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MOSAIC DISEASE OF MAIZE CAUSED BY SUGARCANE MOSAIC POTYVIRUS IN SULAWESI

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ABSTRACT

Mosaic disease of maize and grasses is commonly found in Sulawesi. The symptoms resemble the common mosaic symptoms of virus infection, but the pathogen has not been identified. The objective of this study was to identify the causal agent of the mosaic disease of maize and grasses in Sulawesi. Transmissions of the virus were studied by mechanical inoculation and the insect vector aphid. Serological study was done by using enzyme linked immunosorbent assay (ELISA). Results of mechanical inoculation showed that the disease was caused by a virus which was transmitted from diseased maize and grasses to healthy sweet corn seedlings. The disease was also transmitted by aphid (*Rhopalosiphum maidis*). Serological study indicated that the virus was closely related to the sugarcane mosaic virus (SCMV). Based on these results, it can be concluded that the maize and grass mosaic disease was caused by SCMV.

[*Keywords*: maize; plant diseases; sugarcane mosaic potyvirus; disease transmission; hosts; Sulawesi]

INTRODUCTION

Mosaic disease of maize and grasses was found in several places in Sulawesi, Indonesia, including the Experimental Farms for Secondary Crops, i.e., Bulukumba, Maros, Bontobili, and Lanrang as well as in farmer's fields. The total infected area of the disease has not been reported yet, however, the intensity of the disease was approximately 5-10%.

Viral diseases of maize reported in Indonesia include sugarcane mosaic virus (SCMV), cucumber mosaic virus (CMV), and maize dwarf mosaic virus (MDMV) (Saleh *et al*., 1989; Semangun, 1993; Widodo, 1993). In 1922, Wilbrink *in* Semangun (1993) reported that SCMV was transmitted by the aphid *Rhopalosiphum maidis* from diseased sugarcane to healthy maize plants. There were no reports about the disease in Indonesia until MDMV was reported in Java by Saleh *et al*. (1989) and Widodo (1993). SCMV is now widely distributed in India (Ravindranath, 1978), Brazil (Fernandes and Schaffert, 1978), and Australia (Teakle and Grylls, 1973). In Sulawesi, mosaic symptoms were observed in different places, i.e., Maros, Barru, Bontobili (Gowa), Bantaeng, Bulukumba, and Manado (Wakman and Kontong, 1997). Leaf chlorotic disease of *Rottboellia* sp. (ichy grass) found in Bontobili Experimental Farm in 1997 was similar to maize mosaic with respect to transmission into sweet corn seedlings, symptoms, and host range. The objective of this study was to identify the causal agent of mosaic disease affecting maize and grasses.

MATERIALS AND METHODS

Field Observation and Sample Collection

Infected maize plants were collected from Manado, North Sulawesi, and Maros Experimental Farm, South Sulawesi, whereas *Rottboellia* sp. was collected from Bontobili Experimental Farm, South Sulawesi. The plants were transplanted into pots and grown in a glasshouse of Research Institute for Maize and Other Cereals (RIMOC) in Maros. Natural disease incidence on nine different maize cultivars showing disease in Bulukumba was examined. The incidence was recorded at 35 days after planting on 18 March 1998.

Disease Transmission

Mechanical inoculation

Diseased plants collected from Manado and Maros were grown in pots and put in separated cages in a glasshouse. Ten sweet corn seedlings were used for the mechanical inoculation test. Leaves of one-week old seedlings were dusted with carborundum 600 mesh. A cut of the mosaic infected maize leaf was

crushed homogenously in a mortar with a pestle, and the leaf extract (sap) was rubbed on the seedling leaf surface. The treated leaves were washed with distilled water. The inoculated maize seedlings were kept in a shaded place (room) to protect from direct sunlight for one day. Mosaic symptoms were observed weekly for one month. Inoculation was also done from grass to maize.

Diseased grasses (*Rottboellia* sp. and *Digitaria* sp.) grown in pots were used as the source of inoculum. The sap and inoculation methods were the same as mentioned above.

Aphid transmission

Infected maize plants were grown in pots and kept in an insect-proof cage. Aphids (*R. maidis*) reared in the cage were fed on the infected plants for one day. Five aphids were then transferred onto two-week old healthy sweet corn seedlings grown in plastic trays in screened cages. Each tray contained 40 seedlings. After one day (24 hours) inoculation feeding, the aphids were killed by broadcasting insecticide. Two days later, the seedlings were transplanted into a wooden tray 1 m x 2 m x 20 cm containing soil. Disease symptoms were observed at 1, 2, and 3 weeks after the inoculation.

