B_2F_1-1 had many resistant plants. When the difference of maternal parent

TABLE 21. Response of varieties to

Strain No.		Strain	Infection Type				
			0	1	2	3	4
		D. M. A.	*				
		Manyo				*	
		Suzukaze				*	
		H × D	*				
		4n (H × D)	*				
B_1F_1-1		4n (H × D) × Manyo		*			
-2				*			
B_2F_1	-2- 5	Manyo × B_1F_1-1				*	
	-5- 2	Manyo × B_1F_1-2		*			
	- 3			*			
	- 4						*
	- 5						*
	- 6						*
	-4- 1	4n Manyo × B_1F_1-1					*
	- 2						*
	- 3						*
	- 6						*
	- 7			*			
	- 9				*		
	-10						*
	-12						*
	-20						*
	-3- 2	Suzukaze × B_1F_1-1			*		
	- 3					*	
	- 4		*				

is ignored, about a third of the B_2F_1s were found to be resistant to the rust disease, and about two fifths of them were more severely rusted than the cultivated varieties.

c. Discussion

NELSON (1950 a, b) introduced the rust resistance of *M. crispa* to the Scotch spearmint through interspecific hybridization and described that the resistance to rust disease is conditioned by a single dominant factor. If this is so and its gene is located in each of the eight gemomes of D. M. A., we

infection of *Puccinia Menthae* PERS.

Strain No.		Strain	Infection Type				
			0	1	2	3	4
B_2F_1	-3- 5	Suzukaze x B_1F_1-1				*	
	- 6						*
	- 8				*		
	-12			*			
	-13					*	
	-15						*
	-19						*
	-20						
	-22						*
	1- 1	4n Suzukaze × B_1F_1-1			*		
	- 3				*		
	- 4				*		
	- 6					*	
	- 7				*		
	-10			*			
	-11				*		
	-17						*
	-18				*		
	-21					*	
	-22						*
	-27						*
	-28					*	
	-29						*
	-33				*		
	-39					*	
	-40					*	
	-42						*

TABLE 22. Infection Type in B_2F_1s

Strain	Infection Type				
	1	2	3	4	Total
B_2F_1-2	0	0	1	0	1
B_2F_1-5	2	0	0	3	5
B_2F_1-4	1	1	0	7	9
B_2F_1-3	2	2	4	4	12
B_2F_1-1	1	7	5	5	18
	6	10	10	19	45

TABLE 23. Content of

Strain No.		Strain	Sample Weight (g)	Oil Volume (cc)	cc/Sample Weight×100
		D. M. A.	50	0.60	1.20
		Manyo	50	1.69	3.38
		Suzukaze	50	1.30	2.60
		H×D	50	1.13	2.26
		4n (H×D)	45	1.05	2.33
B_1F_1-1		4n (H×D)×Manyo	50	1.39	2.78
-2			50	1.09	2.18
B_2F_1	-5- 5	Manyo×B_1F_1-1	50	1.12	2.24
	-4- 2	Manyo×B_1F_1-2	50	1.46	2.92
	- 3		50	1.23	2.46
	- 4		50	1.08	2.16
	- 5		50	0.86	2.26
	- 6		50	1.07	2.14
	-4- 1	4n Manyo×B_1F_1-1	50	1.30	2.60
	- 2		50	1.12	2.24
	- 3		50	1.40	2.80
	- 6		50	1.27	2.54
	- 7		50	—	—
	- 9		50	—	—
	-10		50	1.48	2.96
	-12		50	1.00	2.00
	-20		50	0.98	1.96
	-3- 2	Suzukaze×B_1F_1-1	50	0.85	1.70
	- 3		50	1.40	2.80
	- 4		50	1.34	2.68

would be able to expect all B_2F_1 plants to be resistant. However, as far as the present experiment is concerned, a completely immune strain to rust disease could not be obtained in the backcrossed progenies. Furthermore, the large variation of disease resistance in B_2F_1s suggests that D. M. A. is not so simple nor uniform in regards to this character. In addition, the weakened resistance in B_1F_1 and B_2F_1 may indicate that it is controlled by a more complicated genetic constitution.

As far as this experiment is concerned, no inclination based on the difference of the maternal parent could be detected in the infection type as shown

Essential Oil (1960)

Strain No.		Strain	Sample Weight (g)	Oil Volume (cc)	cc/Sample Weight×100
B_2F_1	-3- 5		50	1.33	2.66
	- 6		50	—	—
	- 8		50	1.19	2.38
	-12		50	1.53	3.06
	-13	Suzukaze×B_1F_1-1	50	—	—
	-15		50	1.03	2.06
	-19		50	1.26	2.52
	-20		50	—	—
	-22		50	0.95	1.90
	-1- 1		50	0.96	2.13
	- 3		50	1.10	2.20
	- 4		50	0.90	1.80
	- 6		50	1.30	2.60
	- 7		50	1.46	2.92
	-10		50	1.34	2.68
	-11		50	0.77	1.54
	-17		50	1.23	2.46
	-18	4n Suzukaze×B_1F_1-1	50	1.50	3.00
	-21		50	1.05	2.10
	-22		50	0.80	1.60
	-27		50	1.22	2.44
	-28		50	0.96	1.92
	-29		50	0.90	1.80
	-33		50	1.24	2.48
	-39		50	1.10	2.20
	-40		50	—	—
	-42		50	—	—

in Table 21.

The fact that disease resistance occurs in the second backcrossed progenies to the susceptible varieties is very important and promising for the improvement of Japanese mint..

3. Content of Essential Oil

a. Experimental Method

The harvest of plants was made three times in accordance with the flowering time on Sept. 21, 29 and Oct. 3 in 1960. The harvested plants were dried at room temperature for about 30 days. The analysis of oil content was made on the dry leaves with the usual equipment for essential oil, and the scale reading was made after 24 hrs.

b. Experimental Results

The results of analysis of oil content are shown in Table 23.

The variety D. M. A. had the lowest oil content and was considerably higher in the F_1 hybrid (H × D). In the amphiploid, 4n (H × D), the oil content was higher than in the original (H × D).

In the B_1F_1s one (B_1F_1–1) had a higher oil content than that in 4n (H × D) and it was higher than that in the variety Suzukaze; on the other hand, B_1F_1–2 showed a lower content.

In the B_2F_1s, the oil content showed a very wide range from 1.54 per cent in 1–11 to 3.06 per cent in 3–12. None of B_2F_1s possessed a higher or comparable content to the variety Manyo in which the oil content is the highest among cultivated varieties. Tweve out of B_2F_1s, however, were found to have a higher or comparable content to that in the variety Suzukaze. The plants in which the oil content was below 2.00 per cent were frequently found in B_2F_1–3 and –1 strains in which the maternal parent was 2n or 4n Suzukaze. The numbers of plants in which the oil content was higher than that in their pollen parent were follows: 3 out of 5 in B_2F_1–5 (60 per cent), 2 out of 7 in B_2F_1–4 (about 30 per cent), 2 out of 9 in B_2F_1–3 (about 22 per cent), and 2 out of 16 in B_2F_1–1 (about 13 per cent).

c. Discussion

From the results mentioned above, the content of essential oil appears to be controlled by a complicated genetic constitution.

