Low pH soil inhibits transmission of *Melon necrotic spot virus* by its fungal vector *Olpidium bornovanus*

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Summary

Influence of cultivation pH of melon seedlings on transmission efficiency of *Melon necrotic spot virus* (MNSV) by the fungal vector *Olpidium bornovanus* was investigated. When melon seedlings were grown in plant culture medium, the transmission of MNSV was decreased under low pH values of 5.5 and 4.5. The application of 1% and 2% Ferosand ($\text{FeSO}_4 \cdot \text{H}_2\text{O}$) to decrease soil pH in artificially infested soil that was contaminated with *O. bornovanus* and MNSV, also decreased the transmission efficiency of MNSV. These results indicate that the transmission of MNSV by *O. bornovanus* is inhibited under low pH soil conditions.

Melon necrotic spot virus (MNSV) causes a necrotic spot disease on infected melon and watermelon plants, leading to a significant economic loss in fruit production (Kishi, 1966; Ohki et al., 2008). Importantly, MNSV is soil-transmitted by zoospores of soil-inhabiting obligate chytrid fungi, *Olpidium bornovanus*, and the necrotic spot disease spreads throughout the field (Campbell et al., 1995; Furuki, 1981; Ohki et al., 2008). Although soil fumigation by methyl bromide is the only chemical treatment to prevent this disease, the use of this chemical in Japan was completely suspended in 2013 by the Montreal Protocol. The activity of *Olpidium* is affected by the soil pH. The infectivity of *O. virulentus*, which is a fungal vector for lettuce big-vein disease caused by *Mirafiori lettuce big-vein virus*, decreases under low pH conditions, and disease control via the application of pH-lowering substances has been performed (Nishiguchi et al., 2014). However, the influence of the soil pH on melon necrotic spot disease remains unclear. Here, we compared the transmission efficiency of MNSV by *O. bornovanus* among different pH conditions.

Materials and Methods

MNSV Chiba strain and *O. bornovanus* Q1 strain (Mochizuki et al., 2008) were used in this study. *O. bornovanus* with

MNSV was maintained in makuwa (*Cucumis melo* cv. makuwa) in a commercial soil (Genki-kun No. 1, CO-OP Chemical Co., Tokyo, Japan) containing 0.5% (w/w) hydrated lime. For transmission tests, cultivating soil containing the diseased makuwa roots was used as artificially infested soil.

For transmission test on plant culture liquid medium adjusted to pH, four melon seeds (*Cucumis melo* cv. Miyabisyunjuukei; Yokohama Nursery Co., Yokohama, Japan) were sown in a 50-ml conical tubes that were filled with vermiculite and Hoagland basal medium (Sigma, St. Louis, MO, including 0.05 M MES, pH 7.5, 6.5, 5.5, or 4.5) containing 0.05 g of makuwa roots that was infected with *O. bornovanus* and MNSV. For transmission test on cultivating soil, different amounts {0, 0.5, 1, 2, 4, or 6% (w/w)} of Ferosand ($\text{FeSO}_4 \cdot \text{H}_2\text{O}$, Ishihara Techno Co., Osaka, Japan), which decreased the soil pH, were applied to the artificially infested soil described above. Five melon seeds were sown in an 8-cm plastic pot that was filled with the artificially infested soil with Ferosand. Fifty melon seeds were sown per experimental group, and then 13–50 melon seedlings were used for transmission test because germination percentage was varied among repetitions. The melon seedlings were maintained in growth chambers at 27°C, and MNSV infection in their stem under cotyledons was assessed 4 weeks after sowing using a DAS-ELISA set for MNSV (Japan Plant Protection Association, Tokyo, Japan).

Soil water was collected by Mizu-Toru (Daiki Rika Kogyo Co., Konosu, Japan) from the artificially infested soil, and the pH of soil water was measured using a B-211 compact pH meter (Horiba Co., Kyoto, Japan).

Results and Discussion

The results of the transmission test on plant culture liquid medium are shown in Table 1. The infection rate of MNSV was similar between pH 7.5 and 6.5. In contrast, the

infection rate was lowered in the melon seedlings that were grown at pH 5.5 and pH 4.5, indicating that the transmission efficiency of MNSV was decreased under low pH cultivating conditions. When purified *O. bornovanus* zoospores containing MNSV were used for inocula, all of the melon seedlings that were grown in a low-pH culture medium (pH 5.5 and 4.5) were infected with MNSV (data not shown), indicating that low pH conditions did not inhibit the activity of the released zoospores.

