Maize Dwarf Mosaic Virus Infection in Susceptible and Resistant Corn: Virus Multiplication, Free Amino Acid Concentrations, and Symptom Severity

J. C. Tu and R. E. Ford

Postdoctoral Research Associate and Professor, respectively, Department of Botany and Plant Pathology, Iowa State University, Ames 50010. Present address of senior author: University of Alberta, Edmonton, Alberta, Canada.

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ABSTRACT

We measured changes in free amino acid (AA) concentrations in inoculated and newly emerged leaves of Illinois-A (Ill-A = resistant) and in inoculated and newly emerged systemically infected leaves of Seneca Chief (SC = susceptible) corn infected with maize dwarf mosaic virus strain A (MDMV-A). MDMV-A can multiply only in inoculated leaves of Ill-A corn. MDMV-A can multiply both in inoculated and newly emerged (systemic) leaves of SC corn. Generally, free AAs increased in MDMV-infected inoculated leaves of both Ill-A and SC corn, but the increased AA concentration in newly emerged leaves was significant only in SC, in which systemic virus movement occurred. The dilution end point of MDMV in inoculated leaves at sampling time was the same for both varieties. No infectivity was detected by assay of newly emerged leaves of the Ill-A corn. Older healthy corn leaves always contained less of each of the respective free AAs than younger ones (12% in SC, 13% in Ill-A), and the difference was narrower after MDMV infection (11% in SC, 4% in Ill-A). SC consistently contained less of each of the respective free AAs than Ill-A in healthy leaves (32% in old, 33% in young leaves) as well as in MDMV-infected ones, although the margin of difference was narrower (25% in inoculated [= older], 19% in systemically infected [= younger] leaves). Mosaic symptoms are not induced by an imbalance of free amino acids and/or virus multiplication in mature leaves with mature chloroplasts. Phytopathology 60:1605-1608.

Probably in every virus-host combination, free amino acid (AA) concn change, usually by increasing (1, 5). Changes in leaves of virus-infected stone-fruit trees are usually minor, except in Western X-diseased peach (4). The degree of alteration of host physiology after virus infection depends on host susceptibility, host growth habit differences, and the virulence of the virus. The free AA concn may change quite specifically for each virus-host combination, even though some of these changes are so small that they are undetected by current methods of measurement.

In annual crops AA concn seems to change more drastically with virus infection than do some of the woody plants (4, 5). The quicker growth habits and generally more rapid turnover of the metabolism of annual plants as compared with woody perennial plants could explain this difference.

Changes in free AA concn in corn are virus-specific. Corn infected with different maize dwarf mosaic virus (MDMV) strains varied considerably in free AA concn that were correlated with symptom severity and virus multiplication in systemically-infected leaves (3, 6, 11).

Free AA concn could also be host-specific with a given virus strain. MDMV-resistant and -susceptible corn should show differences in concn if the changes are indeed host-specific.

It is not known if AAs in healthy leaves (leaves showing no symptoms and from which no virus can be recovered) can be altered by virus infection limited to inoculated leaves.

The objectives of this study were to compare changes in the concn of free AAs in inoculated leaves of healthy and MDMV-infected plants within the same variety and between resistant and susceptible varieties, their relationship to virus multiplication, and finally, to study the concn changes in newly emerged leaves with (susceptible) or without (resistant) systemic mosaic symptoms.

MATERIALS AND METHODS.—Seneca Chief (SC) and Illinois-A (Ill-A) corn (*Zea mays* L.) were used. SC sweet corn is highly susceptible to MDMV infection and produces severe systemic mosaic symptoms. Ill-A is a symptomless host in which MDMV can be recovered from inoculated leaves but not from leaves formed after inoculation. Only rarely does a mosaic streak appear in new leaves of an infected Ill-A plant; such plants were discarded in this study.

Inoculum for all tests was the Iowa MDMV-A isolate (2). Inoculum was obtained from MDMV-infected Ohio W-49 corn in the four-leaf stage. Systemically-infected leaves were ground in 0.01 M neutral potassium phosphate buffer 1:1 (w/v) with a mortar and pestle. The sap was strained through two layers of gauze. A small amount of 600-mesh Carborundum was mixed with the inoculum before inoculation. Inoculations were made by rubbing the third leaf of three-leaf-stage corn with a pestle dipped in the inoculum. Controls were rubbed with phosphate buffer, with Carborundum added to simulate inoculation.

