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Effect of Wheat Streak Mosaic Virus Infection on Total DNA and Chloroplast Ribosomal RNA in Wheat Leaves

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With 2 figures

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Abstract

The amount of chloroplast 23s rRNA relative to either DNA or to cytoplasmic 28s rRNA was reduced in young wheat leaves infected with wheat streak mosaic virus.

Chlorophyll was reduced in infected leaves. Fresh weight per leaf and DNA content per leaf were reduced in infected leaves, but DNA per g was increased.

Cytoplasmic ribosomal RNA appeared to degrade more slowly during senescence in infected leaves than in uninfected. Virus was undetectable by density gradient centrifugation in systemically infected leaves less than 6 cm long and reached its highest concentration when young leaves reached their maximum size. Mosaic developed in leaves that became infected when 5 cm long or less. Since the entire leaf eventually developed mosaic, the events leading to mosaic occur after cell division, which is limited to the basal cm of young leaves.

Zusammenfassung

Einfluß einer Wheat-Streak-Mosaikvirusinfektion auf die Gesamte-DNS und die Chloroplasten Ribosomale-RNS in Weizenblättern

Festgestellt wurde eine Verminderung der Chloroplasten-23s r-RNS entweder bezüglich der DNS oder der cytoplasmatischen 28s r-RNS in jungen Weizenblättern, die mit dem Wheat-Streak-Mosaikvirus infiziert wurden.

Der Chlorophyllgehalt, das Blattfrischgewicht und der Blatt-DNS-Gehalt wurde in befallenen Blättern reduziert, der DNS-Gehalt pro Gramm Blattmasse dagegen erhöht.

Der cytoplasmatische ribosomale RNS-Abbau während der Blattseneszenz schien langsamer in befallenen als in nichtbefallenen Blättern.

Das Virus konnte in systemisch infizierten Blättern, die kürzer als 6 cm lang waren, nach Dichtegradientenzentrifugation nicht festgestellt werden. Die höchste Viruskonzentration wurde in jungen Blättern, die ihre volle Größe erreicht haben, ermittelt. Mosaiksymptome entwickelten sich in Blättern, die zur Zeit der Infektion bis zu 5 cm lang waren. Da das ganze Blatt schließlich Mosaiksymptome aufweist, müssen die Ereignisse, die zur Mosaikentwicklung führen, nach der Zellteilung, die im untersten cm eines jungen Blattes stattfindet, eintreten.

Wheat streak mosaic virus (WSMV) causes disease in winter wheat in North America, Europe and Jordan (SLYKHUIS 1967). The virus, a flexuous rod 700 nm long (BRAKKE 1971), is transmitted by eriophyid mites, *Eriophyes tulipae* Keifer in North America and France (SLYKHUIS 1953, POINSON *et al.* 1980) and in Yugoslavia by *E. tosichella* Keifer (TOSIC 1973). The virus causes stunting of roots and tops of plants (LARSEN *et al.* 1985) and yield losses up to 100% in infected areas of a field. The highest loss reported for a whole state was 7% for Kansas in 1981 (BRAKKE 1987).

Little is known about the physiological and cellular changes the virus causes in wheat plants despite the importance of wheat as a crop. Leaves with a mosaic have reduced amounts of chloroplast-associated proteins (WHITE and BRAKKE 1983). Ultrastructural studies have shown cytoplasmic cylindrical inclusions but few structural changes in chloroplasts (LEE 1965, MCMULLEN and GARDNER 1980). The cylindrical inclusions have a protein of Mr 66,000 (BRAKKE *et al.* 1987).

Mosaic is a development-related symptom that appears only in leaves that are systemically infected when very young. The expanding wheat leaf is a dynamic system with dividing cells in the basal cm followed by zones of chloroplast division and chloroplast maturation with mature cells at the leaf tip (DEAN and LEECH 1982). The pattern of development has been closely followed only for the first leaf (DEAN and LEECH 1982), but should be similar for subsequent leaves, though the absolute values of various parameters may differ. WILLIAMS and RIJVEN (1965) reported that the amount of DNA per leaf increased with each succeeding wheat leaf. There is no published information on variation of cellular parameters such as numbers of chloroplasts and ribosomes with leaf position or from plant to plant. Since mosaics appear only when developing leaves are systemically infected with virus, they may be the result of interference of the virus infection with metabolic processes (or genes expressed) unique to developing leaves.

