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Ultrastructural Comparison of Soybean Differentials Infected with a Virulent SMV Strain

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병독성 콩모자이크바이러스계통에 감염된 콩판별품종의 미세구조의 비교

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ABSTRACT : Two soybean cultivars, Kwanggyo and Hwanggeum (soybean mosaic potyvirus (SMV)-resistant cultivars), that had been inoculated with a virulent strain (G-5H, 4) of soybean mosaic potyvirus produced necrotic lesions on inoculated leaves as well as on upper trifoliate leaves. Cells in the lesion area contained sparse numbers of virus particles and very few characteristic pinwheel inclusions. Although a hypersensitive-like cellular response occurred in the two resistant cultivars, this response did not prevent the virus from spreading systemically in these resistant hosts, indicating a different mechanism from the general hypersensitive reaction in relation to host resistance.

Key words : soybean, soybean mosaic potyvirus.

Soybean mosaic potyvirus (SMV) causes various types of symptoms in soybeans depending upon combinations of soybean genotype and virulence of soybean mosaic virus strains (1-3). The present study was undertaken to compare differences in ultrastructural responses of host cells including reactions of cellular organelles and the occurrence of characteristic cylindrical cytoplasmic inclusions in resistant and susceptible soybean cultivars.

MATERIALS AND METHODS

The isolate of soybean mosaic potyvirus (SMV) maintained in the greenhouse at University of Arkansas was used and its classification was determined by the Good man and Cho's system (1). The two resistant soybean cultivars used were Kwanggyo and Hwanggeum which are high yielding and highly resistant cultivars. The two susceptible soybean cultivars showing some differences in severity with mosaic symptoms.

Soybean seeds of Kwanggyo, Hwanggeum, Eunha, and Milyang #4 were obtained from Drs. Seog-dong Kim and Du-chul Shin, Department of Upland Crop of Suwon Crop Experiment Station, and Youngnam Crop Experiment Station, Milyang, Korea, respectively.

Soybean seeds used as differentials were Buffalo, Marshall, Ogden, York, and Davis. These soybean cultivars were used for classification of soybean mosaic virus strains and were obtained from Dr. R. Nelson, USDA soybean germplasm collection, Urbana, Illinois, USA.

Soybean seeds were planted in Redi-Earth and grown in a greenhouse maintaining at a 14-hour-growing period at 20~24°C.

Seedlings were inoculated at the primary leaf stage by dusting carborundum (silicon carbide powder, Buehler, LTD, Lake Bluffm IL) on the leaves and carefully wiping the inoculum on the upper leaf surface. Inoculum was prepared by homogenizing trifoliate leaves from systemically infected plants in 0.05 M phosphate buffer solution (PBS), pH 7.2, using a mortar and pestle.

Samples (approx. 2~3 mm) were taken from leaves of control and systemically infected plants, and fixed in a modified Karnovsky's fixative for 2 hours in a week vacuum. Samples were rinsed in 0.5 M cacodylate buffer solution, postfixed with 1% osmium tetroxide in 0.5 M cacodylate buffer for 2 hours, rinsed with 0.5 M cacodylate buffer and presented overnight with 0.5% aqueous uranyl acetate. Samples were then dehydrated in a graded ethanol series, processed through propylene oxide and em-

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bedded in Spurr's medium (6). Embedded tissue was trimmed and sectioned with a Diatome diamond knife using a Sorvall MT-2B ultramicrotome. Sections were stained with 2% uranyl acetate and lead citrate, then viewed on a JEOL 100 CX electron microscope.

RESULTS AND DISCUSSION

Ultrastructural examination of SMV infection has revealed very striking differences in plant/pathogen interactions between susceptible and resistant cultivars of soybean. Cells of the two susceptible cultivars that were used in this study, exhibited characteristic ultrastructural features of SMV systemic infection (Figs. 1, 2), including great abundance of SMV particles and cylindrical cytoplasmic inclusions in systemically infected cells. A very unusual structure feature discovered in one of the susceptible cultivars Eunha, was the appearance of invaginated plasmalemma (vesicles) closely decorated with SMV particles near the cell wall (Fig. 3).

The decoration was linearly arranged SMV particles (Fig. 4). Such cellular feature has not been noted before in case with SMV infection.

In the two resistant soybean cultivars infected with SMV, there was no great abundance of SMV particles or inclusions as was observed in the two susceptible cultivars. although SMV infection was obvious. However, cellular signs of viral infection were obvious, such as degradation of cellular nucleus (Figs. 5~10). Obvious indications of organelle degradation were: clumping of chromatin in the cell nucleus (Figs. 5 & 6), increased number and size of starch grains in chloroplasts (Fig. 5), and also the occurrence of crystalline phytoferritin

