Aggregates of Chloroplasts in Local Lesions induced in *Chenopodium quinoa* Wild. by Turnip Mosaic Virus

*(Accepted 24 May 1973)*

**SUMMARY**

Aggregates of up to 20 chloroplasts occur in parenchyma cells from local lesions induced in *Chenopodium quinoa* Wild. by turnip mosaic virus. Electron microscopic examination revealed a single layer of virus particles parallel to the surface of chloroplasts between most of the aggregated chloroplasts.

Cruciferous crops grown in São Paulo State, Brazil, are commonly infected with turnip mosaic virus (TuMV) (Costa, Kitajima & Nagai, 1972), an elongated, aphid-borne virus belonging to the potato virus Y (PVY) group (Tomlinson, 1970). *Chenopodium quinoa* Wild. is a useful indicator plant, producing conspicuous chlorotic local lesions after mechanical inoculation with sap from TuMV-diseased hosts. This communication reports a peculiar, virus-mediated aggregation of the chloroplasts in the cells of these local lesions.

*Chenopodium quinoa* plants, about 2 to 3 weeks old, were inoculated mechanically with an isolate of TuMV, obtained from and maintained in kale (*Brassica oleracea* L. var. *acephala*). Local chlorotic lesions were discernible in the inoculated leaves 5 to 7 days after inoculation.

Samples from these local lesions were fixed in 3% glutaraldehyde and post-fixed in 1% OsO₄ (both in phosphate buffer), dehydrated in acetone and embedded in Epon. Sections were stained with uranyl acetate and lead citrate, and examined in a Siemens Elmiskop I. For comparative purposes the following leaf material was also processed and examined as described above: kale and radish (*Raphanus sativus* L.) systemically infected with TuMV; local lesions in *Chenopodium amaranticolor* Coste & Reyn. and tobacco (*Nicotiana tabacum* L.) inoculated with TuMV; local lesions in *C. quinoa* inoculated with tobacco mosaic, Brazilian tobacco rattle, potato virus X, potato virus S, bidens mosaic (Kitajima, Carvalho & Costa, 1961), lettuce mosaic, and celery yellow mosaic (Kitajima & Costa, 1968) viruses.

Light-microscopic examination of the tissues from the local lesions were made on hand-cut unstained sections floated on buffered 3% glutaraldehyde as well as on thick (1 to 2 μm) sections on Epon-embedded tissues, stained with a mixture of Azur II and methylene blue. Most palisade and spongy mesophyll cells contained chlorotic chloroplasts forming large clumps (Fig. 1, inset), instead of being uniformly distributed along the cell periphery. Chloroplast aggregation was rare in vascular parenchyma cells.

Chloroplast aggregates were best seen in thin sections examined in the electron microscope (Figs. 1 to 4). Under low-power magnification they were seen to consist of up to 20 chloroplasts, tightly apposed to each other in a chain or irregularly grouped. Individual chloroplasts usually contained several large osmiophilic granules, a poorly developed lamellar system, few or no starch grains, and also had an irregular profile, depending on the surrounding chloroplasts.

At higher magnifications, a layer of elongated particles was seen between most of the adjacent chloroplasts in the aggregates. These particles were arranged parallel to each other, with their long axes parallel to the chloroplast surface (Figs. 2 to 4). They also appeared
Figs. 1 to 4. For legends see facing page.
between the chloroplast and the tonoplast where these structures were closely apposed (Fig. 3), as well as in narrow strands of cytoplasm projecting into the vacuole (Fig. 2). In several instances they were seen at the surfaces of the chloroplasts that were in direct contact with the cytoplasm and not apposed to other chloroplasts (Figs. 2, 3). Whenever elongated particles were associated with the surface of the chloroplast they always appeared in a single layer.

The diam. of the elongated particles was 10 to 12 nm but their length was not measurable. They probably represent TuMV because they are similar to the TuMV particles observed in sections of purified preparations (Hill & Shepherd, 1972) and those pelleted by ultracentrifuging (Fig. 4, inset).