Host Range Test

Sixteen species of grasses including maize (*Zea mays*), sorghum (*Sorghum bicolor*), and pearl millet (*Pennisetum glaucum*) were inoculated mechanically with the sap of diseased maize leaves followed the method described before. Young fully developed grass leaves and seedling leaves of maize, sorghum, and pearl millet were dusted with carborundum and rubbed with the sap of maize infected leaves as mentioned above. Disease development was examined weekly for one month, starting one week after the inoculation.

Serological Identification of Maize SCMV

Diseased leaf samples of the six different species of grasses showing mosaic symptoms collected from Maros were disinfected in 5% chlorine solution and put in separated envelopes. Samples were immediately sent to the Queensland Department of Primary Industries (QDPI), Australia. The six samples from Indonesia and five leaf samples from Australia were tested serologically using the ELISA test in QDPI Indooroopily. Leaf sap of each sample was extracted in 0.05 M sodium carbonate buffer solution pH 9.6 (coating buffer), and added to microtiter plates (200 µl per well). The plates were incubated overnight at 4°C, then rinsed three times 10 minutes with phosphate buffered saline containing 0.05% Tween 20 (PBS-T), followed by the addition of a 1/1000 dilution of rabbit anti SCMV and JGMV polyclonal IgG conjugated with alkaline phosphatase. Plates were incubated for 2 hours at room temperature then rinsed three times for 10 minutes with PBS-T. The enzyme substrate (p-nitrophenyl phosphate 1 mg ml^{-1}) in 0.01 M glycine buffer containing 1mM $MgCl_2$ and 1 mM NaCl, pH 10.4 was added (200 µl per well). The ELISA plate reader recorded quantitatively the changing colour of the substrate solution in each microplate well.

RESULTS AND DISCUSSION

Disease Incidence

Digitaria sp. infected by mosaic disease virus was commonly found in Bulukumba. Nine maize cultivars, i.e., Antasena, Arjuna, Bisma, Lagaligo, Rama, Semar-2, Semar-3, Wisanggeni, and sweet corn were also naturally infected by the virus. Disease intensity ranged from 3.3% to 7.3% (Table 1). *Digitaria* sp. was thought to be the source of infection. The virus was first transmitted to maize plants (primary infection) then to new young maize seedlings growing nearby the infected maize plants (secondary infection). The secondary infection caused more serious loss, especially when the population of the aphid vector was high.

Table 1. Mosaic disease incidence on nine varieties of maize naturally infected in Bulukumba, South Sulawesi in 1998.

Maize variety	Total of plants		Percentage of plants infected
	Examined	Infected	
Antasena	1,997	145	7.3
Arjuna	1,772	100	5.6
Wisanggeni	2,001	113	5.6
Rama	1,925	102	5.3
Sweet corn	1,714	89	5.2
Semar-3	2,141	99	4.6
Semar-2	1,829	77	4.2
Lagaligo	1,909	78	4.1
Bisma	1,683	56	3.3

Disease Transmission

Mechanical inoculation

Plant sap of diseased maize samples from both Manado and Maros, and grasses from Bontobili and Batukaropa (Bulukumba) when inoculated mechanically into one-week old sweet corn seedlings, produced similar mosaic symptoms (Fig. 1). The incubation period of the plants infected by the inoculum from maize was shorter than that from grasses. Mechanical transmission with the sap from *Rottboellia* sp. showing chlorotic mosaic symptoms and *Digitaria* sp. showing mosaic symptoms to sweet corn seedlings both produced mosaic symptoms.

Thirteen grasses and three cereal plants inoculated mechanically with the sap from maize mosaic leaves produced mosaic symptoms (Table 2). However, their symptoms varied from mild to severe depending on the type of leaves. The broad leaf type showed more severe mosaic symptoms than the narrow leaf type. *Digitaria* sp., *Rottboellia* sp., *Ischaemum* spp., and *Brachiaria* spp. produced clearer mosaic symptoms compared to the other grasses tested. The incubation period in *Digitaria* sp. was the shortest compared to the other grasses, and the longest was in *Dactyloctenium* sp. (more than a month). Inoculated *Dactyloctenium* sp. produced slightly different symptoms which appeared on the leaves of branches grown from the same node where the leaf was mechanically inoculated. On other plants, disease symptoms appeared on the new leaves above the inoculated one. That is why the disease incubation on *Dactyloctenium* sp. took longer.

Aphid vector transmission

Aphid transmission using *R. maidis* was very effective in transferring the virus from the infected maize to healthy sweet corn seedlings. Not all maize seedlings inoculated produced symptoms, only 60 seedlings were infected from 113 seedling inoculated. This was caused by distribution of the aphid vectors which was not the same on each maize seedling.

