Electron Microscopy of Developmental Stages of Northern Cereal Mosaic Virus in Wheat Plant Cells

Shigemitsu Toriyama*

Abstract

Northern cereal mosaic virus (NCMV) initially infects phloem cells of the wheat plant. It spreads to mesophyll cells adjacent to the phloem and finally infects all cell types including phloem parenchyma cells, companion cells, sieve elements, developing xylem cells, mesophyll cells, guard cells and epidermal cells. NCMV was usually found accumulated within cisternae of the endoplasmic reticulum (ER) system in the cytoplasm. In some instances, the presence of NCMV particles within the nucleoplasm and perinuclear spaces was observed. A few particles of inner nucleocapsides ('immature' NCMV) were also seen within the cytoplasm. Budding profiles were rarely observed. NCMV seems to be enveloped by membranes almost simultaneously as inner nucleocapsides are formed. Initial appearance of NCMV in the cytoplasm was always associated with the formation of a viroplasm like inclusion. This viroplasm-like area (VpA) appeared as an electron dense matrix of granular or fibrous materials, which formed and distributed throughout the cytoplasm and sometimes occupied a large portion of it. At an early stage of infection, virus particles frequently appeared within the 'non-denatured' cytoplasm rather than at the periphery of the VpA. A few 'immature' particles were only occasionally observed within the VpA, while mature particles of NCMV were never seen scattered in the VpA. However, mature particles in aggregates were rarely found enclosed by the VpA. A second membraneous inclusion was also found in the cytoplasm of infected cells which exhibited an ellipsoidal structure and virus particles were frequently localized at the periphery of this inclusion. In infected mesophyll cells, membranes which protruded into central vacuoles were occasionally observed. Many 'naked' particles of NCMV were seen arranged in parallel between the membranes. Abnormal proliferation of Golgi vesicles (about 50-200 nm in diameter) with an electron dense core were seen in the cytoplasm of young developing wheat leaves infected with NCMV, but disappeared as leaf stage progressed. The relation of these Golgi vesicles to stunting of wheat plant caused by NCMV infection is discussed.

(Received March 29, 1976)

Introduction

Northern cereal mosaic virus (NCMV) is a rhabdovirus which infects wheat, barley, oat, rye and some grasses. It is transmitted by the planthopper, Laodelphax striatellus Fallén, but not by sap inoculation11,12). Four other species of planthoppers are known to be vectors of NCMV. Virus particles in purified preparations are bacilliform, 350×68 nm in size. RNA and lipids (phospholipids and sterols)

* Faculty of Agriculture, The University of Tokyo, Bunkyo-ku, Tokyo, Japan.
東京大学農学部
have been detected in NCMV\textsuperscript{28,29}. Some plant rhabdoviruses appear to accumulate in perinuclear spaces, while others have been observed scattered throughout the cytoplasm\textsuperscript{6,9}. NCMV particles surrounded by a membrane have been observed in the cytoplasm of mesophyll and phloem cells but not in the nucleus\textsuperscript{24,31}. Barley yellow striate mosaic virus (BYSMV) in Italy\textsuperscript{2} has been regarded as a synonym of NCMV based upon similarities of host range, vector species and morphology of virus particles\textsuperscript{28}. Francki\textsuperscript{6} has also suggested that BYSMV might be a synonym of NCMV. Conti and Appiano\textsuperscript{3} reported the presence of inclusions (viroplasm) in the cytoplasm of BYSMV-infected barley cells. Similar inclusions were also observed in the case of NCMV. The present paper describes results of a detailed study of NCMV in wheat plant cells, with particular emphasis on the development of virus particles and cytoplasmic inclusions following virus inoculation.

