STUDIES ON BARLEY STRIPE MOSAIC IN JAPAN

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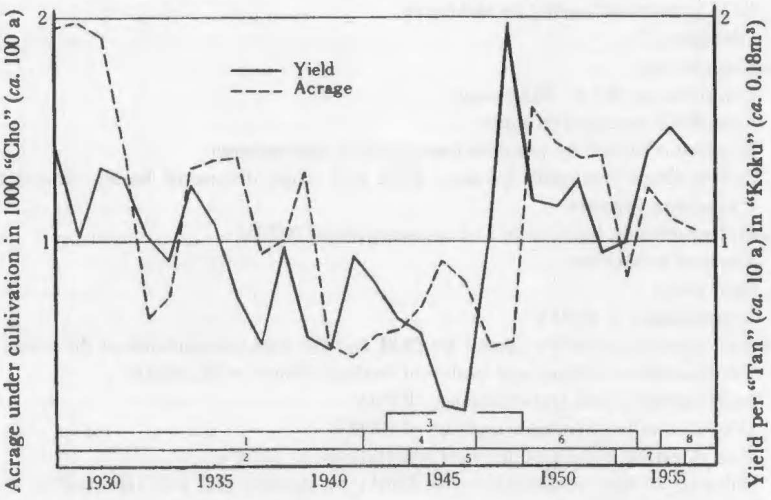
CONTENTS

I. INTRODUCTION

As the materials for malt, some two-rowed varieties of barley, such as

Chevalier and Hokudai No. 1, had long been grown in Hokkaido with invariable success. However, in about 1930 some of the growers became aware of the fact that semi-sterility accompanied with inferior growth of plant and incomplete emergence of head from the sheaths occurred very commonly in these varieties, which resulted in an appreciable decrease in yield. And, this had become before long so marked that some of the farmers gave up growing barley for malt. Under such circumstances, the renewal of the variety in question was the only effective measure to recover the previous level of the yield (Meguro, 1948). In fact, several renewals were made only to see that those new varieties were found to be effective only temporarily, because the semi-sterility became so serious even in those new varieties within several years (Fig. 1). Many efforts had been devoted to clarify the cause of the semi-sterility from the physiological viewpoint. Although some hypothesis have been presented, the major cause of the semi-sterility has long been left unknown.



1 : Hokudai No. 1 2 : Chevalier 3 : Hakata No. 2 4 : Bando-shu
5 : Moravia 6 : Harbin 2-rowed 7 : Nissei 8 : Shunsei

The data from Nippon Beer Co. mentioned that, "marked degeneration in Hokudai No. 1 and Chevalier in 1936 and 1943 respectively," and also "prevalent occurrence of sterility in Harbin 2-rowed in 1951 were noticed." These facts suggest that some part of the yield reduction noticed in this figure (1932–1946, and 1949–1952) was attributed to the "Chochin-bo" (semi-sterility of barley).

Fig. 1 The acrage and the yield of barley for malt in Hokkaido for the period of 1928–1957. (According to the data from Nippon Beer Co.)

Takahashi and Akaki made a genetical experiment using fertile and semi-sterile strains of Moravia variety of barley for the period 1954 to 1956. A crossing experiment with the use of these strains suggested that the semi-sterili-

This article was compiled from the data published in Nogaku Kenkyu (Japanese report of the Ohara institute) Vols. 44–49, 1957–1961, together with unpublished data accumulated since 1956.

ty might be inherited through maternal cytoplasm. However, some phenomena incompatible with this hypothesis were found: viz., considerable differences in fertility among the semi-sterile parent, M. 14 and the F_1 and F_2 of a M. 14 × M. 40 cross, and also a tendency of variability in fertility to increase as the sterility gets higher. It was also found that such characters as incomplete emergence of heads from the sheaths, light weight of kernels, loss of vigor, uneven plant height and heading, these characteristics of the semi-sterility of barley prevalent in Hokkaido, were generally accompanied by the semi-sterility. In the spring of 1956, pronounced mosaic and necrotic stripe symptoms happened to be found in the majority of the plants of the semi-sterile lines and a hybrid population. These facts indicated that the semi-sterility was not attributable to a genetic cause but to a kind of seed-borne disease. Therefore, the author made further inquiries from the pathological viewpoint, and identified this disease to barley stripe mosaic (BSM) that has been reported in the North America, but new to Japan at that time.

This study includes the investigations on the seed transmissions of the virus and the sterility caused by this disease, together with some other descriptions on this disease.

II. HISTORICAL REVIEW

A. STUDIES ON THE SEMI-STERILITY OF BARLFY AND ON BSM IN JAPAN

Yamamoto, Y. and Terada (1940), and Suto (1942) demonstrated experimentally that rainfall at the time of anthesis caused sterility of barley. Nevertheless, Suto (1942) made an opinion that the semi-sterility then prevalent in Hokkaido might not be wholly attributed to such a simple cause, but he estimated that the principal cause for it might be the unfavorable conditions of the soil. He also pointed out in his paper that the semi-sterile plants were stunted in general; their height below normal, their appearance slender, their roots underdeveloped and most of the heads did not emerge completely from the sheaths. Yamamoto, T. (1950, 1952, and 1955), using two strains with high and low fertility isolated from Moravia, investigated the physiological mechanism of the semi-sterility. As a result, it was confirmed that the semi-sterility was mostly due to the unsplitted anthers and also that the degree of sterility was affected by the sowing time and other physiological factors. Yamamoto, Y. (1942) and Takano (1942) ascertained the fact that, even under the same growing condition, the degree of sterility differed considerably among different varieties or strains. Takano, on this basis, emphasized the necessity of the renewing the variety in order to overcome the difficulties experienced in barley growing in Hokkaido at that time.

Takahashi and Akaki made a genetical experiment on fertile and semi-sterile strains of Moravia. The results suggested maternal inheritance of the semi-sterility. However, some illegitimate behaviors of the hybrids and the parental

lines were also noticed. Moreover, in the course of another genetic experiment conducted in 1956, a prevalent occurrence of morbid symptoms similar to those of the so-called yellow-mosaic and stripe disease of barley was observed on some of the genetic materials. In view of these facts, it was presumed that the semi-sterility might be caused by the infection of a certain disease, which was to be later identified by the author to be barley stripe mosaic.

Since the author's discovery of BSMV, several workers in Hokkaido began to study on this disease. Oshima, Goto, Goto, and Sato (1958) reported that BSM was responsible to the yield reduction and the sterility of barley and wheat. Murayama, Nemoto, and Yokoyama (1959) made a serological study, and reported the physiological properties of this virus. The electromicrograph of the virus particles was reported by Shikata, Murayama, and Nemoto (1959). Oba (1959) reported the occurrence of BSM in two-rowed barley for malt in Hokkaido in 1957. Further, Oba and Sugiyama (1959) surveyed the prevalence of this disease in 1958 and 1959 in Hokkaido, and studied the effect of the disease on sterility, plant height and other characters of barley.

B. STUDIES ON BSM IN FOREIGN COUNTRIES

BSM, which had long been known as a non-parasitic disease "false stripe of barley", was first identified by McKinney in 1951 as a seed-borne virus disease. According to McKinney, the "false stripe" had been noticed since about 1910 in U. S. A., and the symptoms on the pressed specimens of "false stripe" plants, prepared by A. G. Johnson, were found to be the same as those induced by BSMV. Hagborg (1951) found that, "false stripe" in Canada, which had been known since the description by L. I. Conner in 1924, was also caused by the same virus as BSMV. The occurrence of the disease in England and Germany was first reported by Bawden (Slykhuis, Watson, Mulligan) in 1957 and Klinkowski and Kreutzberg in 1958, respectively.

Slykhuis (1952) investigated the host species of BSMV using various gramineous plants, and Singh and others (1959) reported the susceptible plant species in and outside the *Gramineaceae*. In addition to these reports, the host range or susceptible host plant of this virus was studied by McKinney (1951, 1953, 1954), Hagborg (1951, 1954), Kahn and Dickerson (1957), Hollings (1957), and Bawden (Kassanis, Slykhuis (1958)). Physical properties of the virus were first recorded by McKinney (1951, 1953). Hagborg (1955) reported on the heat tolerance of this virus. Kassanis and Slykhuis (1959) studied on the virus properties by inoculation and serological tests. The size and the shape of the virus particles were described by Gold and others (1954), Bawden (Kassanis, Slykhuis, 1958) and Klinkowski and Kreutzberg (1958). Moorhead (1956) made a serological study of the virus by means of the complement-fixation technique. Shalla (1959) reported that, the rod shaped particles were easily found in cytoplasm of mesophyll and epidermal cells of BSM infected barley leaves by the electron microscopic study.

Since the identification of BSMV by McKinney (1951), many workers confirmed the seed transmission of this virus. McKinney (1954) reported that some of the seed-borne symptoms of this disease were difficult to detect in some conditions. Then, Hampton and others (1957) found the optimum light intensities and temperatures for the seed-borne symptom expression. Crowley (1959) presented an opinion on the mechanism of seed transmission of the virus, studying on the time of embryo infection by the virus, and confirmed the results which had been obtained by Eslick and Afanasiev. McKinney (1951) obtained negative results on the insect and soil transmission of the disease. Pollen transmission of this virus was proved by Gold and others (1954). The occurrence of plant-to-plant contact transmission of the disease was closely investigated by McKinney (1954). Fitzgerald and others (1957) reported that the virus was transmitted from infected spring barley to winter wheat when crops were grown in adjacent rows with leaves in contact. Hagborg (1960) obtained the evidence that both skim milk and whey were effecteve in reducing the plant-to-plant contact transmission of the disease.

Effects of BSM infection in barley and wheat on the plant yield and other characteristics were studied by Eslick (1953), McKinney (1953), Hagborg (1954), Eslick and Afanasiev (1955), McNeal and Afanasiev (1955, 1956), and Arny and others (1958). Singh and others (1960) investigated the effects which soil and air temperatures and the age of the plant have on the symptom expression and seed transmission.

Timian and Sisler (1955), and Sisler and Timian (1956), found several varieties of barley resistant to this disease from the Abyssinian barleys, and studied the inheritance of resistance of Modjo and C. I. 3212–1.

III. BARLEY STRIPE MOSAIC VIRUS FOUND IN THE SEMI-STERILE STRAIN OF 2–ROWED BARLEY FOR MALT AND THE STERILE BARLEY PLANTS COLLECTED FROM HOKKAIDO

A series of pathological surveys were commenced by the author, to follow to the genetical study on the semi-sterility of barley by Takahashi and Akaki as already mentioned, in order to ascertain whether the mosaic and necrotic symptoms observed in semi-sterile strains of Moravia in 1956 were caused by a virus disease, and whether these symptoms were related to the semi-sterility of barley prevalent in Hokkaido.

A. TRANSMISSION OF THE MOSAIC SYMPTOMS AND IDENTIFICATION AS A VIRUS DISEASE

1. X-body

The characteristic X-body is generally found in the epidermal cells of cereal crop plants infected with the known virus diseases in Japan. The epidermis of infected seedlings and mosaic leaves of F_2 hybrids with M. 14 were stripped and stained by orange GG, safranin, basic fuchsin, eosin or methylen-blue, and

examined under the microscope. However, no X-body was found.

2. *Seed transmission*

Many of the seedlings grown from the seeds of M. 14 obtained in 1955 showed mosaic symptoms: yellowish green or whitish yellow or grayish white spots and streaks developed on the primary leaves of these seedlings, and these were seen at the tip or the base of the leaves. These mosaic seedlings were generally stunted in some degree. The seeds from some plants taken at random from the F_2 of a M. 14 (♀) × Aohadaka (♂) cross cultivated in 1956 were sown, and the seedlings with mosaic symptoms were counted. As seen in Table 1, 454 out of 670 seedlings (67.8%) carried mosaic symptoms. Therefore, it was recognized that these mosaic symptoms of the seedling were found to be seed-borne.

Table 1. Seed-borne infection of seedlings grown from the seeds of the F_2 plants of a M.14 (♀) × Aohadaka (♂) cross

Seedling	Observed	Healthy in appearance	Mottled			
			Severe	Moderate	Mild	Total
Number	670	116	117	240	97	454
%	100	32.2	17.5	35.8	14.5	67.8

Final count was made at the 3rd leaf stage

3. *Transmission by plant juice*

As stated before, mosaic symptoms were observed on a single plant of Aohadaka stood closely to F_2 hybrids between M. 14 and Aohadaka, which had expressed mosaic symptom. This suggested that the transmission of the symptoms to the Aohadaka plant might be due to the inter-plant contact. Healthy seedlings were inoculated with the use of carborundum by juice expressed from mosaic seedlings of M. 14 and mosaic leaves of some F_2 hybrids of M. 14 grown in the field (July, 1956, under glasshouse condition). After 3–4 days, necrotic spots and streaks appeared on the inoculated leaves and the mosaic symptoms developed on the youngest leaves. Infections were obtained also in the case of the inoculation without carborundum.

4. *Transmission by soil*

There was no evidence accruing to soil transmission of this disease according to the observation in the field occurrence of the diseased plants of M. 14 and its hybrids in 1956. To see whether the disease was transmitted by soil or not, all the artificially infected seedlings grown in pots were removed, then disease-free seeds of barley were sown there. Furthermore, the field soil taken from the barley field, where the semi-sterile strain of Moravia had been grown in 1956, was examined also for its infectivity to healthy seedlings. All the test gave negative results.

5. Insect transmission

Insect transmission of the disease under discussion was examined, using a kind of aphid and planthopper, which migrated on barley grown in glasshouse. These insects, after fed on diseased barley seedling, transfered to some healthy plants and fed for 1–3 days. The results proved to be negative.

6. Inoculation test to several plant species other than barley

Inoculation test was made to know the susceptibility of several plant species other than barley to this disease. Mosaic symptoms were observed in the inoculated seedlings of wheat, oat, broom corn millet, green foxtail and perennial ryegrass. Subinoculation tests proved the susceptibility of these plants. However, no infection was observed in orchard grass, *Agropyron semicostatum*, tobacco and *Nicotiana glutinosa*.

The results of the experiment described above showed that a virus disease was a possible cause of the semi-sterility of M. 14 barley. The disease under discussion seemed to be identical with barley stripe mosaic (barley false stripe) which had been prevalent in North America as described by Mckinney (1951) but new to Japan, in view of its mode of transmission, symptoms and susceptible plant species. Table 2 shows the comparison among the virus disease under consideration, virus diseases of barley known in Japan, and BSM described by McKinney as to their modes of transmission and presence or absence of X-body.

Table 2. Comparisons among the disease under consideration, virus disease of barley and wheat known in Japan, and BSM reported by McKinney

Virus disease	X-body	Transmission			
		Soil	Insect	Plant juice	Seed
Weat green mosaic	+	+	−	+	−
Barley yellow mosaic	+	+	−	+	−
Northern cereal mosaic	−, +	−	+	−	−
Barley stripe mosaic	−	−	−	+	+
The disease under consideration	−	−	−	+	+

B. DETECTION OF BSM IN THE SEEDS TAKEN FROM THE NATURALLY OCCURRED SEMI-STERILE PLANTS AND THE SEMI-STERILE STRAINS OF 2-ROWED BARLEY GROWN IN HOKKAIDO

Several experiments were carried out to see whether BSM found in the semi-sterile strain of Moravia barley, M. 14, was also detected in the seeds taken from the semi-sterile 2-rowed barley grown in Hokkaido.

1. Materials and methods

In one experiment, the following materials were used which were supplied by Sapporo Factory, Nippon Beer Co., in Hokkaido: (a) matured 10 semi-sterile plants of Shunsei variety obtained from Furano, Hokkaido, in 1956, (b) seed samples of Shunsei and Nissei varieties produced at the nurseries at Naebo and

Shin-Kotoni, Hokkaido, in 1956. In another experiment, seeds from 60 lines of 6 semi-sterile strains, A, B, C, D, E and F, isolated from Harbin 2-rowed were used, which were supplied by the Kitami Branch of the Hokkaido Agricultural Experiment Station, Memanbetsu, Hokkaido, in 1956. The semi-sterility per cent of these 6 strains in the 3-year period, 1954–1956, are presented in Table 4. Seeds were shown 7 × 7 cm apart in wooden boxes. Seedlings grown had been examined daily, until the third leaves were unfolded. Infected seedlings were discarded whenever they appeared, so as to avoid plant-to-plant contact.

Table 3. Detection of seed-borne infection in the seedlings grown from the seeds of semi-sterile plants of Shunsei barley collected at Furano, Hokkaido, in 1956

No. of plant	Sterility %	Seed sown	Seed germinated	Seedling diseased	Seed-borne infection %	Sub-inoculation
1	71	32	30	27	90.0	+
2	64	46	43	40	93.0	+
3	52	53	53	50	94.3	+
4	56	50	49	43	87.8	+
5	68	29	25	25	100.0	+
6	28	50	47	37	78.8	+
7	55	50	48	41	85.4	+
8	0	50	49	26	53.0	+
9	69	31	31	29	93.5	+
10	44	37	35	31	88.5	+
Total	49.1	428	410	349	81.5	
Shunsei*		50	49	0	0	–
Shunsei**		50	50	0	0	–
Nissei**		50	50	0	0	–

Collected from the nurseries of * Naebo, ** Shin-kotoni, Hokkaido. Seeds were sown on Sept. 5. Final count on Sept. 28, 1956.

Table 4. Sterility of semi-sterile strains of Harbin 2-rowed (%)

Year	Barley strain					
	A	B	C	D	E	F
1954	4.1	23.0				8.0
1955	3.0	15.9				9.6
1956	0.7	14.5	32.6	32.2	29.3	10.0

Strains C, D, and E were isolated from strain B in 1955

2. *Results*

Table 3 shows that the rate of sterility and the infected seedlings were 0 to 71 and 53 to 100, respectively, in the 10 semi-sterile Shunsei plants. Correlation between both figures was as high as +0.92, which proved to be significant on 1 % level, but no correlation was found between the semi-sterility and the

germination rate. All the cross inoculations between seed-borne infected seedlings and healthy ones gave positive results.

The results of the seedling test with semi-sterile lines of Harbin 2-rowed are shown in Table 5. Seed-borne infections of the seedlings did not occur at all in A and E. But in C and D strains, they occurred in 8 and 9 out of 10 lines respectively, and in both B and F strains, in all lines. Both A and E strain were not immune to the virus, since they were susceptible to all of the viruses isolated from B, C, D and F strain of Harbin 2-rowed, semi-sterile Shunsei barley and M. 14 strain. Therefore, these two strains might be virus-free or their percentages of seed infection were very low. There was no correlation between the sterility and seed-borne infection in 4 diseased strains. Relation of the sterility per cent of the lines with their seedling infection could not be surveyed, for the sterility of each line was not known.

Table 5. Detection of seed-borne infection with the use of 6 strains of Harbin 2-rowed (c. f. Table 4)

Barley strain	Sterility %	Seed sown	Seed germinated	Diseased seedling	Seed transmission (%)	
					Average	Individual plant
A	0.7	489	478	0	0	
B	14.5	500	480	343	71.5	38.0—86.0
C	32.6	422	392	269	68.6	60.5—83.3
D	32.2	497	440	272	61.8	36.8—86.0
E	29.3	500	462	0	0	
F	10.0	500	487	354	72.7	64.0—85.7

About 50 seeds from 10 plants of each strain of barley. Seeds were sown on Sept. 19. Final count on Oct. 4, 1956.

3. Discussion

It was possibly supposed that, the major part of the semi-sterility of 2-rowed barley in Hokkaido should be caused by BSMV, according to the investigation of this disease in semi-sterile barley strain of Moravia in Kurashiki as mentioned in the previous section. The results obtained in this section also gave some information supporting this assumption, although the semi-sterility of barley was not caused by a single factor as already discussed by Suto (1948). The characteristic effects of the semi-sterility on barley growing in Hokkaido were considered to be as follows: (a) marked yield reductions caused by the semi-sterility in successive years, (b) inferior growth of semi-sterile plants, and (c) essential but temporary effect of the renewal of the variety to secure the normal yield. These characteristics may easily be recognized when one remembers that the seed transmissible disease BSM has been the major cause of the semi-sterility under discussion.

IV. OCCURRENCE AND DISTRIBUTION OF BARLEY STRIPE MOSAIC IN JAPAN

The prevalence of BSM in farmer's fields has so far been known in U. S. A., Canada and Japan (Hokkaido). According to the reports from England and Germany, the disease has been found only in a few varieties in the collections of barley (Bawden, 1957; Klinkowski and Kreutzberg, 1958). In 1957, the author made the inquires to the Experiment Stations and workers in many districts of Japan except Hokkaido about the occurrence of BSM, but could not obtained any positive information. Several barley samples carrying some doubtful symptoms had been sent, but all the inoculation tests with them gave negative results. From this, and from the results of the field survey in Hokkaido, the author are now led to think that the natural occurrence of BSM in Japan is only in Hokkaido at the present time.

A. THE FIELD SURVEY OF BSM IN TWO-ROWED BARLEY FOR MALT IN HOKKAIDO

As described in Chapter III, the author discovered in 1956 BSMV in the semi-sterile barley sent from Hokkaido. Since the occurrence of BSM had not up to that time been reported nor ascertained in Hokkaido, the author naturally wanted to make a field servey in Hokkaido to see if the semi-sterility of barley in Hokkaido was really attributed to BSMV. For this purpose, toward the end of June in 1957, the author made a field survey of BSM in two-rowed barley for malt in Hokkaido with a cooperation of Dr. R. Takahashi, the Ohara Institute, Okayama University, and Mr. K. Oba, Nippon Beer Co..

After this joint field survey, Mr. Oba remained in Hokkaido for further three to four weeks to make more extensive surveys on the field. Further to these field surveys, investigations were made at Kurashiki on the semi-sterility and the seed-infection with the samples of the matured plants and the seeds obtained from the major infected fields in Hokkaido.

1. Experimental methods

Twenty-six and eighty-four barley fields were covered under the first and the second survey, respectively. In determining prevalence of BSM, the following standard were employed:

−: No diseased plant was observable at all.
±: Very rare occurrence was observable on a closer examination in the field.
+: Sporadic occurrence was observable on random walkings through the field.
++: Diseased plants were easily found on random walkings through thefield.
+++: More than several diseased plants were found on random walkings through the field.
++++: Very dense occurrence was observable throughout the field over.

Along with this field survey, some of the diseased plants were marked on the spot and these were later sent together with the seeds obtained from the fields to Kurashiki, where the author made the tests to examine the seed infec-

tion with these materials.

2. *Results*

i) Occurrence of BSM in the foundation, the propagation and the malt barley field in various districts of Hokkaido

Two-rowed barley in Hokkaido is grown under the following system of seed production. The breeder's stock field and some of the foundation fields are super-

Table 6. Field survey of the occurrence of BSM in Shunsei variety for malt in Hokkaido (First survey, June, 1957)

Region	Barley field	BSM occurrence						Total
		−	±	+	++	+++	++++	
Ishikari	Propagation			1				1
	Malt barley	1			2		2	5
	(No. 7-5)*	2		1				3
	Total	3		2	2		2	9
Kamikawa	Foundation			1				1
	Propagation				1			1
	Malt barley		1	2	3			6
	Total		1	3	4			8
Abashiri	Foundation		1					1
	Propagation	1	1					2
	Malt barley	3	2	1				6
	Total	4	4	1				9

Number of field surveyed

* A variety under breeding

Table 7. Field survey of the occurrence of BSM in Shunsei variety for malt in Hokkaido (Second survey, July, 1957)

Region	Barley field	BSM occurrence						Total
		−	±	+	++	+++	++++	
Ishikari	Propagation			1				1
	Malt barley					1	1	2
	Total			1		1	1	3
Kamikawa	Foundation		1					1
	Propagation		1	1		2		4
	Malt barley		4	5	4	2		15
	Total		6	6	4	4		20
Abashiri	Foundation		1					1
	Propagation	10	5	6				21
	Malt barley	15	8	11	4	1		39
	Total	25	14	17	4	1		61

Number of field surveyed

vised by the Hokkaido Agricultural Experiment Station, and a limited number of growers appointed by brewery company takes charge of some part of the production of foundation and all of the propagation fields are then made available for individual farmers for the production of barley for malt. Table 6 and 7 show, the occurrence of BSM observed in the foundation, the propagation and the malt barley fields in various regions of Hokkaido. Table 6 shows the results of the first survey carried on at the end of July, 1957, and Table 7 the results of the second survey for the latter half of July of that year. The second survey covered all the fields observed in the first survey together with many other additional fields. Both surveys gave similar results as to the occurrence of BSM. BSM in Shunsei barley was observed in 78.3% and 70.2% of the total fields inspected under the first and the second surveys, respectively. By region, the disease was observed in 83.3% (1st survey) and 100% of the total field inspected in the Ishikari district, 100% for both surveys in the Kamikawa district, and 55.6% (1st survey) and 59.6% (2nd survey) in the Abashiri district. Among the four categories of fields covered under the surveys, most frequent occurrence of BSM was observed in the malt barley fields.

ii) Field occurrence of BSM by differet sources of seeds

Table 8. Occurrence of BSM in the fields by different sources of the seed used

(First survey)

Seeds obtained from	BSM occurrence						Total
	−	±	+	++	+++	++++	
Breeder's stock farm		1	1				2
Foundation field	1	1	1	1			4
Propagation field			1				1
Mlat barley field				1		1	2
Private propagation filed	2	2		1			5
Source unknown	2	1	2	3		1	9
Total	5	5	5	6		2	23

(Second survey)

Seeds obtained from	BSM occurrence						Total
	−	±	+	++	+++	++++	
Breeder's stock farm		2					2
Foundation field	5	5	4				14
Propagation field	12	7	13	3	2		37
Malt barley field					1	1	2
Private propagation field	1	1	4	5	2		13
Source unknown	7	5	3		1		16
Total	25	20	24	8	6	1	84

Number of field surveyed

As the BSMV is seed-borne, the repeated uses of the seeds obtained from BSM infected fields will possibly increase the occurrence of BSM year by year. The results of our field survey in Hokkaido have supported this assumption. Table 8 shows the occurrence of the disease by different sources of the seeds used. The most frequent occurrence was observed in the fields where the seeds used had been obtained from malt barley fields as well as the non-authorized private propagation fields. The seeds obtained from propagation and foundation fields proved to have caused a medium frequency of occurrence.

iii) Semi-sterility and seed infection caused by BSM

From the above symptom observations, we could ascertain that some of the barley fields in Hokkaido was BSM infected. In order to finalize our investigation in Hokkaido to ascertain BSM to be really a major factor that has caused the semi-sterility of the infected barley covered under this survey, it was necessary for us further to see if the semi-sterility in question was particularly large with the BSM infected barley plants as compared with the healthy plants. We had also to see if these diseased plants had been infected by seed-transmissible BSMV. For this purpose, later in Kurashiki, semi-sterility and seed infection together with plant height and some other plant characteristics of the diseased plants were compared with those of the healthy ones.

