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SEED AND POLLEN TRANSMISSION OF A NEW UNIDENTIFIED MOTTLE DISORDER OF MAIZE IN INDONESIA

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ABSTRACT

A new unidentified mottle disorder of maize in Indonesia was found at the Research Institute for Maize and Other Cereals (RIMOC), Maros, South Sulawesi in 1995. Attempts to identify the disorder were made by mechanical inoculation, insect vector (*Rhopalosiphum maidis* and *Peregrinus maidis*) transmission, seed and pollen transmission, electron microscopy, and serological tests. Fifty seeds from each of 22 ears of Arjuna maize plants showing the disorder were planted and symptoms on the seedlings were recorded at 1, 2, and 3 weeks after planting. The percentage of seedlings showing the disorder ranged from 40 to 100. Pollen of affected Arjuna was then used to pollinate four sweet corn female flowers. Hybrid seeds (50 per ear) of the crosses were planted and symptoms were recorded at 1, 2, and 3 weeks after planting. The results showed that percentage of seedlings showing the disorder ranged from 22 to 84. Electron microscopy and ELISA tests on 15 viruses and one phytospiroplasma antiserum however, gave negative results. Therefore, maize disorder at Maros was not identical to any known viral disease of maize. It could be a genetical disorder and has been given the name maize mottle.

[*Keywords*: *Zea mays*; plant diseases; seed; pollen; Indonesia]

INTRODUCTION

A number forms of abnormal plants caused by non-infectious disease organisms were reported occur in cereal plants (Zillinsky, 1983). In maize, leaf mottle similar to downy mildew was observed in Lampung, Indonesia (Sudjadi *et al.*, 1973). However, no study was done on the disease, which was previously though caused by a virus.

Abnormal mottle or mosaic leaf symptoms were also observed on Arjuna maize variety at the Research Institute for Maize and Other Cereals (RIMOC), Maros, South Sulawesi, Indonesia in 1995 (Fig. 1). The symptoms closely resembled to those of the new leaves of maize plants infected by downy mildew fungus following treatment with a fungicide containing metalaxyl active ingredient. Symptoms appeared at the early growth stage (at the first leaf) produced very stunted and sterile plant, while those appeared at the later growth stage caused little stunted plants and produced male and female flower or ears. The ears of the mottle disorder plants are smaller than those of the healthy one. In a preliminary study, the disorder was not transmitted mechanically or by insect vectors, however, seedlings of the affected maize plants developed the similar symptoms (Wakman and Kontong, 1995).

Objective of the study was to identify the causal agent of the maize mottle. Attempts to identify the disorder were continued by mechanical inoculation, insect vector transmission, seed and pollen transmission, electron microscopy, and serological tests. Results of the mechanical and insect transmission always failed to transmit the disorder plant to the healthy one, but seeds from the abnormal maize plant when planted produced similar symptom on some germinated seedling. Pollen from the disorder maize plant could transmit to pollinate seed and seedling

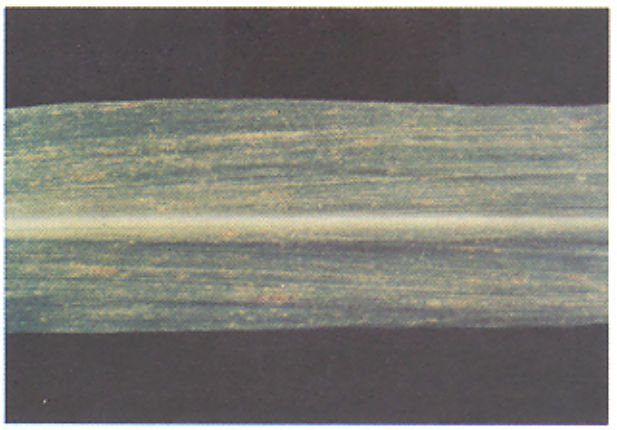

Fig. 1. Mottle leaf disorder of Arjuna maize variety, Maros 1995.

grown from the seeds. No virus particle was found under electron microscope, and ELISA test using 15 maize virus antisera were all negative reaction. The disorder of maize at Maros thought to be genetic anomalies.

MATERIALS AND METHODS

Mechanical Inoculation

Inoculation was prepared from 0.1 g symptomatic small pieces of leaf and homogenized in a mortar with 0.5 ml distilled water using a pestle. Leaves of one-week-old sweet corn seedlings were dusted with carborundum, rubbed with the leaf extract, and washed with distilled water. Observations of symptoms were done three times at weekly intervals, starting from one week after inoculation.