**Materials and Methods**

Seedlings of wheat (\textit{Triticum aestivum} L. cultivar. Fujimi-komugi) at the first leaf stage were inoculated with NCMV by caging viruliferous planthoppers, \textit{Laodelphax striatellus} Fallen, for one day at 25°C. After removal of the insects, the inoculated seedlings were held in a biotron at 20°C under natural light. Small chlorotic spots, an early symptom of NCMV, appeared on the developing 2nd leaf on 4th day after inoculation. Portions of the 2nd leaf were sampled at 2, 3, 4, 6 and 8 days after inoculation. Samples of young 3rd leaf, showing distinct mosaic symptoms, were collected on the 10th and 20th day after inoculation. Small pieces of excised tissue from the leaves were fixed for 3 hours in 5% glutaraldehyde prepared in 0.1 M phosphate buffer at pH 7.0 containing 0.2M sucrose, and followed by several washings in 0.1 M phosphate buffer during an over night period. Thesections were postfixed for an hour in buffered 1% osmium tetroxide, dehydrated in a graded series of ethanol and embedded in resin (Epoxy 812). Leaf samples from healthy wheat plants representing similar leaf stages were collected and prepared in the same manner. Thin sections were cut on an LKB ultratome, stained with uranyl acetate and lead citrate, and examined with either Hitachi HU-12 or JEM 7 A electron microscope.

**Results**

\textit{Development of virus particles in the wheat plant after inoculation}

NCMV was found in 3rd and 4th day samples, but only rarely in 2nd day samples. A few 4th day samples showed progressive profiles of virus accumulation, and most provided good information regarding early stage formation of virus particles. In these samples, virus particles were found either confined to some phloem cells or to both phloem and adjacent mesophyll cells. It seems that viruses first appear in well developed phloem rather than in developing ones. Leaf samples of the 6th day or later showed more advanced profiles of virus accumulation, but no striking differences in development were seen among samples taken at 6, 8, 10 and 20 days after inoculation. In 10 and 20-day leaf samples, viruses were found in all cell types including phloem parenchyma cells, sieve elements, companion cells, developing xylem cells, mesophyll cells, guard cells and epidermal cells. Necrotic cells were rarely observed even in the phloem of leaves at later stages of infection, but the cytoplasm of most cells of the phloem were frequently filled with virus particles and inclusions or slime-like material.
NCMV was usually seen surrounded by a membrane only in the cytoplasm (Fig. 1). Fig. 2 shows various profiles of NCMV particles according to the cutting plane. Only one instance of the presence of NCMV particles within the nucleus of a phloem companion cell was recognized (Fig. 3). Furthermore, virus particles were found to localize within the perinuclear spaces in some instances. Many NCMV particles were occasionally localized in the neighborhood of the nucleus and membrane-enclosed viruses were only rarely found in proximity with outer membranes of the nucleus. In sieve elements, virus particles were frequently observed dispersed or aggregated. Sometimes many spherical vesicles were seen accumulated within the vacuoles of phloem cells. NCMV particles were not present in central vacuoles but were seen in small ones formed in the cytoplasm of phloem cells at more advanced stages. The virus particles in sieve elements and small vacuoles sometimes exhibited a slightly swelled appearance.

**Formation of viroplasm-like area and virus development in the cytoplasm**

At an early stage of infection, bacilliform particles of NCMV were frequently surrounded by a membrane or closely associated with membranes in the 'non-denatured' cytoplasm which contained ribosome particles (Fig. 4, 8 and 9). Observations suggest that the membranes originated from cisternae of the endoplasmic reticulum system (Fig. 5 and 6). Viruses often appeared in the neighborhood of the plasmodesmata. Occasionally, membranes surrounding virus particles were obscure and virus particles seemed to emerge from the cytoplasm. A few particles corresponding in size to inner nucleocapsides of NCMV were sometimes found within the cytoplasm.