Table 9. Comparisons between healthy and BSM infected plants of barley collected from Hokkaido on sterility and other agronomic characters, and also on seed transmission of the disease

		Healthy plant	Diseased Plant
Plant height	cm	96·2	87.3
Uncovered portion of the uppermost internode	cm	14.0	12.2
Length of head	cm	6.6	6.6
Number of kernel per head		25.7	25.6
100 kernel weight	*g	5.1	4.2
Sterility	%	2.8	8.3
Seed transmission	**%	0	48.1

Averages of 15 healthy and 20 diseased plants, * 9 and 13 plants, ** 7 and 25 plants.

As shown in Table 9, the length and the number of kernels per spike of the diseased barley plants proved to be not so different from those of the healthy ones, but the shortening of plant height, incomplete emergence from the sheath, slight decrease of kernel weight and the increased ratio of sterility were observed in comparison with the healthy plants. Seed infection of the diseased barley amounted as high as to 48%. These findings proved to be incomformity with the results previously obtained through the field inoculation test referred to in Chapter VII, and therefore, we came to determine that the disease of the barley under survey in Hokkaido had been caused by BSM as had earlier been ascertained in Kurashiki.

As Table 10 shows, seed infection was observed greater in the case with the seeds obtained from the fields in which BSM prevalence was indicated by ++ and +++ signs, than in the case with the seeds obtained from the fields in which BSM prevalence was indicated by ± and + signs. This should mean that, if seeds obtained from BSM infected fields are sown every year, the seed infection ratio of barley grown out of such origin of seeds tends to be quite a high one as the year renews itself.

Table 10. Seed transmission detected from the seeds obtained from the BSM infected field in Hokkaido

Seed transmission %	Field occurrence of the disease	Seed transmission %	Field occurrence of the disease
2.0***	+	40.8***	++
1.3*	+	31.2**	+++
4.1*	+	66.0**	+++
0*	+	59.0	Uncertain
6.2***	+		

About 100 seeds/sample
Seeds obtained from * foundation or propagation field, ** malt barley field, and *** private propagation field.

B. BSM IN 6-ROWED BARLEY IN HOKKAIDO

Besides the field survey for 2-rowed barley, the preliminary survey was made on the occurrence of BSM in 6-rowed barley not for malt in Hokkaido. The knowledge on the distribution of BSM described here was still unsufficient, but it was very conspicuous that the disease was found so commonly in the old varieties such as Sapporo-Rokkaku and Taiki-Omugi in Ishikari district. There was also prevalent occurrence of the disease in many varieties of the barley collection by Kitami Branch of Hokkaido Agricultural Experiment Station. Oba (1958) stated that 0.5–25.7% seed infection was detected in the seeds of 3 varieties (Rokkaku-Ozeki, Sapporo-Rokkaku, Taiki-Omugi) out of 5 barleys collected from the Branches of Hokkaido Agricultural Experiment Station. These proved the wide occurrence of the disease in 6-rowed barley, especially in the old varieties, as with the case in 2-rowed barley in Hokkaido.

C. DISCUSSION

Since BSM is seed-borne, it is easily supposed that the disease may begin to show its occurrence in such districts where the disease had never occurred, whenever the infected seeds are introduced. It is said that BSM in England occurred in Gloire du-velay imported from France (Bawden, 1957), but there is no report on the disease from France so far. The author found the occurrence of the disease in several varieties in the barley collection grown in the field of the Ohara Institute for Agricultural Biology, Okayama University. These diseased plants were difficult to regard as the seed contamination of the author's experi-

mental materials, and it was reasonably considered that these varieties had already been infected by the disease before they were imported. Barley varieties in Hokkaido, especially some old varieties for malt, had been imported from Germany, Sweden, U. S. A. (Meguro, 1948), but now it is very difficult to know whether the disease had been carried by some of those imported seeds. While, one of the most remarkable difference of barley cultivation between Hokkaido, where the disease may have long been prevalent, and other region of Japan is sowing season, the symptoms of the seed-borne infected spring barley from Hokkaido sown in autumn at Kurashiki were similar to those sown in spring in Hokkaido. The natural occurrence of BSM is still restricted in Hokkaido, though the interchange of the seeds between Hokkaido and other regions of Japan is reasonably possible. And, it is difficult to think that the inoculum sources (diseased seeds) has never been introduced from Hokkaido.

Our field survey in Hokkaido in 1957 revealed the following facts:

(1) The disease was observed in every barley growing district, though the prevalence differed somewhat by region. The semi-sterility of barley by the disease was also observed.

(2) The occurrence of the disease was not so prevalent in the foundation fields and the fields where the seeds used had been obtained from right sources. The yeild reduction caused by the disease estimated to be almost fatal, because the barley Shunsei had only been introduced several years before. On the other hand, heavy occurrence of the disease was observed in the fields where used the seeds obtained from the malt barley field and the private propagation field had been sown.

In view of the above-mentioned facts, the following measures may be recommended for checking the prevalence of BSM in two-rowed barley for malt in Hokkaido. Since the lack of control checking the repeated use of BSM infected seeds will naturally results in the increased cases of seed-infection, it is necessary for us to check the disease contamination in the breeder's stock farm and the foundation field. For this purpose, it is desirable that close seed-testings, and also close inspection of the barley fields be carried out in order to pick out as many diseased plants as possible. It is also important that the barley growers should be obligated to use the seeds obtained from the right propagation fields.

Fortunately, the disease in Shunsei, which is grown commonly in Hokkaido, is not yet so destructive, and the complete renewal of the variety may not be urgently necessary at this situation. But, if we do not take appropriate measures right now, the variety Shunsei will follow the same fate as those Chevalier, Hokudai No. 1, Moravia or Harbin 2-rowed in the near future. To break down the sources of infection, the sweeping renewal of the old 6-rowed varieties should be made as soon as possible.

Oba and Sugiyama (1959) surveyed the disease in 2-rowed barley in Hokkaido in 1957 and 1958, and investigated the inferiority of many of the agronomic characters of the diseased plants. They stated further "—the sterility of

BSM plants is rather variable, while the sterility of the healthy plants is very low in all cases—". This remark agrees with the results stated in Chapter VII, and supports the author's opinion on the sterility of barley for malt in Hokkaido is attributed to the BSMV.

V. SYMPTOMATOLOGY

The symptoms of BSM gave already been dealt with by McKinney (1953), Hagborg (1954) and some other workers. Experimenting on the disease since 1956, the author observed that BSM shows symptoms slightly different according to the modes of transmission of the disease. This characteristic was also ascertained on his field survey in Hokkaido referred to in Chapter IV. Indeed, among a number of cases observed, there have been quite many in which BSM showed such characteristics of symptoms as were very apt to be taken for the symptoms observable on the barley which was infected by *Pyrenophora graminea*. In order to clearly distinguish this from that, therefore, the author found it necessary to work out some appropriate measures. In this chapter, the author describes, with due consideration to the routes of infection, various symptoms observed on the barley infected by BSM. He also describes symptoms observed on the barley infected by stripe disease of barley on the other, so that the both types of the symptoms were compared with each other.

A. SYMPTOMS IN SEEDLING STAGE

1. Symptoms for seed-borne infection

The clearest symptom expression in the seedlings grown from BSM infected seeds generally appear on the first leaves of the seedlings (Plate I, 1). The chlorotic markings on mottled leaves appear as spots, narrow or wide, and as broken or long stripes in white, grayish white, yellowish white or in yellow. The chlorotic markings are so weak with certain varieties of barley and with certain strains of virus, as well as under certain environmental conditions during the period of tests, that the diseased seedlings can hardly be distinguished from the healthy. The seedling symptom expression in 6-rowed barley is generally easier to be detected than in 2-rowed barley. It has been observed that higher temperatures (21°–27°C) are favorable to the symptom expression as reported by Hampton and others in 1957. In most cases, the symptoms tend to be milder on later leaf stage, but in the cases of certain strains of virus, the characteristically distinct leaf mottling is retained down to the later leaf stages. In the glasshouse conditions, the characteristic necrotic symptoms develope only occasionally. Diseased seedlings get stunted in various degrees.

2. Symptoms for sap-inoculation

After 4 to 10 days from the inoculation on the first leaf stage, yellow spots appear on the second or the third leaf stage, to be followed by chlorotic mottling. The first necrotic symptom frequently appear along the borderline bet-

ween the normal green part and yellow part, giving the necrotic "V"-area (Hagborg, 1954). It is observed that the higher the temperature, the severer the symptoms appear, so much so that, in some cases, most part of the leaf gets necrotic, resulting in the death of the whole leaf. Local necrotic lesions (Plate III, 1) appear on the inoculated leaf just about the time when the first mosaic symptoms appear on the youngest leaf. In most cases, the inoculated leaf gets entirely necrotic and dies shortly. The symptoms on the artificially infected seedlings tend to get milder as the growth of the leaf stage, and they take an appearance similar to that of the symptoms for the seed-borne infection. In the sap-inoculation, too, the diseased plant gets markedly stunted as compared with the sound plant.

B. SYMPTOMS ON MATURED PLANT

The leaves of diseased matured barley plant finally carry similar symptoms regardless of the routes of infection either through seed-borne infection, sap-inoculation or contact transmission. Typical symptoms on the leaves of matured barley plant are seen in Plate II, 2. Mosaic symptoms are also observed on the sheaths, young stem, hoods, glumes, and, in some instances, on the epidermis of inmatured seeds. Necrotic symptoms (stripes, dashes or V-shaped) are not so common on the plants diseased by seed-borne infection. But, on the plants infected by the artificial inoculation and the plant-to-plant contact transmission, the characteristic necrosis appears in early stages of the symptom development. Although the various types of the early symptoms developed with different infection routes, they finally get to show similar symptoms, as has already been described. Diseased barley plants are generally stunted with the reduction in plant height, and the incomplete head emergence from the sheaths. Further, the diseased barley field gets to give uneven appearance. Characteristic "Chochin-Bo"—"Lantern head" (semi-sterility)—results from the unpolinated flowers, which is caused by the disorders of anther in diseased barley. The kernel weight and the width of grain from diseased plants are reduced generally various degrees. Shrivelled grains are commonly observed in diseased naked barley (Plate VI, 1).

C. SYMPTOMS CAUSED BY PLANT-TO-PLANT CONTACT TRANSMISSION

The early symptom expression in plant-to-plant contact transmission in the seedling stage is similar in appearance to that in mechanical inoculation. Yellowish flecks and necrotic "V"-area or lines followed by leaf mottling appear on the youngest leaf. In glasshouse conditions, the linear brownish necrosis, which suggests the invasion of the causal virus, is easily recognized along the midrib or the margin of the lower leaf.

Stripe disease-like necrotic symptoms are observed commonly in matured barley (Plate I, 2; III, 2). Necrotic lines and dashes or V-shaped necrosis, by which the normal green and mottled portions are seperated in most cases, de-

velop along the midrib or leaf margins as they do in the seedling stages. This necrosis extends over the 1–3 leaf stages in the early symptom expression, and this is followed by mottling. Necrosis appears not only on leaves but also on sheaths, stems or hoods as well with the advance of symptom development (Plate III, 3).

D. DIFFERENCE OF SYMPTOMS BETWEEN BSM AND STRIPE DISEASE OF BARLEY CAUSED BY PYRENOPHORA GRAMINEA

It is valuable to distinguish the symptoms between BSM and stripe disease of barley, because some of the symptoms of these two diseases are very resembled from one to the other, as has been described above. From the following observation, the author has an opinion that these two diseases are distinguishable in many cases, except in the case of double infection.

1. In seedling stage

When seeds are sown in winter or in early spring, it is rather difficult to notice the difference of symptoms between the two disease. Though the symptoms of the both diseases on primary leaf stage are quite resembled, the differences are usually noticeable by successive observations for several days. It is nearly impossible to distinguish them from one to the other under field conditions in winter. In Table 11, the differences of symptoms between the two diseases under glasshouse conditions are described.

Table 11. Difference of symptoms between BSM and stripe disease of barley in the seedling stage

Chlorotic lesion	Stripe disease	BSM
Color	Light green~yellow~grayish white, at first. It turns to orange yellow, then to necrotic.	Similar to that of stripe disease, but no marked alteration in color tone.
Shape	Stripes, lines, spots, dots. They develop larger. Fine thread-like branches appear at the stripe to the ractangular direction; many of them are joined each other.	Stripes, lines and oblong spots. No marked development (growth) of lesion.
Portion	Lesions are seen along the midrib and leaf margin; frequently they are on the entire surface. Necrotic symptom tends to develop at the tip or along the margin of leaf.	Comparatively irregular distribution. Lesions are not usually resticted with veins.

2. In matured plants

Differences of symptoms between the two diseases get to be noticeable with less difficulty (Plate I, 3). Table 12 shows the differences. If caused by stripe disease of barley, matured plants get entirely necrotic in many cases, and so, there will be no mistaking this from that.

Table 12. Difference of symptoms between BSM and stripe disease of barley in adult plant

	Stripe disease	BSM
Chlorotic lesion		
Color	Orange yellow.	Yellowish green~yellowish white~graysh white.
Shape	Similar to that in seedling stage. It looks like to be composed by small rectangle.	Similar to that in seedling stage. It looks like to be composed by oval and oblong fusiform.
Portion	Similar to that in seedling stage.	Similar to that in seedling stage.
Necrotic lesion	Yellow lesion turns to brown~black, gradually. Long necrotic veinal stripes develope along the midrib and the leaf margin. Many fungus spores are produced on the surface of the necrotic lesions. Later, the leaf or the entire plant is killed.	Necrotic lines, dashes or V-shaped areas appear at the boaderline of the chlorotic lesions. Partial necrosis of chlorotic lesion, in some cases. Brown ~purplish red brown in color.

VI. PHYSICAL PROPERTIES, HOST RANGE AND TRANSMISSION OF BSMV

A. PHYSICAL PROPERTIES

1. Thermal inactivation

McKinney (1951) stated that the thermal inactivation point of this virus in expressed sap was near 68°C. According to Hagborg (1955), it was 64.1°C. Murayama, Nemoto and Yokoyama's result (1959) was 55–60°C. Kassanis and Slykhuis (1959) reported that it was 65°C by serological test. As seen in Table 13, the author's result on the thermal inactivation of BSMV in sap was 65°C for 10 minutes.

2. Dilution end point

According to McKinney (1951), dilution end point of the virus was slightly beyond 10^{-4}. But, Murayama and others (1959) stated that it was about 1:500, and in some instances, 1:1,000–2,000. Kassanis and Slykhuis (1959) reported that it was still infective in 1:2,048 dilution by inoculation test, but 1:256 in serological test. The author's result on the dilution end point was 1:2,000–6,000, and 1:8,000 in another test.

3. Aging

McKinney (1951, 1953) reported that the inactivation time of the virus in plant juice was 15–22 days, in laboratory temperatures, and in clipped dried leaf tissue, 35–40 days; but, when the leaves were left on the infected plants, the death of the virus closely followed the death of the leaves. He also stated that the virus seemed not to overseason in the soil. According to Murayama and others (1959), aging in vitro of the virus was 7 days in room temperatures,

Table 13. Thermal inactivation of BSMV in plant juice

Temperature °C	Experiment		
	A	B	C
Room temperature	50/50	30/30	28/30
55	50/50	—	—
60	39/51	4/30	6/30
63	—	2/30	3/30
65	6/50	—	0/30
66	—	0/30	0/30*
68	0/50	0/30	—
70	0/50	0/30	0/30
75	0/50	—	—

* 67°C

Table 14. Dilution end point of BSMV in plant juice

Dilution 1:	Experiment		
	A	B	C
10	50/50	—	—
100	50/50	10/15	29/29
1,000	37/50	2/30	6/28
2,000	16/49	1/50	2/30
4,000	3/50	1/50	0/30
6,000	3/50	0/50	0/30
8,000	1/50	0/50	0/30
10,000	0/50	0/50	0/30

Table 15. Aging of BSMV

Days	Juice stored		Dried leaf tissue
	Refregerator	18–20°C	
5	30*	29	—
10	30	7	15**
15	29	23	—
20	28	6	19
25	21	0	6
30	25	0	3
35	29	0	13
40	30	0	23
45	22	—	—
50	22	—	27
55	21	—	—
60	28	—	14
65	16	—	—
70	22	—	—
80	10	—	3
90	4	—	—
100	4	—	—
110	4	—	—
120	2	—	—
130	2	—	—

* Number of infected seedling out of 30 seedlings inoculated

** 15 seedlings were inoculated

and in dried leaf tissue, it was 37 days. Kassanis and Slykhuis (1959) reported that the virus was still infective after 32 days at 20°C, but it lost the infectivity and the serological activity within 3 days at −20°C. According to the author's experiment, as seen in Table 15, the inactivation time of the virus in expressed sap was 20–25 days at 18–20°C, over 130 days in refregerator, and over 80 days in dried leaf tissue.

B. HOST RANGE

Host range of BSMV in gramineous and other genus was studied, and several new host plants were found. Plants were inoculated in their seedling stages, using carborundum as an abrasive. Small seedlings of many of the gramineous grasses and weeds were inoculated 2 or 3 times in their 2–4 leaf stages. Ten or more plants, at least, were used for the inoculation tests.

1. Cereals

Table 16 shows the host range of this virus in cereals. Wien was the only variety of barley out of the 2,191 varieties which was not infected systemically.

Table 16. Host range of BSMV in cereal crops

	The author	Slykhuis 1952	McKinney 1951
Avena sativa L.	+	−	+
Hordeum vulgare L.	+	+	+
Oryza sativa L.	−		L
Panicum miliaceum L.	+	+	
Secale Cereale L.**	+		
Setaria italica (L.) Beauv.	+	+	
Sorghum vulgare Pers.	−	−	
Triticum durum Desf.	+	+	
T. monococcum L.	+		
T. spelta L.	+		
T. vulgare Vill.	+	+	+
Zea mays L. (Sweet corn)	+	+	+
(Dent corn)	+*	+	
(Flint corn)	+*		

* Occasional infection ** Positive result was reported by Hagborg (1951)
L: Local infection

About 180 varieties of wheat were all susceptible to the virus. The symptoms on wheat were severer than in the case of barley, with the pronounced yellow mottling and leaf curl (Plate IV, 1). Seed infection in wheat was observed as frequently in susceptible varieties of barley. *Triticum spelta* and *T. monococcum* were the new host plants of BSMV in this paper. And, many hybrids between wheat and rye tested were also highly susceptible to the virus.

Slykhuis (1959) reported that his strain of the virus was not infectious to oat. On the other hand, McKinney (1953) and Sill and Hansing (1955) stated that oat was infected by some of strains of the virus. Many of the author's isolates was infectious to oat, but their infectivities were not so high. Symptoms on oat were milder in general than in the case of barley and wheat.

Sweetcorn (Golden Bantum) showed high susceptibility to the virus, but rare and slight infection was observed on dentcorn (White Dent and Yellow Dent) and flintcorn (Koshu). Diseased sweetcorn was severely mottled and stunted (Plate IV, 2). Leaves were curled, crinkled, folded and broken, and the diseased plants could not produce seeds in some instances. On dentcorn and flintcorn, the symptoms were very mild with a small number of chlorotic dashes or short stripes on leaves. The growth was not affected by the infection in most of the cases.

In Italian millet (*Setalia italica*) and Broom corn millet (*Panicum miliaceum*), the symptoms were similar to those in barley. Twisted anomalous growth similar as in sweetcorn was frequently observed in Italian millet. No seed infection was detected in about 2,000 seeds obtained from the diseased plants of these two millets.

McKinney (1951) reported that the virus produced local lesions on rice.

And, according to Kahn and Dickerson (1957), some varieties of rice were systemically infectious to the virus. But, a variety of rice, Asahi, was not infected with the author's virus isolates.

2. *Hordeum plants*

According to Slykhuis's result (1952), *Hordeum jubatum*, showed only local infection. The author did not test on this plant, but tested on the other 14 species of *Hordeum*. The results were shown in Table 17. The stripe mosaic symptoms were produced in all species except *H. bulbossum*, which was not susceptible to the virus.

Table 17. Susceptibility of Hordeum plants to BSMV

Susceptible species		Non-susceptible
H. agriocrithon	*H. murinum*	*H. bulbossum*
H. arizoniacum	*H. nodosum*	
H. depressum	*H. pussilum*	
H. glaucum	*H. spontaneum*	
H. Gussoneanum	*H. spontaneum var. transcaspicum*	
H. maritinum	*H. stebbinsii*	
H. Morinum		

3. *Gramineous grasses and weeds*

In Table 18, the host range of BSMV in 26 gramineous grasses and weeds were shown, in comparison with the Slykhuis's result (1952). Eight species such as *Agrostis alba*, *Agropyron elongatum*, *Bromus inermis*, 3 species of *Lolium*, *Phleum pratense*, and *Setaria viridis* were susceptible to the virus. Many of these plants infected with the virus showed stripe mosaic and stunt symptoms. *Lolium* plants showed chlorotic blotches, stripe mosaic and leaf curl symptoms. *S. viridis* was fairly susceptible to the virus, however, most of these grasses were not so highly infectious, especially in the case of thimothy. Slykhuis (1952) reported that *A. elongatum*, *B. inermis* and *P. pratense* were not susceptible, but *Digitalia sanguinalis* was susceptible. On the other hand, McKinney (1951) stated that *B. inermis* was susceptible to the virus, and the similar result was obtained by the author, as seen in this paper.

4. *Non-gramineous plants*

Several non-gramineous plant species susceptible to BSMV had been reported as follows; *Chenopodium album* (McKinney, 1954), *C. amaranticolor* (Hollings, 1957), *C. capitatum* (Singh and others, 1960), *Beta vulgaris*, *Spinacia oleracea* (Kassanis and Slykhuis, 1959), *Amaranthus retroflexus* (Singh and others, 1960), *Nicotiana tabacum* var. Samsun (McKinney, 1951). As seen in Table 19, all of the plants susceptible to the author's isolate of BSMV were limited in *Chenopodiaceae* with an exception of *Commelina communis*.

Table 18. Host range of BSMV in gramineous grasses and weeds

Plant species	The author	Slykhuis (1952)
Agropyron desertorum (Fisch.) Schut.	−	−
A. elongatum (Host.) Beauv.	+	−, +[1]
A. intermedium (Host), Beauv.	−	−
A. semicostatum Nees.	−	
Agrostis alba L.	−	
Alopeculus pratonsis L.	−	
A. aequalis Sobol.	−	
Bromus catharticus Vahl	−	
B. inermis Leyss.	+	−, +[1, 2]
B. marginatus Nees.	−	
Dactylis glomerata L.	−	−[1]
Digitaria cilialis Pers.	−	+
Ehrharta calycina	−	
Eleusine indica (L.) Gartn.	−	
Festuca arundinacea	−	
Lolium multiflorum Lam.	+	
L. rigidum	+	
L. Perenne L.	+	
Panicum Crusgalli L. var. *submutica* Mey.	−	
Phalaris arundinacea L.	−	−
P. tuberosa	−	
Phleum pratense L.	+	−[1]
Poa pratensis L.	−	−
Poa annua L.	−	
Setaria viridis (L.) Beauv.	+	+

[1] McKinney (1951)
[2] Singh, Arny & Pound (1960)

Spinach showed mild vein clearing followed by slight chlorosis and faint mottling. On *Commelina communis*, the symptoms caused by the virus were the pronounced mottling and necrosis (Plate V, 4), which were somewhat similar to the symptoms on barley, differed from those caused by cucumber mosaic virus. Moderate stunting and the occurrence of sterility were observed on diseased commelina. Susceptible plants in *Chenopodiaceae* other than spinach, showed local infections. On *Chenopodium album*, diffuse chlorotic local lesions were produced in a similar way as reported by McKinney (Plate V, 1). In some instances, smaller and more definite countable necrotic lesions appeared on this plant. Sill and others (1955) and Singh and others (1960) reported that their isolates of the virus were not infectious to Swiss chard or sugar beet. But, the author's virus produced chlorotic or necrotic local lesions on these plant species (Plate V, 3).

Table 19. Susceptible and non-susceptible plant species to BSMV

Susceptible plant species	Symptom
Beta vulgaris L. var. *Cicla* L.	CL
B. vulgaris L. var. *Rapa* Dumort.	NL
Chenopodium album L.	CL
C. amaranticolor Coste & Reyn.	CL(NL)
Commelina communis L.	S
Spinacia oleracea L.	S

Non-susceptible plant species	
Allium fistulosum L.	*Lycopersicon esculentum* Mill.
Amaranthus retulosum L.	*Matthiola incana* R. Br.
Brassica oleracea L. var *capitata* L.	*Nicotiana glutinosa* L.
Cucumis sativus L.	*N. tabacum* L.
Callistephus chinensis Nees.	*Phaseolus vulgaris* L.
Capsicum annus L.	*Raphanus sativus* L.
Centaurea Cyanus L.	*Solanum Melongena* L. var. *esculentum* Nees.
Glycine max Merr.	*Vigna sinensis* L.
Gomphrena globosa L.	*Viola tricolor* L. var. *hortensis* DC.
Lupinus luteus L.	