Four groups of 50 pots each were planted, two groups with Ill-A and two with SC. Eight corn seeds were sown in each 5-inch clay pot. The soil was a steamed mixture of sand:peat:soil in 1:1:2 proportions. The pots were watered with tap water and maintained in a 24 ± 3 C greenhouse. One group each of Ill-A and SC three-leaf-stage seedlings were inoculated with

MDMV; the other groups were the buffer-rubbed controls.

The upper two systemically-infected leaves of five-leaf-stage SC and Ill-A leaves were harvested for AA analysis and for infectivity assays. Inoculated third leaves of all groups also were harvested. Comparable leaves were harvested from buffer-rubbed SC and Ill-A corn. Before harvesting, a dilution end point (DEP) assay was made for inoculated leaves and for the first fully expanded leaves (fifth leaves). DEP infectivity assays were made on W-49 corn seedlings in the three-leaf stage, with 20 seedlings for each dilution. All corn leaves were harvested 17 days after inoculation between 10 and 11 AM. The leaves from each treatment were kept in a plastic bag and stored in a freezer.

This experiment was repeated 1 week later.

The processes of free AA extraction and preparation have been described (6). The AA analysis was conducted using a Technicon (Type A chromobeads) automatic AA analyzer.

RESULTS.—*Free amino acid concn in newly formed leaves.*—Leaves of SC, newly formed after MDMV inoculation, contained high virus titer and had systemic mosaic symptoms, but those of Ill-A had neither virus infectivity nor symptoms. Nineteen AAs were detected in MDMV-infected SC, but only 16 of these 19 AAs were detected in comparable healthy preparations. No citrulline, cysteine, and arginine were detected in healthy SC leaves, but they were found in very low concn in MDMV-infected ones (Table 1). Eleven AAs increased and eight decreased slightly in infected versus healthy leaves. Large increases were observed with aspartic acid, serine-glutamine-asparagine, glutamic acid, glycine, and alanine with a proportionally smaller increase of the other 5 AAs (Table 1). Free AAs increased 33% more in infected than in healthy SC. A DEP of 1/2,000 accompanied this AA concn increase in the systemically-infected leaves (Table 3).

Nineteen free AAs were detected in newly formed leaves of both healthy and MDMV-diseased Ill-A plants. Four AAs, serine, glycine, aspartic acid, and glutamic acid increased considerably (Table 1). Concentration of the other 15 AAs remained unchanged, increased, or decreased slightly. Total free AA contents were 2.510 and 2.738 μmoles/g of the newly emerged leaves of healthy and MDMV-infected Ill-A plants, respectively. The increase was 0.228 μmoles/g of leaf tissue, or 9%. This increase was attributable primarily to the increase in aspartic acid, serine-glutamine-asparagine, glycine, and glutamic acid. The combined total net increases for the 4 AAs that increased considerably were 0.250 μmoles/g of leaf tissue (a 10% increase). The 9% free AA was not directly associated with virus multiplication in situ (Table 3).

Free amino acid concn in inoculated leaves.—Only 17 of 19 AAs in MDMV-inoculated SC leaves were detected in noninfected leaves (Table 2). All 19 AAs, however, were detected in both noninfected and infected inoculated leaves of Ill-A. Proline and methionine were slightly decreased in infected plants of both varieties. Concentrations of citrulline and valine remained unchanged, and lysine and leucine decreased slightly in infected SC, but these 4 AAs were slightly increased in infected Ill-A. The changes in concn in these 4 AAs were, however, slight and probably of no significant importance. AAs that increased in large quantities in infected leaves of both varieties were aspartic acid, serine-glutamine-asparagine, glutamic acid, glycine, and alanine (Table 2).

