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Effect of Maize Dwarf Mosaic Inoculations at Various Growth Stages on Yield of Nakhon Sawan 3 (NS3)

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Introduction

Maize dwarf mosaic virus (MDMV) belongs to the genus Potyvirus in the family Potyviridae. MDMV is widely distributed in many countries where maize is grown. Thirty-years ago in Thailand, an epidemic of the virus spread through the country (Sutabut, 1989). Just a decade later, the virus re-emerged in Nakhon Ratchasima province, (2003 to 2004) with damage found in sweet corn plantings. Analysis of this incident, using nucleotide and amino acid sequence of MDMV isolates collected from three different locations found low-genetic variation, which may have indicated a re-emergence of the same strain of SCMV-MDB. (Reanwarakorn *et.al*, 2006). However, the disease did not cause economic damage on field corn, until 2008, when susceptible commercial maize in Lopburi province resulted in total losses.

Transmission of MDMV occurs mechanically and by a broad range of aphids in a non-persistent manner. MDMV seed transmission occurs at frequencies from 0.007 percent to 0.4 percent (Ford *et al.*, 2004). MDMV-related yield reduction and symptom expression mostly occur during the growth stage particularly in sweet corn, (Gregory and Ayers, 1982), involving a maximum reduction of 34 percent for yield and 14 percent for plant height. One inbred line, H184, showed severe symptoms when inoculated 10 days after planting. However, only mild symptoms occurred when inoculated later. (Genter *et al.*, 1973). When MDMV infected H84, yields only reduced, on average, 2.4 percent for each 10 percent increase in prevalence. (Scott *et al.*, 1988). MDMV infection reduced young plant height, total plant weight, and ear weight. (Fuchs and Griintzig, 1994) most likely due to reduced photosynthetic rate and increased respiration rate in the infected plants, resulting in yield losses (Gates and Gudauskas, 1969)

Nakhon Sawan 3 (NS3) is a drought tolerant hybrid improved by the Nakhon Sawan Field Crops Research Center (NSFCRC), and released in 2009 and promoted across many provinces that grow maize. The technology of hybrid seed production for this cultivar was transferred to farmers through the Department of Agriculture's Maize Seed Village project. NSFCRC provides parental inbred lines to farmers, groups of farmers and local companies who participated in the project and makes available F₁ hybrid seed for non-participating farmers. Through direct-seed production, participating farmers reduced the cost of purchasing seed and, due to reduced cost of production were able to generate more profit from the maize they

grew. This study evaluated the effects of MDMV mechanical inoculation at different growth stages, on yield and disease severity in NS3.

Material and methods

Field design and agronomic management

The field experiment was conducted at Nakhon Sawan Field Crops Research Center during the 2012 rainy season (June through July). The experiment methodology involved a randomized complete block design (RCBD) with four replications. Treatments consisted of inoculations at five growth stages, V1, V3, V6, V9, V12 and a non-inoculated control. The single cross hybrid NS3 was planted in the field on 11 June 2012. Experimental units were six-row plots, five meters long. Spacing between rows and plants were 75 x 20 centimeters (cm). Chemical fertilizer applications were 312.5 kg ha^{-1} of 15-15-15 (nitrogen, phosphorus and potassium [NPK]) at the time of planting and 187.5 kilograms per hectare (kg ha^{-1}) of ammonium sulfate applied as a side-dressing 30 days after planting. Plants in the four middle rows were harvested on 3 October, 2012

Inoculations

The MDMV isolate used was collected from the National Maize and Sorghum Research Center Nakhon Rachasima province, which was maintained at NSFCRC as 'susceptible sweet corn.' The inoculum was prepared by blending thirty grams of symptomatic leaves with 100 milliliters (ml) of 0.01M phosphate buffer (pH 6.9) for 1 minute. Inoculations were made at various maize growth stages according to the treatments. The two youngest leaves of the plants in the four middle rows were manually rub-inoculated. Plants were inoculated on 18 and 25 June, and 2, 9, 19 July for the V1, V3, V6, V9 and V12 growth stages, respectively.

MDMV evaluation

Disease ratings were taken at 80 days after planting according to the following scale: i) no symptoms or a few chlorotic spots on leaves, less than 5 percent of the leaf area affected; ii) chlorotic spots on a few leaves, 6 percent to 25 percent of the leaf area affected; iii) mosaic symptom with leaf yellowing on upper

leaves, 26 percent to 50 percent of the leaf area affected; iv) systemic mosaic symptoms with leaf yellowing on the middle and upper leaves, 51 percent to 75 percent of the leaf area affected; v) systemic mosaic symptoms with all the leaves yellowing, >76 percent of the leaf area affected.

