

Movement of *Sugarcane mosaic virus* in plants of resistant and susceptible maize lines

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Summary

The movement of *Sugarcane mosaic virus* (SCMV) in the leaves and entire plants of three maize lines was studied under greenhouse conditions. One line was considered resistant and the other two showed a different level of susceptibility. The effect of the growth stage at which the maize plants were inoculated on SCMV movement was also examined. None of the lines tested was fully immune, even in the resistant line the inoculated leaves were infected. Nevertheless, SCMV was detected in the inoculated leaves 7 days later than in the susceptible lines and did not spread into the newly developed leaves. In the lines with the different level of susceptibility SCMV moved into the newly developed leaves at a different rate and in the less susceptible line the spread was also affected by the development stage of maize at inoculation. The spread of the virus from the inoculated parts of the leaves into the non-inoculated ones was also considerably faster in the highly susceptible line, compared with the susceptible and the resistant line, whereas in these two lines the rate of spread was dependent on the development stage of the plants under inoculation and also on the position of the leaf on the plant.

Keywords: maize; *Sugarcane mosaic virus* (SCMV); resistance; movement

Introduction

Systemic infection of susceptible plants by virus pathogens usually involves the successful replication of virus nucleic acid and entire particles in the initially infected cells, spreading from the infected cells into the adjacent uninfected ones through plasmodesmata (cell-to cell movement). One of the critical stages seems also to be the long-distance transport through the vascular system during which the virus must enter and exit bundle sheath cells, phloem parenchyma and companion cells, and sieve elements (SE) and subsequently after the transport it must be unloaded from SE at remote sites to establish further infection by cell-to-cell postphloem movement (OPARKA and SANTA CRUZ 2000; WEIGMANN et al. 2004).

Sugarcane mosaic virus (SCMV), a widespread pathogen of maize in the Czech Republic (POKORNÝ and PORUBOVÁ 2000), belongs to the genus *Potyvirus*. Plants have developed a wide range of defense mechanisms to the virus pathogens of the genus. Besides others, they also include the restriction of long-distance transport (REVERS et al. 1999). These mechanisms were also determined in maize. In one line resistant to *Maize dwarf mosaic virus* (MDMV) LAW et al. (1989) suppose that the transport of the virus from the roots to the newly developing leaves is limited. KOVÁCS et al. (1998) found in the experiments that in resistant lines MDMV and SCMV were confined only to the inoculated leaves and did not spread into the upper leaves. Also, KUNTZE et al. (1997) reported that in resistant lines SCMV was

limited to the inoculated leaves. It has not yet been determined what the time course of SCMV spread in the inoculated and the non-inoculated leaves of maize plants with a different level of resistance is and what is the influence of the development stage on the spread. This paper is to elucidate some of the questions.

Material and methods

Plant material, growth conditions and virus inoculum: On the basis of the previous experiments (POKORNÝ and PORUBOVÁ 2001) three maize lines were selected – a highly susceptible line TR65, a susceptible line TR51 and a resistant line TR42. The plants of the respective lines were grown in plastic pots in the greenhouse in May and June with no supplemental lightening and with the temperature of 18 to 37°C.

A SCMV 190Tr isolate was maintained for a long time in *Sorghum guinense* Stapf. and 4 weeks before inoculation it was multiplied in maize plants cv. Ceklad. The inoculum was prepared by grinding 1 g of maize leaves showing symptoms of mosaic in 7 ml 0.01 M sodium phosphate buffer, strained through the cheesecloth and on the corresponding date it was mechanically applied by using polyurethane sponge to the upper side of maize leaves, following the inoculation protocols, as they are described in particular experiments.

SCMV detection: The virus was detected in individual parts of maize plants by DAS - ELISA (CLARK and ADAMS 1977) with commercial kits (Bioreba). The procedures of sample collection are described in particular experiments. The preparation and dilution in all experiments were in accordance with the manufacturer's instructions (1g of herbage per 20 ml of extraction buffer). Incubation with enzyme substrate always lasted 45 min. The resultant absorbance was assessed by using the photometer MR-700 (Dynatech) at a wavelength of 405 nm.

Experiment A: At two time replications five plants of each maize line were inoculated at the stage of the third and the fourth leaf or at the stage of the fifth and the sixth leaf. At both stages, the two youngest leaves were always inoculated in such a way that the inoculum was applied approximately to one half of leaf area. The samples for virus detection by DAS - ELISA were taken on day 4, 11, 18, and 28 after inoculation (dpi). On the first three sampling dates the entire longitudinal halves of the two inoculated leaves of individual plants were taken, homogenized in extraction buffer and used for SCMV detection. On the last sampling day the inoculated leaves could not be collected because they died off in most plants. For SCMV detection in the non-inoculated leaves the youngest (4 and 11 dpi), possibly the two youngest (18 and 28 dpi), newly developing leaves were taken from individual plants.