We have investigated the content of chlorophyll, DNA, and cytoplasmic and chloroplast rRNAs in segments of infected and uninfected wheat leaves at various stages of development to obtain further information on some of the cellular changes associated with mosaics.

Materials and Methods

Virus maintenance and assay

The Type strain of WSMV (PV57; ATCC 1981) and an isolate collected from the field in 1981 were maintained in wheat plants (*Triticum aestivum* L., cv. 'Michigan Amber'). Plants in the two-leaf

stage were inoculated by leaf rubbing with juice from infected plants. Control plants were rubbed with water. Plants were grown, 5 per 10-cm pot, in a glasshouse kept at 25–30°C. Unless otherwise stated, all assays were of combined leaves from 12 to 25 plants per experiment. Leaves were cut into 1-cm long segments, weighed, mixed, and randomly selected samples used for chlorophyll, rRNA, and DNA assays. The first leaf to appear, the coleoptile leaf, was called number 1, the second, number 2 and so on. Virus concentration was estimated from the ultraviolet absorbance of the virus zone after density gradient centrifugation of extracts clarified by heating at 40°C and addition of Triton X-100 to a final concentration of 1% (BRAKKE and BALL 1968). Virus concentration in roots was compared with that in leaves by infectivity assay as described by BRAKKE (1958). The time needed for virus to become systemic was determined by removing the inoculated leaves from a portion of the plants at daily intervals and observing the plants for symptom development.

Ribosomal RNA assay

Leaves were ground in a Virtis homogenizer in 18 volumes (ml/g) of 110 mM $(\text{NH}_4)_2\text{CO}_3$, 330 mM NH_4Cl , 11 mM EDTA (ethylene diamine tetraacetic acid), which had been adjusted to pH 9.3 with NaOH (BRAKKE 1972). The extract was filtered through cheesecloth, added to 2 volumes (ml/g) of 10% SDS (sodium dodecyl sulfate) and incubated at 25°C for 30 min. Most proteins were removed by precipitation as potassium dodecyl sulfate complexes by addition of 5 M potassium acetate (pH 8) to a final concentration of 0.3 M, incubation in an ice bath for 20 min and low-speed centrifugation (e.g. 10 min at 10,000 rpm; $9,000 \times g$ average RCF). Portions (50–200 μl , depending on leaf age) of the clarified extract were centrifuged for 3.5 h at 54,000 rpm ($300,000 \times g$ average RCF) at 15°C in a Beckman sw60 rotor through a linear 7.5–30% sucrose gradient in NaGPS buffer (50 mM Na_2HPO_4 , 100 mM glycine, 300 mM NaCl, 1 mM EDTA, adjusted to pH 9.4 with NaOH). RNA concentrations were calculated from areas under peaks of ultraviolet absorbance scans of the gradients.

DNA and chlorophyll assay

DNA was determined by the diphenylamine assay (BURTON 1968) of extracts prepared by grinding leaves in a Virtis homogenizer in 20 volumes (ml/g) of 2 M NaCl, 10 mM EDTA, 1% SDS, pH 7.0. After addition of an equal volume of chloroform, the mixture was again homogenized in the Virtis, and centrifuged to separate the emulsion. A portion of the aqueous layer was added to an equal volume of isopropanol, incubated for 2 h at –20°C, and centrifuged to pellet the precipitate of nucleic acid. The pellet was washed once with 70% ethanol, dried in a partial vacuum, and suspended in 0.5 M perchloric acid for the diphenylamine reaction.

Chlorophyll was determined from absorbance at 663 and 645 nm of an extract in 80% acetone (ARNON 1949).

Results

Concentration of virus and development of symptoms

The first systemic symptoms appeared in leaf 3 at 5 days after inoculation of plants at the 2-leaf stage. Leaf 3 was 2–5 cm long at 3 days after inoculation when the virus became systemic in half of the plants, as determined by excising the inoculated leaf from a portion of inoculated plants at daily intervals after inoculation. Therefore, events leading to mosaic occurred after this stage of leaf development.