CL: Chlorotic local lesion S: Systemic infection
NL: Necrotic local lesion

5. The seed transmission of BSMV in diseased plants other than barley

The seed transmission of the virus was known in wheat and oat as well as barley (Mckinney, 1953; and other workers' reports). In the experiment for this paper, the author also ascertained the seed transmission of the virus in wheat and oat as well as in the seeds from mechanically infected plants of the following plant species; *Hordeum glaucum* (1.9%), *H. stebbinsii* (1/4), *H. depressum* (2.5%), three species of *Lolium* (2.5–8.0%), *Agropyron elongatum* (1/9), *Bromus inermis* (8.2%), and *Commelina communis* (4.3%).

C. TRANSMISSION OF BSMV

BSMV is transmitted by (1) seed, (2) plant juice, (3) plant-to-plant contact, and (4) pollen. Seed-borne infection and sap transmission are both well known. In this study, these routes of transmission are to be confirmed althogether. However, since the transmission by seed or plant juice was described on many occasions throughout this paper, only the results of the several experiments carried out to ascertain plant-to-plant contact and pollen transmission are to be described in this section.

1. Plant-to-plant contact transmission

A detailed study on plant-to-plant contact transmission was made by McKinney (1954). He stated that the transmission occurred through the inter-plant contact except root contact, and he pointed out the possibilities of infection by

hand contaminated with juice of diseased plants. Infection caused by inter-plant contact between spring barley and winter wheat grown in adjacent rows was observed by Fitzgerald and others (1957). Hagborg (1954) used oat plants, which are resistant to his virus strain, as the buffer to be grown between experimental plots to avoid the plant-to-plant contact transmission. Further, he found (1960) that skim milk and whey have some good effect to prevent the spread of the disease under field conditions.

As have been described in Chapter III of this paper, stripe mosaic-like symptoms on several Aohadaka plants adjacent to the infected strains of Moravia and it's hybrids made the author to think of the possibility of contact transmission. Furthermore, many cases of infection attributable to inter-plant contact transmission, were observed from the experiments in glasshouse. In order to ascertain this possibility, the following experiments were carried out:

i) Transmission by the natural inter-plant contact under glasshouse conditions

Two wooden boxes were prepared, in which the seeds taken from the diseased Akashinriki were sown apart 2.5 × 4 cm. Fifteen days after sowing, one of wooden boxes (B) was set in the artificial wind for 7–8 hr. a day for the following one week to increase the inter-plant contact, alternating the directions of the wind every other day. The second box (A) was carefully kept in the closed glasshouse so as to avoid inter-plant contact.

In another experiment, three boxes were prepared. Each six seeds of Akashinriki (49/210 infected) was sown 4 × 5 cm apart in the first box (C). And, the seeds of Akashinriki (48/183 infected) and Chevalier (10/135 infected) were sown separately 1 × 5 cm in the second (D) and the third box (E) respectively. These boxes were placed in the glasshouse and were kept without special care. The results were shown in Table 20.

Contrary to the author's expectation, several infections attributable to the in-

Table 20. Contact transmission of BSM in glasshouse

	Variety	Treatment	Seed transmission	Infected seedling by contact transmission	
				33*	41*
A	Akashinriki	None	30/250	1	3
B	Akashinriki	Wind	36/245	6	11
				25*	33*
C	Akashinriki	None	49/210	8	15
D	Akashinriki	None	48/183	5	9
E	Chevalier	None	10/135	2	6

A: 25 seeds/row were sown separately 2.5 × 4cm apart in 10 rows.
B: 〃 , applied fan for 15–22 days after sowing.
C: Each 6 seeds were sown at 12 places/row apart 4 × 5 cm in 3 rows.
D, E: 30 seeds/row were sown seperately 1 × 5 cm apart in 6 and 5 rows.
* Days after sowing

ter-plant contact transmission were observed in the box (A) to which a special care had been paid to avoid the plant contact. When the inter-plant contacts were promoted by fan, the apparent increase of infected seedlings occurred in the box B. The number of the cases of seedling infection by contact transmission was large indeed for the boxes C, D and E, in which the seedling was grown with no special care. Above all, in the case of the box C, in which each 6 seeds were sown in one place, considerable number of infection was observed. Many of the infected seedlings caused by inter-plant contact, as it was a matter of course, were those adjacent to the seed-borne infected seedlings in that or the next rows.

ii) Experiments on the artificial transmission in various modes of plant contact

The artificial contacts were applied between healthy and diseased seedlings or other inoculation sources in various modes (Table 21). Infections occurred in every trial except in the case of leaf clipping with scissors. Number of infection was increased by the application of carborundum on the surface of healthy seedling before the contact with inoculum. Infections occurred when healthy seedling was rubbed gently with the air-dried ground leaf tissue or healthy leaf which had been soaked into the diseased plant juice and then air-dried. Moreover, the infections occurred in the wounded portion of the leaf, which had been snapped one day before. These results apparently show the chances of infection in natural contact between healthy and diseased plants. That is to say, the infective virus particles in plant juice, which has been exuded over the surface of leaf from the wounded tissue by some mechanical cause, may have the chances to invade through the wounded portion on the surface of healthy plants. Chances of infection seemed to be somewhat related with the extent of wound or contact portion. In the test "rubbed gently", in the table, healthy seedlings were allowed to bend, without any support by hand all through the contact inoculation with the light touches at the midrib or leaf margin.

iii) Activity of the virus in air-dried plant juice

To approach the knowledge on the activity of the virus particles in plant juice exuded over the leaf surface from wounded portion, inactivation time of the virus was examined in air-dried plant juice. As seen in Table 22, activities of the virus were observed in the residues from air-dried plant juice. Although the inactivation time was varied with the conditions, such as temperatures and the time for drying, in some instances, it was 10–15 days. This is a smaller value than with the case of dried leaf tissue. But, if the infectivity of the virus under discussion may agree with the virus activity in the wound exudate, the results obtained in the experiment ii) should be natural.

iv) Plant-to-plant contact transmission under the field conditions

A field observation of BSM was made to see if the disease was transmitted by natural inter-plant contacts. Healthy seedlings of Akashinriki (6-rowed,

Table 21. Transmission of BSM with various modes of contact

Modes of contact	Healthy seedling	Inoculum	Time of contact	Carborundum application	
				−	+
I	A	Clipped leaf	1	1/30*, 2/30	
〃	〃	〃	2	1/30	
〃	〃	〃	3	3/30, 2/30, 8/18	
〃	〃	[1]	3		1/30
〃	〃	Cut end of leaf	1	4/30, 4/12	2/14
〃	〃	〃	3	9/30, 7/30, 4/18	12/30, 7/16, 11/30
〃	〃	[2]	1	1/15	
〃	〃	〃	3	7/15	12/30
〃	〃	[3]	1		24/30, 10/30
〃	B[4]	[5]	1		23/30
II	A	[6]	1	2/30	11/30, 11/30
III	〃	Crumple leaf with finger	2	4/30	9/30
〃	〃	〃	6	4/30	
IV	B[7]	Plant juice		2/30	
〃	〃[8]	〃		2/30	
V				0/30, 0/30, 0/30	

* Infected seedling/inoculated seedling

I : Rubbed gently the leaf of healthy seedling with inoculum.

II : Leaf of diseased seedling over the healthy, and pressed lightly them with finger.

III : Brushed roughly the healthy seedlings with the inoculum.

IV : A drop of diseased plant juice is smeared on the wounded portion of healthy seedling.

V : Alternate leaf clipping of both healthy and diseased seedling with scissors.

[1] : Healthy leaf which immersed in diseased plant juice

[2] : Ground tissue of diseased leaf, immediately.

[3] : 〃 , 3 hr. later.

[4] : Healthy seedling is dusted carborundum, and is rubbed with cotton ball.

[5] : Ground tissue of diseased leaf, 3 hr. later.

[6] : Healthy and diseased seedlings were grown in adjacent rows. Primary leaves of both healthy and diseased seedlings were made to approach.

[7] : Clipped healthy leaf. Inoculum was applied just after the leaf clipping on wounded portion.

[8] : 〃 . Inoculum was applied on the next day on wounded portion.

Table 22. Activity of BSMV in air dried plant juice

Temperature		Days								
		2	3	4	6	8	9	10	12	15
17—18°C	A		5/7		20/30		0/30		0/30	0/30
	B		11/30		10/30		3/30		1/30	0/30
	C	1/15	5/15	1/12		0/22		0/30	0/30	
25°C	A		5/8		0/30		0/30		0/30	0/30
	B		3/30		0/30		0/30		0/30	0/30
	C	14/18	4/16	1/15		3/18		7/30		

A, B : 10 ml of plant juice (× 10) in petri dish (diam. 57 mm) was air- dried

C : 8ml of plant juice in petri dish (diam. 140 mm) was dried by fan

naked), Harbin 2-rowed and Chevalier (2-rowed) were transplanted singly in rows apart 6 cm at their two-leaf stage (November, 1959). Seed-borne infected seedlings were planted in places of the same varieties. They were grown and allowed to mature without special treatment. Observations of infection were made monthly until April (1960), but, no diseased plant was recognized other than the inoculum source plants. However, as seen in Figure 2, the number of infected plants gradually increased later in mid-April. At the last observation (May, 26), there were many infections, especially most of all the plants adjacent to the inoculum source plants were infected. Infections were also observed in the plants which had grown in the area adout 30 cm away from the inoculum. Necrotic or chlorotic lesions on stems, hoods and glumes were commonly

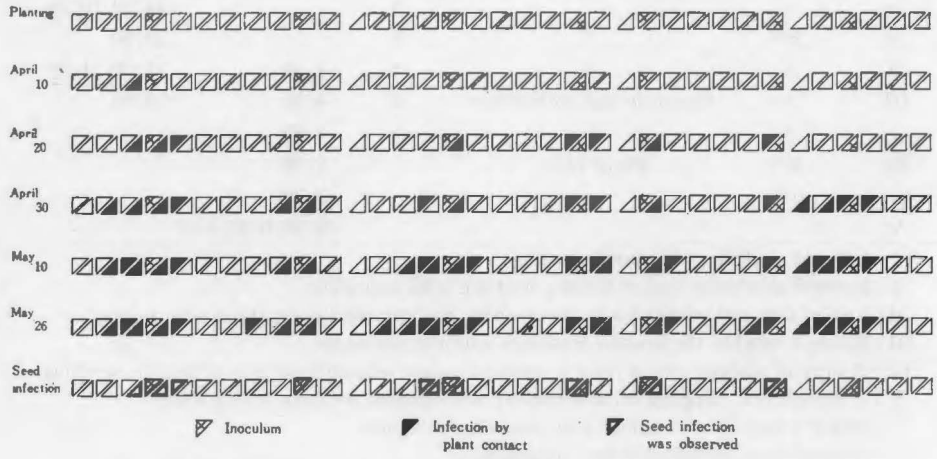


Fig. 2. Natural contact transmission of BSM in barley field (Akashinriki).

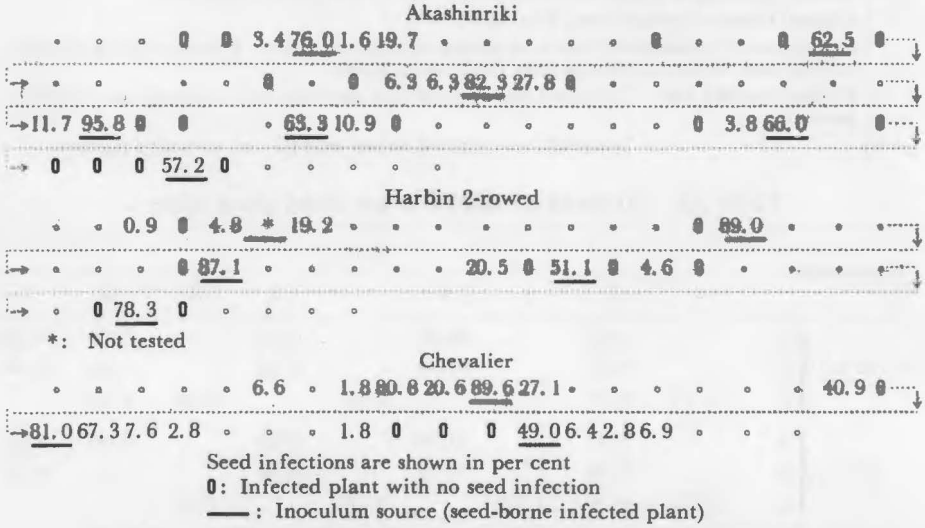


Fig. 3. Seed infection found in the seeds taken from the plants infected by natural contact transmission of BSMV.

recognized in the plants which had got affected toward the end of May (after heading time), and these were similar in symptoms to the case of mechanical inoculation at the heading stage. In other two varieties, the infections caused by plant-to-plant contact transmission occurred also, but the number of infections were less than in the case of Akashinriki.

Seed infections were examined with each about 100 seeds taken from each plant in the experiment. As seen in Figure 2 and 3, the seed infection was not always found in the seeds taken from the plants infected by contact transmission, but was observed generally in the seeds from the plants infected in the early stages.

The earliest symptom expression caused by natural contact transmission was seen on the fourth leaf counted from the uppermost in the case of Akashinriki. Each 5–10 heads were sampled from the culms which had expressed the initial symptom on the following portions; the third, the second leaf from the uppermost, flag leaf and the uppermost sheath, head, and the stem just below the head. Then, the size, the weight and the virus infection of the seeds taken from these heads were examined. In Table 23, the results were shown. Compared with the healthy plants, the reductions in kernel weight and large seeds

Table 23. Influence of time of initial expression of symptoms with natural contact transmission of BSM on kernel weight, seed size and seed infection

Initial expression of symptom on	100 kernel weight g	Number of seed examined	Proportion of large seed (>2.0 mm) %	Seed transmission	
					%
The 3rd leaf from the uppermost	1.4	476	23.6	46/47	97.8
The 2nd leaf from the uppermost	1.4	209	25.2	45/53	84.8
The uppermost leaf and sheath	1.7	383	34.4	19/45	42.3
Head	1.5	413	24.2	5/51	9.8
The stem just below the head	1.3	1210	10.2	0/94	0
Seed-borne infected plant	1.5	200	27.0	61/70	87.2
Healthy plant	2.0	572	58.2	0/108	0

Variety : Akashinriki

were observed in the seeds from the infected heads. The semi-sterility caused by the disease infection was rather few in case, but the reduction of the seed size was so marked in the case of the late infection, i.e. the initial symptoms appeared on the stem just below the head. Much seed infections were detected in the cases of earlier infections, but very rare in the cases of late infections. The necrosis on the stem seemed to show that the plant had been infected later than plant which showed symptoms on hoods or glumes.

The plant-to-plant contact transmission of BSM was proved as mentioned above. For the contact transmission of plant viruses, the concentration and the stability of the virus in plant tissue seemed to be an essential factor. Only a few viruses have been known as the contact transmissible viruses, such as TMV,

which is highly infectious and stable. It is difficult to believe that BSMV is one of the most stable plant viruses, according to its aging and dilution end point. Therefore, it may be said that BSM is an exceptional example of plant virus in contact transmission. The secondary spread of the disease caused by the natural contact between healthy and diseased plants was ascertained in the field observation. And, the results of the experiment on the effects of contact infection on the seeds agreed with those obtained in the field inoculation test in Chapter VII, 5 of this study.

2. *Pollen transmission*

Pollen transmission of viruses in self-pollinate plants is considerd to have no practical importance in the secondary spread of diseases except in the case of artificial pollination. But, the pollen transmission of seed-borne viruses should be fully investigated to approach the mechanism of embryo and seed infection. Gold and others (1954) reported that BSMV particles were found in pistiles, anthers, pollen, embryos and endosperms of diseased barley, as well as in the seeds which were derived from diseased pollen and healthy pistils. Moreover, they found the seed-borne symptoms on approximately 10 % of the seedlings from diseased pollen and healthy pistils.

Susceptible varieties of barley, Akashinriki, Shunsei, Harbin 2-rowed and Kenyoshi No. 3, and resistant varieties, Imperial and Wien were used for the experiment of pollen transmission of BSMV. Artificial pollinations were made between healthy and diseased (seed-borne infected) plants of susceptible varieties, and healthy plants of resistant varieties in various combinations. The seeds derived from these crossing were sown to examine the symptom of the seedlings.

Table 24 shows the results. Compared with the seedling infection found in the crosses between healthy pistils and diseased pollen, high percentage of seed

Table 24. Pollen transmission of BSMV

Cross ♀		× ♂		Fertility %	Seed transmission	%
Akashinriki	(H)	× Akashinriki	(D)		29/112	24.8
Shunsei	(H)	× Shunsei	(D)		5/112	4.1
Shunsei	(D)	× Shunsei	(H)		19/ 35	54.3
Harbin 2-rowed	(H)	× Harbin 2-rowed	(H)	98.1	0/106	0
Harbin 2-rowed	(H)	× Harbin 2-rowed	(D)	91.8	32/ 90	35.6
Harbin 2-rowed	(D)	× Harbin 2-rowed	(H)	84.7	103/111	92.8
Harbin 2-rowed	(D)	× Harbin 2-rowed	(D)	91.9	54/ 64	84.4
Imperial	(H)	× Harbin 2-rowed	(D)	94.1	18/229	7.9
Harbin 2-rowed	(D)	× Imperial	(H)	83.9	33/171	19.3
Wien	(H)	× Kenyoshi No. 3	(D)	89.5	33/111	29.7
Kenyoshi No. 3	(D)	× Wien	(H)	83.2	104/162	64.2

H : Healthy plant
D : Diseased plant caused by seed transmission

infection caused by pollen transmission was observed throughout the experiment. Even in the crosses with the resistant varieties Imperial and Wien, positive results on the pollen transmission were also obtained. Infectivity of diseased pollen was ascertained by an inoculation test in which the pollen of diseased Kenyoshi No. 3 barley was used as an inoculum.

VII. THE SEMI-STERILITY OF BARLEY CAUSED BY BSM AND THE SEED TRANSMISSION OF THE VIRUS

The seed transmission and the sterility in the diseased plants are the marked characteristics of BSM, and these were the inducement of the identification of the disease in the semi-sterile barley in Hokkaido.

A. THE DISORDER OF ANTHER AND POLLEN OF BARLEY INFECTED WITH BSMV

It has been shown in the early part of this paper, that BSM was the major cause of the semi-sterility of barley, so-called "Chochin-bo"—"Lantern head". Yamamoto, T. (1951) came to the conclusion on the semi-sterility of barley in Hokkaido, that the sterility was caused by the morophorogical and physiological disorder of the anther and pollen. In this section, the author is going to make several observations to see whether the sterility caused by BSM is similar to the disorder which had been reported by Yamamoto.

1. Materials and methods

While the details of materials and methods of experiments had had to be slightly different as the case required, spikes taken from Harbin 2-rowed and Akashinriki plants diseased by seed infection were used in the most of the experiments. The virus of Harbin 2-rowed was a severe symptom expressing isolate, and the virus of Akashinriki was a moderate one.

2. Results

i) Size of anther, and the amount of pollen in anther

Length and width of the anthers in 10 flowers in the middle part of each 3 spikes taken from 10 plants were measured under the microscope with low magnification ($\times 21$). As seen in Table 25, compared with the healthy anthers, the diseased anthers were small in length and width.

Table 25. Length and width of BSM infected barley

Variety		Length mm	Width mm
Harbin 2-rowed	Healthy	4.06 ± 0.1032	0.86 ± 0.0261
	Diseased	3.84 ± 0.1496	0.77 ± 0.0305
Akashinriki	Healthy	3.12 ± 0.1122	0.78 ± 0.0235
	Diseased	2.86 ± 0.0791	0.72 ± 0.0333

Averages of 300 spikelets

Differences of measurement among healthy and diseassed anthers are significant.

Most of the pollen in anther was made to shed on the surface of gelatine smeared slide glass, by way of trembling gently the anther about to open. The number of pollen on the slide glass was counted under the microscope. Although the counting was not repeated so often as to make certain of the accuracy of the number counted, it proved abundantly certain that the number of pollen was markedly smaller with the diseased anther than with the healthy anther, as shown in Table 26.

Table 26. The amount of pollen in anther of BSM infected barley

Variety	Healthy plant	Diseased plant
Harbin 2-rowed*	2862	1509
Akashinriki**	1744	1271

* Averages of 5 and 2 anthers in healthy and diseased plant.
** Averages of 12 and 2 anthers in healthy and diseased plant.

ii) Number of pollen on the pistil just after the flowering

Pistils which had been pollinated under the natural field conditions were stained with cotton blue just after flowering. The number of pollen on the pistils was counted under the microscope. The results were given in Table 27. The number of pistils on which pollen was not found proved to be as large as 9.7% in diseased spikelets of Harbin 2-rowed, while it was 0.3% in healthy ones. In Akashinriki, the proportion was 0% in healthy and 0.3% in diseased spikelets. The number of pollen on the pistils of diseased plants was apparently observed to be smaller than that of healthy plants.

Table 27. The amount of pollen observed on pistil of BSM infected plant just after the flowering

Variety		Number of flowers observed	Flowers (%)					
			0*	1–5	6–10	11–19	20–29	>30
Harbin 2-rowed	Healthy	299	0.3	2.7	3.3	4.7	9.7	79.3
	Diseased	290	9.7	21.4	12.7	10.3	9.3	36.6
Akashinriki	Healthy	360	0	0.3	0.3	1.4	6.9	91.1
	Diseased	338	0.3	1.2	2.9	2.1	5.9	87.6

* Number of pollen per pistil

iii) Dehiscence of anther

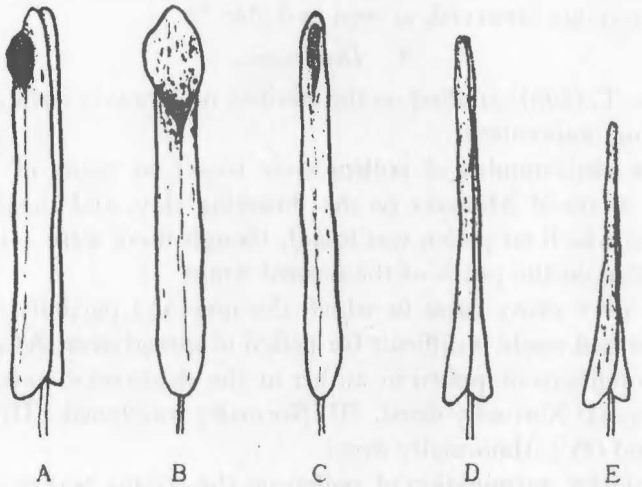
The number of spikelets in the middle part of the heads, containing non- and partially-dehiscent anthers was counted. As shown in Table 28, the number of these abnormal dehiscence of anther in diseased plants was much greater than that in healthy plants. Further, the larger number of abnormal anther was counted in Harbin 2-rowed infected with a severe isolate of the virus than in

Table 28. Frequency of abnormal anther in BSM infected plant

Variety			Number of flower observed	Flowers with abnormal anther (%)			
				0*	1	2	3
Harbin 2-rowed	Healthy	A	70	97.1	2.9	0	0
		B	412	97.3	1.9	0.5	0.2
	Diseased	A	140	86.4	10.0	3.6	0
		B	398	28.1	21.6	20.1	30.1
Akashinriki	Healthy	A	180	99.4	0.6	0	0
		B	293	95.9	3.1	0.7	0.3
	Diseased	A	120	95.0	5.0	0	0
		B	513	67.6	21.8	7.0	3.5

A : Non-dehiscent anther B : Incomplete dehiscent anther
* Number of abnormal anther per flower

the case of Akashinriki infected with a moderate isolate. The number of the abnormal non-dehiscent anthers which contained no pollen was as large as 12.5 % against the total of the non-dehiscent anthers. In Fig. 4, non- or partially-dehiscent anthers were shown.



A–C : Incomplete dehiscent anther (After Yamamoto, T.)
D–E : Non-dehiscent anther

Fig. 4. Disorder of anther in BSM infected barley.

iv) Germination of pollen

Healthy and diseased pollen of Harbin 2-rowed, Akashinriki and Hosogara No. 1 were germinated on the artificial media (starch 10%, sucrose 15%; Hozumi and Igarashi, 1957) in van Tieghem cells, and using acetocarmine stain, the germination of pollen was examined. The results shown in Table 29 had failed to show the evidence with which to estimate the germination in natural condi-

Table 29. Germination of pollen of BSM infected plant

Variety		Number of pollen observed	Germination %
Harbin 2-rowed	Healthy	3849	14.8
	Diseased	2527	4.3
Akashinriki	Healthy	1099	18.3
	Diseased	907	5.5
Hosogara No. 1	Healthy	2530	10.1
	Diseased	2233	1.8

Germination media : 10 g starch, 15 g sucrose per 100 ml

tions, as the tests did not give the uniform and good germinations, however, it was at least supposed that the diseased pollen gives the inferior germination.

v) Morophorogy and fertility of pistil

So far as the author observed, the appearance of the pistils in diseased plants was apparently normal. The fertilizing percentages were examined on the artificial crosses between the healthy and the diseased plants of Harbin 2-rowed, Kenyoshi No. 3, together with the healthy plants of Wien and Imperial in various combinations. Marked differences between the fertilizing percentages in these crosses were not observed, as seen in Table 24.