Experiment B: In five plants of individual lines only apical parts (c. 5 cm) of the two youngest leaves were inoculated at the stage of 3 or 4 leaves or at the stage of 5 and 6 leaves. On day 11 and 18 after inoculation the apical (inoculated) and the basal (non-inoculated) parts of the leaves were collected from individual plants, the presence of SCMV was detected by DAS - ELISA separately in the lower and upper inoculated leaves.

Data analysis: In individual experiments the infected plant parts were determined and expressed as a number. The virus concentrations were expressed as mean absorbance value and their standard deviations. These values were calculated in individual experiments just only for the infected plant parts.

Results

Experiment A - Movement of SCMV in the whole plants

The results of the experiment are given in Table 1. In both susceptible lines SCMV was detected in the inoculated leaves as early as 4 dpi. Whereas in the very susceptible line TR65 there was almost no difference in the number of infected leaves between the two dates of inoculation, in the susceptible line TR51 the number of leaves with SCMV was slightly higher when inoculation was conducted at the stage of 5 to 6 leaves. In the resistant line TR42 at that time infection of only one leaf was detected when inoculation was done at the higher development stage. On the newly developing leaves 4 dpi the symptoms of infection occurred in some plants of the line TR65 and also the virus was only detected in this line, whereas the number of infected plants was slightly higher on the second date of inoculation. In both susceptible lines 11 dpi 100 % infection was detected in the inoculated leaves of maize, in the resistant line TR42 infection on both dates of inoculation was 80%. In the highly susceptible line TR65 the symptoms of infection occurred on all the newly developing leaves and SCMV was also detected by ELISA. Both in the resistant line TR42 and the susceptible line TR51 no symptoms of infection were observed on the newly developing leaves and the virus was not detected by ELISA. On the other date of assessment (18 dpi) the infection of the inoculated leaves was 100% in all the lines. At this time the symptoms of infection already occurred and SCMV was detected by ELISA in the newly developing leaves in the susceptible line TR51, whereas the number of infected plants was higher with inoculation done at the lower development stage. In both susceptible lines 28 dpi of SCMV was determined in the newly developing leaves of all the plants, only in one plant of the line TR51 inoculated at the stage of 3 to 4 leaves no symptoms occurred. Even on this date no virus was detected in the new leaves of the resistant line TR42.

As for absorbance values, which express the relative concentration of SCMV, there were differences in the inoculated leaves of individual lines on some dates (Table 1), but only in several cases they were considerable. With inoculation at the stage of 3 to 4 leaves, on day 11 after inoculation absorbance was significantly higher in the line TR65 compared with the lines TR51 and TR42. With inoculation at the higher development stage on all dates of evaluation there were significant differences in absorbance between the highly susceptible line TR65 and the resistant line TR42. On day 11 after inoculation there were differences between the lines TR42 and TR51 and also between the lines with a different degree of susceptibility. As far as the virus was detected in susceptible lines in the newly developing leaves the differences in absorbance values were not significant.

Experiment B - Movement of virus within inoculated leaves

The results of this experiment are presented in Table 2. In all three lines SCMV was detected almost in all the inoculated apices of leaves on both dates of evaluation. An exception was one plant of the line TR42 inoculated at the stage of 5 to 6 leaves, in which 11 dpi no virus was present in the upper leaf. In the highly susceptible line TR65 SCMV spread into the non-inoculated leaf bases even before day 11 after inoculation on both dates of inoculation irrespective of their position on the plant. In the susceptible line TR51 the spread of SCMV in the inoculated leaves was faster with inoculation at the lower development stage and also depending on the position of leaves. SCMV was detected in the non-inoculated base at 100% 11 dpi only in the lower leaves and 18 dpi in leaves in both positions with inoculation at the

Table 1 - Virus pattern movement of SCMV in plants of three maize lines inoculated in two development stages (5 plants in two time replications were inoculated in each line at each stage)