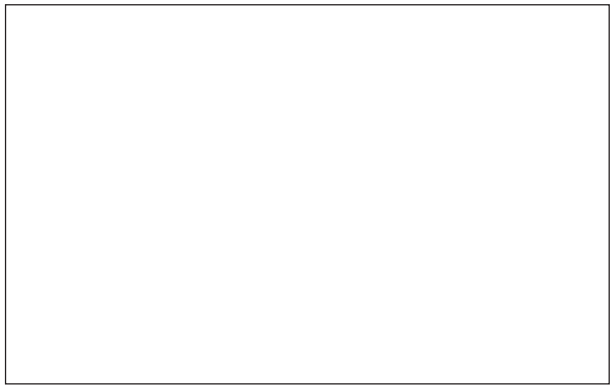

Fig. 1. Mosaic viral disease symptoms on sweet corn after mechanical inoculation.

Table 2. Various plant species found to be infected mechanically by mosaic disease of maize in Sulawesi.

Plant species	Infection
Brachiaria paspaloides (Presl.) C.E.Hubb.	+
Brachiaria sp.	+
Dactyloctenium aegyptium (L.) Beauv.	+
Digitaria longiflora (Retz.) Pers.	+
Digitaria nuda (Schumach.)	+
Digitaria ciliaris (Retz.) Koel.	+
Echinochloa colona (L.) Link.	+
Echinochloa crusgalli (L.) Beauv.	+
Ischaemum rugosum Salisb	+
Ischaemum timorense Kunth.	+
Leptochloa chinensis (L. Nees)	+
Paspalum distichum L.	+
Rottboellia exaltata Lf.	+
Pennisetum glaucum (L.) R. Br	+
Sorghum bicolor (L.) Moench (BG 38)	+
Zea mays (L.) (sweet corn)	+

Enzyme Linked Immunosorbent Assay (ELISA) Test

The ELISA test using conjugated antisera to sugarcane mosaic potyvirus (SCMV) and Johnson grass mosaic virus (JGMV) indicated that all sample plants (pearl millet, sorghum, *Rottboellia* sp., *Digitaria* sp., *Dactyloctenium* sp., and sweet corn) from Indonesia showed positive reaction to the antiserum specific to SCMV, but not to JGMV (Table 3). Very strong ELISA reactions were obtained for several samples which reacted to the SCMV antiserum ranging from 1.89 to more than 3.0, compared to the control buffer absorbance (0.03), healthy sorghum (0.05), and healthy maize (0.04). On the contrary, reaction to the JGMV antiserum was weak with intensity absorbances in the range 0.04-0.15. These results indicated that sample plants from Maros were all infected by SCMV, not by JGMV.

Teakle *et al.* (1989) showed MDMV differed serologically from SCMV. JGMV was also serologically distant from MDMV and SCMV (Shukla and Teakle, 1989). The maize virus in Sulawesi has the properties of a potyvirus and was transmitted mechanically and by aphid vectors in nonpersistent manner. Sero-

Table 3. Results of ELISA test of plant samples from Indonesia and Australia against sugarcane mosaic virus (SCMV) and Johnson grass mosaic virus (JGMV) antisera.

Samples	Absorbance reading to antisera					
	SCMV			JGMV		
	I	II	Mean	I	II	Mean
Healthy sorghum[1]	0.05	0.05	0.05	0.03	0.03	0.03
Healthy maize[1]	0.04	0.04	0.04	0.05	0.05	0.05
JGMV[1]	0.40	0.40	0.40	2.00	1.85	1.92
Millet[2]	2.97	>3.00	>3.00	0.04	0.03	0.04
Sorghum[2]	2.21	1.98	2.10	0.04	0.04	0.04
Rottboellia sp.[2]	2.57	2.19	2.28	0.04	0.04	0.04
Digitaria sp.[2]	2.52	2.33	2.42	0.07	0.06	0.07
Dactyloctenium sp.[2]	1.93	1.85	1.89	0.07	0.06	0.07
Sweet corn[2]	>3.00	2.74	>2.87	0.17	0.15	0.16
SCMV isolate 366[1]	2.00	2.44	2.52	0.03	0.03	0.03
JGM GH[1]	0.34	0.34	0.34	1.94	1.80	1.87
Buffer	0.03	0.03	0.03	0.05	0.05	0.05

[1]Australian samples
[2]Indonesian samples

logical tests indicated that the maize mosaic virus in Sulawesi is more closely related to SCMV than to JGMV. This is the first report of SCMV causing mosaic disease of maize in Sulawesi and the second in Indonesia, following the report from Cirebon, West Java by Wilbrink in 1922 (Semangun, 1993).

CONCLUSION

Mosaic disease of maize, *Rottboellia* sp., and *Digitaria* sp. in Sulawesi was caused by sugarcane mosaic virus (SCMV) and transmitted mechanically to maize, as well as from maize to grasses, sorghum, and pearl millet. The disease was also transmitted by the aphid (*R. maidis*) from maize to maize seedlings.

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