The concentration of virus in immature leaves increased with leaf size, reaching a maximum of about 75 μg per g of leaf in the youngest fully expanded leaf. Virus could not be detected by density gradient centrifugation in young leaves 4 and 5 when they were less than 6 cm long even though they developed subsequent to the first leaf to show mosaic and should have been systemically

infected when they were a few mm long. The limit of detection was about $2 \mu\text{g}$ of virus per gm of leaf. The virus concentration always decreased in leaves after they had stopped growing. Virus frequently could not be detected by density gradient centrifugation from old but still green leaves with mosaic symptoms. Therefore, virus multiplied more rapidly than it was degraded while the leaves were growing, but after the leaves had stopped growing, the reverse was true. Virus multiplication may have ceased in the old leaves.

The infectivity titre of virus in leaf extracts was 20-fold higher (average of 4 experiments) than in root extracts of the same plants harvested 3 weeks after inoculation. Virus could not be detected in root extracts by density gradient centrifugation.

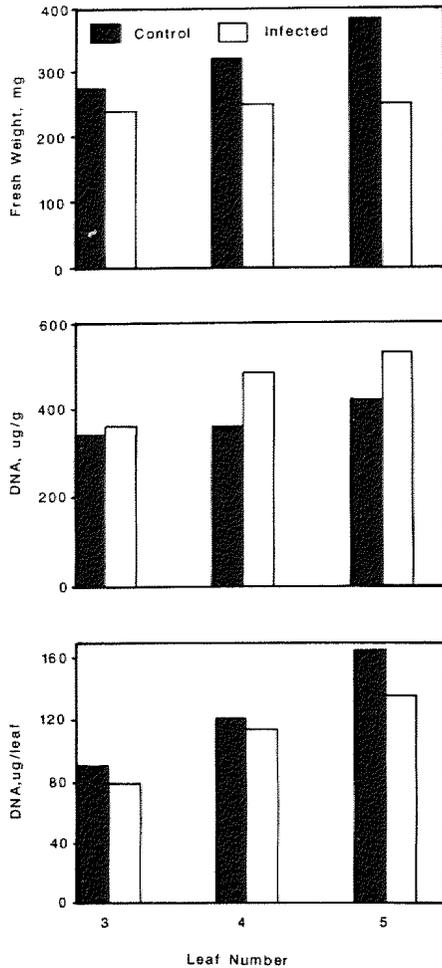


Fig. 1. Weight and DNA content of fully grown wheat leaves infected with WSMV. Values are averages of 3 experiments with 14, 25, and 26 plants per experiment, except for DNA content of leaf 3 which is based on 2 experiments. All differences between infected and uninfected leaves are significant, $P < 0.05$, except for DNA per g for leaf 3 and DNA per leaf for leaf 4

Changes in DNA and weight of infected leaves

Infected leaves had increased concentrations of DNA ($\mu\text{g/g}$), but decreased fresh weight and decreased amounts of DNA per leaf (Fig. 1). Differences were generally greater in leaves 4 and 5 than in leaf 3 which was the first leaf to show symptoms. The data in Fig. 1 are from 3 experiments with a total of 65 plants. Leaves were combined for assay in each experiment, a procedure which gives average values but no measure of variation from plant to plant in values for a given leaf. To obtain an estimate of the variation from plant to plant for use in statistical analysis, DNA and fresh weight of 36 individual leaves, each from a separate plant, were determined. The coefficients of variation were 12% for DNA content per gram or per leaf and 11% for fresh weight per leaf.