Line/DPI	Inoculation in stage of					
	3. - 4. leaf			5.- 6. leaf		
	I		N	I		N
	No	A ₄₀₅	S No	No	A ₄₀₅	S No
TR42	0	-	0 0	1	0,216+0,000	0 0
	4	0,180+0,071	0 0	8	0,183+0,047	0 0
	11	0,352+0,084	0 0	10	0,332+0,074	0 0
	18	-	0 0	nt	-	0 0
	28	-	0 0	nt	-	0 0
TR51	5	0,142+0,059	0 0	8	0,266+0,066	0 0
	4	0,310+0,090	0 0	10	0,349+0,078	0 0
	11	0,401+0,039	7 8	10	0,372+0,070	4 4
	18	-	9 10	nt	-	10 10
	28	-	2 5	10	0,315+0,090	1 8
TR65	9	0,249+0,081	10 10	10	0,440+0,006	10 10
	4	0,438+0,005	10 10	10	0,420+0,010	10 10
	11	0,411+0,030	10 10	nt	-	10 10
	18	-	10 10	10	0,441+0,004	10 10
	28	-	10 10	10	0,436+0,006	10 10

DPI - days after inoculation

I - inoculated leaves

N - noninoculated leaves

S - number of plants with symptoms on newly developed leaves

No - number of infected particular parts determined by DAS-ELISA

A₄₀₅ - mean absorbance of infected parts and its standard deviation: + is for +/-

Table 2 - Virus pattern movement in leaves of three maize lines inoculated in two development stages (5 apexes of upper and lower leaves were inoculated in each line at each stage)

Line/DPI	Leaf position	Inoculation in stage of									
		3. - 4. leaf		N		I		5. - 6. leaf		N	
		No	A ₄₀₅	No	A ₄₀₅	No	A ₄₀₅	No	A ₄₀₅	No	A ₄₀₅
TR42	U	5	0,312+0,107	0	-	4	0,304+0,164	0	-	0	-
	L	5	0,365+0,030	1	0,261+0,000	5	0,396+0,082	1	0,138+0,000	1	0,138+0,000
	U	5	0,446+0,006	5	0,388+0,074	5	0,430+0,023	1	0,161+0,000	1	0,161+0,000
	L	5	0,440+0,008	5	0,445+0,004	5	0,431+0,007	3	0,259+0,116	3	0,259+0,116
TR51	U	5	0,381+0,043	2	0,244+0,020	5	0,434+0,005	0	-	0	-
	L	5	0,397+0,039	5	0,293+0,090	5	0,433+0,014	2	0,132+0,062	2	0,132+0,062
	U	5	0,444+0,005	5	0,445+0,004	5	0,442+0,002	3	0,400+0,071	3	0,400+0,071
	L	5	0,445+0,007	5	0,443+0,004	5	0,430+0,023	5	0,392+0,103	5	0,392+0,103
TR65	U	5	0,346+0,049	5	0,425+0,022	5	0,437+0,008	5	0,411+0,002	5	0,411+0,002
	L	5	0,415+0,037	5	0,391+0,038	5	0,440+0,008	5	0,438+0,005	5	0,438+0,005
	U	5	0,444+0,005	5	0,444+0,004	5	0,431+0,017	5	0,437+0,002	5	0,437+0,002
	L	5	0,447+0,002	5	0,438+0,011	5	0,444+0,003	5	0,434+0,002	5	0,434+0,002

DPI - days after inoculation

U - upper leaf

L - lower leaf

I - inoculated apexes of leaves

N - noninoculated bases of leaves

No - number of infected particular parts determined by DAS-ELISA

A₄₀₅ - mean absorbance of infected parts and its standard deviation: + is for +/-

stage of 3–4 leaves. If the plants of this line were inoculated at the stage of 5 to 6 leaves the virus was present in 100% of the non-inoculated bases of only the lower leaves 18 dpi. Also, in the resistant line TR42 the spread of SCMV was faster with inoculation at the lower development stage and 100% spread into the leaf bases was achieved only 18 dpi inoculation irrespective of their position.

Absorbance values did not differ much in the inoculated leaf apices of all the lines, irrespective of the date of inoculation and the position of leaves on the plant (Table 2). Nevertheless, significant differences were found in the non-inoculated leaf bases between some lines, depending on the date of inoculation and the position of leaves. With inoculation at the lower development stage on day 11 after inoculation there were significant differences in this trait between one infected lower leaf of the line TR42 and the lower leaves of the line TR65 and between the upper leaves of the lines TR51 and TR65. If maize plants were inoculated at the stage of 5 to 6 leaves absorbance values were significantly higher in the infected leaf bases of the line TR65 in comparison with the line TR42 on both dates of evaluation regardless on the position of leaves. Significant differences were also found between absorbance values in the lower leaves of the lines TR65 and TR51 on day 11 after inoculation and the upper leaves of the lines TR51 a TR42 on day 18 after inoculation.