In the B_2F_1 generation, the oil content in 5 out of 13 plants (about 38 per cent) in which the maternal parent was 2n or 4n Manyo was higher than that in the pollen parent; on the other hand, only 4 out of 25 plants (16 per

cent) in which the maternal parent was 2n or 4n Suzukaze showed higher values. Furthermore, the mean value of oil content in the former group was higher (2.41 per cent) than that in the latter one (2.31 per cent). Although the difference between these values is not statistically significant, it may be possible to say that the variety Manyo is more useful for the recovery of the oil content. The fact that the oil content in this variety is the highest among the cultivated varieties may be helpful for this estimation.

4. Content of Menthol in the Essential Oil

a. Experimental Method

As the quantity of oil samples was insufficient, they were analyzed only for free menthol by liquid chromatography designed by HAMARHEH, et al (1956). The standard curve used in this experiment was drawn by the present author with aid of Hitachi photoelectric photometer, Type EPO-B, filter No. 55. Although the standard curve for menthol content to be a straight line going throught the O point, despite several trials, it traced a curved line at the points of lower and higher menthol contents. Therefore, the author diluted the oil sampe in chloroform so that the scale reading might fall on the straight portion of the curve as shown in Fig. 23. Other analytical methods were used in accordance with the directions by HAMARHEH, et al.

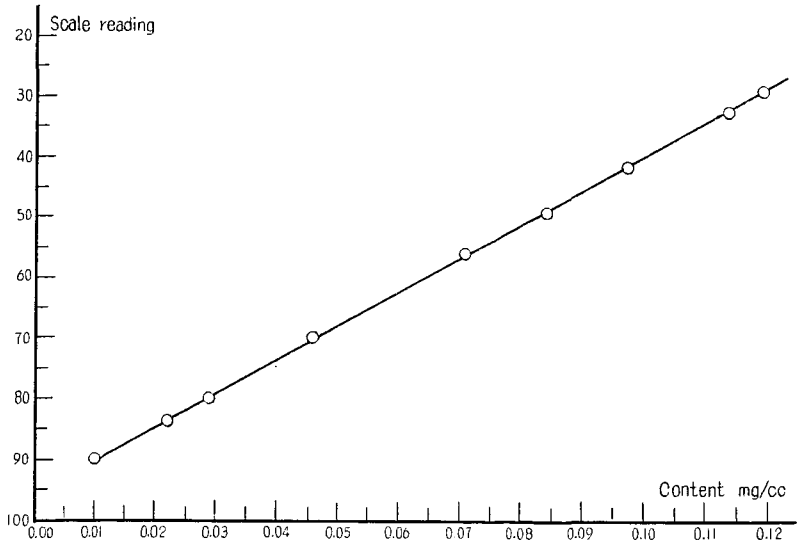


Fig. 23. Standard curve for menthol in chloroform by means of Hitachi Photo-electric Photometer Type EPO-B with filter No. 55.

TABLE 24. Menthol Content (1960) (* Dilution failed)

Strain No.	Strain	Sample Weight (mg)	Menthol Weight (mg)	Per cent
	D. M. A.	665	72	10.8
	Manyo	1,014	727	71.7
	Suzukaze	667	499	74.8
	H×D	725	392	54.1
	4n (H×D)	576	294	51.0
B_1F_1-1 -2	4n (H×D)× Manyo	1,051	588	55.9
		608	369	60.7
B_2F_1 -2- 5	Manyo×B_1F_1-1	615	360	58.5
-5- 2	Manyo×B^1F_1-2	748	459	61.4
* - 3		—	—	—
- 4		501	300	59.9
- 5		351	227	64.7
- 6		517	331	64.0
-4- 1	4n Manyo×B_1F_1-1	601	449	74.7
- 2		614	429	69.9
- 3		685	444	64.8
- 6		555	426	76.8
-10		734	485	66.1
-12		379	238	62.8
-20		308	201	65.3

Strain No.	Strain	Sample Weight (mg)	Menthol Weight (mg)	Per cent
B_2F_1 -3- 2	Suzukaze×B_1F_1-1	366	265	72.4
- 3		124	81	65.3
- 4		789	597	75.7
- 5		497	361	72.6
- 8		712	436	61.2
-12		874	591	67.6
-15		492	409	83.1
-19		536	320	59.7
-20		232	152	65.5
-22		307	206	67.1
-1- 1	4n Suzukaze× B_1F_1-1	450	330	73.3
- 3		460	379	82.4
- 4		327	219	67.0
* - 6		—	—	—
- 7		777	556	71.6
-10		861	597	69.3
-11		198	141	71.2
-17		529	344	65.0
-18		858	567	66.1
-21		445	281	63.1
* -22		—	—	—
-27		514	384	74.7
-28		381	239	62.7
-29		398	293	73.6
-33		558	416	74.6
-39		449	317	70.6

b. Experimental Results

The results are shown in Table 24. The content of free menthol in D. M. A. was very low and it was considerably higher in the F_1 hybrid (H × D), but was far lower than that in Japaneses mint which exceeds 70 per cent. In the amphiploid of this hybrid, 4n (H × D), it was slightly reduced.

In B_1F_1s, it increased to about 60 per cet, but the free menthol did not crystalize by cooling, and in this generation, the content differed from plant to plant.

In B_2F_1s, the content of free menthol showed a very wide range from 58.5 per cent in 2–5 to 83.1 per cent in 3–15, and all of the plants except for 5–4 had a higher content than their pollen parents. None in the B_2F_1–5 had comparable content to its maternal parent (the variety Manyo), and 2 out of 7 B_2F_1–4s (about 30 per cent) had a higher content than the same variety which is the original variety of their maternal parent. The content in 2 out of 10 B_2F_1–3s (20 per cent) was higher than that in their maternal parent (the variety Suzukaze), and 3 out of 14 B_2F_1–1s (about 21 per cent) had a higher or comparable content to that in the same variety which was the original variety of their maternal parent.

c. Discussion

The wide range of menthol content in B_2F_1 suggests its complicated genetic constitution.

It is an interesting observation that not only a comparable menthol content to that in cultivated varieties, but also a higher content was found in some B_2F_1s.

If many individuals were examined in F_1 or B_1F_1 generation, the recovery of menthol content might be realized in these generations. However, experience indicates that the menthol content in the F_1 hybrids between Japanese mint and other species is always reduced. IKEDA and UDO (1955, 1957) found a few plants with rather high menthol content in F_1 hybrids of Japanese mint × *M. spicata* var. *crispa* and Japanes mint × corn mint, but they were still low as compared with that in Japanese mint. Furthermore, as far as this experiment is concerned, all 13 B_1F_1s were found to have a low menthol content based on the taste and odor of their leaves.

The number of plants having a comparable or higher menthol content than that in the variety Manyo was as follows : 2 out of 12 (16.7 per cent) in the group in B_2F_1 in which maternal parent was 2n or 4n Manyo, and 11 out of 24 (about 45.8 per cent) in the group of which maternal parent was 2n or 4n Suzukaze. As compared with that in the variety Suzukaze, 2 out

of 12 (16.7 per cent) in the former group had a higher content, and 5 out of 24 (20.8 per cent) in the latter group. Furthermore, the mean value of menthol content was higher in the latter group (69.8 per cent) than in the former group (65.7 per cent). Although these differences between two groups were not statistically significant, the Suzukaze variety appears to be more useful as a maternal parent for the purpose of recovery of menthol content. The fact that the menthol content in this variety is the highest among the cultivated varieties may be helpful for this estimation.

5. General Discussion and Conclusion

Intergeneric and interspecific hybridizations have been made by many plant breeders. There appears to be two different objectives in their intentions. One of them is the establishment of a new crop by means of amphiploid between two different cultivated crops (KAGAWA 1957). The other aim is the transference of a desirable character from another species to a cultivated species (HOLMES 1936, POEHLMAN 1959, WATANABE 1956, SEARS 1956, STOREY and others 1957, etc).