The initial appearance of NCMV particles in the cytoplasm was always associated with the formation of a cytoplasmic area which appeared externally as a dissolution of ribosome particles in the cytoplasm. This area appeared as a rather electron dense cytoplasmic matrix, composed of granular or fibrous materials (Fig. 8-9 and 13). Occasionally, a network of fine fibrillar strands was found within the matrix. Materials in this area were initially present in a loose configuration, but as infection progressed, the area expanded and sometimes exhibited a more compact appearance (Fig. 8 and 13). Appearance and formation of this area was not restricted to any particular region of the cytoplasm and occasionally occupied a large portion of the cytoplasm. Membranes were neither observed to delineate the area from cytoplasm nor were they apparent within the area itself. In some instances, a patch of cytoplasm was observed in which ribosome particles were found localized within the area (Fig. 13). EM profiles indicated that the cytoplasmic area may be the same as the "viroplasm inclusion", "dark staining masses", "virogenic stroma" or "inclusion bodies" which have been described in various vertebrate animal, insect and plant viruses. However, no evidence of the presence of viral protein or nucleic acid in the area has been shown for NCMV, and consequently description in this report is limited to "a viroplasm-like area (VpA)". Although NCMV was localized in the 'non-denatured' cytoplasm rather than at the periphery of the VpA at an early stage of infection, pviruses were sometimes found close to the VpA (Fig. 8). Occasionally, a few uncoated particles were found within the VpA. However no mature particles were dispersed individually within it (Fig. 8 and 10). Moreover, virus aggregates surrounded by membranes were rarely observed inside the VpA (Fig. 10).

**Membraneous inclusions and filamentous inclusions**

Membraneous inclusions exhibiting round to ellipsoidal structures were found in the cytoplasm together with virus particles (Fig. 11). These inclusions appeared to be enclosed by a membranous sheath consisting of many layers of paired parallel
membranes. These membranes were sometimes tightly packed and membraneous inclusions demonstrated an appearance similar to that of the VpA (Fig. 13). The origin of the membraneous inclusions is unknown. Fig. 12 suggests that abnormal proliferation of the ER might be induced by virus infection following the formation of membraneous inclusions. Virus particles were localized between the parallel membranes at the periphery of the inclusions (Fig. 11 and 12).

Occasionally filamentous inclusions were found in NCMV infected cell cytoplasm and were very similar to inclusions described in wheat striate mosaic virus infected wheat32).

Naked particles of NCMV formed between membranes which occasionally protruded into a central vacuole

Membranes which protruded into vacuoles were sometimes seen in the infected mesophyll cells (Fig. 14). Frequently, 'naked' particles, corresponding in size to the inner nucleocapsides of NCMV, were observed between the parallel two membranes (Fig. 14 and 15). Sometimes, large numbers of 'naked' particles of NCMV were seen arranged between these membranes (Fig. 16). It seems possible that the protruding membranes are parts of a tonoplast and its formation is induced by virus infection. Mature virus particles were found in a small patch of cytoplasm which was enclosed by these membranes (Fig. 17). Occasionally, 'naked' particles also were seen close to or nearby a 'normal' tonoplast (Fig. 15). Sometimes, vesicles were formed in the cytoplasm near a tonoplast and relatively large numbers of 'naked' particles were seen between these vesicles (Fig. 17).

*Increase of Golgi vesicles in infected cells*

Many spherical vesicles (about 50-200 nm in diameter) with an electron dense core were found in 2nd leaf cells of samples taken 4 days after inoculation (Fig. 18-20). These vesicles were regarded as Golgi vesicles on the basis of the following observation: 1) frequent localization around cisternae of Golgi apparatus, and 2) presence of these vesicles together with the Golgi apparatus in the cells of healthy wheat plants. The number of Golgi vesicles in infected cells was greater than that in cells of corresponding parts of the 2nd leaf of healthy plants at the same growth stage. The profiles of Golgi vesicles of infected cells were more distinct than those of healthy ones. In healthy plants, a slightly more frequency of vesicles was seen in some cells of the vascular system and guard cells which underwent strong cell wall thickening. Even in infected plants, relatively numerous vesicles were frequently observed in cells of the vascular system and guard cells. Many vesicles were occasionally seen in mesophyll cells together with virus particles (Fig. 9). The Golgi vesicles were observed to localize together with the VpA and / or virus particles at an early stage of virus infection. These Golgi vesicles were seldom observed in completely developed 2nd leaf cells 8 days after inoculation.