Discussion

The results of our experiments showed that individual lines with different levels of susceptibility differed in the spread of SCMV in the inoculated leaves and the entire plants. The line TR42 was not immune, as SCMV multiplied and spread in the inoculated leaves, but it was not able to expand into the newly developing leaves. Nonetheless, even in the inoculated leaves of this line the virus was detected later than in both the susceptible lines and as far as the entire leaves were tested its concentration increased more slowly with time, predominantly in comparison with the highly susceptible line TR65. These differences were more evident as long as the plants of individual maize lines were inoculated at the higher development stage. With inoculation at this stage the spread of SCMV in the inoculated leaves was considerably slower even in the susceptible line TR51 than in the highly susceptible line TR65. The lower concentrations of SCMV in the resistant line were probably caused by the slower spread of this virus in the inoculated leaves from the apices into the bases, because all the lines in experiment B differed neither in the number of infected apices nor in the concentration of SCMV in these parts. There were also differences between the two susceptible lines in the spread of SCMV into the newly developing leaves. In the highly susceptible line TR65 the virus was detected by ELISA as early as 4 dpi and on this day the symptoms of infection even occurred on several leaves, the line was fully infected 11 dpi. In the susceptible line TR51 the virus did not spread into the new leaves until 18 dpi and all plants were completely infected only 28 dpi, which corresponds to our previous findings (POKORNÝ and PORUBOVÁ 2001).

On the basis of the results from experiments A and B it is supposed that in the resistant line TR42 the long-distance movement of SCMV from the infected leaves to the newly developing plant parts is blocked. This mechanism is common not only in maize resistant to some virus pathogens of the genus *Potyvirus* (LEI and AGRIOS 1986; LAW et al. 1989; KUNTZE et al. 1997; KOVÁCS et al. 1998), but also in many other combinations of plant species with a virus pathogen from this genus, (GERMAN-RETANA et al. 2000; GRUMET et al.

2000; ULLAH and GRUMET 2002). In some cases the resistance to virus long-distance movement is affected by the development stage, at which the plants are inoculated. GARCIA-RUIZ and MURPHY (2001) concluded that *Cucumber mosaic virus* (CMV) in *Capsicum annuum* plants inoculated at the later development stage propagated only in the inoculated leaves and did not spread into the newly developing leaves, which was common in the plants inoculated at a lower stage. Similar conclusions were also made by RAJAMÄKI and VALKONEN (1999) in resistant plants of *Nicandra physaloides* inoculated by PVA and SOLER et al. (1999) in *Capsicum chinense* infected by *Tomato spotted wilt virus* (TSWV). In many cases, however, the development stage does not affect long-distance movement in the sense of go or not go, but it affects the level of spread. For example, ULLAH and GRUMET (2002) have reported that the resistance of some *Cucumis sativus* origins to *Zucchini yellow mosaic virus* (ZYMV) was developmentally regulated - the virus multiplies in the inoculated cotyledons and is transported only into the leaves, which develop rapidly at the time of inoculation and does not spread on from these leaves. Our experiments have also revealed the effect of the development stage of maize plants on the spread of SCMV in the inoculated leaves and the entire plants. In the resistant line TR42, SCMV spread in the inoculated leaves faster, as far as inoculation was done at the stage of 3 to 4 leaves. Also, in the susceptible line TR51 the virus spread in the leaves faster and expanded into the newly developing leaves somewhat faster as far as inoculation was performed at the lower development stage. On the other hand, in the strongly susceptible line TR65 the development stage did not affect the spread of SCMV in the leaves and in the entire plant. In the lines TR42 and TR51 the effect of the position of the inoculated leaf on the plant on the spread of the virus was also found. In the lower (or older) leaves of these lines the virus spread faster from the apex to the base, which was evident especially with inoculation at the higher development stage. After entering the phloem, the particles of virus pathogen are likely to move passively along with assimilates (OPARKA and SANTA CRUZ 2000). The flow of assimilates in the plants is directed from the source to the sink, whereas there is a change in the flows of assimilates during the development of leaves. At first the young leaf attracts assimilates from other parts of the plant, soon, however, it becomes self-sustaining and changes into the source of assimilates for the other parts of the plant (PROCHÁZKA et al. 1998). So, it is most likely that in all the lines SCMV moved more slowly in the younger leaf but in the TR42 and TR51 lines its concentration was not sufficient for its multiplication on the leaf base whereas in the susceptible line TR65 even with the slower spread of virus in the younger leaf there was faster multiplication at the base.

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