3. Discussion

Yamamoto, T. (1951) studied on the sterility of Moravia barley, and reported the following observation:

1) Only a small number of pollens were found on many of the pistils of sterile strain of Moravia on the flowering day, and there were many cases in which no pollen was found, though there were a large number of pollens on the pistils of the normal strain.
2) There were many cases in which the non- and partially-dehiscence of anthers had made it difficult for pollen to spread over the pistils.
3) The conditions of pollen in anther at the dehiscence were classified as follows; (I) Normally dried, (II) Normally moistened, (III) Collapsedly wet, and (IV) Abnormally dried.
4) The inferior germination of pollen on the stigma was observed in the sterile strain of barley by the artificial pollination.
5) The normality of pistils in fertility was proved by the cytological examination and the artificial pollination.

The sterility caused by BSM was closely resembled to the sterility observed in the sterile strain of Moravia, and was resulted by the incomplete shedding of pollen from the abnormal anthers. Compared with the case of healthy plant, the amount of normal pollen was scarce, and the aspects of pollen in most of the abnormal anthers was not favorable for shedding. The high percentage of fertilizing in the artificial pollinations with the use of diseased pollen indicates the

fact that diseased pollen, however inferior the germination may be, is potent for fertilization, if only the diseased pollen is available in ample quantities.

To summarize the foregoing statement, immatured anthers, abnormal dehiscence and the reduction of normal pollen were the immediate causes of the sterility of stripe mosaic infected barley. The similarity in the sterility between the stripe mosaic diseased barley and the sterile barley strain is an important evidence suggesting the author's opinion on the semi-sterility of barley in Hokkaido which has been given in chapter III.

B. MECHANISM OF SEED TRANSMISSION OF BSMV

High percentage of seed transmission is a marked characteristic of BSMV. Crowley (1959) found the infectivity of the embryo of the diseased barley with inoculation test, and observed the fact that the seed transmission in the seeds from the diseased plants gets to be smaller, if the plants are inoculated after flowering. This finding confirmed to the result which had earlier been obtained by Eslick and Afanasiev (1955).

The experiments described here were carried out to determine the amount of embryo infection together with the distribution of the virus in various stages of the seed development.

1. Materials and methods

Diseased plants of Chevalier barley, with which the seed-borne infection had been ascertained at their seedling stage, were grown in the field for the 1959's experiment. For the 1960's experiment, diseased plants of Chevalier and Shunsei were grown. The dates of flowering at the middle part of the heads were recorded. All the seeds removed from one side of 9–10 heads on the given

Table 30. Effect of 'Lipon F' solution on the infectivity of BSMV at the surface of dissected embryo of barley

Treatment	Inoculation test*	Embryo culture test**
'Lipon F' solution × 100 (20 min. immersion) × 500	0/16, 0/28 0/20, 0/27	0/32
× 1000	0/20, 0/25	
Diseased plant juice (× 10) made from phosphate buffer (pH 7.0)		15/15
1% Lipon solution		0/26
0.7% Na_2SO_3		20/20
A	7/22, 5/25	4/26, 0/41
A→immersed embryo in water (5 min.)		2/26
A→ 〃 (5 min. × 2)		1/26
A→immersed embryo in 1% Lipon (5 min.)		1/29
A→ 〃 →immersed in water (5 min. × 2)		1/26

A : Immersed embryo in diseased plant juice
* : Seedling infected/seedling inoculated
** : Embryo infected/embryo examined

days after flowering (c.f. Table 31, 32) were used for the embryo culture. The dissected embryos were rinsed with the sterile water and placed on solidified White's media (White, 1954) in petri dishes in the 1959's experiment. In the 1960's experiment, the embryos were rinsed with sterile water after immersion to the 1:100 solution of "Lipon F" (Table 30) for several minutes. Germinated embryos, which had been cultured in petri dishes at 28°C, were transplanted into the test tubes containing the same media and cultured under the glasshouse conditions. The seedlings carrying doubtful or no symptom were transferred to virmiculite or soil at their 2–3 leaf stage for further symptom observation. Then, the symptomless seedlings were tested, in the same way as described in Chapter VII, 3, to see whether they were virus-free or not.

The matured seeds taken from the spikes, from which, the seeds at the opposite side had been removed for the use of the embryo culture, were sown for the seed transmission test. The test was carried on under the glasshouse conditions or under the flourescent light at 24–25°C.

For the seed dissection and inoculation test in 1959, the dissected tissues were washed in water and ground in neutral phosphate buffer, and then inoculated to susceptible barley seedlings. The similar techniques were used in the 1960's experiment with Shunsei barley, but the dissected tissues were rinsed with 1:100 solution of "Lipon F" before the washing in water.

2. Results

i) Embryo culture test

Table 31 and 32 show the results obtained in 1959 and 1960 respectively. It was noticed that the embryo infection did not tend to increase or decrease with the stage of seed maturation. Moreover, the significant differences were not noticed between the data on the embryo infection examined in various stages of seed maturation and the seed transmission throughout the experiments. It was also true even in the case of the extremely young embryos dissected on 12th day after flowering with much difficulties, as seen in the result of the 1960's experiment. (The early development of seeds were somewhat suppressed in this year, because of the unusual low temperatures during the middle and the later

Table 31. Embryo infection with BSMV in various stages of seed maturation of barley (1959)

Days after flowering		14	17	21	28	36	42	56
Right half Embryo culture	Sterility	58/143	46/123	40/134	54/132	62/124	23/104	40/125
	%	40.6	37.4	29.8	40.9	50.0	22.1	29.6
	Diseased embryo	65/74	69/73	89/94	53/62	45/52	62/72	67/76
	%	87.8	94.5	94.7	85.5	86.5	86.1	88.2
Left half Matured seed sown	Sterility	49/142	56/122	37/136	57/129	57/124	23/104	36/135
	%	35.5	45.9	27.3	44.2	46.0	22.1	26.7
	Seed transmission	58/68	45/49	69/75	49/57	50/57	65/74	74/83
	%	85.3	91.8	92.0	86.0	87.7	87.8	89.2

Variety : Chevalier. Matured seeds were harvested 33 days after flowering

Table 32. Embryo infection with BSMV in various of seed stages maturation of barley (1960)

Variety			Days after flowering				
			12	15	20	35	50
Shunsei	Right half Embryo culture	Sterility	51/201	50/170	55/166	61/163	57/153
		%	25.7	29.4	33.1	37.1	37.3
		Diseased embryo	63/76	68/80	82/100	67/79	61/66
		%	82.9	85.0	82.0	84.8	92.4
	Left half	Sterility	60/200	39/165	44/165	46/162	60/155
		%	30.0	23.6	26.7	28.4	38.7
	Matured seed sown	Seed transmission	99/113	87/194	87/98	73/87	81/91
		%	87.7	92.5	88.8	84.0	89.0
Chevalier	Right half Embryo culture	Sterility	96/167	110/166	89/161	85/161	75/158
		%	57.5	66.3	55.3	52.8	47.5
		Diseased embryo	19/22	27/31	59/70	33/38	52/66
		%	86.3	87.2	84.3	86.8	78.8
	Left half	Sterility	104/165	117/168	92/165	88/162	79/156
		%	63.0	69.7	55.8	54.3	50.7
	Matured seed sown	Seed transmission	39/44	31/36	36/41	32/37	44/55
		%	88.7	86.0	87.8	86.5	80.0

part of April.)

ii) Seed dissection and inoculation test

Table 33 and 34 show the results of the seed dissection and inoculation test. The virus was detected from all of the tissues of the dissected immature seeds taken from the diseased Akashinriki plants 11–21 days after flowering in the 1959's experiment. In the 1960's experiment with Shunsei, the infectivity was also noticed in all of the dissected tissues of young seeds (12–20 days after flowering) containing testa and carp. However, the virus was recovered only from embryos and endosperms in the cases of matured seeds (35 days after flowering). The results of inoculation test with the matured seeds suggested that the percentages of embryo infection were quite similar to those of seed in-

Table 33. Distribution of BSMV in barley seed —Dissection and inoculation test—(1)

		Akashinriki				Chevalier	
Date / Days after flowing	May 4 (Pre-flowering)	May 11 6	16 11	21 16	26 21	May 17 19	26 28
Leaf	10/10						
Stem	10/10						
Glume	9/10						
Ovary	10/10						
Anther	10/10						
Epidermis		7/10	10/10	10/10	10/10	7/10	6/10
Testa and carp		5/10	10/10	9/10	9/ 9	3/ 9	5/10
Endosperm		0/10	9/10	9/ 9	10/10	8/10	7/10
Embryo		0/10	1/10	5/10	9/ 9	6/10	6/10

Infected seedling/inoculated seedling

Table 34. Distribution of BSMV in barley seed
—Dissection and inoculation test—(2)

	Days after flowering			Matured seed					
	12	20	35	A	B	C	D	E	Total
Embryo	2/28	25/30	22/25	8/10	6/10	7/10	9/10	9/10	39/50
Endosperm	30/30	26/29	20/30	7/10	6/10	6/10	7/10	7/10	33/50
Testa and carp	30/30	8/30	0/25						
Glume			0/29						
Leaf	13/13								
Seed transmission*				8/10	8/10	8/10	7/10	8/10	39/50

Seeds sampled 12–35 days after flowering: Infested seedling/ inoculated
Matured seed: Seed from which BSMV was detected/ seed tested
A–E showed the repeation
* Seed transmission in seed sample for the control

fection, but the infection percentages of endosperm were somewhat lower. Either of the embryo and endosperm infection was noticed in 10 out of 50 seeds tested, and, in 6 seeds, the virus was detected from endosperm only.

3. Discussion

The rate of infection in young embryos just as developed as to be dissectable proved to be very similar to that of the more developed embryos and fully matured seeds. In other words, the rate of the seed infection seems to be determined by the embryo infection. But, on the other hand, according to the results of inoculation tests, it was noticed that the frequency of the endosperm infections was almost equal to the embryo infections. Attached young seeds (15 days after flowering) were inoculated at the top end with a needle, in order to see whether the following supposition was true: The embryos were virus-free at the early stage of seed development, but, they became to be infected by the virus invasion from the other tissues in the seed at the later stage. The inoculated seeds were tested for the infection by embryo culture with the use of young (7 days after inoculation) and fully matured seeds, as well as by the seed dissection and inoculation test. Another seed dissection and inoculation test was also made with the seeds 25 days after the inoculation. The infectivity was not detected from any dissected tissue of the inoculated seeds. It was not known whether the negative results were caused by the technical failure or by some virus inhibitive substances in inmatured endosperm. In another test, healthy embryos were grafted on the diseased endosperms by Yamasaki's method (1939) to see whether the seedling infection occurred in those seeds, in which the virus had not been presented in embryo at the germination time. All the seedlings grown from such grafted seeds were apparently healthy. Notwithstanding the facts that the virus was detected only in endosperm in many instances, considering the uniformity of the results in embryo culture tests, the virus in the endosperm did not seem to be responsible for the seed transmission.

Crowley (1959) studied on the time of embryo infection with some viruses, such as barley stripe mosaic, southern bean mosaic and tobacco ring-spot. He confirmed that some seed transmitted viruses can infect the embryos of their host either by way of the pollen or, to some extent, by infecting the young embryo in the early stages of its development. Pollen transmission or the infectivity of pistil and pollen were also confirmed in this paper (Chapter VI, 3). From the foregoing analysis, the author agreed with Crowley's opinion on the seed transmission in BSMV.

The several observations made in this study on the mechanism of seed transmission of BSMV are summarized as follows:

1) The seeds can be infected either pollen or oval.
2) The rate of embryo infection or the rate of seed transmission is determined already in the early stages of seed development.
3) Marked increase and decrease of seed infection does not occure at the later stages of seed development and at the germination time. In other words, it is not likely that new invasion to or inactivation of the virus in the seed takes place at these stages.
4) The virus in endosperm seems to be not responsible for the seed transmission.

C. DETECTION OF SEED-BORNE SYMPTOMS OF BSMV

Seed-borne symptoms of BSMV in barley seedling were very weak and faint under some experimental conditions. Hampton and others (1957) found that the optimum condition was light intensities of 10,000 F. C. and temperatures 70–85°F for the seed-borne symptom expression. On the other hand, McKinney (1954) stated, "testing barley seedlings for seed-borne virus of type culture of stripe-mosaic virus has presented no striking difficulty in the varieties tested, as chlorotic markings are detectable over a wide range of culture conditions. However, difficulty is encountered with the mild and the very mild symptom-inducing viruses that predominate in the seed of certain varieties of barley,...".

According to McKinney (1954), to detect the trace infections in seed samples when they occurred at the rate of 1.562% or 6.25% of the population, within the limits of the 0.1% level of probability, 442 or 107 seedlings must be tested, respectively. Hampton and others (1957) reported that at least 100 seeds of a sample unit should be used in a seed certification program, if possible.

In this paper, several investigations were made on the relationship between the leaf stage and the initial expression of the seed-borne symptom, on the relationship between the light intensity and the seed-borne symptom expression, on the interference of the virus in the seed-borne infected seedling, and also on the scale of seed sample unit for the seedling test.

1. Relationship between the initial expression of seed-borne symptom and the leaf stage of seedling

Seed-borne symptoms of BSMV does not always appear in the primary

leaves. The initial expression of seed-borne symptom was observed daily until 3 leaf stage, using the seedlings grown from the seeds which had been obtained from the artificially diseased barley plants. The varieties of barley, of which seeds were used in the experiment, were as follows:

Naked barley Kairyo-bozu-mugi, Ehime-hadaka No. 2, Shiroto, Akashinriki, Kobinkatagi

6-rowed barley Kinai No. 42, Wase-hosogara, Kenyoshi No. 3, Nihon-san, Sangatzu, Kaikei No. 44, Aizu No. 5, Aizu No. 6, Nakaizumi-zairai, Hoso-mugi, Hosogara No. 1, German summer, Miyagi No. 123, Mukade-mugi, Kuromugi No. 148, Date No. 2, Kachidoki, Omugi No. 2, Chevron, Trebi I, Atlas, Peruvian, Vaga, Abyssinia, Arlington, Nepal

2-rowed barley Hokudai No. 1, Hokudai No. 4, Hokudai No. 9, Russian No. 8, Russian No. 12, Russian No. 50, Russian No. 59, German No. 17, German No. 61, Moravia, Harbin 2-rowed, Hatakaze, Nissei, Shunsei, Ebisu, Sulton, Sydney, Sächsender, Asahi No. 5, Kyotonakate, Chevalier, Two-rowed Chevalier, Chevalier winter Frederikson, Golden Melon No. 1, Kagawa Golden, Hakata No. 2, Victorie, Gold Foil, Nigrinudum

In Table 35, part of the results was shown. All of the seed-borne infected seedlings of naked and 6-rowed barley showed the seed-borne symptoms at their first leaf stage just after the emergence. There was no diseased seedling which did not express the symptoms until 2 or 3 leaf stage. In 2-rowed barley, many of the seed-borne symptoms were also detectable at their 1st leaf stage. However, some of the diseased seedlings showed no symptom until the 2nd, the 3rd or more advanced leaf stages. Since some of the experimental conditions seemed to be far from the optimum condition reported by Hampton and others (1957), it seemed that the milder symptoms had failed to detect in some cases. But, since the seed-borne symptoms of the virus isolates used in this study were de-

Table 35. Relation between the initial expression of seed-borne symptom of BSM and the stage of seedling growth

Variety		Seedling examined	Seedling expressed the initial symptom on			Seed-borne infection %	Detection of seed-borne infection in the 1st leaf stage %
			1st leaf	2nd leaf	3rd leaf		
Akashinriki	6-rowed, naked	790	494	0	0	62.5	100
Omugi No. 2	6-rowed	861	406	0	0	47.2	100
Atlas	6-rowed	664	465	0	0	70.0	100
Harbin 2-rowed	2-rowed	791	458	10	0	59.2	97.8
Shunsei	2-rowed	1171	705	52	1	64.8	93.0
Chevalier	2-rowed	1492	940	12	2	64.0	98.5

tectable over the wide range of the conditions, as seen in the further experiment in below, most of the experimental conditions may well be adequate for the seedling test.

2. Effect of light intensity on the seed-borne symptom expression of BSMV

The seed-borne symptoms of the author's virus isolates seemed to be detectable over a wide range of the conditions as similar in the case of the type culture reported by McKinney (1954). Since the high light intensity as was noticed by Hampton and others (1957) had not always seemed to be essential, the effect of light intensity on the symptom expression was studied. Moreover, the individual differences among observers, who have been experienced in various degrees in the detecting seed-borne symptoms, were investigated.

Seeds taken from the diseased plant of Akashinriki, Kenyoshi No. 3, Chevalier, Harbin 2-rowed, and Shunsei were sown in glasshouse (light intensities at the soil level on a bright and cloudy day at 10:00 AM were 3,400 and 2,600 lux respectively; average temperature was about 23°C) and in the temperature controlled room (24–26°C) under flouresent light. The seed-borne symptoms on the primary leaf were examined 8–10 days after sowing. Then, the sap-inoculation was applied on all the seedlings. Further observations of symptoms caused by both seed transmission and sap-inoculation to the 1st and the 2nd leaf were made 7–10 days after the first observation. Results were shown in Table 36. There was no significant difference among the seed transmission per cent detected in seedlings grown under the different light conditions. Mild symptom expressing strain of the virus in Chevalier was detectable by careful observations, though it produced only faint symptoms at low light intensities.

Table 36. Relation between light intensity and seed-borne symptom expression of BSM

Variety	Glasshouse	Light intensity (lux)		
		8700	7200	5600
Akashinriki	61.6	68.5	65.5	71.6
Kenyoshi No. 3	77.5	69.7	78.5	71.0
Harbin 2-rowed	81.6	80.2	84.5	83.8
Chevalier	74.3	76.5	65.5	69.6

Seed-borne symptom detected in lst leaf stage (%)
Differences among the data from 3 light intensities were not significant.
Symptoms in Chevalier under the artificial light conditions were very weak.

Seed samples taken from the same source were tested seperately by 4 observers, who had been experienced in various degree. Diseased seeds of Moravia and Shunsei, which had been taken from the diseased field in Hokkaido, were sown in glasshouse and temperature controlled room (5,600 and 3,400 lux), and the seed-borne symptoms on primary leaf were examined. Final count of seed transmission was made with the similar way as mentioned in the foregoing ex-

Table 37. Detection of seed-borne symptoms by different observers in lst leaf stage of seedling

Variety	Light intensity* lux	Seedling observed	Seed-borne infected seedling**	Infected seedling detected by individual observer			
				A	B	C	D
Shunsei	Glashsouse	53.5	43.0	41.0	8.5	42.0	31.5
	5,600	54.5	42.0	38.5	37.0	38.0	37.5
	3,400	54.5	45.0	44.5	32.5	43.5	41.0
Moravia	Glasshouse	51.5	42.0	39.5	7.5	30.0	11.5
	5,600	51.0	41.5	37.5	9.0	38.5	22.0
	3,400	52.0	41.5	33.5	9.0	34.0	9.5

Average of two experiments (Number of seedling)
* : White-and daylight fluorescent light
** : Inoculation-testing method with the use of seed samples from the same seed source

periment. As seen in Table 37, the results obtained by different observers were so markedly differed from each other because of the unequality in experience in the symptom observation. Inexperienced observers may well have been apt to overlook the faint symptoms, and their results were found to be unreliable. As similar to the results obtained in the former experiments, light intensity seemed not to be a limiting factor on the symptom expression. The seed-borne symptoms in Moravia were faint but detectable at 2,800 lux of light intensity. This was the result quite unexpected by the writer, since the symptoms in Moravia is so mild as to be detectable even under the glasshouse conditions. In several seedlings the initial symptom expressions were noticed at the 2nd or more advanced leaf stages in both Shunsei and Moravia, and this agreed with the result shown in Table 35.

3. Virus interference in seed-borne infected barley seedlings

McKinney (1956) studied on the interference among strains of BSMV and stated that once systemic infection was established by an avirulent strain, further infection by the virulent strain used was effectively blocked. Some of the seed-borne infected seedlings were still symptomless (at their primary leaf stage, at least) according to barley varieties, virus strains and the environmental conditions. To detect these symptomless infected seedlings, it was necessary to observe the symptom development over comparatively a long period of time or the subinoculation test for the virus latency. The author felt it necessary to make a supposition that, seed-borne infected seedlings without visible symptoms should be detected by a further inoculation test to these seedlings: if the interference among strains of the virus in young barley seedlings certainly takes place, they should not produce any symptom to be resulted by the inoculation. For the purpose of verifying this supposition, experiments were conducted to confirm the interference among isolates of BSMV in seed-borne infected seedlings. The foregoing experiment on the light intensity and the symptom ex-

pression was also carried out for the same purpose.

Diseased seeds of Harbin 2-rowed, Chevalier, Kenyoshi No. 3 and Akashinriki were grown under the light intensities at 6,400 and 2,800 lux, and the seed-borne infection on the primary leaves and the infection caused by a further inoculation were examined. Symptoms by these two types of infection were easily distinguishable (c.f. Chapter V). Table 38 shows the results. All of the

Table 38. Interference of BSMV in seed-borne infected barley seedling

Light intensity lux	Variety	Seedling examined	Seed-borne infection observed in lst leaf stage	Infection caused by inoculation	Seed transmission*
5,600	Harbin 2-rowed	20	18	2	18
	Chevalier	21	2	5	16
	Kenyoshi No. 3	29	22	7	22
	Akashinriki	30	24	6	24
3.400	Harbin 2-rowed	52	49	3	49
	Chevalier	42	1	12	30
	Kenyoshi No. 3	52	38	11	41
	Akashinriki	52	34	14	38

All the seedlings were cultured under 5600 lux after inoculation

*: The seedlings which experessed seed-borne symptoms at the inoculation time.

infected seedlings by the inoculation which had been symptomless at their 1st leaf stage showed typical local necrosis on inoculated leaf (primary leaf) and both mosaic and necrosis on young leaves (2nd or 3rd leaf) caused by the mechanical inoculation. All the inoculated seedlings, which had showed the seed-borne symptoms at their 1st leaf stage at the inoculation time, still showed the seed-borne symptom alone at their 2nd or more advanced leaf stages. Moreover, subinoculation test proved that, the virus isolate used for the inoculation, Im-strain, which was a characteristic yellow mottling producing strain obtained from the seed-borne infected seedling of resistant variety Imperial (Plate VI, 3), was not recovered from the inoculated seedlings which had expressed the seed-borne symptom at their 1st leaf stage. Im-strain was not recovered also from the inoculated seedlings, which had been symptomless at their 1st leaf stage, but had showed seed-borne symptoms on their 2nd or 3rd leaf. These results suggested that, the interference among strains of BSMV was found not only in the seedlings, on which seed-borne symptoms were presented at their 1st leaf stage, but also in the symptomless seed-borne infected seedlings.

4. Scale of seed sample unit for the accurate detection of seed transmission

Detection of the higher population of infected seed in samples is not so difficult, but, it is rather difficult to detect traces of seed infections. In the latter cases, a large number of seedlings should be tested as McKinney (1954) pointed out. Figure 5 was obtained by a seed-sampling test: each 30 seed sample was

drawn separately 100 times from 3,000 seed mixture which included dyed (0.2–10.0%) and non-dyed seeds, and the number of seeds to be used in seed testing was calculated by the following:

$$n = \frac{s^2 t^2}{l^2}$$

s : standard deviation
l : $0.2p$ in this case
p : % of seed transmission

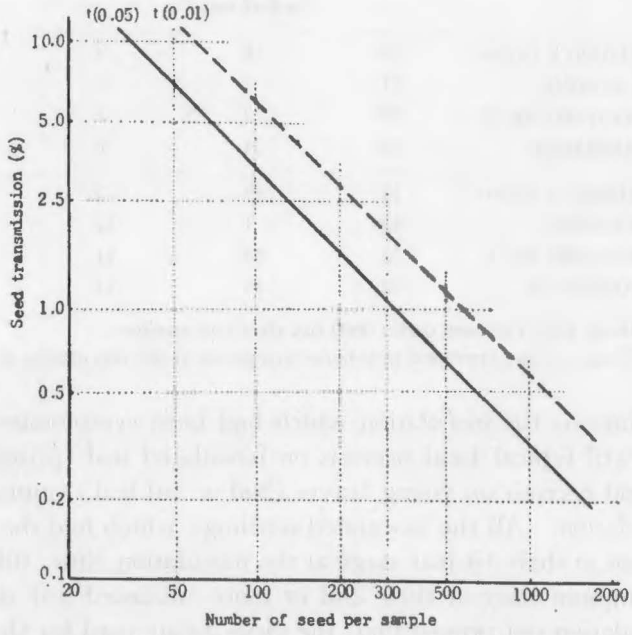


Fig. 5. Scale of seed sample for the detection of seed transmission of BSMV.

As apparently seen in Fig. 5, at least 350 or 650 seeds must be tested to detect the rate of 1% of seeds transmission within the limits of 5 or 1% level of probability. The more accurate rate of seed transmission may be expected by the larger scale of seed sample unit, though it seems to be limited by many factors for practical used. A rough estimation of the rate of seed transmission with the use of 30–50 seedlings at their 1st leaf stage is advisable prior to the accurate estimation, if possible, when the rate of seed transmission in the seed sample is not known. The use of 50–100 seeds per sample unit will be enough to detect the 20% or more rate of seed transmission.

D. A SEED TESTING TECHNIQUE FOR SEED TRANSMISSIN OF BSMV

The use of certified virus-free seeds is most important for the control of field occurrence of the disease, as BSMV is seed-borne by nature. Therefore, it is

necessary to establish the seed testing technique for practical uses.

McKinney (1954) pointed out that, the difficulties are encountered with seed-borne symptom expression in barley seedlings in some cases. And, Hampton and others (1957) showed that high light intensity and 70–80°F of temperature was the optimum condition for the seed testing technique.