In the latter case, a backcross to the cultivated crops is necessary and the number of backcrosses may vary from one to eight, depending upon how completely the breeder wishes to recover the genes from the recurrent paren (POEHLMAN 1959). In the present experiment however, it appears to be rather easy to recover the desirable character of the cultivated varieties, since the author has obtained several strains in B_2F_1 generation which are promising from a practical point of view. In Table 25, rust resistant strains in B_2F_1s were examined for the content of essential oil and menthol content in com- parison with those in the cultivated varieties. From this table, we can estimate that 3–4 (Fig. 24) and 1–7 are very promising, and 3–12 and 5–3 are useful as breeding mate- rials because of their high disease resistance and their capacity for seed setting.

Fig. 24. B_2F_1–3–4 (resistant to rust) disease and high menthol strain)

Although the author did not investigate the chemical components in the essential oil

except for menthol, B_2F_1s were found to have very different odors and tastes in their oils when compared with each other. Some of them, for example, 4–1, has a very pleasant odor comparable to European peppermint. This fact is interesting in the improvement of residual oil in Japanese mint.

TABLE 25. Contents of Essential Oil and Menthol in Rust Resistant B_2F_1s

Strain	Infection Type	Oil Content (cc/Sample Weight) ×100	Menthol Content (per cent)
5— 2	1	2.92	61.4
5— 3	1	2.46	—
4— 7	1	—	—
3— 4	1	2.68	75.7
3—12	1	3.06	67.6
1—10	1	2.68	69.3
4— 9	2	—	—
3— 2	2	1.70	72.4
3— 6	2	2.38	61.2
1— 1	2	2.13	73.3
1— 3	2	2.20	82.4
1— 4	2	1.80	67.0
1— 7	2	2.92	71.6
1—11	2	1.54	71.2
1—18	2	3.00	66.1
1—33	2	2.48	74.6
Manyo	3	3.38	71.7
Suzukaze	3	2.60	74.8

Depending upon these results mentioned above, we may be able to conclude as follows: When the segmental allooctoploid is used as a non-recurrent parent, fertility can be maintained even in B_2F_1, and the combination of the desirable characters of the recurrent and non-recurrent parents into a plant may be expected at least in the B_2F_1 generation.

The fact that completely immune strains could not be obtained in backcrossed progenies naturally suggests that the rust fungus may become adapted to these resistant strains in the near future. Dr. LOCKWOOD suggests to the author in a private letter, however, that the imcomplete and multigenic resistance is often more stable to different races of the fungus than complete, single gene resistance. Accordingly, we might expect to find a completely immune plant

TABLE 26.

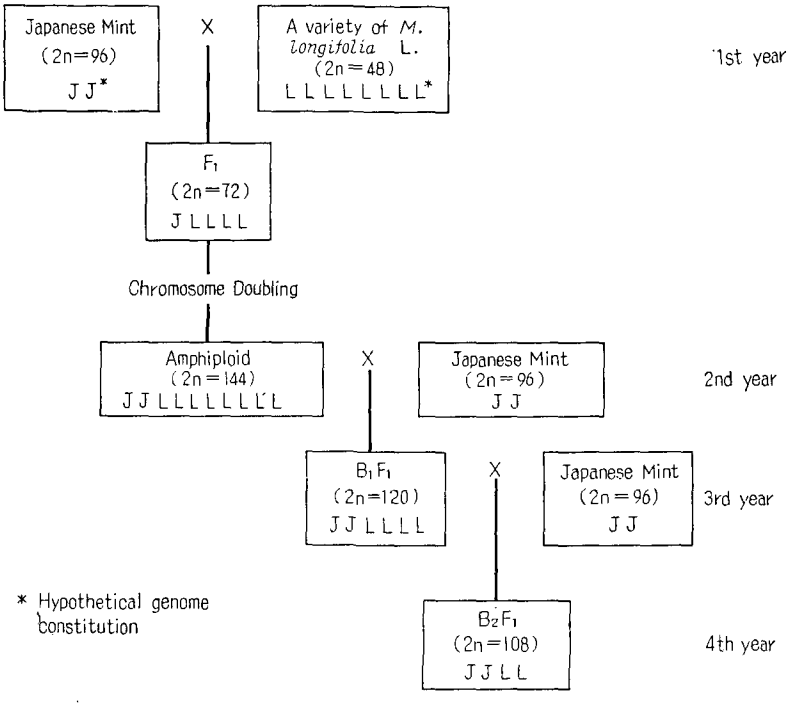
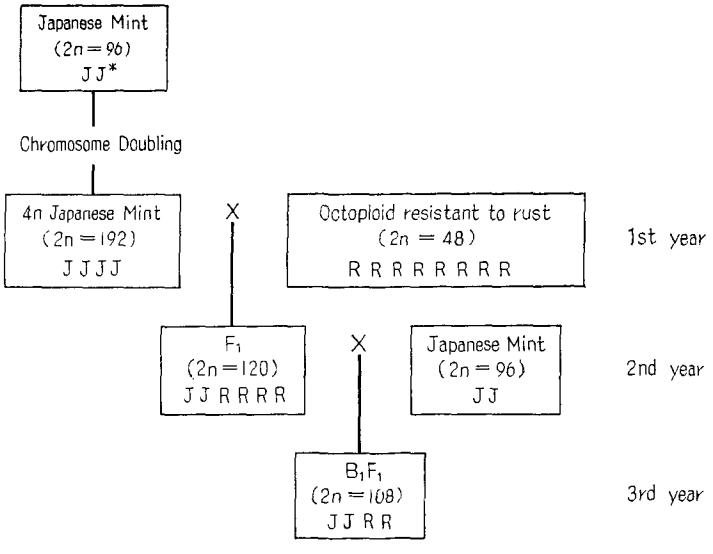


TABLE 27.



in backcrossed progenies through extensive experiments because the amphiploid was completely immune to the disease.

Furthermore, there are many octoploid plants showing similar chromosome behavior to that in D. M. A. which are completely immune to the rust disease. Extensive studies with these plants may break new ground for the improvement of Japanese mint.

Table 26 explains schematically the process of this experiment. When the artificial autopolyploid of Japanese mint is used in the original interspecific hybridization, this process may be shortened as shown in Table 27, and it may be more efficient than the induction of amphiploid of F_1 hybrid which will show segregation in regards to the characters.

IV. Investigations on Some Available Characters of Artificially Induced Polyploids in Japanese Mint

1. Experimental Method

a. Material

Japanese mint (*Mentha arvensis* var. *piperascens* MALINV.)
Variety Manyo
Suzukaze

These commercial varieties are hybrids between Japanese mint and Japanese mint introduced from China (KASANO 1953, 1954). Although the author has not confirmed it, it is claimed that these original varieties each belong to a different variety of *M. arvensis* L.. In any event, Manyo and Suzukaze show hybrid vigor.

b. Technique for Induction of Artificial Polyploids

Preparatory Treatment: The vigorous rhizomes (Fig. 25, A) were washed with tap water, then cut into 3–5 cm. lengths to include unsprouted buds at the middle of the rhizome (Fig. 25, B). They were put in sterilized sand at 20°C in a petri-dish and grown for 3–4 days (Fig. 25, C).