TABLE 1. Concentration of free amino acids (μmoles/g leaf tissue) in newly formed leaves in healthy and maize dwarf mosaic virus (MDMV-A)-infected Seneca Chief (with systemic symptoms) and Illinois-A (without symptoms) corn plants

Amino acid	Seneca Chief		Illinois-A	
	Healthy	Infected	Healthy	Infected[b]
	μmoles/g	*μmoles/g*	*μmoles/g*	*μmoles/g*
Aspartic acid	0.129[a]	0.191	0.148	0.237
Threonine	0.061	0.075	0.073	0.075
Serine-glutamine-asparagine	0.240	0.487	0.471	0.535
Glutamic acid	0.101	0.133	0.205	0.221
Citrulline	N.D.	0.003	0.002	0.002
Proline	0.038	0.032	0.039	0.035
Glycine	0.297	0.402	0.372	0.459
Alanine	0.560	0.660	0.990	0.972
Valine	0.051	0.059	0.042	0.043
Cysteine	N.D.	0.003	0.003	0.003
Methionine	0.010	0.008	0.012	0.010
Isoleucine	0.034	0.037	0.027	0.028
Leucine	0.035	0.029	0.031	0.028
Tyrosine	0.033	0.031	0.019	0.020
Phenylalanine	0.024	0.021	0.016	0.011
Ornithine	0.019	0.014	0.010	0.013
Lysine	0.033	0.029	0.034	0.030
Histidine	0.013	0.013	0.009	0.009
Arginine	N.D.	0.002	0.007	0.007
Total	1.678	2.229	2.510	2.738

[a] The figures are an average of two runs in a mixture of two replicate samples.

[b] Apparent healthy leaves of Illinois-A, which are comparable to systemically infected leaves of Seneca Chief. Illinois-A was infected by MDMV only in inoculated leaves; systemic movement seldom occurs.

Total free AA concn increased 33 and 21%, respectively, in inoculated infected leaves of SC and Ill-A (Table 2). The percentages of increase seemed correlated with virus multiplication. MDMV-infected inoculated leaves of SC and Ill-A had DEP's of 1/1,000 and 1/500, respectively (Table 3).

Amino acids in new and old leaves.—The inoculated leaves were 15-17 days older than the newly formed leaves when they were harvested for infectivity assay and AA measurement. Inoculated leaves contained 11 and 4% fewer free AAs (SC and Ill-A, respectively) than newly formed leaves (Tables 1, 2).

Symptoms did not appear in inoculated leaves even though these leaves supported virus multiplication (DEP 1/1,000 in SC, 1/500 in Ill-A) and free AA concn were altered considerably (33% increases in SC, 21% in Ill-A) (Tables 2, 3). Severe mosaic symptoms appear in newly formed leaves of SC after inoculation. MDMV in these systemically-infected leaves has a DEP of 1/2,000, and these leaves had an

TABLE 2. Concentration of free amino acids (μmoles/g leaf tissue) in healthy and maize dwarf mosaic virus (MDMV-A)-inoculated leaves of Seneca Chief and Illinois-A corn plants. Virus was recoverable from inoculated leaves of both hosts

Amino acid	Seneca Chief		Illinois-A	
	Healthy	Infected	Healthy	Infected
	μmoles/g	*μmoles/g*	*μmoles/g*	*μmoles/g*
Aspartic acid	0.112[a]	0.151	0.132	0.188
Threonine	0.056	0.069	0.066	0.077
Serine-glutamine-asparagine	0.213	0.400	0.426	0.492
Glutamic acid	0.096	0.117	0.177	0.243
Citrulline	0.002	0.002	0.003	0.005
Proline	0.035	0.031	0.035	0.033
Glycine	0.228	0.341	0.327	0.441
Alanine	0.512	0.628	0.824	0.922
Valine	0.051	0.051	0.041	0.051
Cysteine	N.D.	0.003	0.002	0.004
Methionine	0.010	0.007	0.011	0.010
Isoleucine	0.030	0.033	0.024	0.033
Leucine	0.031	0.029	0.029	0.031
Tyrosine	0.031	0.034	0.017	0.026
Phenylalanine	0.021	0.023	0.013	0.016
Ornithine	0.015	0.013	0.009	0.010
Lysine	0.032	0.028	0.033	0.033
Histidine	0.010	0.012	0.009	0.011
Arginine	N.D.	0.002	0.005	0.008
Total	1.485	1.974	2.183	2.634

[a] The figures are an average of two runs made to a mixture of two replicate samples.

AA increase of 11%. These values are nearly the same as those for Ill-A, even though no MDMV was recovered from leaves of Ill-A formed after inoculation (Tables 1, 3).