Insect Transmission

Corn planthoppers (*Peregrinus maidis* Ishmed) and corn leaf aphids (*Rhopalosiphum maidis* Fitch) were given with acquisition access period of one day on Arjuna maize plants showing mottle disorder. Thirty capped test tubes were prepared. A two-day-old sprouted maize seedling and five corn planthoppers were put into a test tube. There were ten-test tubes used for planthopper inoculation test. The other case ten tubes were used for aphid inoculation test. A two-day-old sprouted maize seedling and five aphids were put into a test tube. The remaining ten tubes were used as control, without inoculation of insect, only with maize seedling. After 24-inoculation access period, the seedlings were planted in a tray containing soil in a glasshouse. The maize plants were observed four time at weekly intervals.

Seed Transmission

Twenty two ears of 22 Arjuna maize plants showing the disorder were harvested and sun dried for two days. Fifty seeds per ear were then planted in soil in trays in a glasshouse. The number of seedlings showing symptoms was recorded at 1, 2, and 3 weeks after planting.

Pollen Transmission

Sweet corn was used as female plant. The reason was easy to detect the pollinated seeds which were fully developed same as male parent Arjuna seeds, different with unpollinated sweet corn seeds which were dent or unfully developed.

Three pollen transmission tests were conducted. The first, pollen of Arjuna maize plants showing mottle symptoms were used to pollinate an ear of four healthy sweet corn plants. The ears were covered with paper bags before and after pollination. Fifty seeds of each of the four cross-pollinated ears were planted in trays in a glasshouse. The number of seedlings showing symptoms was recorded weekly for 3 weeks, starting one week after planting. The second, pollen of Arjuna maize showing mottle leaf disorder symptoms was dusted on one ear of a healthy sweet corn plant having two ears. The cross-pollinated ear was then covered with a paper bag. Mature ears were harvested and the seeds from each ear were planted separately in a glasshouse. Seedlings were examined for symptoms three times, weekly interval, starting from one week after planting. The last, pollen from Arjuna maize showing mottle leaf disorder symptoms was dusted on the ear of each of three healthy sweet corn plants when the silk had partly emerged. The ears were left uncovered to allow the silk to come out further and then the ear was self-pollinated. The dry, mature ears were harvested and the seeds of each ear were planted separately. Symptoms in the resulting seedlings were recorded at 1, 2, and 3 weeks after planting.

Electron Microscopy Tests

Electron microscopy observations were conducted at the Bogor Research Institute for Food Crop Biotechnology, Indonesia and the University of Queensland, Australia. A sap extract of a leaf showing symptoms was prepared by crushing 0.1 g leaf and 0.5 ml phosphate buffer, pH 7, in a mortar, and the extract was centrifuged at 10,000 rpm (8.000 g) for 5 min. The supernatant was then collected and a drop put on a formvar coated grid. The grid was drained using filter paper, and then viewed under an electron microscope.

Enzyme-Linked Immunosorbent Assay (ELISA) Test

Serological tests by ELISA were conducted at the Ohio State University, USA. Seeds of maize plants with mottle symptoms were sent from Maros (Indonesia) to Ohio (USA) and planted. Sap of seedlings showing symptoms was tested by ELISA for the presence of brome mosaic bromovirus, maize white line mosaic virus, maize chlorotic mottle

machlomovirus, maize rayado fino marafivirus, maize subtle mosaic virus, maize streak mastrevirus, maize stripe tenuivirus, maize mosaic nucleorhabdovirus, maize rough dwarf fijivirus, maize chlorotic dwarf waikavirus (type and mild isolates), maize dwarf mosaic potyvirus (strains A, B, and O), wheat streak mosaic rymovirus, and corn stunt spiroplasma.

RESULTS AND DISCUSSION

The mottle disorder of maize at Maros was not transmitted either mechanically or by insect vectors (*R. maidis* and *P. maidis*) to maize seedlings according to symptom observations. However, symptoms were observed on plants from seed of symptomatic Arjuna maize and on plants from sweet corn pollinated with pollen from symptomatic Arjuna maize, ranging from 40 to 100% (73.6% on average) and from 22 to 84% (57% on average), respectively (Tables 1 and 2).

Pollen of maize plants with mottle symptoms transmitted the disorder through the cross-pollinated ear, but not to the second open-pollinated ear on the same maize plant (Table 3). An ear of sweet corn having mixed, cross-pollinated seeds and open-pollinated seeds, indicated that only cross-pollinated seeds transmited the symptoms (Table 4).

No virus particles were detected by electron microscopy at Bogor and Australia. ELISA serological tests at The Ohio State University using 15 maize viruses and the corn stunt phytospiroplasma anti-serum also gave a negatif result.

Symptoms of a maize mottle similar to downy mildew were previously observed in Jabung District, Lampung, Indonesia (Sudjadi *et al.*, 1973). However, no study was done on the disease, which was previously thought caused by a virus. The affected maize found at Maros also showed symptoms similar to downy mildew disease following treatment with metalaxyl fungicide. However, our study indicated that the disorder was seed and pollen borne (Wakman and Kontong, 1995).