Treatment with Colchicine: When growing buds reached 0.5–1 cm. in length with no node (Fig. 25, D), the rhizomes were removed from the sand and immersed in an aqueous colchicine solution of determined concentration in a petri-dish (Fig. 25, E). The colochicine treatment was carried out at room temperature or at 20°C for a determined period.

Planting: The rhizomes were removed from the solution and the excess solution adhering to the rhizomes was removed by filter paper. Then rhizomes

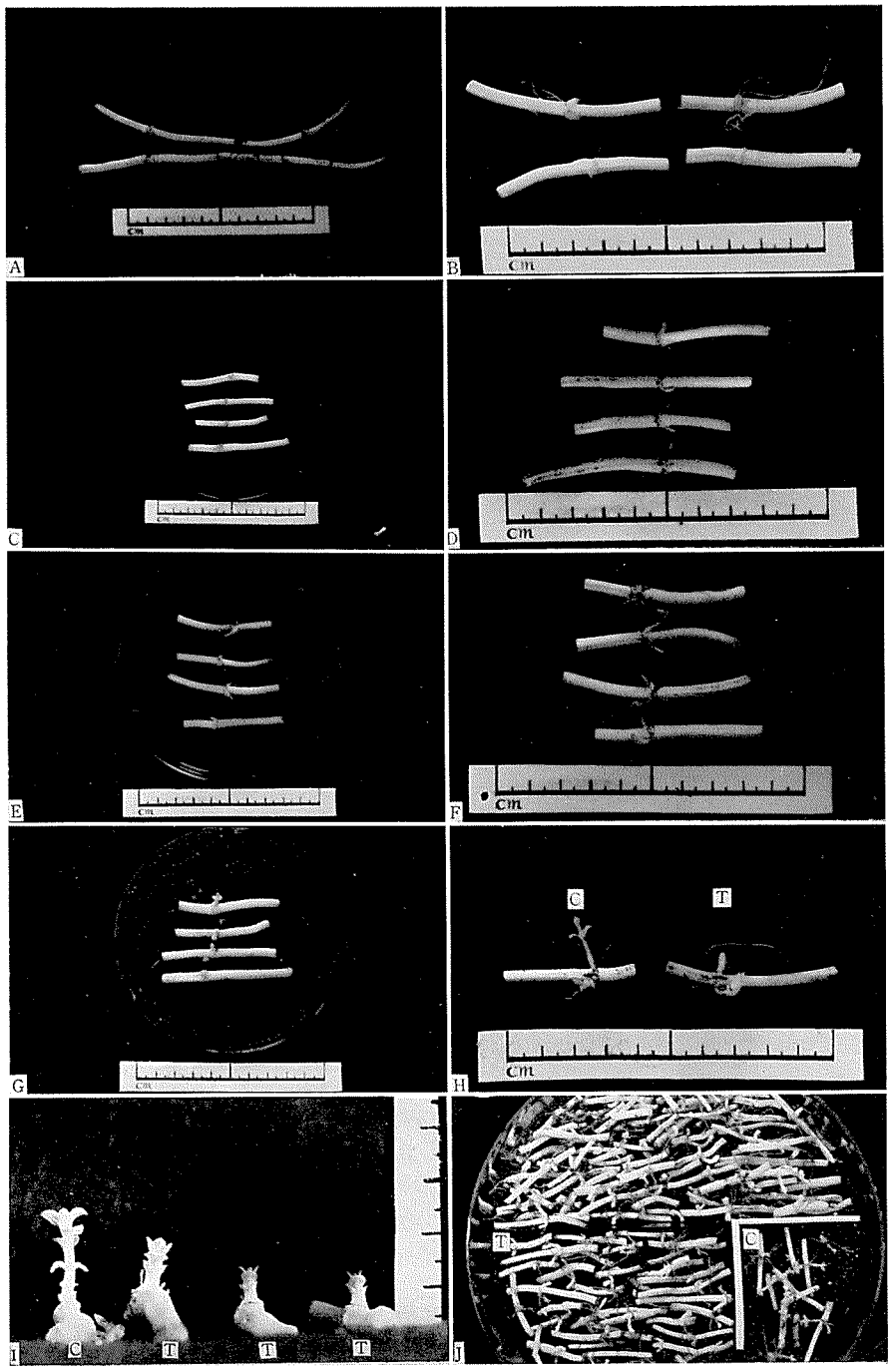


Fig. 25. Process of colchicine treatment. (See text for detailed explanation)

were planted in sterilized sand (Fig. 25, F, G) at 20–25°C for one or two weeks until the effects of colchicine could be detected. This effect was noticed by a swelling of buds (Fig. 25, H, right). The affected sprouts were transplanted into sterilized soil in an unglazed pot covered with a glass plate with an opening for ventilation. The sprout, except for the top, was covered with soil to prevent drying.

The growth of buds affected by colchicine was delayed in comparison with those of check plants (Fig. 25, I). A month or more was required for the bud to begin elongation. During this period, thick small leaves developed.

After the stem length reached 3–5 cm., the plants were transplanted into unglazed pots, so that 2–3 nodes of the stem were covered with the soil to induce polyploid rhizome formations from the nodes.

c. Determination of Chromosome Doubling[1)]

The criterion of chromosome doubling, except for the chromosome counting, is ordinarily the enlargement of stomata. In mint plants, however, chromosome doubling causes an increase in diameter of the oil glands on the epidermis of the above-groud parts. Because of their ease of measurement, the author compared the oil glands on the leaf.

The oil glands on fully grown leaves were printed by the 'Sump' method. The diameters of the long axis of 10 oil glands were measured with a micrometer ocular (40×8).

The measurements of pollen grains and chromosome counts in root tips were also obtained for the confirmation of chromosome doubling. The chromosome number in a polyploid plant is so high that determination in all the plants was virtually impossible. The author counted the chromosome number in a plant selected at random from those having similar morphological characteristics. The technique of observation was the same as that in the cytogenetical work described in the previous section.

d. Morphological Comparison and Yield Test

Morphological comparisons were made on the plants in a yield test plot. The items of comparison are described in the experimental results.

The comparison of yields between original varieties and their polyploid plants was conducted on C_1, C_2 and C_3 lines from 1956 to 1958 in the Experimental Field of the Hokkaido University. As the regular method of yield testing was impossible for several reasons, a modified method was used. The detailed method will be described in the results of experiment, though the items of cultural practice which were used commonly through these experiments

1) Cited from POEHLMAN (1959)

are as follows:

Area of 1 plot	2 m. × 3 m.
Width between row	0.5 m.
Number of rows	6

Fertilizer

Base Fertilizer per 1/10 ha

Ammonium Sulphate	18.75 kg.
Calcium Superphosphate	30.00 kg.
Potassium Sulphate	9.40 kg.

Ammonium Sulphate at 5 kg. 1/10 ha was side-dressed at the end of June.

The field was plowed in the previous fall and tilled in the early spring of 1956. Weeding and spraying with insecticides and fungicides were conducted as required.

The recommended harvest time for mint plants is at blooming. Because of convenience of observation, the author harvested the plants singly, when a flower in the fourth verticil from the bottom opened.

The quantitative analysis of oil was done with the usual equipment for essential oil. The free menthol was crystalized by storage for 24 hours in a low temperature room of −20°C at the Low Temperature Science Institute, Hokkaido University. Afterwards, the menthol crystals were separated from the oil by a suction pump, and the oil adhering to the crystals was absorbed by filter paper. The crystals were dried on filter paper for 24 hours at room temperature and weighed. The residual menthol in the dementholized oil was determined by ordinary chemical analysis according to The Japan Pharmacopoeia.