**Discussion**

Fourth day samples showing early symptoms of NCMV depicted various profiles of virus growth stages from an early condition to slightly more advanced one. Moreover, viruses first appeared in the more developed vascular system rather than in developing ones. Virus particles were first seen in a few phloem or mesophyll cells adjacent to the phloem. In some instances, many virus particles were found accumulated in sieve elements at a relatively early stage of infection. These observations indicate that NCMV initially infects phloem cells during feeding by viruliferous planthoppers and that viruses may be translocated to a distant region through
the phloem. Lee\textsuperscript{17}) also examined the developmental stage of a leafhopper-transmitted virus—wheat striate mosaic virus—in wheat plants and showed that virus particles were limited to the vascular conducting system in those plants which showed no external symptoms.

It has been reported that NCMV is confined to the cytoplasm, and it has never been found in the nucleus\textsuperscript{24,31}). In this study, virus particles of NCMV were also seen in the nucleoplasm and perinucleolar spaces. Numerous particles of NCMV were sometimes localized in the neighborhood of the nucleus; also virus aggregates surrounded by membranes were occasionally found close to the outer membranes of the nucleus.

Many electron micrographs showed that enveloped particles of NCMV are within the cisternae of the ER. Although a few 'immature' particles of NCMV were occasionally present in the cytoplasm, there is little evidence to indicate how these 'immature' particles are enveloped. Fig. 7 suggests that 'immature' particles in the cytoplasm may acquire their envelope from ER elements by means of budding. However, intermediate stages reflecting this condition were seldom seen and in many cases, NCMV seemed to emerge into the cytoplasm without "budding". NCMV may become enveloped by membranes almost simultaneously as inner nucleocapsids are formed.

Enveloped viruses generally mature by obtaining membranes derived from the host. Maturation of animal rhabdoviruses occurs by budding from plasma membranes and / or intracytoplasmic vacuolar membranes (ER)\textsuperscript{8,20}). Plant rhabdoviruses also become enveloped by using inner nuclear or ER elements\textsuperscript{6}). Enveloped tomato spotted wilt viruses appear to undergo assembly by using membranes of the ER system\textsuperscript{14}). It has been reported that the lipid composition of the viral envelope closely resembles that of the host cell\textsuperscript{13,15,16,18}). NCMV has an envelope consisting of phospholipids and sterols\textsuperscript{28}). It has been shown that the composition of sterols are broadly resemble that of the whole host plant and its microsomal fraction. The content of cholesterol was about ten times higher in NCMV\textsuperscript{29}). This suggests that NCMV obtains host membranes which have been slightly modified by virus infection or NCMV incorporates selectively lipid components into its envelope. Occasionally, membranous inclusions and proliferation of ER were observed in NCMV infected cytoplasm. Virus particles were localized between the parallel membranes at the periphery of these membranous inclusions and suggests that active synthesis of membrane materials may occur as a result of virus infection.

An increase in the frequency of membranes which protruded into vacuoles was found in NCMV-infected mesophyll cells. It seems likely that a tonoplast might be induced abnormal development into the vacuole. Another possibility is that vacuolation, as shown in Fig. 17, which occurred in the cytoplasm near the tonoplast may result in the formation of protruding membranes. Reasons are unknown as to why mature particles cannot form on the membranes. It may be impossible for these particles to bud from the membranes and therefore they may remain 'naked'. NCMV has never been found in central vacuoles and this may be due to properties of the tonoplast itself which may prevent particles from budding into the vacuole. Furthermore, Fig. 17 shows that cytoplasm may also be necessary for the formation of mature virus particles.