In addition to the chloroplast aggregates, most cells contained many lamellar inclusions, with varied configurations depending on the plane of sectioning, which are characteristic of cells infected with viruses of the PVY group (Edwardson, Purcifull & Christie, 1968) (Fig. 1).

Similar, although much less conspicuous, chloroplast aggregates were observed in local lesions from TuMV-infected Chenopodium amaranticolor, but not in tobacco (local lesions) or radish and kale (systemically infected leaves). Chloroplast aggregates were not detected in local lesions, from C. quinoa leaves induced by other elongated viruses (tobacco mosaic, Brazilian tobacco rattle, potato viruses S and X) including some other members of the PVY-group (bidens mosaic, lettuce mosaic, celery yellow mosaic). Previous studies on cytological aspects of TuMV-infection in cruciferous hosts (Hayashi, Matsui & Yamaguchi, 1965; Kamei, Honda & Matsui, 1969; Edwardson & Purcifull, 1970) do not mention aggregation of chloroplasts.

Some degree of plastid aggregation associated with virus particles was reported in chinese cabbage infected with turnip yellow mosaic virus (Chalcroft & Matthews, 1966) and in barley infected with barley stripe mosaic virus (Carroll, 1970), but it was much less conspicuous than the aggregation described here. Moreover, the rod-shaped particles of barley stripe mosaic virus were attached to the chloroplast surface by their ends and not by their sides. A similar difference was reported between a Brazilian strain of tobacco rattle virus (Harrison & Roberts, 1968; Kitajima & Costa, 1969) and henbane mosaic virus (Kitajima & Lovisolo, 1972) in their association with mitochondria. The former attach to the mitochondrial surface by their ends, whereas the latter attach by their sides. Thus the pattern of plastid aggregation in TuMV-infected Chenopodium quinoa is similar to the mitochondrial aggregate in henbane mosaic virus-infected Datura stramonium L. (Kitajima & Lovisolo, 1972) and the mechanism of aggregation proposed for that system is probably applicable here. The specific interaction between the outer surface of the chloroplast and the TuMV particles probably involves specific receptor sites. However, in preliminary trials, chloroplasts isolated

---

Fig. 1. Electron micrograph of a thin section of a local lesion in Chenopodium quinoa caused by TuMV. Cells are somewhat shrunken and most chloroplasts (P) are aggregated. They usually contain large osmiophilic granules, underdeveloped lamellar system, and irregular contour. Lamellar inclusions (LI), typical for viruses of the PVY group can be seen abundantly in the cytoplasm. In the inset a light micrograph of a similar but thicker section, showing chloroplast aggregates in parenchyma cells close to the vascular bundle. X, xylem; IS, intercellular space; vc, vacuole; W, cell wall.

Fig. 2. A chloroplast aggregate in which a layer of TuMV particles can be seen 'sandwiched' between adjacent plastids (P). Some virus particles can be seen in cytoplasmic strands projecting into the vacuole (arrow).

Figs. 3, 4. A higher magnification of a chloroplast aggregate. Virus particles (arrows) can be seen in transverse or longitudinal orientation. Inset of Fig. 4 shows a section of a purified TuMV preparation, pelleted by ultracentrifuging.
from healthy C. quinoa failed to aggregate when allowed to react with purified TuMV in vitro. Possibly the physiological state of the chloroplast in the infected cell is important.

Because hand-cut sections may easily be prepared and examined the detection of chloroplast aggregates in TuMV-infected Chenopodium quinoa may have a useful specific diagnostic value, provided that this phenomenon could be demonstrated with other strains of TuMV.

This work received financial support from Conselho Nacional de Pesquisas-CNPq (TC 12275) and Fundação de Amparo a Pesquisa do Estado de São Paulo (C. Agron. 72/376). The authors are research fellows of CNPq.

Virus Department
Instituto Agronômico
13.100-Campinas, SP
Brazil

E. W. KITAJIMA*
A. S. COSTA

REFERENCES

(Received 29 March 1973)

* Present address: Dept. Biologia Celular, ICB, Universidade de Brasília, 70.000 Brasília, DF, Brazil.