2. Experimental Results

a. Efficiency of Induction Method of Chromosome Doubling

The efficiency of chromosome doubling by previous workers is rather low (IKEDA and KONISHI 1956). As shown in Table 28, the efficiency of the immersion technique used by the present author was 32 per cent at maximum, and is almost ten times greater than that of other techniques. However, the determination of the most effective concentration of colchicine solution and of the treatment length required more detailed experiments.

Despite careful handling the author found that some of C_1 plants propagated from polyploid plants by rhizomes reverted to 2n. They may have originated from the remaining unaffected nodes in treated C_0 plants. It is necessary, therefore, to examine C_1 plants for chromosome doubling. The

TABLE 28. Efficiency of Colchicine Treatment

Variety	Treatment		Number of Treated Plants	Number of Polyploid Plants Obtained	Efficiency (per cent)
	Concentration of Colchicin Solution (%)	Length (hrs.)			
Manyo	0.025	40	37	0	0.0
	0.025	24	71	4	5.6
	0.2	5	83	10	12.0
	0.2	4	68	15	22.1
	0.2	14	67	9	13.4
Suzukaze	0.2	6	37	12	32.4
	0.025	46	36	9	25.0

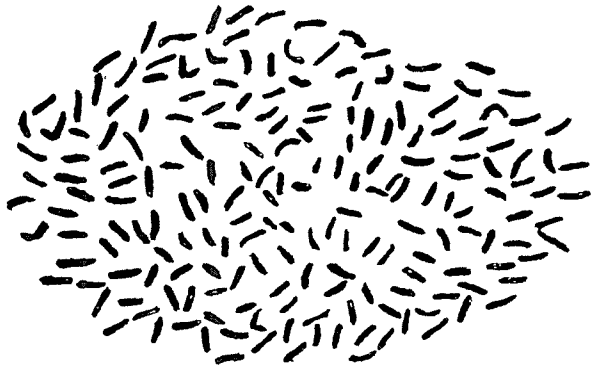


Fig. 26. Somatic chromosomes of 4n Manyo. (2n=192) (×3000)

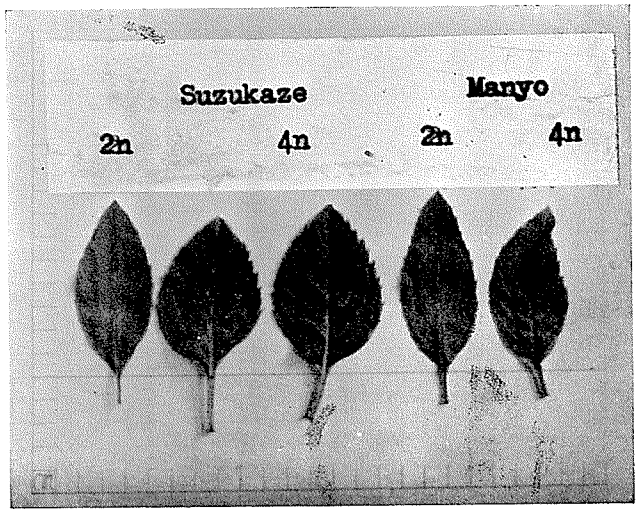


Fig. 27. Shape of leaves of 2n and 4n plants.

thickness and short internodes of polyploid rhizomes in C_0 plants seem to be helpful for their isolation.

b. The Characteristics of Polyploid Japanese Mint

Both 2n varieties have somatic chromosme numbers of 96. Accordingly, the chromosome numbers of both poly ploids would be expected to be 192, and the results of the observations met this expectation (Fig. 26).

The polyploid Japanese mints have thicker stems, thicker leaves, deeper serrations of leaves (Fig. 27), larger flowers, and thicker rhizomes. The induced polyploid Japanese mint shows the gigas type as expected, excluding the stem length.

Although the diameter of oil glands was different from variety to variety, it was increased by chromosome doubling as shown in Table 29 and Fig. 28.

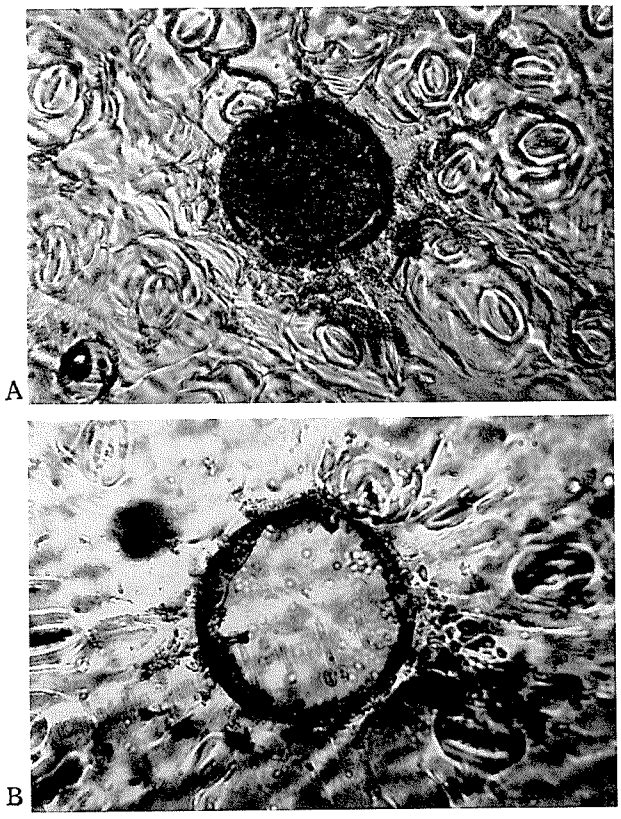


Fig. 28. Size of oil glands in A. 2n and B. 4n Suzukaze. (×1000)

TABLE 29. Size of Oil Gland (Scale numbers of ocular micrometer in magnification of 40×8)
(1 scale=3.8 μ)

	Manyo		Suzukaze	
	2n	4n	2n	4n
	26.0	30.0	20.0	28.5
	24.5	30.5	19.0	27.5
	24.0	28.0	19.5	30.0
	23.0	32.0	21.0	29.5
	25.5	32.0	21.5	27.0
	23.0	26.0	21.0	26.5
	22.5	29.0	21.0	27.5
	25.0	28.5	19.5	28.5
	27.0	27.5	19.5	27.5
	25.0	32.0	22.0	30.0
Average	24.55	29.55	20.40	28.25
μ	93.3	112.3	77.5	107.3

F=97.9**, L. S. D. (P=0.01)=0.58=2.2 μ

Table 30 reveals that the flowering time was delayed in polyploid mint as in many other induced autopolyploid plants.

The changes in leaf number caused by chromosome doubling were observed in 1956. As the leaves of mint plants are opposite, the number of nodes represents half of the leaf number. As shown in Table 30, the number of nodes in the variety Suzukaze were reduced significantly by chromosome doubling. On the other hand, the number of nodes in 4n Manyo seems to be increased.

Although there was no significant difference in the number of branches between 2n and 4n in both varieties, we might expect the same tendency as thatshown in the number of leaves (Table 31).

Contrary to these results, chromosome doubling prevented leaf abscission (Table 31). This is one of the most remarkable characteristics of 4n Suzukaze, and 4n Manyo also showed the same tendency.