Data collection and analysis

Data on agronomic traits, grain yield, symptoms and disease incidence (percentages) of MDMV infection was obtained from the middle four rows of each plot and were subjected to an analysis of variance (ANOVA).

Results and Discussion

The growth stages of NS3 at the time of inoculation of V1, V3, V6, and V12 occurred at 7, 14, 21, 28, and 38 days after planting, respectively (Table 1). Inoculation at various growth stages of NS3 significantly affected plant height, ear height, MDMV severity and yield. Days-to-silking ranged from 55-57 days when inoculations were made at the V1-V12 stages. Silking was delayed three days when infections were made at the V1 and V3 growth stages, compared to the non-inoculated treatment. Days-to-tasselling ranged from 55-56 days in the inoculated treatments. Tasselling was delayed one to two days when inoculations were made at the V1 and V3 growth stages compared to the non-inoculated treatment. Days-to-silking and tasselling of NS3 in the non-inoculated treatment was 54 days after planting. The mean plant height and ear height of the inoculated plants were 175 and 100 cm respectively, whereas the non-inoculated control was 194 and 113 cm.

There were no significant differences in disease incidence, among the inoculations made during the five growth stages. Prevalence of MDIMV in the inoculated treatments were 98-99 percent. Although natural infections were found in the non-inoculated control treatment, only small chlorotic spots developed on the leaves. Plants inoculated at various growth stages were distinct based on the intensity of mosaic symptoms expression. Inoculations made at the V1, V3 and V6 growth stages showed the highest level of disease expression. MDMV rating scales were 4.2, 4.7, 5.0, 3.8, and 2.0 when inoculations were made at the V1, V 3, V6, V9 and V12 growth stages, respectively. Plants inoculated at the V1-V9 growth stages showed mosaic symptoms with leaf yellowing.

NS3 had grain yields of 4.19, 3.83, 4.34, 5.02 and 5.66 tons per hectare ($t\ ha^{-1}$) when inoculations were made at the V1, V3, V6, V9 and V12 growth stages, respectively. The non-inoculated control treatment had a yield of $6.31\ t/ha^{-1}$. The greatest reduction in yield was 38.6 percent when inoculated at the V3 growth stage. The yield obtained from non-inoculated control treatment and when inoculated at the V12 stage were not significantly different (Table 2). However, correlations between disease severity, yield, plant height, ear height and stalk lodging were statistically significant (Table 3). Increased disease severity decreased yield, plant height, ear height. Data also indicated that MDMV caused stalk lodging up to 30 percent, suggesting that plant infected by MDMV have reduced stalk strength.

Table 1 Agronomic traits of hybrid maize Nakhon Sawan 3 inoculated with MDMV at various growth stages at NSFCRC, in 2012.

growth stage at time of inoculation	day after planting	day to silking (day)	day to tasselling (day)	ear height (cm)	plant height (cm)
V1	7	57.25	56.00	100	172
V3	14	57.50	56.25	91	171
V6	21	55.50	54.50	96	172
V9	28	55.25	55.00	104	170
V12	38	55.25	55.00	109	189
non-inoculated		54.25	54.25	113	194
mean		55.88	55.17	102	178
CV (%)		2.8	1.9	10.6	5.9
LSD (0.05)		2.33	1.56	16.4	16.0

Table 2 Grain yield and MDM severity of hybrid maize Nakhon Sawan 3 inoculated with MDMV at various growth stages at NSFRC, in 2012.

growth stage at time of inoculation	stalk lodge (%)	MDM incidence (%)	severity	yield (tonha ⁻¹)	yield reduction (%)
V1	17.6	99.2	4.2	4.19	32.7
V3	30.2	99.2	4.7	3.83	38.6
V6	25.1	99.5	5.0	4.34	30.6
V9	3.0	98.0	3.8	5.02	20.5
V12	1.3	99.5	2.0	5.66	9.3
non-inoculated	3.1	4.6	1.1	6.31	
mean	13.5	83.5	3.5	4.89	
CV(%)	83.7	1.1	8.4	11.6	
LSD (0.05)	17.1	1.4	0.4	0.85	

Table 3 Correlation coefficients between MDM severity and some agronomic traits.

	yield	day to silking	day to tasselling	plant height	ear height	stalk lodge
MDM severity	-0.953**	0.705	0.530	-0.957**	-0.933**	-0.812*

* ,** significant at p=0.05 and p=0.01 N = 6

Conclusion

MDMV is most damaging to NS3 at critical growth stages and, depending on the severity of prevalence, results in yield reductions. To minimize yield loss, avoidance of disease infection using methods available for the control of NIDMV could be considered particularly during the V1-V9 growth stages, up to approximately 30 days after planting.

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