Five of 24 maize viral diseases are known to be seed borne (Shurtleff, 1980), i.e., chloris striate mosaic geminivirus (CSMV), maize dwarf mosaic potyvirus

Table 1. Percentage of seedlings showing mottle symptoms grown from seeds of 22 ears of Arjuna showing mottle disorder.

Ear number	Number of seeds planted	Number of seeds germinated	Seedlings showing mottle (%)		
			7 das[1]	14 das[1]	21 das[1]
1	50	48	6.3	33.3	39.6
2	50	44	29.5	40.9	43.2
3	50	50	24.0	38.0	46.0
4	50	50	40.0	44.0	46.0
5	50	32	6.3	25.0	46.9
6	50	45	22.2	46.7	60.0
7	50	38	57.9	60.5	60.5
8	50	34	35.3	52.9	61.8
9	50	32	57.5	56.3	65.6
10	50	50	64.0	68.0	68.0
11	50	35	22.9	62.9	80.0
12	50	26	34.6	69.2	80.8
13	50	46	19.6	65.2	82.6
14	50	50	18.0	70.0	84.0
15	50	46	71.7	80.4	84.8
16	50	50	26.0	80.0	90.0
17	50	50	64.0	92.0	92.0
18	50	50	16.0	60.0	94.0
19	50	50	80.0	94.0	96.0
20	50	45	33.3	97.7	97.7
21	50	50	58.0	82.0	100.0
22	50	43	20.9	55.8	100.0
Average	50	43.8	35.8	73.6	69.5

[1]das = days after sowing

(MDMV), sugarcane mosaic potyvirus (ScMV), maize ring mottle virus (MRMV), and barley stripe mosaic hordeivirus (BSMV) (Shurtleff, 1980; Brunt *et al.*, 1990). Among the five, only BSMV was reported to be pollen borne (Brunt *et al.*, 1990). However, BSMV was transmissible mechanically and had rigid rod-shaped particles, whereas the Arjuna mottle leaf disorder was not transmitted mechanically, and no particles were found. Another possibility was that the mottle leaf disorder of Arjuna maize at Maros was

Table 2. Percentage of seedlings showing mottle, grown from hybrid seeds of healthy sweet corn pollinated by pollen of Arjuna with mottle disorder.

Ear number	Number of seeds planted	Number of seeds germinated	Seedlings showing mottle (%)		
			7 das[1]	14 das[1]	21 das[1]
1	50	50 (100)	74.0	82.0	84.0
2	50	50 (100)	34.0	42.0	48.0
3	50	50 (100)	70.0	70.0	74.0
4	50	46 (92)	21.7	21.7	21.7
Average	50	49 (98)	49.9	53.9	56.9

[1]das = days after sowing
Numbers in parentheses are percentage of seeds germinated to seeds planted.

Table 3. Effect of cross-pollination or self-pollination on transmission of mottle disorder of Arjuna maize to healthy sweet corn.

Sweet corn plant[1]	Number of seedlings grown	Number of seedlings with mottle
Ear 1: Cross-pollinated with pollen from maize with disorder	48	38
Ear 2: Self-pollinated	71	0
Ear 3: Self-pollinated	96	0

[1]Ears 1 and 2 were from one sweet corn plant; ear 3 was from another sweet corn plant.

Table 4. Pollen transmission of the mottle leaf disorder of Arjuna maize to part of an ear of a healthy sweet corn.

Ear number	Number of pollinated seeds	Number of seedlings grown	Number of seedlings with mottle
Ear 1	60[1]	53 (88.3)	2 (3.8)
	96[2]	50 (52.1)	0 (0)
Ear 2	81[1]	77 (95.1)	8 (10.4)
	88[2]	86 (97.7)	0 (0)
Ear 3	44[1]	26 (59.1)	3 (11.5)
	80[2]	79 (98.8)	0 (0)
Mottled Arjuna (affected control)	100	79 (79.0)	50 (63.3)
Sweet corn (healthy control)	100	95 (95.0)	0 (0)

[1]Cross-pollinated with pollen from Arjuna maize affected with mottle leaf disorder
[2]Open-pollinated with pollen from healthy sweet corn
Numbers in parentheses are percentages

not an infectious disease. Genetic stripe, leaf spots and flecks of maize were reported by Shurtleff (1980). Seed and pollen borne transmission are the characteristics of genetic disorders, and it was possible that the mottle leaf disorder of maize at Maros was also genetically based. The disorder on cereals was observed in the early generations of breeding program, due to chromosomal instability of genetic combination (Zillinsky, 1983).

CONCLUSION

The new leaf disorder of Arjuna maize at Maros characterized by mottle symptoms is transmitted by seed and pollen. It is most likely a genetically-based abnormality or less likely, a new unidentified viral disease. The disorder is conditionally named maize mottle. It is still little information on the maize mottle disorder, so more studies were needed particularly on yield losses due to the disorder, and control methods.

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