TABLE 30. Date of Harvest (Date when a flower in the fourth verticil from the bottom opened) (1956)

Manyo		Suzukaze	
2n	4n	2n	4n
Sept. 3	Sept. 10	Sept. 6	Sept. 10
Sept. 3	Sept. 26	Sept. 6	Sept. 18
Sept. 6	Sept. 27	Sept. 10	Sept. 19
Sept. 29	Sept. 28	Sept. 13	Sept. 21
Sept. 25	Sept. 28	Sept. 13	Sept. 21
Sept. 25	Sept. 29	Sept. 18	Sept. 21
Sept. 25	Oct. 1	Sept. 20	Sept. 24
Sept. 26	Oct. 1	Sept. 20	Sept. 24
Sept. 26	Oct. 1	Sept. 21	Sept. 24
Oct. 1	Oct. 2	Sept. 26	Sept. 25
Oct. 1	Oct. 2		Sept. 25
Oct. 2	Oct. 2		Sept. 25
Oct. 2	Oct. 5		Sept. 26
Oct. 3	Oct. 5		Sept. 26
			Sept. 28
			Sept. 28
			Sept. 29
			Sept. 29
			Oct. 1
			Oct. 3
			Oct. 3
			Oct. 3

TABLE 31. Changes of Characters Caused by Chromosome Doubling

	Manyo			Suzukaze		
	2n	4n	Significance of Difference	2n	4n	Significance of Difference
Plant Length (cm)	92.5	93.0	n. s.	84.4	83.4	n. s.
Number of Branches	20.5	21.6	n. s.	31.6	21.8	n. s.
Number of nodes of Main Stem	25.9	26.4	n. s.	29.0	28.4	n. s.
Number of nodes of Blanches	277.6	196.3	n. s.	293.3	173.4	*
Number of Fallen Leaves	134.6	150.2	n. s.	93.8	42.2	**
Number of Harvested Leaves	472.4	540.4	n. s.	550.8	361.2	*
Fallen Leaves (per cent)	19.2	16.0	n. s.	13.9	8.2	*
Number of Observed Plants	28	16		18	17	

The number of harvested leaves decreased significantly in 4n Suzukaze, and seemed to increase in 4n Manyo (Table 31).

c. Yield Test

In 1955, the rhizomes which had grown from C_0 and check plants were transplanted to pots, which were buried in the soil out-of-doors through winter. In the early spring of 1956, these rhizomes were planted in rows. After the shoots emerged, all of the plants in the polyploid plot were examind again for polyploidy and the 2n plants were discarded. As the plants reverting to 2n were discarded, it was impossible to maintain a definite spacing between plants in the row in 4n plots. Because of this situation and other factors, the variation in the weight of plants and the number of leaves within a plot became so large that the author drew regression lines of the weight of leaves on their number. Based on these lines, he compared the rate of variation in leaf weight which corresponds to the increase or decrease in the number of leaves. As shown in Fig. 29 A and B it was found that the fresh weight of the polyploid is heavier than that of the diploid when the leaves of 2n and 4n are the same number. The same tendency was shown in the weight of dry leaves, as shown in Fig. 29, C and D.

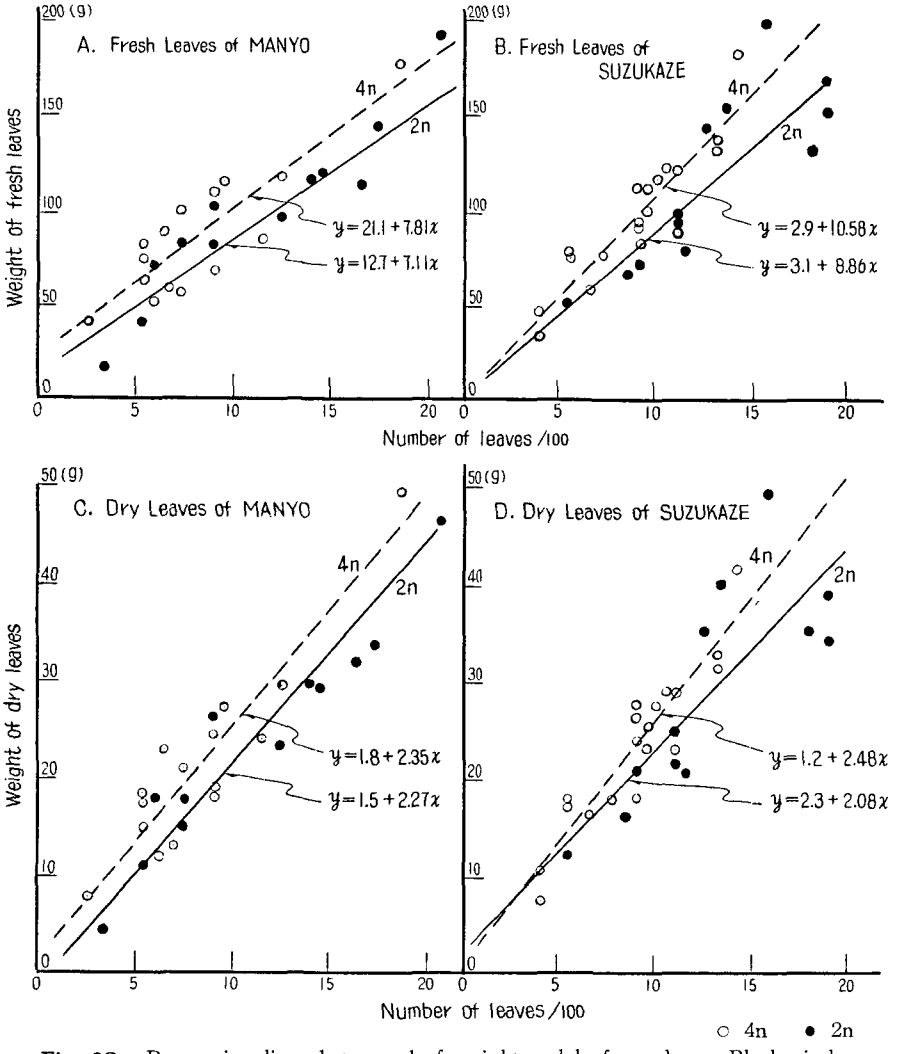


Fig. 29. Regression lines between laef weight and leaf number. Black circles mark 2n individuals and white circles mark 4n individuals.

TABLE 32. Yield Test in 1957 (kg. per row of 2 meters)

Variety	Manyo		Suzukaze	
Row Number	2n	4n	2n	4n
1	2.377	2,986	2.482	1.678
2	3.162	2.963	2.539	2.514
3	2.526	2.747	2.248	1.712
4	3.127	3.418	3.180	2.670
Total	11.192	12.114	10.449	8.574

F=3.55 Non-significant

TABLE 33. Yield Test Based on the Individual Plant

1. Weight of Fresh Plant (g)

Year	Manyo					Suzukaze				
	2n		4n		Significance of Difference	2n		4n		Significance of Difference
	Observed Number	Average	Observed Number	Average		Observed Number	Average	Observed Number	Average	
1956	12	194.3	13	183.1	n. s.	12	117.2	19	97.6	n. s.
1957	55	121.7	55	141.9	n. s.	48	138.3	48	114.0	n. s.

2. Weight of Dry Plant (g)

Year	Manyo					Suzukaze				
	2n		4n		Significance of Difference	2n		4n		Significance of Difference
	Observed Number	Average	Observed Number	Average		Observed Number	Average	Observed Number	Average	
1956	12	54.0	13	52.1	n. s.	12	65.4	19	57.5	n. s.
1957	31	29.1	16	41.6	n. s.	18	29.7	30	25.8	n. s.