Next, the influence of a lower soil pH on the transmission efficiency of MNSV by *O. bornovanus* was examined using the artificially infested soil containing *O. bornovanus* and MNSV. The effect of the application of different amounts of Ferosand (0–6%) to the artificially infested soil on soil pH was measured for 10 days after Ferosand application (Figure 1).

Table 1. Effect of pH on the transmission of MNSV in melon seedlings that were grown in plant culture liquid medium.

pH	Repetition	Number of test plants	Number of infected plants	MNSV infection rate
7.5	1	40	6	15.0
	2	46	17	37.0
6.5	1	42	7	16.7
	2	45	13	28.9
5.5	1	48	5	10.4
	2	48	0	0.0
4.5	1	46	0	0.0
	2	48	1	2.1

The soil pH was immediately decreased by Ferosand application; 0.5% and 1% Ferosand maintained the soil at pH 6.5. The application of 2% to 6% Ferosand decreased the soil pH to ca. 5.5, ca. 4.5, and ca. 3.5, respectively, which inhibited the transmission of MNSV. In the transmission test, the transmission rate of MNSV significantly decreased in the artificially infested soil containing 2% Ferosand (Table 2). Although there was no significant difference, the application of 1% Ferosand tended to decrease the fugal transmission rate of MNSV. On the other hands, the transmission rate of MNSV in the artificially infested soil with 0.5% Ferosand was similar to that of the control soil (0%), although soil pH of 0.5% Ferosand was similar to that of soil with 1%

Fig. 1. Fluctuation of soil pH of artificially infested soil by the application of different amounts {0–6% (w/w)} of Ferosand ($\text{FeSO}_4 \cdot \text{H}_2\text{O}$).

Table 2. Effect of Ferosand application on the transmission of MNSV in melon seedlings that were grown in artificially infested soil.

Amount of Ferosand (w/w)	Repetition	Number of test plants	Number of infected plants	MNSV infection rate	Average MNSV infection rate with SD ^(a)
0%	1	50	8	16.0	36.5 ± 17.7
	2	36	17	47.2	
	3	26	12	46.2	
0.5%	1	48	1	2.1	32.6 ± 28.0
	2	42	24	57.1	
	3	13	5	38.5	
1%	1	46	0	0.0	7.6 ± 9.2
	2	40	2	5.0	
	3	28	5	17.9	
2%	1	43	0	0.0	0.9 ± 1.7*
	2	35	1	2.9	
	3	40	0	0.0	

(a) Three independent experiments were performed, and significant difference between the control (0%) and the other experimental groups was assessed by Dunnett's multiple comparison test (*, $p < 0.05$).

Ferosand (pH 6.5, Figure 1). In addition, the transmission of MNSV was not inhibited in culture medium pH 6.5 (Table 1). Thus, in the transmission test on cultivating soil, direct effects of Ferosand on *O. bornovanus* infectivity have not been ruled out. Melon seedlings did not germinate in the artificially infested soil with 4% and 6% Ferosand application, most likely due to the extremely low pH of the soil.

Taken together, these results indicate that low pH soil conditions inhibit the transmission of MNSV by *O. bornovanus*. However, there are several problems for practical application; (1) Application of 2% (w/w) Ferosand correspond to ca. 4 t per 10 a (20 cm depth). This enormous amount is unreasonable in practical melon cultivation. (2) Effect of a lower pH on MNSV transmission in commercial melon fields has not been investigated. (3) Influence of low soil pH on melon fruit production and other important fungal diseases, such as Fusarium wilt, are not clear. In the control of lettuce big-vein disease, spot application (60 kg/10 a) of fertilizer containing the phosphorous acid material and iron sulfate near the root decreased soil pH around the lettuce root, and then inhibited *O. virulentus* infectivity and incidence of big-vein

disease (Nishiguchi et al., 2014). Thus, for the practical application of low soil pH in melon cultivation, further studies, such as optimizations of material and application method, are required to solve these problems.

Acknowledgments

This study was supported by a Grant-in-Aid as part of the Development of practical technologies to implement new agricultural, forestry, and fishery policies by the Ministry of Agriculture, Forestry and Fisheries in Japan.

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