It seems likely that the best way to examine the seed transmission of the virus is to experiment under the optimum light and temperature condition in which all of the seed-borne diseased seedlings produce visible symptoms. Even so, however, a difficulty remains that the equipment to regulate the environmental factors would not always be available. Moreover, further technical difficulties will arize when a great number of seed samples must be tested simultaneously. The author carried out the seedling test by the "inoculation-testing method", in which comparatively large number of seed samples were testable. Though, on account of some trouble in inoculation process, the inoculation-testing method may not be said to be the best way, this method assured us good results. The technique of this method and some of the experiments by seedling test were described below.

1. Step to be taken for inoculation-testing method

Inoculation-testing method for seed-borne infection of BSMV includes the

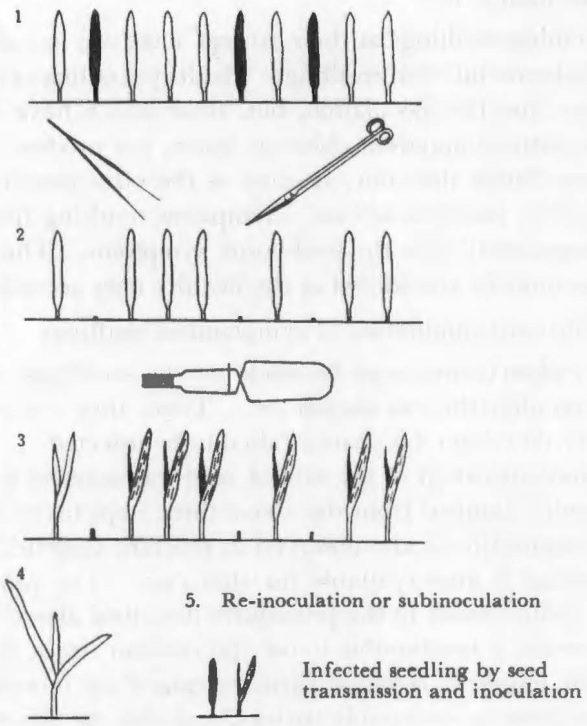


Fig. 6. Inoculation-testing method for the detection of seed transmission of BSM.

following 3 successive steps of examination; observation of the seed-borne symptom expression at the primary leaf stage, the inoculation to the healthy seedlings in appearance at the primary leaf stage (detection of healthy seedling), and the re-inoculation or the subinoculation with symptomless seedling. Since the seed-borne symptoms of the stripe disease of barley is very alike to those of BSM in some instances, as seen in Chapter V, the seeds which could be supposed to have been infected by the stripe disease, must be sown after the disinfection. Figure 6 shows the inoculation-testing method.

i) Examination of seed-borne symptom at the primary leaf stage

According to McKinney (1954), he sowed the seeds with wide spaces to avoid the inter-plant contact transmission. But, the author sowed the seeds with the spaces of 2×3 cm in wooden boxes, because the plant-to-plant contact transmission is to be easily distinguishable from the seed-borne infection by the symptoms characteristic to contact transmission, though its occurrence is rather rare in case. To avoid the unfavorable occurrence of contact transmission, it is advisable to keep the glasshouse free from the wind from outside. Careful observation and roguing of infected seedlings must be made at least two times until the primary leaves fully develop. Infected seedlings are carefully nipped off with a long thin scissors at the soil level on every occasion, avoiding the violent contact with the neighboring seedlings.

ii) Inoculation test

Symptomless seedlings at their 1st leaf stage are inoculated after the removing of seed-borne infected seedlings. Healthy seedlings get to be infected within 4–7 days after the inoculation, but, those which have already been infected seed-borne without apparent chlorotic lesion, not produce any additional symptoms by inoculation this time, because of the virus interference which has been metioned in the previous section. Symptoms resulting from the inoculation are easily distinguished from the seed-borne symptoms. The seedlings infected by inoculation must be considered as the healthy ones according to this method.

iii) Further examination of symptomless seedlings

Further observation must be made on the seedlings, which are still symptomless even after the inoculation test. Then, they are re- or subinoculated to see whether they have by chance failed to be infected.

The final estimation of the rate of seed transmission is made by accumulating the results obtained from the above three steps taken for the test.

Seed transmission is also observed in resistant varieties, and the inoculation-testing method is also available for that case. The procedure of the testing method is quite similar to the procedures described above. As an inoculum for inoculation-test, it is advisable to use the virulent strain of the virus. Chlorotic and necrotic lesions in resistant varieties caused by inoculation are milder and fewer than those in susceptible barleys, and also the development of symptoms is delayed. However, the seed-borne symptoms are easily distinguishable from

those of mechanical inoculation.

2. Experiment results by the inoculation-testing method

i) Experiments with susceptible varieties

Table 39 shows part of the results of experiments by inoculation-testing method on the seed-transmission of BSMV with susceptible 2-rowed, 6-rowed and naked varieties of barley. No difference was observed among the percentages of the seed-borne infection observed at their 1st leaf stage and those of seed transmission examined by the inoculation-testing method. The results showed that, all of the seed-borne symptoms of infected seedlings of 6-rowed or

Table 39. Seedling test by inoculation-testing method for the detection of seed transmission of BSM in susceptible varieties

	Variety	Number of seedling				Seed transmission (%) detected by		Detection of Seed-borne symptoms**	
		Tested	Seed-borne symptom in 1st leaf stage	Inoculated	Infected by inoculation	1st leaf observation	Inoculation testing	*	
6-rowed, naked	Akashinriki	96	75	21	21	78.2	78.2	0	A
	Shiroto	38	5	33	33	13.2	13.2	0	〃
	Kobinkatagi	80	34	46	46	42.5	42.5	0	〃
	Ehimehadaka No. 2	96	4	92	92	4.2	4.2	0	〃
6-rowed	Hosogara No. 1	99	40	59	59	40.4	40.4	0	A
	Omugi No. 2	89	41	48	48	46.1	46.1	0	〃
	Mukade-mugi	89	9	80	80	10.1	10.1	0	〃
	Kinai No. 42	98	2	96	96	2.0	2.0	0	〃
	Nihonsan	97	64	33	33	66.0	66.0	0	〃
	Miyagi No. 123	100	8	92	92	8.0	8.0	0	B
	Date No. 2	100	21	79	79	21.0	21.0	0	A
	Aizu No. 5	95	36	59	59	37.9	37.9	0	〃
	Kenyoshi No. 3	99	88	11	11	88.8	88.8	0	〃
	Atlas	55	38	17	17	69.1	69.1	0	〃
	Chevron	99	48	51	51	48.5	48.5	0	〃
2-rowed	Sulton	50	18	32	31	36.0	38.0	1	B
	Golden Melon	95	20	75	73	21.1	23.2	2	C
	Harbin2-rowed	97	21	76	71	21.6	27.8	5	〃
	Kyoto-nakate	99	19	80	76	19.2	23.2	4	D
	Hokudai No. 1	84	13	71	69	15.5	17.9	2	C
	Houkudai No. 4	96	54	42	42	56.3	56.3	0	B
	Moravia	98	32	66	64	32.6	34.7	2	C
	Asahi No. 5	99	24	75	75	24.3	24.3	0	B
	Hatakaze	80	34	46	46	42.5	42.5	0	〃
	Shunsei	94	30	64	61	31.9	35.1	3	C

* Number of seedling which expressed the initial expression of seed-borne symptom in 2nd leaf or more advanced stage of seedling.

** A : Easy, B : Slightly difficult, C : Difficult, E : Very difficult

naked barleys appeared just after the emergence without exception, as similar to the results shown in Table 35. In most of the 2-rowed barleys, there was a large difference in the results between the 1st leaf observation and the inoculation-testing method. Namely, some of the initial expression of the seed-borne symptoms did not take place even after the 2nd leaf or more advanced stages of seedlings developed.

ii) Experiments with resistant varieties

Seed-borne infection was examined with the seeds obtained from the artificially diseased plants of resistant varieties. The results were shown in Table 40. All of the healthy seedlings of most of the Turkish barleys (T 101- T 573), which were resistant to BSM in various degree, were detected by an inoculation. A re-inoculation was enough for the estimation of the rate of seed transmission in resistant barley, Ko-ran. Several seedlings of highly resistant Imperial was still symptomless even after the re-inoculation test. However, they were found to be virus-free by the result of a subinoculation test.

Table 40. Seedling test by inoculation-testing method for the detection of seed transmission of BSM in resistant varieties

Variety	Number of seedling Tested	Seed-borne symptoms in lst leaf stage	Infection caused by		Number of seedling infected by inoculation*	Seed trans-mission %	Symptom
			lst inocultiaon	2nd inoculation			
T 101	60	16	48/48	—	48	26.7	M-S
T 121	86	40	46/46	—	46	46.5	S
T 169	88	46	42/42	—	42	52.3	M
T 239	50	30	20/20	—	20	60.0	M-S
T 245	113	18	95/95	—	95	15.9	M-S
T 251	115	56	59/59	—	59	48.7	M-S
T 433	115	19	96/96	—	96	16.5	M-S
T 434	105	34	71/71	—	71	32.4	M, S
T 462	90	26	64/64	—	64	28.9	S
T 508	65	23	42/42	—	42	35.4	F-M-S
T 567	28	8	22/22	—	22	28.6	M
T 573	87	17	69/70	1/1	70	19.6	S
Modjo	115	60	54/55	1/1	55	52.2	M-S
Ko-ran	229	3	221/226	5/5	226	1.3	M-S
Imperial	122	8	107/114	5/7**	112 (114***)	6.6	M-S

* This must be considered as the healthy seedling in the test.

** Two seedlings without visible symptom were determined to be healthy by subinoculation.

*** Includes with 2 seedlings of (**)

F, M, S: Faint, moderate and severe symptoms

3. Discussion

Detection of seed-borne symptoms of BSMV is affected by the following fac-

tors: (1) Experience of the observer, (2) temperature, (3) light intensity, (4) barley variety, and (5) virus strain. The symptoms caused by inoculation can be detected easily, as they have marked characteristics as mentioned in Chapter V. Moreover, since these symptoms by inoculation are detectable under the wide range of conditions of light intensities and temperatures, and since they are distinct even in the case of mild symptom producing strain, no special experience may be required to detect them, compared with those of the seed-borne infection. In this connection, the inoculation-testing method has the following advantages:

(a) No specialized skill necessary to find out the seed-borne infection at the 1st leaf stage.

(b) No special experience is required to detect the symptoms caused by inoculation.

(c) Seed-borne infected seedlings can be detected even though they give no symptom of infection.

(d) This method is available under the glasshouse conditions throughout the year except in summer.

(e) This method is available for the test with a comparatively large scale of seed sample unit.

But, on the other hand, there are some difficulties as follows:

(a) Inoculation-test is rather troublesome. Several observations per sample seedling must be repeated.

(b) Seed propagation from virus-free seedlings is impossible, because all of them are to be inoculated.

This method is particularly effective, when it is applied on 2-rowed barleys, in which some of seed-borne infections are not detectable at their 1st leaf stage. In the case of resistant varieties, inoculation-testing method is also available, although more effective and simple method for testing may be required.

E. INFLUENCE OF TIME OF INFECTION WITH BSMV ON STERILITY AND SEED TRANSMISSION

The secondary dissemination of BSM is caused by the plant-to-plant contact transmission, as seen in Chapter VI. Since the symptoms caused by the contact transmission are similar to those caused by inoculation (not by seed-borne infection), influence on barley of infection time of BSM caused by natural contact transmission may be assumed by the field inoculation test in various stages of plant growth.

Hagborg (1954), Eslick and Afanasiev (1955) reported that, influence on plant yield and seed transmission differed with infection time of this disease. And, Crowley (1959) ascertained the marked difference of the rate of seed transmission between the infections in pre- and post-flowering stage of barley. The author carried out the field inoculation tests, in 1956–7 and also 1959–60, to see the effects of BSM inoculation time on sterility, and plant yield of barley and

wheat, and also on the seed transmission of the virus.

1. Materials and methods

Three 2-rowed varieties of barley, Chevalier, Golden Melon No. 1 (Tochigi), and Hakata No. 2, and a wheat, Norin No. 52 were used for the experiment in 1956–7. Each 20 germinated seeds were sown about 8 cm apart in the field (November 18, 1956). Plots I, II and III were inoculated on February 19, April 9, and April 23, 1957, respectively. Plants in plot IV were rubbed with healthy plant juice on February 19, and plot V was left with no treatment. Three to five leaves per plant were rubbed with thumb and forefinger, which wore with finger stalls and immersed them in the diseased plant juice ($\times 10$). A small amount of 600 mesh carborundum was added to the inoculum as an abrasive.

Averages of heading time, plant height, length of the uppermost internode and sheath, length of uncovered portion of the uppermost internode, kernel weight, and sterility were examined with 5 initially headed culms taken from 10 plants in all plots. In order to estimate the sterility of wheat, the number of fertiled kernels of the 10 spikelets were counted. Since the wheat plants in plot III produced extremely poor yield because of their systemic necrosis together with the infection with *G. zeae*, their kernel weight, fertiled seed number and yield were not estimated. Field inoculation test was carried out with 3 replications, and all the data were examined statistically.

The seeds obtained from 10 plants in each plot were mixed throughly and were sown in the glasshouse. Seed transmission of the virus in about 500 seedlings in each seed sample unit were observed until their 3rd leaf stage.

In the experiment in 1959–60, two-rowed variety Chevalier was used. Barley plants in 10 experimental plots were inoculated at their various stages of growth. The dates of inoculation were as follows: January 5, February 20, March 31, April 12, 18, 22, 26 and 29, and May 2, in 1960. The methods of inoculation and the scale of experimental plots were quite similar to those of the experiment in 1956–7. Plant height, length of the uncovered portion of the uppermost internode, and sterility were examined with 5 initially headed culms of 10 plants in all plots. And, the rate of large and small grain, 1,000 kernel weight, plant yield, and seed transmission were examined with seeds which were collected from 10 plants and then throughly mixed. Seed transmission was tested for 3 times using 1,000 seeds per plot.

2. Results

Table 41 shows the results of the experiment in 1956–7. Plant height, sterility and other characters of the diseased plants varied markedly with each plant. Moreover, the uneven occurrence of sterility of head was observed generally in even the same diseased plant (Table 42). The results in Table 41 were as follows:

Heading time: Heading time of the plant in plot I (early infection) was markedly delayed, especially in the case of Hakata No. 2 and wheat. No effect

on heading time was observed in the case of the late infection (plot III), which had been inoculated just before the heading time.

Plant height: This was apparently reduced 7–8 cm in the plots I and II, but not in the plot III of Golden Melon. The difference in the plant height among

Table 41. Influence of infection time with BSM to various agronomic characters of barley and wheat, and also to seed transmission of the disease (1)

Variety	Plot	Retardation of heading Day	Plant height cm	The uppermost Internode (A) cm	The uppermost Sheath (B) cm	The uppermost (A) − (B) cm	Sterility %	100 kernel weight g	Plant yield g	Seed transmission %
Chevaleir	I	−3.3	89.2	29.6**	24.3	5.0**	45.6**	3.6	7.4**	52.1
	II	−0.3	93.9	32.2	24.5	7.8	12.3	3.7	13.2	59.7
	III	+0.4	98.2	33.6	24.0	9.2	7.3	3.0	9.5*	0
	IV	May1	93.9	33.1	24.5	8.5	6.3	3.7	16.7	0
	V		96.8	33.5	24.2	9.1	6.3	4.0	17.3	0
	LSD	(0.05)		2.3		1.7	12.1	0.4	4.2	
		(0.01)		3.3		2.5	17.6	0.6	6.2	
Golden Melon No. 1	I	−2.0	94.2**	37.9**	22.5	14.9**	20.0**	4.0	13.5**	22.9
	II	−1.5	95.4**	36.8**	22.1	15.6**	17.4**	3.9	13.6**	34.2
	III	−0.9	100.1	40.4*	22.8	17.6*	4.5	2.8**	10.1**	2.2
	IV	May4	103.2	44.8	23.0	22.2	1.8	4.5	25.7	0
	V	(−0.1)	103.7	43.2	22.8	20.5	2.8	4.3	19.4	0
	LSD	(0.05)	4.9	2.9		2.9	4.1	0.6	3.2	
		(0.01)	7.2	4.2		4.2	5.9	0.9	4.6	
Hakata No. 2	I	−2.8**	80.2	31.8**	20.8	10.9**	36.1**	4.0	12.0**	34.2
	II	−0.9	85.4	35.3*	21.1	13.7**	7.3	4.0	13.3*	40.3
	III	+0.5	90.9	37.1	20.8	16.4	4.0	2.9**	10.6**	0.1
	IV	May2	91.7	39.2	21.0	18.2	1.9	4.2	20.3	0
	V	(+0.1)	90.6	38.9	20.9	18.1	3.0	4.5	19.9	0
	LSD	(0.05) 1.8		3.2		3.2	4.2	0.6	5.3	
		(0.01) 2.5		4.6		4.6	6.0	0.9	7.7	
Wheat(Norin No.52)	I	−1.4**	80.9	30.1**	17.8*	14.0**	13.2**	2.5**	6.2**	47.9
	II	−0.6	86.5	33.9*	19.5	14.0**	21.0**	2.0**	9.9**	43.9
	III	+0.6	90.5	34.2	18.8	15.2*	—	—	—	—
	IV	May4	90.6	36.0	19.3	16.6	26.6	3.4	20.7	0
	V	(−0.5)	91.5	36.4	19.6	16.8	26.2	3.4	21.9	0
	LSD	(0.05) 0.9		2.3	1.5	1.7	2.0	0.3	4.1	
		(0.01) 1.3		3.8	2.1	2.5	3.0	0.5	6.2	

Plots I, II, III were inoculated on Feb. 19, April 9, and 23, respectively
Plants in plot IV were rubbed with healthy plant juice
Plot V was untreated check
**, *: Significant in 1 and 5% level
Sterility of wheat was indicated by the fertiled kernels in 10 spikelets

the plots of other varieties were not significant statistically, however, there was a tendency that the plant height was reduced by the early infection.

Length of the uppermost internode: The earlier the infection time was, the more the effect was noticed on the length of the uppermost internode. The reduction in the length of barley was observed to be significant in plots I and II of all the varieties except Chevalier (II). Marked reduction of this character was also noticed in the case of late infection (III) of Golden Melon.

Table 42. Culm length and sterility of different tillers in BSM infected plant

V		I		II		III	
Culm length cm	Sterility %	Culm length cm	Sterility %	Culm length cm	Sterility %	Culm length cm	Sterility %
103	3.8	95	7.1	106	7.7	102	0
101	0	92	76.9	104	36.4	101	0
100	4.0	91	100.0	103	8.0	101	8.3
99	0	91	91.7	101	30.8	98	12.5
99	10.7	90	23.1	100	36.8	97	0
98	6.9	89	50.0	99	53.6	96	4.6
98	0	87	50.0	96	3.7	96	0
95	0	86	25.0	96	6.9	96	0
94	7.7	84	100.0	96	0	95	17.6
94	0	84	26.9	96	15.4	94	4.2

Variety: Chevalier
I, II, III, V: See Table 41
Ten culms from the longest were taken

Length of the uppermost sheath: This was shortened about 2 cm by early infection (I) in wheat, but not in all of the other cases.

Length of the uncovered portion of the uppermost internode (=Length of the uppermost internode — the uppermost sheath): As similar to those of the length of the uppermost internode, the earlier the infection time, the more the effect was observed.

Number of head: Reduction of head number caused by infection was commonly observed. As seen in Table 42, the number of head could not be estimated, because of the uneven length of culms and also the high sterility of the head in infected plants.

Sterility: High sterilities were observed in early infected plants. It amounted to 20% in Golden Melon, 36% in Hakata No. 2, and 45.6% in Chevalier. Reductions in the number of the fertiled seeds in wheat amounted to 50% and 20% in those experimental plots I and II respectively.

100 kernel weight: Reduction in kernel weight caused by the shrivelled grains was observed to be significant in the case of the late infection (III) in barleys, but not in the early infection. Reduction in kernel weight was marked

in all the inoculated plots of wheat, especially in the late inoculated plots.

Plant yield: Plant yield was reduced in all the inoculated plots of all varieties except Chevalier (II). Large effect of infection time on yield reduction was noticed in order of the plots I, II and III in Chevalier. However, it was noticed in order of III, I and II in other varieties of barley and wheat. In some cases of the late infection (III), plant yield was reduced by 50%. The yield reduction in the late inoculated plots (III) of wheat was observed to be extremely destructive, because of the severe necrosis caused by this disease together with the scab infection.

Seed transmission: A large amount of seed transmission was observed in the seeds taken from the field plot I and II in all varieties. However, only occasional or no seed transmission was observed in the late inoculated plot (III). The seeds from uninoculted plots were all found to be virus-free.

Most of the results obtained from the field experiment in 1959–60 (Table 43) were very alike but not quite similar to those obtained in 1956–7, as follows:

Plant height: Marked reduction of plant height was resulted by the early infections before April 12. This was found to be quite similar to the previous experiment that, the earlier the inoculation time was, the more the effect of infection was noticed.

Length of the uncovered portion of the uppermost internode: This was reduced in the early inoculated plots, especially in those inoculated before February 20.

Sterility: Marked reduction in fertility was resulted by the early infections, especially before March 31. This was also similar to the results of the previous experiment.

Proportion of the large seeds: Proportion of large seeds (more than 2.5 mm width) was reduced markedly by the disease infection at any infection time.

Proportion of the small seeds: Proportion of the small seeds (less than 2.0 mm in width) was increased in the early inoculated plots or the plots inoculated at the flowering time.

Kernel weight: The earlier the infection time was, the more the reduction in kernel weight was observed. Above all, the reduction in the kernel weight was found to be significant in those plots inoculated early before April 12, and also in the late inoculated (May 2) plots. These were somewhat differed from the results of the previous experiment.

Yield: The yield in most of the inoculated plots was reduced. And, the earlier the infection time was, the more effect on the yield reduction was noticed.

Seed transmission: Compared with those found in the early inoculated plots, the amount of seed transmission was noticed to be very small in those plots inoculated after April 12, especially after the heading time.

Table 43. Influence of time of infection with BSM to agronomic characters of barly, and to seed transmission of the disease (2)

Plot	Date		Occurrence of the disease	Plant height	Length of the uncovered portion of the uppermost internode	Sterility	Proportion of		1000 kernel weight	Yield (10 plants)	Seed transmission
	Inoculated	Heading					Large seed (>2.5mm)	Small seed (<2.0mm)			
				cm	cm	%	%	%	g	g	%
1	Jan. 5	April 28	A	115.6**	13.7**	48.2**	38.3**	16.1	34.5*	61.2**	34.4
2	Feb. 20	〃	A	107.9**	11.2**	59.9**	28.7**	16.5*	33.5*	44.4**	37.4
3	March 21	April 26	B	120.0**	16.6	24.0	37.5**	15.3	34.7*	86.6**	31.6
4	March 31	April 25	B	121.7**	16.9	25.0*	35.2**	12.1	34.9*	92.3**	28.0
5	April 12	April 24	C	125.5*	18.1	17.2	42.1	12.2	34.9*	112.6**	3.7
6	April 18	〃	C	126.7	17.3	17.3	43.0	9.6	36.8	110.4**	1.7
7	April 22	〃	D	131.4	18.5	13.7	39.4*	9.4	36.2	129.7	0.6
8	April 26	〃	D	131.6	20.2*	17.0	42.7	9.5	36.6	125.6*	0.1
9	April 29	〃	D	129.4	19.6	14.2	40.2*	14.2	36.2	128.4	0.1
10	May 2	〃	D	131.4	18.4	15.9	38.2*	13.6	34.8*	123.4*	1.1
Control	—	〃	—	129.6	18.1	17.1	54.7	10.1	38.5	145.6	0
LSD (0.05)				3.87	1.75	11.44	12.89	6.44	3.58	18.44	
(0.01)				5.28	2.39	15.60	17.57	8.78	4.87	25.15	

A : Systemic occurrence

B : Symptoms appeared on 1–3 leaf from the uppermost, tillers emerged early were symptomless.

C : Symptoms were found only on flag leaf and head, tillers emerged early were symptomless.

D : Symptoms were found only on stem just below the head, tillers emerged early were symptomless.

3. Discussion

Quite similar effects of infection time on heading time, plant height, and length of the uncovered portion of the uppermost internode, were observed in both of the field experiments carried out in 1956–7 and 1959–60. But, some differences on the kernel weight and yield were noticed between the results of these two experiments. Hagborg (1954) found that, using two varieties of spring wheat, the reductions of plant height, yield and kernel weight were 13, 75 and 31%, respectively, in the early inoculated plots, though these effects of infection were not observed in the late inoculated plots. He found also, that, the reductions of yield and kernel weight of Plush barley were 65.5 and 8%, respectively, in the case of early infection. Eslick and Afanasiev (1955) reported that, reduction in barley yield was most marked when plants were inoculated 1–3 weeks before heading.

A large amount of sterility was found in the early inoculated plots in both experiments carried out in 1956–7 and 1959–60. On the other hand, very rare sterility was noticed in the case of late infection when plants inoculated heading time or the after. These suggested that the fetility was not affected by the disease, because the fertilization was finished before the plant was invaded systemically by the virus.

According to Hagborg's result (1954), seed transmission of the virus (71% in wheat and 81% in barley) was found only in the early inoculated plots. On the other hand, Eslick and Afanasiev (1955) stated that, seed transmission was greatest when inoculations were made 10 days before heading, but very rare in those inoculated after heading. Crowley (1959) reported also, that very small amount of seed transmission was found in the plants inoculated after heading, compared with those inoculated before heading. The author's results in this study were similar to those of Eslick and Afanasiev, and Crowley. Comparatively large amount of seed transmission (3.4%) was found in one plot inoculated after heading (May 2) in the experiment of 1959–60. However, it might be caused by some young tillers with highly fertiled head in this plot, or the contamination of diseased plants or diseased seeds. Since the initial symptom expression appeared after the flowering time in the plants inoculated after heading time except the case of young tillers, seed transmission of the virus may not have occurred to those plants. Further argument on this problem with reference to the mechenism of seed transmission will be discussed in Chapter X.