As described above, the early appearance of NCMV infected cells was always associated with the formation of the VpA. In insect-borne plant pathogenic viruses, inclusions similar to the VpA have been observed in cells of host plants and insect vectors infected with various viruses (Table 1). Large conspicuous inclusions have
<table>
<thead>
<tr>
<th>Viruses</th>
<th>Insect vectors</th>
<th>Examined tissues</th>
<th>Cytoplasmic inclusions and texture</th>
<th>Presence of mature or immature particles in the inclusions</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tomato spotted wilt virus (TSWV)</td>
<td><em>Thrips tabaci</em></td>
<td>tomato*</td>
<td>viroplasm: amorphous, densely stained material dark diffuse masses</td>
<td>not observed</td>
<td>19</td>
</tr>
<tr>
<td>Lettuce necrotic yellows virus (LNYV)</td>
<td><em>Hyperomyzus lactucae</em></td>
<td>cucumber cotyledon*</td>
<td>viroplasm: masses of granular and fibrillar material</td>
<td>small spherical particles</td>
<td>10</td>
</tr>
<tr>
<td>Sowthistle yellow vein virus (SYVV)</td>
<td><em>Hyperomyzus lactucae</em></td>
<td><em>Nicotiana glutinosa</em></td>
<td>viroplasm (virogenic area)**</td>
<td>uncoated particles (very infrequently)</td>
<td>34</td>
</tr>
<tr>
<td>Rice dwarf virus (RDV)</td>
<td><em>Nephotettex cincticeps</em></td>
<td>insect vector</td>
<td>masses of filamentous or granular substances</td>
<td>not observed</td>
<td>23</td>
</tr>
<tr>
<td>Rice tungro virus (RTV)</td>
<td><em>Nephotettex cincticeps</em></td>
<td>rice plant, insect vector</td>
<td>masses of filamentous or finely granular material</td>
<td>mature particles</td>
<td>5</td>
</tr>
<tr>
<td>Wound tumor virus (WTV)</td>
<td><em>Agallia constricta</em></td>
<td>sweet and crimson clover, insect vector</td>
<td>viroplasm: electron dense materials of filamentous and granular elements</td>
<td>mature particles, doughnut shells (incomplete particles)</td>
<td>25, 26</td>
</tr>
<tr>
<td>Barley yellow striate mosaic virus (BYSMV)</td>
<td><em>Laodelphax striatellus</em></td>
<td>barley</td>
<td>viroplasm: electron dense intracytoplasmic masses of granular or finely fibrous</td>
<td>mature virus aggregates</td>
<td>3</td>
</tr>
<tr>
<td>Northern cereal mosaic virus (NCMV)</td>
<td><em>Laodelphax striatellus</em></td>
<td>wheat</td>
<td>viroplasm-like area: electron dense matrix of granular or fibrous materials</td>
<td>few immature particles, mature particles in aggregates (infrequently)</td>
<td>present paper</td>
</tr>
<tr>
<td>Maize rough dwarf virus (MRDV)</td>
<td><em>Laodelphax striatellus</em></td>
<td>maize</td>
<td>viroplasm: dense masses of thin fibrils</td>
<td>many immature particles</td>
<td>7</td>
</tr>
<tr>
<td>Rice black-streaked dwarf virus*** (RBSDV)</td>
<td><em>Laodelphax striatellus</em></td>
<td>maize, insect vector</td>
<td>electron dense granular structure of fine filamentous substances</td>
<td>small particles</td>
<td>23</td>
</tr>
</tbody>
</table>

* The virus was inoculated by sap inoculation.
** Inclusions were seen only in the nucleus.
*** RBSDV is a synonym of MRDV.
been found in RTV, WTV, MRDV, BYSMV and NCMV. In many cases, the inclusions have been interpreted as the site of viral assembly or the synthesis of viral components\(^1,3,25,26\). Developmental profiles of the VpA of NCMV suggest a possibility that the VpA of NCMV might be an expression of cytopathogenic effects by virus infection. In order to clarify the relationship of the inclusions in NCMV infected cells to virus assembly or synthesis of viral components, it is necessary to conduct cytochemical and autoradiographical tests.