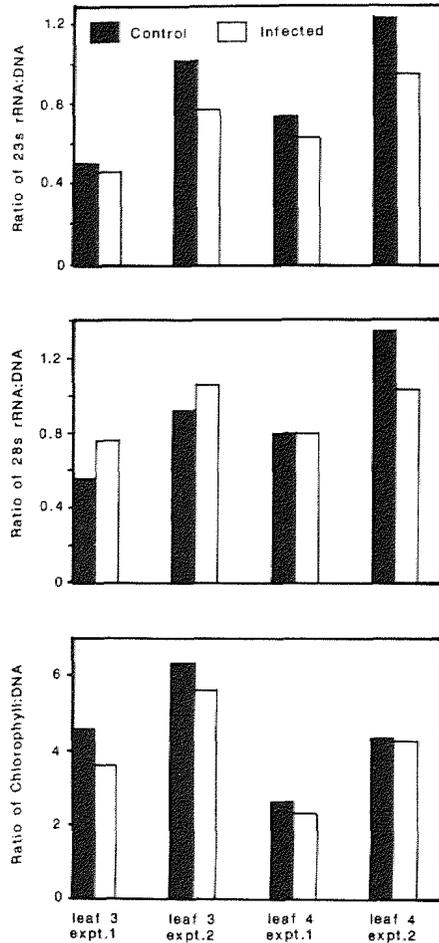
The effect of WSMV infection on rRNA

Sucrose density gradient centrifugation resolves chloroplast 23s rRNA and cytoplasmic 28s rRNA, but not the chloroplast 16s and cytoplasmic 18s rRNAs. Therefore, the 23s and 28s rRNAs were measured to follow the changes in chloroplast and cytoplasmic rRNAs, respectively. Immature leaves 4, 5 and 6 of matched lengths from infected and uninfected plants were assayed for rRNA at 3 weeks after inoculation. The ratio of 23s/28s rRNA was less in the infected than in the uninfected leaves in 19 of 21 such comparisons. The probability of obtaining 19 of 21 ratios that are less in the infected than in the uninfected is only 0.0002 if it is hypothesized that there is no difference. Therefore, the hypothesis is rejected. The ratio of 23s/28s rRNA averaged 30% less in the infected than in the uninfected leaves.

The time course of change in concentration of both 23s and 28s rRNA with leaf age differed subtly between infected and uninfected leaves. The maximum concentration of rRNA was 2—2.5 mg per g of leaf for very young leaves less than 7 cm long, either infected or uninfected. The concentration of rRNA per g of leaf decreased continuously as the leaf grew and then senesced. The total amount of rRNA per leaf increased as the leaf grew, reached a maximum when the leaf reached its maximum length and then decreased. The ratio of chloroplast 23s rRNA to cytoplasmic 28s rRNA increased with growth of immature leaves, but less so in the infected than in the uninfected. After the leaf had reached full size, rRNAs decreased less in the infected than in the uninfected. For example, in early experiments, both infected and uninfected leaves no. 4 had more of both rRNAs per leaf than corresponding leaves no. 3, which were older and somewhat smaller. However, the difference between leaves 4 and 3 was greater in the uninfected than in the infected (data not shown). The difference could have been the result of slower degradation of rRNA in infected than in uninfected leaves, or a reflection of the greater degree of stunting of infected leaf 4 than of leaf 3.

To eliminate ambiguities due to comparisons based on weight or on a per leaf basis, and to find if the decreased 23s/28s rRNA ratio resulted from decreased 23s rRNA or increased 28s rRNA, the ratio of rRNA to DNA was determined in two experiments. Combined leaves from 12 to 20 plants per experiment were assayed for rRNA, DNA, and chlorophyll. The ratio of 23s chloroplast rRNA to total

Fig. 2. Chlorophyll, chloroplast 23s rRNA, and cytoplasmic 28s rRNA in infected and uninfected wheat leaves harvested 12 days after inoculation. Symptoms appeared 5 days after inoculation. Leaf 3 was mature and weighed 236 mg (uninf) and 233 mg (inf) in expt. 1, and 187 mg (uninf) and 184 mg (inf) in expt. 2. Leaf 4 was immature and weighed 128 mg (uninf) and 107 mg (inf) in expt. 1 and 162 mg (uninf) and 118 mg (inf) in expt. 2. DNA concentrations in μg per g for leaf 3 were 339 (uninf) and 338 (inf) in expt. 1, and 352 (uninf) and 334 (inf) in expt. 2, and for leaf 4 were 425 (uninf) and 534 (inf) in expt. 1, and 400 (uninf) and 415 (inf) in expt. 2. Leaves from 20 plants were combined for each extraction, except for leaf 3 of experiment 1 for which 12 leaves were used



DNA was less in virus infected than in uninfected plants in both mature leaf 3 and immature leaf 4 (Fig. 2). Chlorophyll was also reduced, but not as much as the 23s rRNA. The ratio of 28s rRNA to DNA was higher in infected than in uninfected leaves in 2 of 4 comparisons and unchanged in a third. In both experiments, the amount of 28s rRNA per unit DNA was the same for leaves 3 and 4 of infected plants. However, in the uninfected plants, leaf 3 had less 28s rRNA per unit DNA than leaf 4. Old infected leaves must have either a slower rate of degradation of 28s rRNA than uninfected leaves or an increased rate of synthesis.