The effect of BSMV on the yield of barely and wheat, when the aspects of yield are splitted into the 3 factors; viz., number of head, fertility and kernel weight, may be explained as follows: The earlier the infection time is, the less the head number and the lower the fertility get to be. The reduction in kernel weight occurs not only in the case of early infection, but also in the late infection at heading or flowering time. Late infection does not affect to head number and fertility.

As already shown in Figure 2 in Chapte VI, 3, most of the infections

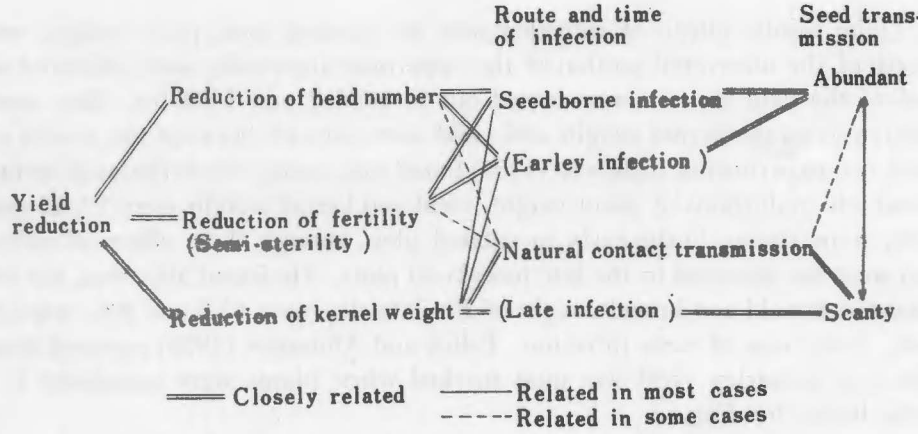


Fig. 7. Effect of BSM infection on the yield reduction of barley.

caused by natural plant-to-plant contact transmission occurred in late stages of growth (after heading time—middle of April). Moreover, compared with those in the case of early infection, the reduction in the proportion of larger seeds and the rarity of seed transmission were noticed in the plants which had expressed the initial symptom on the spikes or the uppermost internodes, as seen in Table 23. The late infected plants in natural contact transmission were corresponded to the field plots "inoculated after heading time", and the infection by seed transmission and the early infection by natural contact transmission were corresponded to those "early inoculated plots". Figure 7 shows diagrammatically the effects of BSMV infection on the yield reduction of barley on the basis of the above discussed.

VIII. BSMV RESISTANCE IN BARLEY

The renewal of variety had been the only solution to cope with "Chochinbo"—sterility of barley in Hokkaido—until BSMV, the cause of this disorder, was discovered in 1956. The use of resistant varieties as well as the seed testing will be effective for the control of BSM. However, only a few resistant barley was reported by Timian and Sisler (1955) and Sisler and Timian (1956). They selected several varieties from Abyssinian barleys, and also investigated the inheritance of resistance in Medjo and C. I. 3212–1.

A. SELECTION OF THE RESISTANT VARIETIES

The investigation was carried out to select the resistant barleys with the series of inoculation test. The degree of resistance was determined at the second step of the screening for BSMV resistance.

1. Materials and methods

i) The first screening for BSMV resistance

About 2,200 varieties of barley were used at the 1st screening for BSMV resistance. Table 44 shows the sources or the origins of barley varieties used. With the use of "inoculation forecept" (Plate VI. 2), 10–30 seedlings of these barleys grown in wooden box were inoculated at their primary leaf stage. Infections were observed 10–15 days after inoculation. Non-infected varieties were selected together with those varieties expressed mild symptoms. Further inoculation tests with these selected varieties were repeated to ascertain their resistances.

Table 44. The first step of screening for BSMV resistance

Origin or source	Number of variety	Selected varieties
Japan	518	
6-rowed	263	
6-rowed, naked	200	
2-rowed*	55	Imperial
The Far East	531	
Korea	388	
China and Manchuria	136	Ko-ran, Sanfunga, Harbin-zairai, Chamusu
Formosa	7	
The Middle Asia and other part of Asia	1009	
Nepal	151	T 40, 101, 122, 126, 167, 169, 239,
Turkey	792	241, 245, 246, 251, 270, 381, 427,
Other parts	66	433, 434, 462, 463, 467, 470, 471,
		501, 508, 556, 567, 653, 658
Other part of the world	133	Wien
Europe, Africa, North and South America, Australia		(Modjo, C. I. 3212–1)**
Total	2191	

* : Imported varieties

** : Abyssinian varieties resistant to BSM sent from Dr. R. G. Timian, U. S. A.

T : Turkish barleys

ii) The second screening for resistance

To determine the grade of BSMV resistance, a set of 3 standard varieties were used in all the inoculation tests. Harbin 2-rowed, Ko-ran and Imperial were used for the susceptible, the resistant and the highly resistant standard, respectively. About each 30 seedlings of 7 varieties and 3 standard varieties were sown in a wooden box. Seedlings were grown as uniform as possible. About a week after sowing, inoculation was made by inoculation forecept, immersing it in the inoculum with each 5-seedlings inoculation. As the inoculum source, diseased leaf juice ($\times 30$) of Hosogara No. 1 was used. Every rubbing on both sur-

faces of the leaf was applied as even as possible. Experiments were carried out in 4 replications. Daily observation was made until the most of the infection was detected. Mean incubation periods in Harbin 2-rowed, Ko-ran, and Imperial were evaluated to 1.0, 3.0 and 5.0 of "resistance-grade", respectively. Figure 9 shows the relation between the mean incubation period and the resistance-grade with 10 experiments carried out in different time. The average incubation period in other varieties was compared with those of standard varieties, and the resistance-grade was estimated from the figure.

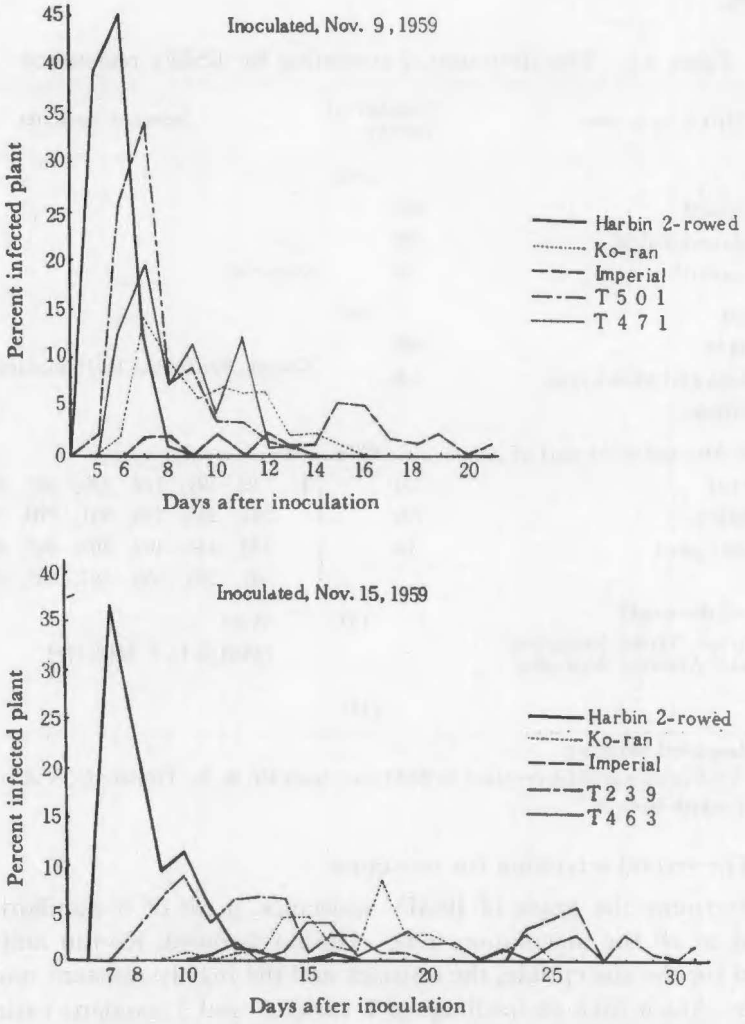
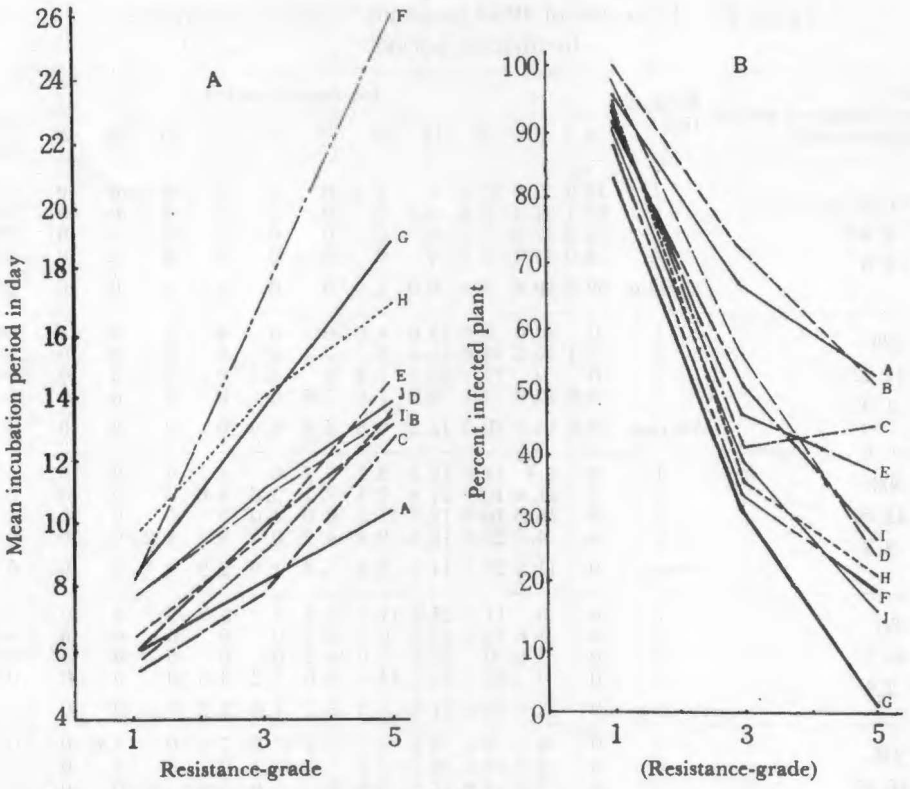


Fig. 8. Infection of seedlings of various varieties resistant to BSM by mechanical inoculation.



A-H showed different experiments (c.f. Table 48)
The data correspond to the resistance-grade 1, 3, and 5 were obtained from the data of Harbin 2-rowed, Ko-ran, and Imperial.

Fig. 9 Relation among the resistance-grade and the mean incubation period (A) and the infection percent (B) of three standard vtarieties.

2. *Results*

i) The 1st screening

Table 44 shows the varieties which were noticed to be resistant in the 1st screening. Various resistant varieties were found frequently in barleys collected from Turkey and Manchuria. All of the tested varieties from the other sources were found to be susceptible to the disease. However, Wien and Imperial were found exceptionally to be highly resistant.

ii) The 2nd screening (determination of resistance-grade)

In Table 47, the results obtained from the experiments carried out during spring and autumn of 1959 were summarized. Resistance-grades of a variety obtained from several experiments in different time were resembled each other among different experiments. Moreover, there was no instance, in which the resistance-grades differed from each other 1.0 or more, and, the order of resistance-grades of varieties reversed greatly.

Table 45. Infection of BSM resistant varieties in various incubation period

Variety / Mean incubation period / Resistance-grade	Replication	Incubation period											
		8	10	12	14	16	18	20	22	24	26	28	30 day
		%											
Harbin 2-rowed	1	32.0	32.0	12.0	0	4.0	0	0	0	0	0	0	0
	2	57.1	21.4	3.6	3.6	0	0	0	0	0	0	0	0
8.40	3	75.0	17.8	7.2	0	0	0	0	0	0	0	0	0
1.0	4	74.0	11.1	7.4	0	0	0	0	0	0	0	0	0
	Average	59.5	20.6	7.6	0.9	1.0	0	0	0	0	0	0	0
T 239	1	0	20.0	8.0	12.0	4.0	0	0	0	0	0	0	0
	2	7.4	29.6	14.8	14.8	0	7.4	0	0	0	0	0	0
12.22	3	0	0	11.1	22.2	7.4	0	3.7	0	0	0	0	0
1.9	4	3.8	10.9	7.1	3.8	7.6	7.6	0	0	0	0	0	0
	Average	2.8	15.1	10.3	13.2	3.8	3.8	0.9	0	0	0	0	0
T 508	1	0	3.7	14.8	11.1	3.7	3.7	0	3.7	0	0	0	0
	2	0	21.4	10.7	21.4	7.1	7.1	3.6	3.6	0	0	0	0
13.66	3	0	16.0	16.0	12.0	12.0	8.0	4.0	0	0	0	0	0
2.3	4	0	4.2	29.2	12.5	8.4	4.2	0	4.2	4.2	0	0	0
	Average	0	11.3	17.7	14.3	7.8	5.8	1.9	2.9	1.1	0	0	0
T 427	1	0	0	11.1	22.2	11.1	7.4	0	3.7	3.7	0	0	0
	2	0	7.6	15.3	15.3	0	7.7	0	0	0	0	0	0
14.32	3	0	3.6	0	21.4	3.6	14.2	0	0	0	0	0	0
2.4	4	0	0	14.3	25.0	14.3	3.6	7.2	3.6	0	0	0	0
	Average	0	2.8	10.2	21.0	7.3	8.2	1.8	1.8	0.9	0	0	0
T 246	1	0	0	3.6	3.6	0	7.2	17.9	7.1	0	3.6	0	0
	2	0	3.7	14.8	18.5	7.4	7.4	11.1	0	0	0	0	0
16.47	3	0	7.2	3.6	7.1	3.6	0	3.6	3.6	3.6	0	0	0
2.9	4	0	0	7.7	3.8	3.8	3.8	7.7	7.7	0	3.8	0	0
	Average	0	2.7	7.4	8.3	3.7	4.6	10.1	4.6	0.9	1.8	0	0
T 471	1	0	0	3.7	22.2	7.4	0	0	0	0	0	0	0
	2	0	0	7.4	18.5	0	0	3.7	7.4	3.7	3.7	0	0
16.07	3	0	0	11.1	7.4	11.1	7.4	3.7	3.7	0	3.7	0	0
2.8	4	0	0	0	15.3	7.6	7.7	3.8	11.5	0	0	0	0
	Average	0	0	5.6	15.9	6.5	3.8	2.8	5.7	0.9	1.9	0	0
Ko-ran	1	0	0	0	7.7	3.8	19.2	3.8	0	0	0	0	0
	2	0	4.0	0	12.0	4.0	4.0	0	8.0	0	0	0	0
16.86	3	0	0	3.7	0	14.8	11.1	7.4	0	3.7	0	0	0
3.0	4	0	0	0	3.8	3.8	3.8	7.7	0	7.6	0	0	0
	Average	0	1.0	0.9	5.9	6.6	9.5	4.7	2.0	2.8	0	0	0
T 462	1	0	0	0	4.6	4.6	4.6	0	4.6	4.6	4.6	0	0
	2	0	0	0	0	4.0	4.0	0	12.0	4.0	0	4.0	0
18.82	3	0	0	4.6	0	4.6	0	9.1	9.2	0	0	0	0
3.4	4	0	0	0	8.7	4.3	4.3	0	4.3	0	4.3	0	0
	Average	0	0	1.2	3.3	4.4	3.2	2.3	7.5	2.2	2.2	1.0	0
T 463	1	0	0	4.2	0	4.2	4.2	20.9	4.2	0	0	0	0
	2	0	0	0	0	7.7	0	0	0	0	3.8	0	0
18.54	3	0	0	4.3	0	13.0	4.3	0	4.3	4.3	8.7	0	0
3.4	4	0	0	4.2	4.2	8.4	4.2	0	0	0	8.3	0	0
	Average	0	0	3.2	1.1	8.3	3.2	5.2	2.1	1.1	5.2	0	0
Imperial	1	0	0	0	0	0	0	0	0	0	16.0	4.0	0
	2	0	0	0	0	0	0	0	0	0	7.6	0	0
25.98	3	0	0	0	0	0	0	0	0	14.8	14.8	0	3.7
5.0	4	0	0	0	0	0	0	0	0	0	3.8	7.7	0
	Average	0	0	0	0	0	0	0	0	3.7	10.6	2.7	0.9

Table 46. Mean incubation period and infection percent of BSM resistant varieties

Experiment	Variety	Mean incubation period Day	Infection %	Experiment	Variety	Mean incubation period Day	Infection %
A	T 40	6.01*	82.3	B	T 239	7.70	74.8
	T 101	7.13	96.1*		T 241	8.68	66.8
	T 121	5.89*	96.0*		T 245	9.43	51.9
	T 122	6.74	91.3*		T 246	9.58	47.4
	T 126	8.07	76.1		T 251	8.68	59.3
	T 167	7.65	97.6*		T 270	7.08*	71.0
	T 169	7.72	81.2		Harbin 2-rowed	6.09	93.6
	Harbin 2-rowed	6.20	96.3		Ko-ran	10.07	46.6
	Ko-ran	8.41	66.5		Imperial	13.37	36.7
	Imperial	10.74	51.4				
C	T 381	8.57	61.7	D	T 471	8.54	67.6
	T 427	7.51	54.6		T 501	7.39	88.3
	T 433	5.99*	83.7*		T 508	8.27	81.1
	T 434	6.88	70.9		T 556	6.03*	92.3*
	T 462	10.08	57.2		T 567	8.87	73.4
	T 463	9.69	45.0		T 573	8.88	64.2
	T 470	8.96	59.7		T 653	8.50	66.9
	Harbin 2-rowed	5.54	93.8		Harbin 2-rowed	5.76	98.1
	Ko-ran	8.08	40.4		Ko-ran	9.19	55.2
	Imperial	12.99	44.5		Imperial	13.76	25.0
E	T 640	6.63*	95.0*	F	T 239	12.22	49.3
	T 658	9.36	87.3		T 246	16.47	44.2
	Harbin-zairai	8.09	91.2		T 462	18.82	27.2
	Sanfunga	9.54	96.2*		T 463	18.54	29.3
	Modjo	9.28	97.3*		T 471	16.07	43.0
	C. I. 3212-1	9.33	100.0*		T 508	13.66	62.6
	Wien		0		T 427	14.32	51.4
	Harbin 2-rowed	6.53	100.0		Harbin 2-rowed	8.40	88.8
	Ko-ran	9.84	71.2		Ko-ran	16.86	33.6
	Imperial	14.52	50.3		Imperial	25.98	18.1
G	T 101	11.62	41.0	H	T 126	13.40	51.7
	T 121	8.36*	88.1*		T 239	12.14	62.1
	T 245	13.36	38.7		T 246	14.26	48.2
	T 433	9.52*	82.3*		T 463	14.72	44.2
	T 467	7.89*	96.0*		T 470	13.13	32.5
	T 573	12.52	58.2		T 653	14.42	60.1
	T 658	12.73	47.2		Harbin zairai	12.61	67.6
	Harbin 2-rowed	8.60	83.4		Harbin 2-rowed	9.74	95.7
	Ko-ran	13.67	31.0		Ko-ran	14.37	35.3
	Imperial	19.0	1.0		Imperial	17.11	21.0
I	T 194	8.02*	81.3*	J	T 40	8.51*	75.1
	T 381	9.58	63.8		T 167	10.40	78.3
	T 462	11.13	76.2		T 251	9.98	62.1
	T 471	11.00	36.0		T 270	9.02	87.8*
	T 501	9.80	77.6		T 508	9.47	89.4*
	T 640	8.12*	86.1*		Sanfunga	10.41	87.8*
	T 658	10.61	67.4		Modjo	11.79	51.0
	Harbin 2-rowed	8.05	91.8		C. I. 3212-1	10.51	58.7
	Ko-ran	11.09	50.6		Harbin 2-rowed	7.99	90.5
	Imperial	13.64	27.3		Ko-ran	11.33	38.6
					Imperial	14.00	16.1

* No significant difference compared with Harbin 2-rowed

Correlation co-efficient between mean incubation period and infection percent

A : $r = -0.8284$ $(0.01 > p > 0.001)$
B : $r = -0.9126$ $(0.001 > p)$
C : $r = -0.7253$ $(0.02 > p > 0.01)$
D : $r = -0.9642$ $(0.001 > p)$
E : $r = -0.8359$ $(0.001 > p)$

Table 47. Resistance-grade of BSM resistant varieties

Variety	Resistance-grade (Average)	Experiment									
		A Oct.** 6	B Oct. 6	C Oct. 6	D Oct. 9	E Oct. 11	F Nov. 15	G March 15	H March 20	I March 31	J April 9
Wien	>5.0	—	—	—	—	5.0	—	—	—	—	—
Imperial	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
T 463	3.5	—	—	3.7	—	—	3.4	—	3.3	—	—
T 462	3.4	—	—	3.8	—	—	3.4	—	—	3.0	—
Ko-ran	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
T 470	3.0	—	—	3.4	—	—	—	—	2.5	—	—
Modjo	3.0	—	—	—	—	2.7	—	—	—	—	3.3
T 246	2.9	—	2.8	—	—	—	2.9	—	3.0	—	—
T 381	2.9	—	—	3.2	—	—	—	—	—	2.5	—
T 653	2.9	—	—	—	2.6	—	—	—	3.1	—	—
C. I. 3212-1	2.8	—	—	—	—	2.7	—	—	—	—	2.8
T 471	2.8	—	—	—	2.6	—	2.8	—	3.0	—	—
T 245	2.8	—	2.7	—	—	—	—	2.9	—	—	—
T 567	2.8	—	—	—	2.8	—	—	—	—	—	—
T 126	2.7	2.7	—	—	—	—	—	—	2.6	—	—
T 573	2.7	—	—	—	2.8	—	—	2.6	—	—	—
T 658	2.7	—	—	—	—	2.7	—	2.6	—	2.7	—
Sanfunga	2.6	—	—	—	—	2.8	—	—	—	—	2.4
T 427	2.5	—	—	2.5	—	—	2.4	—	—	—	—
T 167	2.4	2.3	—	—	—	—	—	—	—	—	2.4
T 169	2.4	2.4	—	—	—	—	—	—	—	—	—
T 241	2.3	—	2.3	—	—	—	—	—	—	—	—
T 251	2.3	—	2.3	—	—	—	—	—	—	—	2.2
T 508	2.2	—	—	—	2.5	—	2.3	—	—	—	1.9
Harbin-zairai	2.1	—	—	—	—	1.9	—	—	2.2	—	—
T 434	2.1	—	—	2.1	—	—	—	—	—	—	—
T 101	2.0	1.8	—	—	—	—	—	2.1	—	—	—
T 501	2.0	—	—	—	1.9	—	—	—	—	2.1	—
T 239	1.9	—	1.8	—	—	—	1.9	—	2.0	—	—
T 270	1.6	—	1.5*	—	—	—	—	—	—	—	1.6
T 122	1.5	1.5	—	—	—	—	—	—	—	—	—
T 433	1.4	—	—	1.3*	—	—	—	1.4*	—	—	—
T 40	1.1	0.8*	—	—	—	—	—	—	—	—	1.3*
T 467	1.1	—	—	—	—	—	—	1.1*	—	—	—
T 556	1.1	—	—	—	1.1*	—	—	—	—	—	—
Harbin 2-rowed	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
T 194	1.0	—	—	—	—	—	—	—	—	1.0*	—
T 640	1.0	—	—	—	—	1.0*	—	—	—	1.0*	—
T 121	0.8	0.7*	—	—	—	—	—	0.9*	—	—	—

* No significant difference compared with standard variety, Harbin 2-rowed, as to the mean incubation period

** Date of inoculation (1959)

Wien was only a variety resistant to the virus extremely, and the resistance-grade of this variety was estimated to be above 5.0. Imperial (resistance-grade 5.0) was the most resistant variety next only to Wien. The resistance-grade of Turkish barleys T 462 and T 463 were estimated to be slightly higher than Ko-ran (3.0). Varieties T 245, T 246, T 381, T 470 and T 471 were found to be as equal as or slightly lower than Ko-ran in resistance. Resistances of Abyssinian varieties Medjo (C. I. 3212) and C. I. 3212–1, which had been selected to be resistant to California "E" and other strains of BSMV in U. S. A. by Timian and Sisler (1955), were found to be as resistant as Ko-ran to the author's virus strain.

3. Discussion

A discussion must be made here to see whether the resistance-grades of standard varieties given for the above experiments are appropriately indicated. As seen in Figure 8 and Tables 45–46, compared with the case of susceptible varieties, delayed and scarce infections were found in the case of resistant varieties. However, the incubation period and the infection percentage were not uniform with each of the different varieties and the time of inoculation experiment. Therefore, the resistance-grade of barley must be determined in due consideration to the environmental conditions during the individual experiments. For this purpose, a set of 3 standard varieties were used. And, this seemed to be effective to avoid the confusion in estimating the infection which had been caused by variable environmental conditions during the experiment. Further, the author considered that, the resistance-grade of a variety could be estimated from the mean incubation period by comparing its mean incubation period with those of the two standard varieties which gave the nearest values to those of the unknown's. Although Harbin 2-rowed was not extremely susceptible, it was used as the susceptible standard for the efficiency of the resistance screening.

As seen in Figure 9A, there was found approximately linear relationship between the mean incubation period and the resistance-grade of 3 standard varieties is most of the experiments. Therefore, the values of resistance-grade under discussion seemed to be appropriate for the purpose of the present experiment. There were highly significant correlations among the mean incubation period and the infection percentage in all the experiments with various varieties. However, the mean incubation period was found to be more appropriate in character than the infection percentage for the estimation of the resistance-grade, because of the variability of the latter with conditions (Fig. 9B).