DISCUSSION.—Ill-A corn is an excellent variety for studying the metabolic changes in newly emerged noninfected leaves of virus-infected plants. Since MDMV does not reach as high concn (DEP of 1/500) in inoculated leaves, it is unable to move systemically, and since no virus inhibition was found (13), we believe that the genetic resistance of the plant is preventing virus movement. This is reflected in the increase in free AA concn in Ill-A corn, which was small as compared to the increases in concn in susceptible SC infected with the same virus (Table 2). In this instance, the AA changes are host-specific. Ford & Tu (6) suspected that the changes in AA concn in virus-infected corn resulted from alterations in metabolism due to virus multiplication. Although we could show differences in AA concn with different virus strains, we could not relate DEP infectivity (virus titer) with differences in free AA concn because all virus strains had a DEP of 1/200 (6). In these experiments, however, we show that the degree of MDMV multiplication is correlated with changes in AA concn.

The 9% increase in free AA concn in newly emerged leaves of Ill-A had to result indirectly from metabolic alterations due to virus infection rather than to a direct effect of virus multiplication, because no symptoms and no virus occurred in the leaves above those inoculated. Synthesis of noninfective virus materials or particles is unlikely to occur, as the symptomless leaves are capable of supporting viral synthesis upon inoculation. Our results suggest (i) that the increase in concn of free AAs in MDMV-A infected leaves resulted from both direct virus multiplication and metabolic alterations; and (ii) that changes in free AAs in virus-free leaves of virus-infected plants could result indirectly from metabolic alterations which favor free AA accumulation.

General evidence leads us to believe that free AA increases in virus-infected plants may result in large part from increased aerobic oxidation directly related to CO_2 liberation. The responses of the tricarboxylic acid cycle (TCA) liberate most of the CO_2 in virus-infected plants (1, 5, 7, 9, 12), suggesting an increased TCA activity.

Many enzymes in conjunction with TCA and its immediate terminal electron transfer system have been found to increase in quantity or activity (5, 8, 15). The activated TCA cycle could enhance AA synthesis, mainly by aminating α-ketoglutaric acid to form glutamic acid, the precursor of many AAs (14). The free AAs that increased most were aspartic acid, serine, glycine, and alanine. They are more closely related to glutamic acid than the others (14). This could be one explanation of the accumulation of these free AAs in virus-infected plants.

Although symptom severity has been correlated with free AA changes in systemically-infected leaves, there is no correlation of symptom severity with increases of free AA concn in MDMV-infected inoculated corn leaves. MDMV normally does not produce symptoms in inoculated leaves. Systemic mosaic symptoms, however, are produced in newly emerging leaves of susceptible corn. There is no difference in free AA concn between systemically-infected and inoculated leaves of SC (33 and 33%, respectively) (Tables 1, 2). Increases in free AA concn of 21 and 9%, respectively, were noted in MDMV-infected inoculated vs. newly emerged noninfected Ill-A leaves. These results indicate that increases in free AA concn up to 33% did not incite mosaic symptoms in mature corn leaves. Some of the AA decreases could have been due to age

TABLE 3. Dilution end point for maize dwarf mosaic virus concn in infected corn plants 17 days after inoculation

Corn variety		Dilution						
		10^0	10^{-1}	10^{-2}	2×10^{-3}	10^{-3}	5×10^{-4}	2×10^{-4}
Seneca Chief	Systemic-symptomed fifth leaves	+	+	+	+	+	+	−
	Inoculated third leaves	+	+	+	+	+	−	−
Illinois-A	Inoculated third leaves	−	−	−	−	−	−	−
	Symptomless fifth leaves	+	+	+	+	−	−	−

differences, since the inoculated leaves were 15 days older than the newly emerged leaves. These results agree with Selman et al. (10), who found that the decrease in free AA concn started between 9 and 17 days in healthy tomatoes and between 13 and 17 days in tomatoes infected with tomato spotted wilt virus.

There is no correlation between MDMV multiplication and symptom severity in mature leaves, because virus multiplication occurs in inoculated leaves (Table 3) without inciting any mosaic symptoms. There is a correlation, however, between free AA changes and symptom severity in immature leaves. Virus multiplication causes increased free AA concn during the incipient stage of chlorophyll synthesis and chloroplast development. It allows less chlorophyll synthesis in underdeveloped chloroplasts because of physiological changes in groups of virus-infected cells, which causes symptoms in newly emerged leaves.

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