After the harvest of the above-ground parts in 1956 the rhizomes were dug and replanted in the same row of the experimental plot. At the same time the fertilizer mentioned in the experimental method was applied. In 1957, the cultural practices were almost the same as those in 1956, but the number of plants per row was determined in the middle of May after thinning, (54 plants in a row, about 11 cm apart). The yields of fresh weights of above-ground parts were compared, based on the yield of each row, as shown in Table 32. The variance, however, between rows was still so large that the evaluation was not valid. Though the comparison was carried out on thebasis of individuals selected at random from each plot, no significant results were obtained as may be seen in Table 33. However, from these two comparions, we might be able to estimate that the fresh weight of 4n Suzukaze is reduced, while in 4n Manyo there is a tendency to increase.

In 1958, the cultural practices were the same as in the previous year, but no fungicide was applied so that the plants could be examined for disease

TABLe 34. Content of Essential Oil

1. 1956 (cc in dry leaves of 50 g)

Variety	Manyo		Suzukaze	
	2n	4n	2n	4n
	1.95	2.00	1.30	1.20
	1.85	2.05	1.55	1.10
	1.80	2.10	1.55	1.10
	1.85	2.00	1.65	1.20
	1.95	1.95	1.45	1.22
Average	1.88	2.02	1.50	1.16

F=103.9**, L. S. D. (P=0.01)=0.10

2. 1957 (cc in dry plant of 50 g)

Variety	Manyo		Suzukaze	
	2n	4n	2n	4n
	0.60	0.80	0.65	0.55
	0.70	0.85	0.70	0.60
	0.70	0.80	0.70	0.55
	0.70	0.85	0.60	0.55
	0.65	0.80	0.75	0.50
Average	0.67	0.82	0.68	0.55

F=33.7**, L. S. D. (P=0.01)=0.06

resistance. A yield test was impossible to obtain because all the plants suffered from the rust disease as mentioned later.

The content of essential oil was measured in 1956 and 1957. Table 34 reveals that the oil content in the variety Manyo was increased by chromosome doubling, while it was remarkably reduced in 4n Suzukaze.

The menthol content showed a reverse tendency to that of the essential oil. The chromosome doubling caused a reduction of menthol in Manyo, and an increase in Suzukaze (Table 35). The content of total menthol in the residual oil dementholized by cooling showed the same tendency as that of the crystalized menthol as shown in Table 35.

TABLE 35. Menthol Content

Year		Manyo		Suzukaze	
		2n	4n	2n	4n
1956	Weight of Oil (g)	6.9001	4.2490	5.1535	4.0550
	Weight of Crystalized Menthol (g)	3.2922	1.4047	2.7509	2.3653
	Content (%)	47.7	33.1	53.4	58.3
1957	Weight of Oil (g)	9.2938	9.1382	9.1204	8.8392
	Weight of Crystalized Menthol (g)	4.7823	4.2212	4.9229	4.9112
	Content (%)	51.5	46.2	54.0	55.6

TABLE 36. Content of Total Menthol in the Residual Oil Dementholized by Cooling (per cent in 1956)

Variety	Manyo		Suzukaze	
	2n	4n	2n	4n
	68.0	63.4	68.2	70.2
	65.2	62.5	67.8	69.5
Average	66.6	63.0	68.0	69.9

Fig. 30 makes these results clearer, and it shows the summation of crystalized menthol by cooling and the total menthol in the residual oil dementholized by cooling. Generally speaking, the menthol content in Manyo was always less than that in Suzukaze, and it is further reduced by chromosome doubling. In contrast, the menthol content in Suzukaze is increased by chromosome doubling.

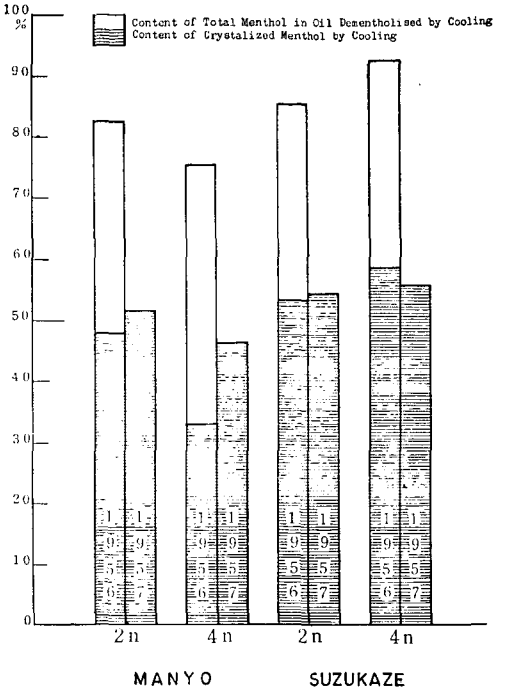


Fig. 30. Menthol content in 4n and 2n Japanese mints.

d. Disease Resistance

The varieties Manyo and Suzukaze were rust resistant when released. At present, however, they suffer severely from this disease. The author was interested in the possiblity that the disease resistance might have changed through chromosome doubling. In 1958, he compared the resistance of these varieties by omitting the spraying with a fungicide. Polyploid varieties showed some resistance in the first stage of disease. However, all the plants were eventually rusted (Fig. 22). Only the young and small leaves were harvestable and rusted plants did not produce rhizomes. Chromosome doubling, therefore, did not significantly improve rust resistance, even though some resistance was expressed early in the season.

3. Discussion and Conclusion

Studies on the artificially induced polyploid of Japanese mint have already been carried out by IKEDA and KONISHI (1954, 1956). To find any inclination of changes which are expected to be caused by chromosome doubling in the character of Japanese mint, it should be taken into account that the

cultivated varieties are highly heterozygous (KASANO 1953, 1954).

The changes in characters of 4n Japanese mints, which were induced by the present author, can be divided into two categories. One of them is a group of changes which occurred in both varieties. These included increases in stem thickness, leaf thickness, and the size of oil glands and stomata. These changes have their origin in increases in cell size. Although this can not be explained satisfactorly, decreases in the number of fallen leaves are within this category.

Another category is a group of changes which occurred differently in two varieties, including the number of leaves, fresh weight, dry weight, and the content of oil and menthol. The fresh and dry weights corresponded to changes in leaf number. This in turn is conditioned by the development of branches. However, we can not explain the fundamental causes of these changes solely by the increase in size or content of the cell through the chromosome doubling.

From the following descriptions, we find that characters which are conspicuous in the two varieties were magnified through the chromosome doubling.

According to KASANO (1953, 1954), the characters of 2n varieties are as follows:

Manyo (Fig. 14,): The growth of lower branches is pro- minent and the whole plant shows a trianglar shape. Consequently, the num- ber of leaves is very high in comparison with the old cultivated variety Hokushin. The content of essential oil is very high, while the menthol con- tent is slightly low.

Suzukaze (Fig. 14,): The growth of branches is not so prominent. The yield of the herb and content of oil are low in comparison with Manyo. The high content of menthol compensates for these weak points.

We have inadequate knowledge of the genetic behavior of the mint plant. It has been suggested that the oil content is influenced greatly by the environment, whereas the menthol content is controlled by genetic constitution. The present experiments suggest that the oil content is also controlled by the genetic constitution. It might be inferred from this experiment that not only the occurrence of menthol in oil, but also its quantity may be conditioned by the genetic constitution. In any event, it is necessary to elucidate this subject completely with biochemical and genetical knowledge of essential oils in the plants.