B. FIELD INOCULTION TESTS WITH VARIOUS RESISTANT VARIETIES

A field inoculation test was made to see whether the varieties selected by the series of seedling inoculation tests were also found to be resistant in the field inoculation test, and whether the values of resistance-grade was still found to be appropriate in the field conditions. This experiment corresponds to the final step of screening for the BSM resistance.

1. Materials and methods

Seven varieties with various resistance-grades and 3 standard barleys were used in the experiment: They were Harbin 2-rowed (resistance-grade 1.0), T 239 (1.9), T 508 (2.2), T 427 (2.5), T 471 (2.8), T 246 (2.9), Ko-ran (3.0), T 462 (3.4), T 463 (3.5), and Imperial (5.0). Seedlings, which had been inoculated 2 days before, were transplanted (November 24, 1959) in 4 replicated field plots consisted of each 23–25 seedlings. Between the plots extremery resistant variety Wien was sown to avoid the natural contact transmission. Further inoculations were made to obtain the good infections with the same way as described in Chapter VII, on January 7 and on March 7, 1960. Infections were observed for the period March to May.

Plant height and sterility of the initial 5 culms in the 10 plants taken from each plot were examined. Seed transmission and 1,000 kernel weight were also tested.

2. Results

Table 48 shows the occurrence of infection for the period early March to early May. Although the results were observed to be somewhat uneven, the higher the resistance-grade was, the earlier the infection and higher the infection percentage was noticed generally. Above all, the varieties Ko-ran, T 462, T 463 and Imperial, of which resistance-grades had been noticed to be above 3.0, showed very rare or no infection.

Table 48. Infection of BSM resistant varieties by the field inoculation

Variety	Resistance-grade	Infection percent					
		March 7	23	April 13	24	May 2	10
Harbin 2-rowed	1.0	26.0	49.9	53.1	67.7	100	100
T 239	1.9	3.2	7.4	42.9	60.2	80.6	84.9
T 508	2.2	0	1.1	9.9	17.5	33.9	36.1
T 427	2.5	0	1.1	17.7	35.8	54.7	57.0
T 246	2.9	0	0	0	0	4.5	4.5
T 471	2.8	0	0	4.3	5.5	19.5	19.5
Ko-ran	3.0	0	0	0	0	2.2	2.2
T 462	3.4	0	0	0	0	0	0
T 463	3.5	0	0	0	0	0	0
Imperial	5.0	0	0	0	0	1.1	1.1

The results of the investigations with harvested materials were summarized in Table 49. Except susceptible Harbin 2-rowed, the varieties, of which characters were markedly affected by the disease infection, were as follows: The reduction in the plant height was found in T 239, plant yield was in T 239, T 508, T 427, and T 246, and also fertility was found in both T 239 and T 427. On the other hand, however, the kernel weight was not reduced markedly in all the resistant varieties. Seed transmission was not always observed in all of the inoculated plants, but only in the infected plants. There was a tendency that

Table 49. Effect of BSM infection of agronomic characters of resistant varieties, and on seed transmission of the disease

Variety (Infected plot, plant)	Resistance-grade	Item	Plant height cm	Sterility %	1000 kernel weight g	Plant yield g	Seed transmission %
Harbin 2-rowed (4, 40)	1.0	A	127.8	2.0	41.8	16.9	0
		B	129.2	7.3	—	10.3**	—
		C	129.2	7.3	33.9**	10.3**	15.7
T 239 (4, 40)	1.9	A	144.6	1.8	25.9	16.3	0
		B	135.1*	5.1*	—	8.8**	—
		C	135.1*	5.1*	23.2	8.8**	6.1
T 508 (4, 26)	2.2	A	139.4	3.4	33.8	19.0	0
		B	130.1	5.2	—	14.6**	0
		C	131.9	6.0	29.8	14.0**	3.8
T 427 (4, 39)	2.5	A	147.2	5.3	27.2	16.0	0
		B	139.6	14.3	—	8.5*	0
		C	139.3	14.3*	24.6	8.8*	3.3
T 471 (3, 4)	2.8	A	136.4	1.6	29.4	15.8	0
		B	135.9	1.6	—	18.5	0
		C	136.7	1.9	28.0	14.3	0.7
T 246 (4, 11)	2.9	A	144.3	1.7	28.0	15.2	0
		B	144.0	2.7	—	13.6	0
		C	140.8	2.5	21.8	9.2*	1.6
Ko-ran (1, 1)	3.0	A	131.4	2.4	27.4	17.3	0
		B	131.6	3.6	—	16.1	0
		C	132.0	3.5	28.7	17.3	1.9
T 462 (0, 0)	3.4	A	134.2	2.5	33.3	18.8	0
		B	134.1	2.9	33.2	18.4	0
		C	—	—	—	—	—
T 463 (0, 0)	3.5	A	138.4	4.3	31.6	18.9	0
		B	136.2	3.7	34.6	18.4	0
		C	—	—	—	—	—
Imperial (2, 2)	5.0	A	132.0	1.4	37.2	16.6	0
		B	—	0.5	—	15.4	0
		C	—	1.4	33.3	14.9	0.2

A : Data from the uninoculated control plot
B : All the plant in inoculated plot ; healthy plants in inoculated plot as to seed transmission
C : Infected plant

the higher the resistance-grade, the less the seed transmission: seed transmission in susceptible Harbin 2-rowed amounted to 15.7%, however, in T 508 (resistance-grade 2.2) and Ko-ran (3.0), it was 3.8 and 1.9% respectively.

3. Discussion

Prior to the above experiment, which was carried out in 1959–60, the two similar field experiments had been made in 1957–8 and 1958–9. However, these experiments had been a failure, since the satisfactory infections had not been obtained in resistant barleys in spite of the duplicated inoculations during March and April. In the view of this failure, in 1959–60′ experiment, barely seedlings were inoculated at their 1st leaf stage in the glasshouse, and further duplicated field inoculations were applied. Good infections were obtained even in resistant varieties by these heavy inoculations.

The results under discussion showed that, the resistance-grade, which was estimated from the seedling inoculation test, was also found to be applicable in the case of field inoculation test. The varieties, of which resistance-grade was around 2.5, were found to be somewhat resistant to the field inoculation. But, the effect of infection on the fertility and the plant yield of these varieties was not negligibly small when the plant was infected. T 471 (resistance-grade 2.8), T 246 (2.9), Ko-ran (3.0) and other more resistant varieties showed high resistances to the heavy inoculation in the field. The effect of the disease infection was apparently negligible, and also the occurrence of seed transmission was very rare even in the case of infected plants of these varieties. Although T 462 (resistance-grade 3.4) and T 463 (3.5) were inoculated at their seedling stages, they were not infected throughout their growth. Since some of the plants of more resistant varieties, Ko-ran (3.0) and Imperial (5.0), had come to be infected after their heading time, further investigations were required to clarify the resistance of T 462 and T 463.

From the results mentioned above, the resistances of Ko-ran, T 462, T 463, and Imperail, as well as Wien, of which resistance-grades at seedling stage were estimated to be above 3.0, seemed to be available for practical use.

C. INHERITANCE OF BSMV RESISTANCE OF WIEN AND IMPERIAL

Wien and Imperial were found to be highly resistant to BSMV from the results obtained in the previous sections. In this section, F_2 segregations were investigated to see the inheritance of resistance of these two varieties.

1. Materials and methods

Crosses were made among resistant (Wien, Imperial) and susceptible (Akashinriki, Hosogara No. 1, Chevalier) varieties. Seedlings of F_1, F_2 plants and parent varieties were inoculated at their primary leaf stage. F_2 plants, in which Wien was used as the resistant parent, were inoculated with 10 times diluted plant juice, and in the case of Imperial as the resistant parent were with 30 times diluted juice. Infected seedlings were recorded daily, and also the occurrence of necrotic symptoms, which was the characteristic initial symptom of susceptibility (Chapter V and Plate VII) in the infected seedlings, was observed.

2. Results

Results were shown in Table 50 and Table 51. Distinct local necrotic lesions

Table 50. F_2 segregation of BSM resistance from crosses among resistant varieties, Wien and Imperial, and susceptible varieties

Cross ♀	Cross ♂	Symptoms on F_1 seedling	Observed numbers in F_2 S	M	R	Total		χ^2 for 3 : 1	p
Imperial	× Akashinriki	S	237	19	50	306	S: (M+R)	0.980	0.5–0.3
Akashinriki	× Imperial	S	227	21	52	300	S: (M+R)	0.071	0.8–0.7
Imperial	× Hosogara No. 1	S	225	26	47	298	S: (M+R)	0.040	0.9–0.8
Hosogara No. 1	× Imperial	S	254	21	51	326	S: (M+R)	1.476	0.3–0.2
Wien	× Akashinriki	R*	77	12	232	321	(R+M) : S	0.174	0.7–0.5
Akashinriki	× Wien	R*	93	12	266	371	(R+M) : S	0.001	1.0–0.9
Wien	× Chevalier	R*	73	53	173	299	(R+M) : S	0.055	0.9–0.8
Chevalier	× Wien	R*	74	10	222	306	(R+M) : S	2.122	0.2–0.1

Symptoms on F_1 seedling
S : Susceptible
R* : Symptoms characteristic to the hybrid with resistant variety, Wien
Symptoms on F_2 seedling
S : Susceptible symptoms with characteristic necrtotic initial symptom
M : Im-type mottling without necrosis (hybrid with Imperial). Similar symotom with R* in F_1 (hybrid with Wien)
R : No symptom — not infected

Table 51. Infection of F_2 hybrid among resistant varieties, Wien and Imperial, and susceptible varieties by sap inoculation

Cross or parent ♀	♂		Seedling examined	Incubaion period 6days	8	10	12	14	16	3weeks	Seedling infected	Seedling uninfected
Imperial	× Akashinriki	F_2	306	123	46	48	20	7	5	7	256	50
Akashinriki	× Imperial	F_2	300	151	41	26	15	6	2	7	248	52
	Akashinriki	P	89	61	13	2	0	0	0	0	76	13
	Imperial	P	97	0	0	0	0	0	1	2	3	94
Imperial	× Hosogara No. 1	F_2	298	129	48	29	24	12	3	6	251	47
Hosogara No. 1	× Imperial	F_2	326	156	55	40	15	5	3	1	275	51
	Hosogara No. 1	P	51	32	14	2	0	0	0	0	48	3
	Imperial	P	104	0	0	0	0	0	0	2	2	102
Wien	× Akashinriki	F_2	321	17	34	20	4	1	1	11	89	232
Akashinriki	× Wien	F_2	371	35	38	11	3	2	1	15	105	266
	Akarhinriki	P	43	29	13	0	0	0	0	0	42	1
	Wien	P	59	0	0	0	0	0	0	0	0	59
Wien	× Chevalier	F_2	299	32	13	17	17	27	9	11	126	173
Chevalier	× Wien	F_2	306	16	21	14	14	11	4	4	84	222
	Chevaleir	P	51	25	9	7	1	0	0	0	42	9
	Wien	P	58	0	0	0	0	0	0	0	0	58

appeared on the inoculated leaf of the seedlings of F_1 crossed among Wien and susceptible barleys. However, systemic development of mild but characteristic symptoms was rather slow (Plate VII, 1). F_2 segregation of these crosses showed

a satisfactory fit to a ratio of 1 plant with susceptible symptoms (S) to 3 plants (R+M) without symptoms (R) and with mild but characteristic symptoms (M) (Plate VII, 2). On the other hand, F_1 seedlings grown from the crosses among Imperial and susceptible barleys showed susceptible symptoms (Plate VII, 1). F_2 families showed a satisfactory fit to a ratio of 3 with susceptible symptom (S) to 1 (R+M) with no symptom (R) and non-necrotic symptoms (M) (Plate VII, 3). As seen in Table 51, the definite segregation of F_2 plants was not noticed by the number of infected seedlings alone.

3. Discussion

From the results obtained in this section alone, the knowledge on the inheritance of BSMV resistance of Wien and Imperial seems to be unsufficient. Further close genetical studies must be required to obtain many other evidences. Resistant varieties except Wien were infectious to the virus in various degrees, and their susceptibilities were variable with the conditions as mentioned in the above sections. Furthermore, most of the infected plants of these resistant varieties showed mosaic symptoms accompanied with necrosis (susceptible type symptom). However, in the seedlings of Imperial, the symptoms were mottling without the characteristic "V" necrosis. Under these circumstances, although the susceptible symptoms were pointed out rather easily, the estimation of seedlings with resistant type symptoms (M in the Table) were found to be difficult, especially in the cases of F_2 crossed Wien as the resistant parent, as seen in Table 51. However, when we regard seedlings without susceptible type necrosis as the phenotype of resistance, even though the seedlings show severe mottling, the inheritance of resistance of Wien and Imperial is likely to be controlled at least by a dominant and a rescessive gene, respectively.

IX. CONTROL OF BSM

On account of the seed-borne and contact transmissbile nature of BSMV, the following types of control measure for the disease will be recommended:

(1) Disinfection of diseased seeds, or selection of virus-free seeds by physical and chemical treatment, or by seedling test.
(2) Roguing and other measures to curf the field occurrence of the disease caused by contact transmission.
(3) Use of resistant varieties, or exhaustive renewal of virus infected variety.

Mckinney (1954) reported that, BSMV was not inactivated by the hot water treatment at 55–58°C. Hagborg (1955) reported also that the virus was not inactivated when barley seed was heated in CCl_4 at 76.8°C for 2 hours. The partial control of the disease by screening was suggested by McKinney (1951) on account of the fact that infected seeds tend to be small in size. The detailed studies on the seed-borne symptoms and the seed testing technique were made by McKinney (1954), and Hampton and others (1957). The author also has in-

vestigated these problems in this study, and presented some applicable information for the seed certification.

Spread of BSM in barley field is caused by the natural contact transmission. Hagborg (1960) reported the interesting results that, both skim milk and whey reduced the spread of field infection when applied as a spray.

Timian and Sisler (1955) and Sisler and Timian (1956) found the BSMV resistance in several varieties of Abyssinian barley and studied further about the inheritance of resistance of Modjo and C. I. 3212–1. As mentioned in the previous Chapter, Wien, Imperial and others were selected for BSMV resistance, and were tested on their field resistances. Further, the author obtained some information on the inheritance of BSMV resistance of Wien and Imperial. Since many difficult problems of breeding for BSMV resistance will be involved, it will be long before the use of resistant barleys comes into practice. Although the renewal of infected variety may be an effective method to control the disease so far as that variety is concerned, it will be no more than the temporary effect, as seen in the cases of semi-sterile barley in Hokkaido, unless any other counterplain is considered.

Availabilities of seedling test and resistant varieties on the control of BSM were already investigated in the previous chapters. In this Chapter, the effects of heat treatment of infected seeds, seed selection through the shieves, and roguing of diseased plants in the field on the control of BSM are to be discussed.

A. HEAT TREATMENT OF INFECTED SEED IN DRIED CONDITION

The thermal death point of barley seed in water is not so higher than that of BSMV in plant juice. However, in dried conditions, heat torelance of barley seed is higher than in the case of hot-water treatment. Thermal inactivation of BSMV in seed was investigated, heating infected seed in dried condition at 70°C and 75°C for 10 minutes, and also at 70°C for 15 minutes. As seen in Table 52, the virus was not inactivated in all the treatments. When barley seed was heated at 75°C for 10 minutes germination rate was found to be normal, but marked retardation of germination was observed.

Table 52. Effect of heat on the seed transmission of BSM infected seed in dried condition

Treatment		Germtinaion	Seed transmission
Temperature °C	Time min.	%	%
Untreated		85.5	30.3
70	10	85.5	30.9
70	15	77.5	26.4
75	10	77.5*	33.0

Averages of 4 replications with 50 seeds of BSM infected Hosogara No. 1
* Retarded germination

B. EFFECT OF SEED SCREENING WITH SIEVE

Stripe mosaic infected barley seed tends to reduce the kernel weight and the proportion of larger seed, but to increase the proportion of small and shrivelled seed, as mentioned in Chapter VII, 5. As already suggested by McKinney (1951), partial control of the disease by seed screening with a sieve should be expected. In this section, the control effect of screening for the seeds which had been infected by various routes of infection was studied.

1. Materials and methods

With 2.7, 2.5 and 2.0 mm or 2.5 and 2.0 mm sieves, 2-rowed barley (Harbin 2-rowed, Chevalier, Shunsei) or 6-rowed barley (Akashinriki, Kobinkatagi, Hosogara No. 1, Kenyoshi No. 3) seeds were screened, respectively. These barley seeds were taken from the diseased plants, which had been infected by seed transmission, natural contact transmission or sap inoculation, and also taken from the field where the disease had occurred. Seed transmission in about 100 seeds passed through the sieves was examined by inoculation testing method.

2. Results

Table 53 shows the result of the experiment with different infection routes and seed sizes. High percentages of seed transmission were detected in the seeds obtained from the seed-borne infected plants irrespective of seed size. In the seeds taken from the plants infected by natural contact transmission, there was no seed transmission in large seeds above 2.5 mm or 2.7 mm in seed width in Akashinriki or Harbin 2-rowed and Chevalier, respectivery. Similary, seed transmission was found to be very rare in large seeds taken from the diseased plants infected by inoculation. It was distinct in the case of Chevalier that, the later the inoculation time, the less the seed transmission in large seeds. In the case of the seeds obtained from the field where the disease had been observed, seed transmission observed in large seeds was not always rare, but it was occasionally observed to be much more than those found in smaller seeds. However, there was a tendency that the seed transmission in large seeds was rare when the percentage of seed transmission in unsieved seeds was small.

3. Discussion

Part of the results in this section may be considered to be self-evident in reference to the results obtained in Chapter VII. Development and maturation of the seed are affected by natural contact transmission and late inoculation, but not so by seed-borne infection and early inoculation. Moreover, the proportion of seed transmission seemed to be decided in the early stages of seed development. Therefore, it must be accepted that the seed transmission found in the seeds obtained from seed-borne infected barley is rather even, irrespective of seed size. Then, by the same reason, the rarity of seed transmission in the seeds from barley plant infected by natural contact transmission or late inoculation also seems to be acceptable.

Table 53. Seed transmission of BSMV in diseased seeds with different size

Route of infection	Variety	Whole sample	Seed size (mm)			
			>2.7	2.7–2.5	2.5–2.0	<2.0
Seed transmission	Akashinriki	65.5		61.7	68.8	63.5
	Kenyoshi No. 3	81.1		68.4	75.3	87.1
	Shunsei	78.8	80.3	73.0	76.8	69.8
	Chevalier	50.0	35.2	49.5	66.7	52.8
	Harbin 2-rowed	72.0	63.3	80.8	79.8	69.7
Contact transmission	Akashinriki	2.7		0	6.1	2.5
	Chevalier	10.6	0	4.0	15.3	7.2
	Harbin 2-rowed	1.9	0	0	6.3	6.4
Inoculation	Akashinriki*			0	4.5	9.4
	Hosogara No. 1*			4.7	51.6	18.1
	Harbin 2-rowed*		1.2	2.2	60.2	66.0
	Harbin 2-rowed	29.5	0	4.0	21.4	49.5
	Chevalier 1)	38.4	0	4.1	50.5	62.0
	Chevalier 2)	33.2	0	0	33.3	58.3
	Chevalier 3)	3.2	0	0	4.0	11.6
Infected field	Akashiriki	18.8		57.2**	22.0	10.3
	Akashiriki	5.6		0	3.2	10.0
	Kobinkatagi	3.1		0**	2.1	2.5
	Kobinkatagi*			3.8	14.3	11.4
	Hosogara No. 1	46.6		66.7**	61.5	40.7
	Kenyoshi No. 3	17.0		0	10.1	29.7
	Kenyoshi No. 3	76.0		44.5**	78.6	71.3
	Chevalier	12.2	1.8	16.0	12.3	12.9
	Chevalier*		3.4	5.6	8.3	7.9
	Harbin 2-rowed	28.6	68.0	56.7	16.2	16.7

Seed obtained in 1960, * seed obtained in 1959, ** less than 30 seeds.
Seeds were taken from the plants which were inoculated 1) February 20, 2) March 31, 3) April 18, 1956.

The most important case in the practical use of seed screening for the control of the disease is not any of the specific infection routes mentioned above, but the case of the seeds obtained from the diseased field, where the diseased plants were infected by various infection routes. The results show that no control effect of seed screening is observed in the seeds in which abundant seed infections are found. However, partial control effect is considered to be expectable in the seeds in which little seed infection is found. And, the use of a sieve with rough eyes above 2.5 or 2.7 mm, if available, seems to be effective to reduce the population of infected seeds.

C. EFFECT OF ROGUING FOR THE CONTROL OF BSM

Since the first occurrence and secondary spread of BSM in the field are

caused by seed- and contact transmission respectively, the removal of diseased plant which is the infection source of contact transmission will be effective for the control of this disease.

1. Materials and methods

Seeds of Akashinriki and Harbin 2-rowed obtained from the diseased field were used for the experiments. Each 5 seeds was sown about 6 cm apart in 27–28 places per plot. Field plots, such as (I) no treatment, (II) rogued on January 9, and (III) rogued several times until March 1, were arranged in order with Latin square. Highly resistant barley Wien was sown for the buffer among plot in the same row. Infected plants were surveyed several times after late April, when the infection caused by contact transmission became to appear. Yield, proportion of large seed, 1,000 kernel weight and the seed transmission were examined with 25 plant-lots per plot.

2. Results

The effect of roguing on the reduction of infected plants was shown in Table 54. No diseased plant was observed after the roguing on March 1 in the plot (III) in both varieties Akashinriki and Harbin 2-rowed. In the case of Akashinriki (II), all of the diseased plants were not completely removable by only a roguing, and the infections amounted to 11.6% at the final observation. On the other hand, all of the diseased plants could be rogued by a field observation in the case of Harbin 2-rowed (II). Infections were markedly increased during April and early May, and they amounted to 84.8 and 45.6% in the non-treated plots (I) of Akashinriki and Harbin 2-rowed respectively.

The effect of roguing on yield and other characters of barley was seen in Table 55. The results obtained here seemed to be somewhat uncertain, because the experimental error was not negligible. However, there was noticed the tendency that, the yield, the proportion of small seed was reduced by the roguing. Especially, the tendency was recognized to be more marked in the plot III than in II. Seeds obtained from the plots III, where diseased plants were removed

Table 54. Effect of roguing on reducing diseased plant in barley field

Variety	Treatment	Number of plant rogued			Number of plant-lot in field plot	Diesased plant (%)			
		Jan. 9	March 1	Total		April 29	May 6	May 12	May 25
Akashinriki	I	—	—	0	26.3	75.8	83.6	84.8	84.8
	II	46.3	—	46.3	26.0	6.4	9.0	9.0	11.6
	III	51.3	0.3	51.7	26.7	0	0	0	0
Harbin 2-rowed	I	—	—	0	27.7	40.8	45.6	45.6	45.6
	II	13.0	—	13.0	27.3	0	0	0	0
	III	10.7	2.0	12.7	26.0	0	0	0	0

I: Non-treated control. II: Rogued on Jan. 9. III: Rogued several times until March 1. Data show the averages of triplicated plots.

Table 55. Effect of roguing on yield and seed infection of barley

Variety	Treatment	Yield (25plant-lots)	Proportion of Large seed (>2.0 mm)	Proportion of Small seed (<2.0 mm)	1000 kernel weight	Seed transmission
		g	%	%	g	%
Akashinriki	I	723*	54.8*	45.2*	19.5	9.7*
	II	877	59.2	40.8	20.7	4.2
	III	925	65.4	34.6	21.5	0
	LSD (0.05)	180.20	10.14	10.14	—	4.49
			(>2.5mm)	(<2.0mm)		
Harbin 2-rowed	I	829	55.4	4.1	38.1	1.9
	II	867	58.2	3.3	40.4	0
	III	903	59.3	3.6	40.4	0

Treatment : See Table 54

completley, were found to be apparently virus-free.

3. Discussion

Roguing for the control of BSM is found to be effective by the results obtained in this section. Early removal of diseased plants from the field also proved to be an effective control measure. Diseased plants infected by contact transmission can be rogued without difficulty according to their characteristic and definite symptoms. However, for the effective control of the disease, it is rather important to rogue the first occurrence, which is seed-borne infected plants and plays a role as the inoculum source of the secondary spread. Since the seed-borne infected plants are missed to detect even with the careful observations in some instances, the field observations should be made as frequent as possible. And, when the infections caused by contact transmission are found, further careful observation to find the seed-borne infected plants should be made with the neighboring plants.

D. GENERAL DISCUSSION ON THE CONTROL OF BSM OCCURRENCE

For the practical use at the present time, selection of virus-free seeds by seedling test and roguing are the effective methods to control the occurrence of BSM. Further, partial control measure of seed screening with sieve is recommendable in some cases, though it is not so in some other cases. Under these circumstances, the use of three methods put together in various combination seems to be the best way for the control of the disease. Inoculation testing method described in Chapter VII, is not available for propagating the virus-free seed from the seedlings tested. For the purpose of propagation of virus-free seeds, either one of the following methods for the symptomless infection must be recommendable; comparatively long period of inspection of seedling, and subinoculation, or examination of seedling under the optimum light and

temperature conditions. Application of whey or skim milk reported by Hagborg (1960) is an interesting method to curf the secondary occurrence of BSM in the field. However, in the case of seed propagation, further seed certification will be needed for the complete control of the disease.

Complete effect of the renewal of variety for BSM control will be recommendable if only the thorough and simultaneous application, for, even the new variety may be susceptible to the disease.

Although some problems for breeding will remain into the future, the use of the resistance of Wien selected in Chapter VIII seems to be recommendable so long as the virus strain infectious do not appear on this variety.