Another outstanding feature of NCMV infected cells is proliferation of Golgi vesicles. These vesicles were especially abundant in cells of the vascular system and sometimes guard cells. The vesicles disappeared as leaf stage progressed. In phloem of various non-infected plants, Esau\(^4\) described that Golgi bodies (dictyosomes) are generally most abundant in young cells of phloem in which the wall is still growing, and disappear or are reduced in number when wall growth is completed. In some cases, abundant vesicles protruding from Golgi apparatus of young sieve elements have been observed\(^4\). When the numbers of Golgi apparatus and vesicles were compared with those of infected wheat plants, Golgi vesicles were more abundant in infected plants. It appears that the abnormal increase of vesicles is due to virus infection. Whaley et al.\(^33\) also reported that the number of small Golgi apparatus–associated vesicles seems to vary with cell activity. No evidence has been obtained for the role of these vesicles in NCMV multiplication or the synthesis of membranes such as ER which seems to be necessary for NCMV assembly. Northcote and Pickett-Heaps\(^21,22\) showed clearly that Golgi vesicles in plant cells are involved in the synthesis of cell wall polysaccharides. NCMV infection causes stunting of host plants. Cell elongation is strikingly suppressed in stunted leaves\(^30\). If abnormal metabolism of cell wall polysaccharides occurs within these Golgi vesicles, it might alter the chemical structure of cell wall and result in the cessation of plant growth. Further experiments are now in progress which are concerned with the relationship between development of these Golgi vesicles during virus infection and suppression of cell elongation.

**Literature cited**

Explanations of Figures

Fig. 1. Aggregates of virus particles and viroplasm like area (VpA) in the cytoplasm of a mesophyll cell.

Fig. 2. Various profiles of NCMV particles are seen according to the cutting plane.
Fig. 3. NCMV particles in the nucleoplasm of a phloem companion cell. N, nucleoplasm.
Fig. 4. NCMV particles surrounded by a membrane in the cytoplasm. bar, 0.25μm.
Fig. 5. and 6. NCMV particles accumulating in cisternae of the endoplasmic reticulum (ER). GA, Golgi apparatus.
Fig. 7. Four 'immature' particles (arrows) appear to be enveloping from the intracytoplasmic membranes by budding.
Fig. 8. A phloem cell at the early stage of infection. Note a large viroplasm like area (VpA) and virus particles in the cytoplasm or close to the VpA. An arrow indicates an 'immature' particle of NCMV (inner nucleocapsides). P, plastid; M, mitochondria.
Fig. 9. A part of mesophyll cell at the early stage of infection. Note viroplasm like area (VpA) and virus particles formed in ribosome particle rich cytoplasm. GA, Golgi apparatus; GV, Golgi vesicles.
Fig. 10. Virus aggregates surrounded by membranes inside the viroplasm like area (VpA) and an 'immature' particle (arrow).
Fig. 11. Membraneous inclusions in the cytoplasm. Virus particles of NCMV localize closely associated with the membranes of outer part of the inclusion.
Fig. 12. Proliferation of endoplasmic reticulum (?) and virus particles localizing between parallel membranes. N, nucleoplasm; VpA, viroplasm like area.
Fig. 13. Membraneous inclusion (mi) and compact viroplasm like area (VpA). Note virus particles close to the membraneous inclusion.
Fig. 14 and 15. Membranes protruding within the vacuole of a mesophyll cell and many 'naked' particles of NCMV distributed between the membranes. Note 'naked' particles close to the tonoplast in Fig. 15. VpA, viroplasm like area; N, nucleoplasm.
Fig. 16. Large numbers of 'naked' particles of NCMV formed arranged in parallel between the membranes within the vacuole of a mesophyll cell.
Fig. 17. Vesicles formed in the cytoplasm near the tonoplast and 'naked' particles of NCMV between these vesicles. Note mature particles (arrow) which present in small patch containing cytoplasm. v, vesicles.
Fig. 18. Many Golgi vesicles in cells of a developing phloem of wheat leaf. CW, cell wall.
Fig. 19. Proliferation of endoplasmic reticulum (ER), Golgi apparatus (GA) and many Golgi vesicles (GV) in the cytoplasm of a phloem cell of wheat leaf taken 4 days after virus inoculation.
Fig. 20. A Golgi apparatus and many Golgi vesicles around it. bar, 0.5μm.