There are numerous literature references in regards to induced polyploids in crop plants. According to ELLIOT (1958), the following rules may apply generally to the adaptability of crop plants to the induction of polyploidy:

1. Plants low in chromosome numbers are more likely to respond well to doubling than those with high numbers.

2. Cross-fertilizing plants are more likely to respond well to doubling than self-fertilizing species.

3. Plants which are grown for their vegetative parts may be more successful as polyploids than those grown for seeds.

In harmony with these rules, the Japanese mint seems to be one of the most adaptable crops to chromosome doubling except for its high chromosome number (2n=96).

Although it requires further detailed investigation to determine whether these polyploid varieties are promising from the practial point of view, it might be expected that the polyploid Manyo will give an increase in the yield of menthol per unit acreage The increases in yield of herb and in oil content seem to outweigh the reduction of menthol content of the oil. On the ot her hand, the increase of menthol in 4n Suzukaze does not seem to compensate the reduction in yield of herb and oil content, as compared with 2n Suzukaze. Furthermore, it may be expected that the desirable characters possessed by these two polyploid Japanese mints can be combined into tetraploid or triploid hybrids from them. The fact that chromosome doubling does not improve the disease resistance satisfactorily, may mean that the direct application of induced polyploidy for the improvement of Japanese mint is limited.

Summary

I. Cytogenetic observations

1. Observations on the chromosome behavior at meiosis indicated that Japanese mint (the variety Hokushin) (*Mentha arvensis* var. *piperascens* MALINV.) (2n=96) is an allopolyploid, and D. M. A. (a variety of *M. longifolia* L.) (2n=48) appears to be an autooctoploid solely based on the occurrence of multivalent chromosomes ranging from tri- to octovalents.

2. An F_1 hybrid between these plants has a somatic chromosome number of 72 and is completely sterile. As the stamens did not develop, the meiotic behavior of chromosomes could not be observed.

3. An artificial amphiploid of the F_1 hybrid mentioned above has a somatic chromosome number of 144. Although the stamens did not develop, the seed setting rate was as high as in the variety Suzukaze (a variety of Japanese mint) which was male sterile.

4. The first back crossed progenies to a variety of Japanese mint (the variety Manyo), B_1F_1-1, and -2 have a somatic chromosome number of 120. At meiosis in B_1F_1-1, the most complete chromosome configuration of 60_{II}

was observed in 6 out of 16 PMCs and multivalent chromosomes ranging from tri- to quadrivalent were observed in 7 PMCs. A possible pentavalent chromosome was found in a PMC. The maximum number of multivalent chromosomes in a PMC was 4. Univalent chromosomes ranging from 2 to 5 were observed in a half of the observed PMCs. The number of chromosomes at MII ranged from 54 to 63, and 60 chromosomes were observed in 10 out of 28 nuclear plates. Consequently, the meiosis was infered to be rather regular as compared with that in D. M. A. The pollen fertility of about 97 per cent was unexpectedly high in comparison with those in D. M. A. and the maternal parent (the variety Manyo), and the seed setting rate of 41.3 per cent was as high as in the variety Manyo.

5. The second backcrosses were made in 1958. The variety Manyo, Suzukaze, 4n Manyo and 4n Suzukaze were used as maternal plants. In some of these backcrossed progenies, 108 and 156 somatic chromosomes were observed depending upon their mother plants. At meiosis in B_2F_1-3-12 (2n Suzukaze $\times$ B_1F_1-1) (2n=108), chromosome numbers ranged from 54 to 60. The most complete chromosome configuration of 54_{II} was observed in 6 out of 65 PMCs with the most incomplete at $48_{II}+12_I$ in 7 PMCs. A tri- and a quadrivalent chromosome were observed and the number of univalent chromosomes ranged from 2 to 12. At M II, chromosomes ranging from 50 to 59 were observed, and 54 chromosomes were observed in 2 out of 8 PMCs. The pollen fertility averaged 41.7 per cent and seed fertility was 11.9 per cent.

6. From these observations, the author concluded as follows:

a. No homologous genome exists between Japanese mint and D. M. A..

b. The univalent chromosomes observed at meiosis in B_1F_1 and B_2F_1 were derived from D. M. A..

c. Four genomes derived from D. M. A. consisted of two sets of two possible isogenomes and there appeared to be also an incomplete homology between these two sets.

d. Consequently, it might be reasonable to infer that D. M. A. should be referred to as a segmental allooctoploid.

II. Investigations on some available characters of progenies of the interspecfic hybrid backcrossed to Japanese mint.

1. The plants used in the cytogenetic observations mentioned above were agronomically evaluated for disease resistance, the content of essential oil and the menthol content.

a. D. M. A., the F_1 hybrid, and its artificial amphiploid proved to be immune to rust disease (*Puccinia menthae* PERS.) and B_1F_1s were highly re-

sistant. The segregation of disease resistance occurred in 45 B_2F_1s : 6 of them were highly resistant, 10 moderately resistant, 10 moderately susceptible and 19 highly susceptible. Consequently, it was revealed that the rust resistance could be maintained to some extent in the second backcrossed progenies to susceptible plants.

b. The content of essential oil in D. M. A., the F_1 hybrid and its artificial amphiploid was rather low, and it increased considerably in B_1F_1. In B_2F_1, it ranged from 1.54 to 3.03 per cent, and, although a higer content than that in the variety Manyo could not be found, 12 out of 38 B_2F_1s had higher contents than that in the variety Suzukaze.

c. The content of free menthol in D. M. A. was only 10 per cent, and the F_1 hybrid had a content of 54 per cent. Through the first backcross to the variety Manyo, it increased to about 60 per cent. In B_2F_1, it ranged from about 60 per cent to 83 per cent, and all of B_2F_1s except for a single strain had a higher content than that in their pollen parent. Four out of 36 B_2F_1s had a higher content than that in the variety Suzukaze of which menthol content is the highest among the cultivated varieties.

d. Three strains in B_2F_1 had resistance to rust disease, with a comparable or higher content of oil and menthol than that in the variety Suzukaze.

2. The yield of herb could not be investigated, though the vigor of B_1F_1s and B_2F_1s appeared to be as high as in the cultivated varieties (Manyo and Suzukaze).

3. These results revealed that backcross breeding, utilizing D. M. A. as a non-recurrent parent, is highly useful and promising for the improvement of disease resistance of Japanese mint.

III. Investigations on some available characters of artificially induced Polyploids of Japanese mint.

1. An immersing technique for the induction of autopolyploid of Japanese mint (the varieties Manyo and Suzukaze) utilizing colchicine, was developed.

2. Respecting the yield of herb, it is estimated that the fresh and dry weight of 4n Suzukaze is reduced, while in 4n Manyo there is a tendency to increase.

3. The content of essential oil was increased by chromosome doubling in the variety Manyo and remarkably reduced in 4n Suzukaze.

4. The chromosome doubling caused a reduction of menthol in the variety Manyo, and an increase in the variety Suzukaze.

5. Rust disease resistance was not significantly increased in artificial autopolyploids.

6. It can be concluded that chromosome doubling magnifies the characters which are conspicuous in these two varieties (the variety Manyo has a higher yield of herb and a higher content of essential oil than the variety Suzukaze, with a reverse tendency in regards to the menthol content). From a practical point of view, polyploid Manyo is promising for the yield of herb and content of essential oil. However, since chromosome doubling does not improve disease resistance, the direct application of induced polyploidy for the improvement of Japanese mint is limited.

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