X. GENERAL DISCUSSION

One of the major purpose of this study was to identify BSMV as the possible factor of the semi-sterility of barley in Hokkaido. The disorder of barley, especially that of 2-rowed barley, which had been called as the degeneration of barley in some cases, had been studied agronomically and physiologically, but the cause had remained unknown for many years. BSMV was first reported by McKinney in 1951 in U. S. A. as the seed transmissible virus disease. This disease, however, had been known as a non-parasitic disease, barley false stripe, in U. S. A. since about 1910. In Canada, the situation had been about the same as in U. S. A. as reported by Hagborg (1951, 1954). It was a very remarkable fact that the cause of this disease had not been determined for a long time both in North America and in Japan, though the situation of the disease in these countries was not quite similar. Further, it was interesting to note that, compared with the case of virus disease of potato in Europe in the past, the successive yield reduction caused by the semi-sterility of barley in Hokkaido supported an opinion of the degeneration in barley in some cases. BSMV had not been in the mind of the author when he started to study on the semi-sterile barley, however, in the cource of the investigation the occurrence of the disorder with stripe disease-like symptoms characteristic to semi-sterile barley strain indicated that a virus was responsible for the disease.

The basis upon which the argument that BSM is the major cause of the semi-sterility of barley in Hokkaido is made are as follows:

1) Seed transmission of BSMV was confirmed in most of the semi-sterile strains of barley which had been used in the studies on barley sterility by Yamamoto, and other workers.
2) Natural occurrence of BSM was ascertained on the field survey in Hokkaido in 1957, and the sterility and the seed transmission in those infected plants were also confirmed.
3) Sterility in BSM infected barley was found to have been caused by the abnormal pollen and anther, just as Yamamoto had earlier described.

Furthermore, on the basis of the following characteristics of the disease, the

author concluded the disease under discussion to be identical to BSM reported in North America: Similarities in (1) symptoms, (2) mode of transmission, (3) shape and size (Shikata and others, 1959), and also (4) physical properties and host range of the causal virus.

It was very curious why the foliage symptoms of this disease had been overlooked for a long time in Hokkaido, which had not been the case with the "false stripe" in North America. Some of the symptoms of BSM (false stripe) are resembled to those of stripe disease of barley caused by *Pyrenophora graminea*. And, in some instances, it is difficult to distinguish between the two diseases by their leaf mottling and necrotic symptoms. Especially, in the case of doubtful symptoms of double infection, the identification seems to be nearly impossible. There was a grower who said to the author on the field survey in Hokkaido that, looking at the BSM symptoms in the field where the stripe disease was not found, he remembered the occurrence of BSM-like foliage symptoms in Moravia grown previously. According to I. Suto, barley plants with stripe disease-like symptoms, which had been found in the experimental field at Suita, Osaka, were rogued when he previously studied on the semi-sterile barley obtained from Hokkaido. Under these circumstances, it was reasonably assumed that, BSM symptoms had not been overlooked in Hokkaido, but had just been mistaken for stripe disease for a long time.

The author came to a conclusion on the mechanism of seed transmission of BSMV, which was similar to those obtained by Crowley (1959) from different viewpoint. Crowley lead an opinion on the mechanism of seed transmission of BSMV and other two viruses, on the basis of the rarity of seed transmission in diseased plants infected after flowering, and also of the knowledge of pollen transmission and virus distribution in diseased seed which had been shown by Gold and others (1954). The author studied to see whether the proportion of embryo infection was varied while the seed developed, in other words, to see whether the embryo which had been virus-free at the early stage of development became to be infected in certain stage of seed development, or to see whether the virus inactivation occurred in developing embryo. The results obtained in this study on pollen and seed transmission was found to agree with Crowley's hypothesis on the seed transmission of this virus. The most important fact about the mechanism of seed transmission found in this study was that, the proportion of seed transmission was already decided in the early stage of seed maturation when the embryo was extremely young but developed as large as dissectable. This was the fact with abundant explanation for the rarity of seed transmission in diseased barley infected after heading time as seen in the case of field inoculation test or natural contact transmission.

XI. SUMMARY

This article deals with the following subjects on which the author has made

investigation since 1956, viz., barley stripe mosaic (BSM) in Japan, and also the other basically related subjects such as seed transmission of the causal virus, sterility of barley induced by BSM, and the control of BSM.

1. Chapter III has dealt with the results of the investigations on BSMV found in the semi-sterile strain of 2-rowed barley for malt and the sterile barleys collected in barley field in Hokkaido.

(1) The stripe disease-like disorder of barley, which had been seen in the semi-sterile strains of Moravia variety in Kurashiki in 1956, was found to be caused by a seed transmissible virus according to its modes of transmission and other examinations. This virus was identical to barley stripe mosaic which had been reported in North America but so far not in Japan.

(2) Seed transmission of this disease was detected frequently from the seeds obtained from the semi-sterile plants of Shunsei barley together with the seeds from the semi-sterile lines of Harbin 2-rowed barley grown in Hokkaido, in 1956. This has indicated that, the semi-sterility of barley, the major cause of which had long been left unknown since about 1930, is chiefly attributable to BSMV.

2. Chapter IV has dealt with the occurrence and the distribution of BSM in Japan.

(3) Occurrence of BSM was observed in all of the barley growing areas in Hokkaido according to the results of the field survey made for the period June to July in 1957. However, this disease was not generally considered to be so serious at that time.

(4) Occurrence of BSM was investigated in different districts where barley was grown, with different sources of seeds used, and on different fields for seeds for foundation and propagation stocks and those for grains for malt. The most frequent occurrence of BSM was found in those fields where the seeds taken from malt barley fields or non-authorized private propagation fields had been used.

(5) Occurrence of semi-sterility in the diseased plants and the seed transmission of BSMV in the seeds taken from the diseased plants and also in the seed samples taken from infected fields covered under the field survey in Hokkaido were ascertained in Kurashiki.

(6) Prevalent occurrence of BSM was noted in some old 6-rowed barley varieties grown in Hokkaido.

(7) Present and future countermeasure against BSM in Hokkaido was discussed.

3. Chapter V has dealt with the various symptoms of BSM as compared with those of stripe disease of barley caused by *Pyrenophora graminea*.

(8) Symptoms of BSM caused by seed-borne or mechanical infections in seedling or adult plant were dealt with closely. Brownish necrotic stripes with various shapes, which were the initial expression of the symptoms caused by

contact transmission or sap inoculation, differed from those caused by seed-borne infection.

(9) Differences between BSM and stripe disease of barley in mosaic and necrotic symptoms at the seedling and the adult stages of barley were dealt with closely.

4. Chapter VI has dealt with the physical properties and host range of BSMV, together with the investigations on plant-to-plant contact transmission and pollen transmission.

(10) Thermal inactivation point of BSMV was 65°C for 10 minutes. Dilution end point was 1:2,000–6,000, and occasionally it was 1:8,000. Inactivation time of the virus in plant juice was 20–25 days at 18°C, over 130 days in refregerator, and it was over 80 days in dried leaf tissue.

(11) Barley, wheat, rye, oat, italian millet, broomcorn millet and sweet corn were found to be systemically susceptible to BSMV, but rice and sorghum were not. All of thirteen species of *Hordeum* plants other than *H. bulbossum* were highly susceptible to the virus. In 25 species in 15 genera of gramineous grasses and weeds tested, tall wheatgrass, smooth brome, green foxtail, red top, thimothy, and 3 species of ryegrass were infected to the virus, but 18 species in 12 genera included *Agropyron semicostatum*, *Aloepeculus aequalis*, *Digitaria cilialis*, *Eleusine indica*, and *Panicum Crusgalli* were not susceptible. In non-gramineous plant species, systemic infection was obtained in *Commelina communis* and spinach, and local infections were in *Chenopodium album*, *C. amaranticolor*, sugar beet and Swiss chard. Tobacco, *N. glutinosa*, tomato, eggplant, radish, cabbage, bean, broad bean, asparagus bean, aster, pansy and other dicotyledonous plant species tested were not infected to the virus.

(12) Seed transmission of this virus was noticed in barley, wheat, oat, many species of *Hordeum*, many kinds of grasses, and *Commelina communis*, but not in italian millet, broomcorn millet, and spinach.

(13) Natural and artificial contact transmission of this virus was confirmed closely by the experiments with various modes of contact among healthy seedling and diseased seedling or other inoculum source. Natural plant-to-plant contact transmission of BSM in barley field apparently increased after the middle of April, and it occurred in those plants distanced 20–30 cm from the infection source plant.

(14) Pollen transmission of this virus was confirmed by the artificial crossing with the use of pollen from diseased plants. The highest ratio in seed infection caused by diseased pollen observed in this experiment was 35%. Pollen transmission was also ascertained even in the cross seeds among healthy pistils of resistant varieties and diseased pollen of susceptible varieties. Infectivity of pollen obtained from diseased plants was confirmed by the inoculation test.

5. Chapter VII has dealt with the results of the experiments on the observation of the disorder of anther and pollen of BSM-infected barley, on the

mechanism of seed transmission of BSMV, on several problems as to the detection of seed-borne symptoms, on a seed testing technique for the BSMV seed transmission, and also on the influence of infection time of BSMV on sterility and seed transmission of barley.

(15) Sterility in BSM infected barley was found to be caused by the abnormal anther and pollen similar to that in semi-sterile strain of Moravia barley which had previously observed by Yamamoto, T. (1951). Compared with those of healthy plants, many of the anthers of diseased plants were observed to be small in size and incomplete in dehiscence, and the normal pollen in those anthers were found to be small in number. Moreover, the amount of pollen found on pistil in diseased barley just after the flowering was found to be less than that in healthy plants. These facts show that the chance of fertilization was reduced in diseased plants, even though the pistil was noticed to be normal in fertility.

(16) Embryo culture test and also dissection and inoculation test were made for the virus infection, using barley seeds and their embryos at various stages of maturation. Approximately the same proportions were noticed between embryo infection and seed transmission throughout the stages of seed maturation even at the extremely young stage when the embryos were hardly to dissect and culture. The virus was also noticed in endosperm of diseased seeds throughout the stages of seed maturation. However, the virus in diseased endosperm seemed to have no relation to the seed transmission so far as investigated.

(17) All of the seed-borne infected seedlings of 6-rowed barley presented the symptoms at their first leaf stage. However, some of the seed-borne infected seedlings of 2-rowed barley did not show any symptom at their first leaf stage, but most of them showed the initial symptoms as their second or more advanced leaf stages.

(18) About 5,000 lux of light intensity was found to be enough for the development of seed-borne symptoms in diseased seedling in most of the virus strains used. But, it was difficult to detect the seed-borne infection with mild symptom expressing strain under the low light intensity. Individual difference among observers was investigated for the detection of seed-borne infection.

(19) Interference reaction of BSMV was ascertained in the seed-borne infected seedlings with or without visible symptoms. Additional symptoms caused by inoculation was not observed on the seed-borne infected seedlings when they were inoculated further.

(20) The number of seeds to be used for the seed testing technique was determined using the seed samples with various seed transmission rate.

(21) A seed testing technique, inoculation-testing method, were devised for the detection of the seed transmission of BSMV. The successive procedure of this technique was as follows: 1) Observation of seed-borne symptoms at the 1st leaf stage of seedling, 2) observation of the symptoms induced by the inoculation to the seedlings without visible symptoms at their 1st leaf stage (detection

of healthy seedling), and 3) further examination by re- or subinoculation to test the virus latency of the seedlings which were symptomless even after the inoculation test. This method was found to be available for the resistant varieties as well as the susceptible varieties.

(22) Effects of infection time with BSM on various kinds of agronomic characters and sterility of barley and wheat, and also seed transmission of the virus were studied. Retardation of heading time, shortening of plant height, incomplete emergence from the sheaths, and also reduction of number of head, kernel weight and proportion of large seeds were resulted by the early infection. Late infection at the heading stage reduced kernel weight and proportion of large seeds. Yield reduction was noticed in all of the plots inoculated. The earlier the infection time was, the more seed transmission was noticed. The amount of seed transmission was greatly reduced when the plants were inoculated after heading time.

6. Chapter VIII shows the results of the investigations on the selection of BSMV resistant varieties from the world-wide collection of barley, on the determination of resistance-grade, on the field inoculation test with resistant varieties, and also on the mode of inheritance of BSMV resistance of Wien and Imperial.

(23) On the first step of screening for BSMV resistance, Wien, Imperial, Ko-ran and three other varieties from Manchuria, and 27 varieties of barley from Turkey were selected from about 2,200 entities of barley by the inoculation test at the seedling stage.

(24) On the second step of BSMV resistance screening, the index of resistance-grade was calculated on the basis of the mean incubation period with the use of Harbin 2-rowed, Ko-ran and Imperial as the susceptible (resistance-grade 1.0), resistant (3.0) and highly resistant (5.0) standard, respectively. Wien, Imperial, T 463, T 462 and Ko-ran were found to be resistant to the virus and their resistance-grades were estimated to >5.0, 5.0, 3.5, 3.4 and 3.0, respectively. Comparatively high value of resistance-grade was also given to those Turkish barleys such as T 245, T 381, T 470 and T 471.

(25) Field inoculation test was made using 7 varieties of barley with various values of resistance-grade, together with the above mentioned 3 standard varieties. The higher the value of resistance-grade, the less effect of the virus infection was noticed, though there were some reverse results as to the order of resistance-grade. The varieties of barley, resistance-grade values of which were higher than 3.0, were found to be resistant practically to the field infection of this virus.

(26) Inocuation test was made using the seedlings of F_2 hybrids among susceptible varieties and resistant varieties, Wien and Imperial. The results obtained may not be conclusive, but the F_2 segregations in these crosses appeared to mean that the resistance of Wien and Imperial may be due to a dominant and a recessive major gene, respectively.

7. Chapter IX shows the results of several experiments for the control measure of BSM.

(27) Thermal inactivation of BSMV in infected seeds was examined, heating them in dried condition at 70° and 75°C for 10 minutes, and also at 70°C for 15 minutes. However, the virus was not inactivated by these treatments.

(28) Effect of seed screening with sieve on reducing the seed transmission of BSMV was examined. Seed screening was found to be not effective in the case of the seeds from seed-borne infected plants, in which the seed transmission in the larger seeds was as much as in those found in small sized seeds. On the other hand, the tendency was noticed in the case of the seeds taken from the diseased plants infected by artificial inoculation or natural contact transmission that, the larger the seeds in size, the less the proportion of the virus infected seeds. Above all, most of the large sized seeds (>2.7 mm in 2-rowed barley, and >2.5 mm in 6-rowed barley) were found to be virus-free. The good effect of seed screening was not always expected in the seeds collected from the infected field, it was possible in some instances when the proportion of infected seeds was small.

(29) Roguing was found to be effective for the control of this disease according to the field experiment using Akashinriki and Harbin 2-rowed barley. Disease occurrence was markedly reduced by only a roguing in the early January before the contact transmission took place. Roguing was also found to be effective to suppress the yield reduction and the seed infection by this disease.

(30) For the practical use, the availability of various measures of BSM control was discussed. And the followings were recommended for the control of BSM at the present time, which should be applied in various combinations by case: Seedling test to obtain the healthy seedlings, seed screening with sieve to reduce the BSMV infected seeds, and roguing in the field.

8. Chapter X has presented the general discussion on this study with special reference to the relation between BSMV and the semi-sterility of barley prevalent in Hokkaido, and also to the seed transmission of this disease, accumulating various information obtained throughout this study.

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EXPLANATION OF PLATES

Plate I.

(1) Seed-borne symptoms of BSM in barley seedling.
(2) Symptoms caused by contact transmission in glasshouse. Healthy plant on the right. Variety: Kobinkatagi.
(3) Foliage symptoms of BSM (3 leaves on the right) and stripe disease (3 leaves on the left) in Shunsei variety.

Plate II.

(1) Semi-sterility of barley (Abyssinian variety) caused by BSM. Two heads on the right from healthy plant.
(2) Typical foliage symptom of BSM on Kenyoshi No. 3.

Plate III.

(1) Necrotic local lesion on primary leaf of Harbin 2-rowed.
(2) Foliage symptoms caused by seed-borne infection (3 leaves on the right) and natural contact transmission (3 leaves on the left). 1, 2, 3: Flag leaf, 2nd leaf, 3rd leaf. Variety: Akashinriki.
(3) Necrotic symptoms on head and stem caused by natural contact transmission. The head on the left from healthy plant. Variety: Akashinriki.

Plate IV.

(1) Foliage symptoms of BSM on wheat (Norin No. 52). The leaf on the left from healthy plant.
(2) Symptoms of BSM on sweet corn (Golden Bantum).

Plate V.

(1) Local chlorotic lesion on *Chenopodium album.*
(2) Local chlorotic lesion on *Chenopodium amaranticolor.*
(3) Local necrotic lesion on sugar beet.
(4) Systemic mosaic symptoms of BSM on *Commelina communis.*

Plate VI.

(1) Discolored and shrivelled grains taken from BSM infected naked barley Kobinkatagi (1, 2) and Akashinriki (3, 4).
1, 3 : Seeds taken from BSM-free plant.
The grains in A, B, and C are >2.5 mm, 2.5–2.0 mm and <2.0 mm in seed size. The grains in D are scab infected seeds.

(2) 'Inoculation forecept'

(3) Foliage mottling on seedling of Harbin 2-rowed infected with various isolates of BSMV. Two leaves on the left are infected with 'Im-strain'.

Plate VII.

(1) Symptoms on the seedling of F_1 hybrid between Wien and Akashinriki (4 leaves on the right), Imperial and Akashinriki (4 leaves on the left).
1 : Inoculated leaf—primary leaf
2~4 : 2nd~4th leaf

(2) The initial symptoms expression on the seedling of F_2 hybrid between Wien and Akashinriki.
R : No symptom—not infected
M : Elongated chlorotic lesions characteristic to the hybrid of Wien.
S : Susceptible type—severe mottling and stripe disease-like necrosis.

(3) The initial symptom expression on the seedling of F_2 hybrid between Imperial and Akashinriki.
R : No symptom—not infected
M : Im-type mottling characteristic to Imperial
S : Susceptible type—severe mottling and stripe disease-like necrosis.

PLATE I.

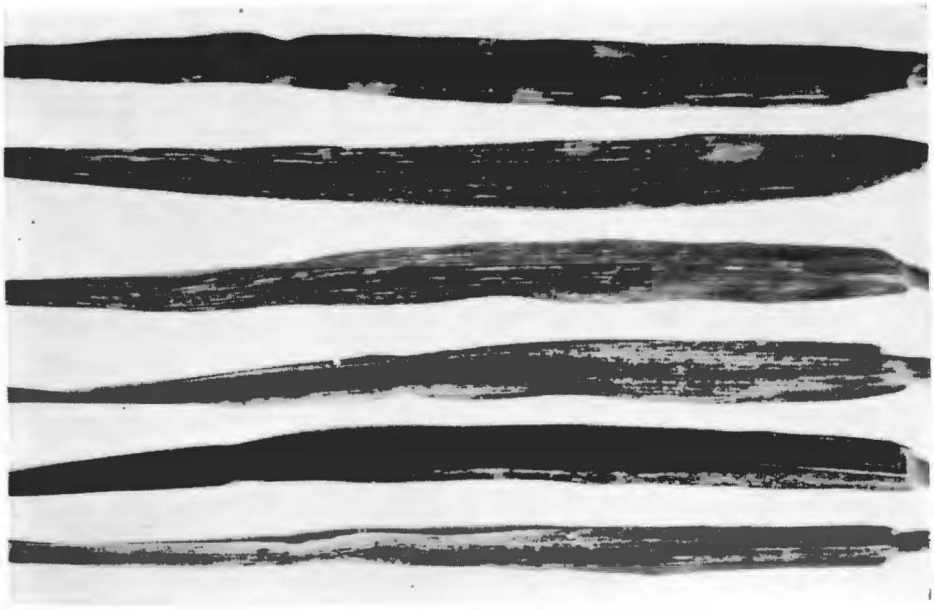
(1)

(2)

(3)

PLATE II.

(1)



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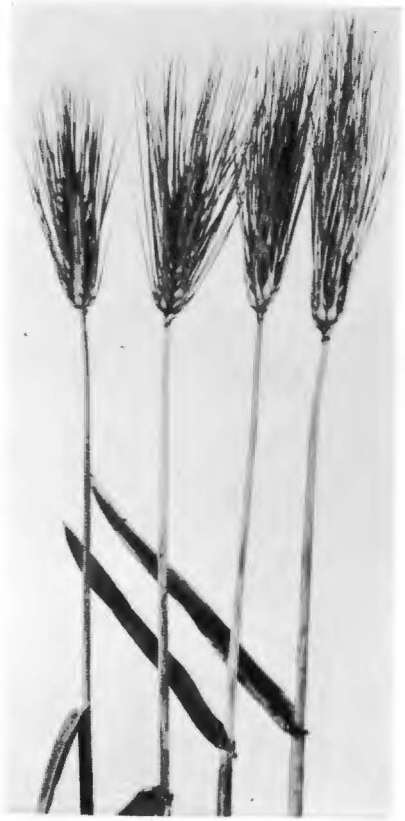

(3)

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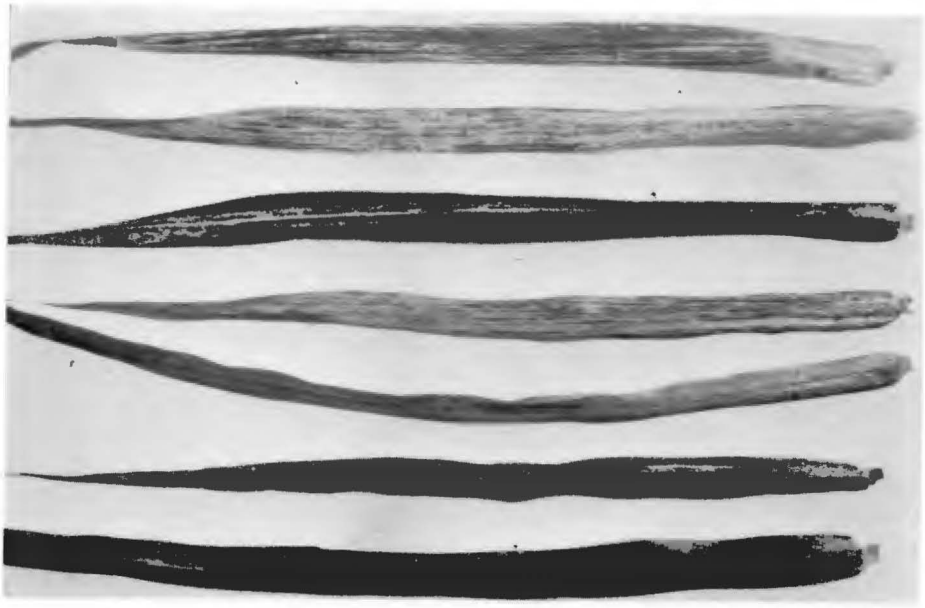
PLATE III.

PLATE IV.



(1)

(2)

PLATE V.

(1)

(2)

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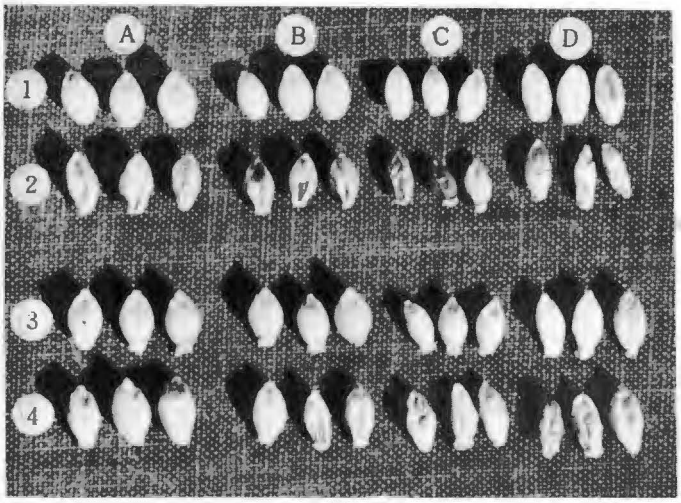
(4)



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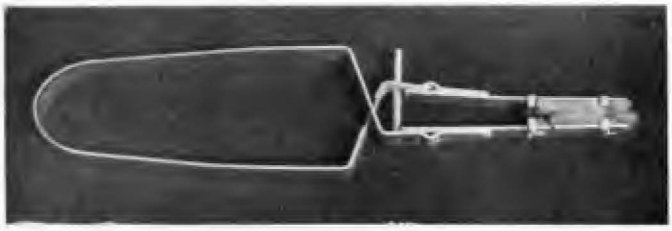

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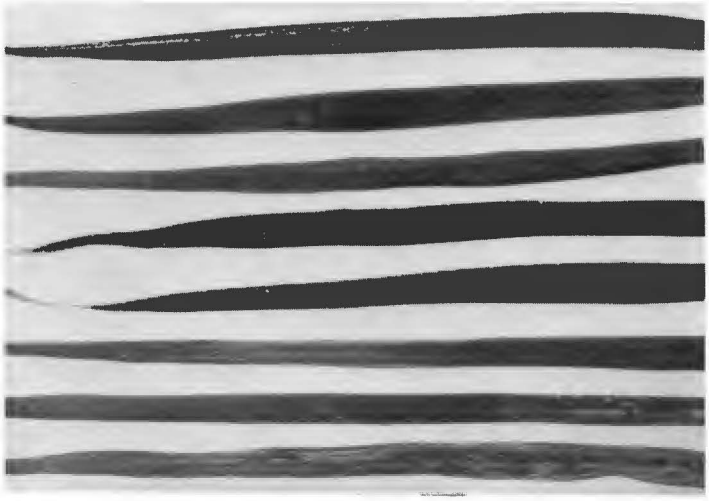

(3)

PLATE VII.