Assays of whole leaves give average values for green and yellow areas. In two experiments, infected leaves fortuitously had larger than usual yellow areas.

Assays of extracts of excised yellow areas showed a 23s/28s rRNA ratio only 40 % that of uninfected leaves, whereas the ratio for green areas of the same leaves was 80 % of the uninfected.

Discussion

These results and the previous report of a reduction in chloroplast associated proteins (WHITE and BRAKKE 1983) supports the conclusion that systemic infection of wheat with WSMV interferes with chloroplast development. With one exception, previous reports on this topic have dealt with dicotyledons and viruses unrelated to WSMV. Decreases in chloroplast ribosomes or ribosomal rRNA on a per leaf or fresh weight basis have been reported for chinese cabbage infected with turnip yellow mosaic virus (REID and MATTHEWS 1966), *Nicotiana glutinosa* L. infected with lettuce necrotic yellows virus (RANGLES and COLEMAN 1970), *N. tabaccum* L. infected with tomato spotted wilt virus (MOHAMED and RANGLES 1972), *N. tabaccum* L. infected with tobacco mosaic virus (HIRAI and WILDMAN 1969, OXELFELT 1971, FRASER 1972), and barley infected with barley stripe mosaic virus (SUZUKI and TANIGUCHI 1973). Decreases were found in young leaves, but not always in old leaves. FRASER (1972) reported that old tobacco leaves from TMV-infected plants had more chloroplast rRNA than similar leaves from uninfected plants. Changes on a cellular basis have not been reported. FRASER (1972) found that DNA and leaf weight were reduced in infected leaves, but did not calculate rRNA as a ratio to DNA.

Our results show that the disruption of chloroplasts is not confined to pigments but extends to macromolecules, the proteins and nucleic acids. Reduction in chloroplast rRNA implies a reduction in chloroplast ribosomes including ribosomal proteins. Under the conditions of these experiments, the content of 28s cytoplasmic rRNA per unit DNA was the same in immature leaves of infected and uninfected plants. However, mature leaves of infected plants had more 28s rRNA per unit DNA than did comparable leaves from uninfected plants. WSMV infection may result in less than normal degradation of cytoplasmic ribosomes as leaves senesce.

Values of chlorophyll and rRNA expressed as ratios to DNA should give values similar to a per cell basis. WILLIAMS and RIJVEN (1965) reported that 90 % of wheat leaf cells are 2C (42 chromosomes), and 80 to 90 % of total leaf DNA in nuclear (ELLIS *et al.* 1983). Since chloroplast DNA may be reduced as a result of virus infection, total DNA probably is not exactly proportional to cell number. Nevertheless, we think DNA is a better basis for comparison than dry weight or wet weight. We attempted to make comparisons on the basis of counts of cells dispersed by chromic acid (BROWN and RICKLESS 1949), but cell counts proved to be less reproducible than DNA assays. Cell counts showed a similar variation from plant to plant for a given leaf as did DNA assays (BRAKKE, unpublished results).

DNA was reduced in infected leaves, but not as much as fresh weight was reduced, suggesting both fewer and smaller cells per leaf. The third leaf to show

symptoms was more stunted in regard to both DNA and weight than the first leaf to show symptoms. The previously reported decrease in dry weight of WSMV-infected plants (LARSEN *et al.* 1985) probably resulted from fewer and smaller cells per leaf as well as fewer leaves. Stunting occurs after mosaic symptoms appear and is a later stage in symptom development than damage to chloroplasts. The damage to chloroplasts is an early phenomenon that occurs in young (from 5 cm long to full length) systemically infected leaves where chloroplasts are developing as virus is rapidly multiplying. The damage to chloroplasts appears to occur after the cell division, which occurs in the basal cm of wheat leaves (DEAN and LEECH 1982).

If virus multiplication interferes with chloroplast development, it is logical to ask if proteins, nucleic acids or other components involved in chloroplast development are necessary for virus replication. The roots contained 5% as much virus as leaves, probably too much to be due to translocation. It may be concluded that chloroplast maturation is not an absolute requirement for virus replication, but roots do contain proplastids and the possibility of a role for proplastid component(s) in virus replication cannot be ruled out.

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