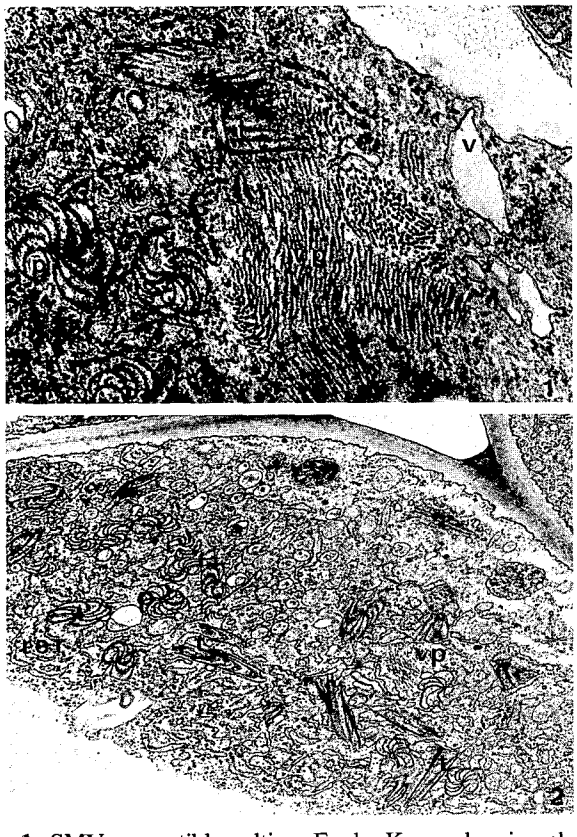


Fig. 1. SMV susceptible cultivar Eunha-Kong, showing abundant SMV particles (vp) and characteristic pinwhells (p) and tubules (t) of cylindrical inclusions. vacuole (v), Mag. 66000X.

Fig. 2. SMV susceptible cultivar, Milyang #4, showing cytoplasm of palisade parenchyma cells, containing various configurations of cylindrical inclusions, including ubule (t), and pinwheels (p), and virus particles (vp). These inclusions are associated with proliferated rough endoplasmic reticulum (rer). Mag. 20000X.

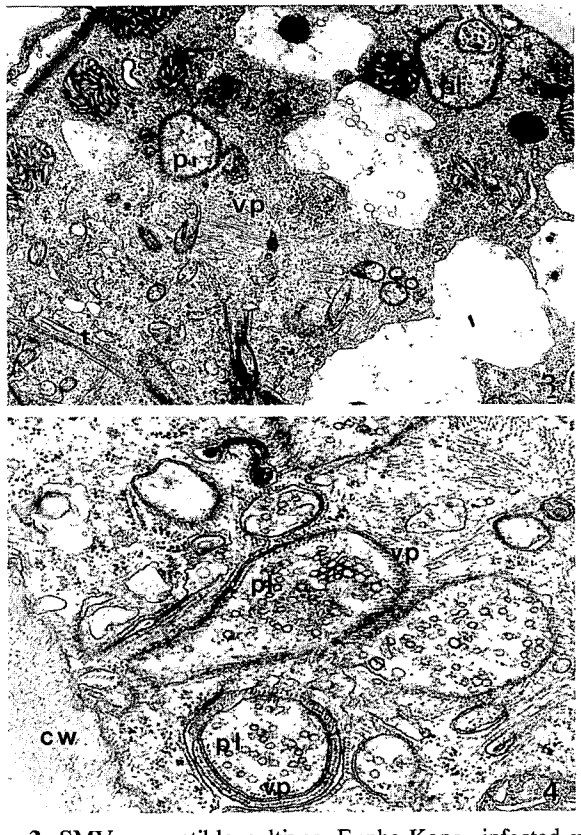
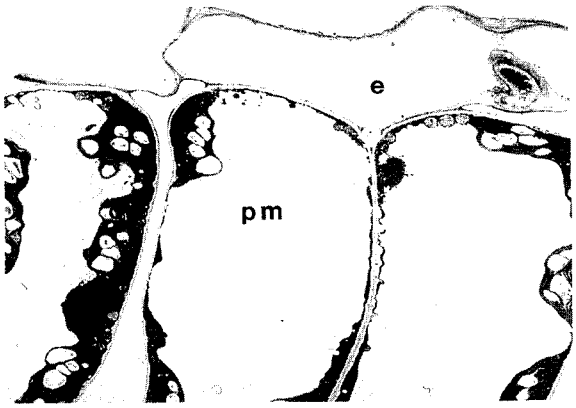


Fig. 3. SMV susceptible cultivar, Eunha-Kong. infected with a virulent SMV strain. Note the cytoplasmic inclusions and virus particles (Vp) and vescicles decorated with SMV particles. Mitochondria (m), vacuole (v). Mag. 26000X.

Fig. 4. SMV-susceptible cultivar Eunha-Kong. Higher magnification of invaginated plasmalemma (pl) showing details of "decoration" of SMV particles (in both cross and longitudinal sections) (vp), surrounding the membranous structure. Note cell wall (cw), verifying origination of membrane. Mag. 52000X.



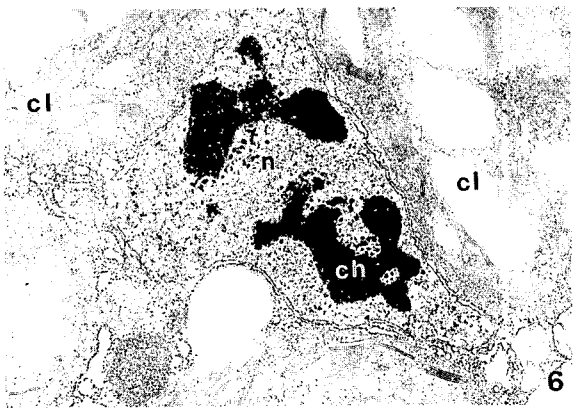


Fig. 5. SMV-resistant cultivar Kwanggyo infected with a virlulent sMV strain. Low magnification of 3 palisade mesophyll cells. Note the clumping of chromatin material and degradation of nucleus. Mag. 4000X.

Fig. 6. SMV-resistant cultivar Kwnaggyo. Higher magnification of Fig. 5 with nucleus (n) in a mesophyll cell (ch), indicating degradation of nucleus. Chloroplast (cl). Mag. 20000X.

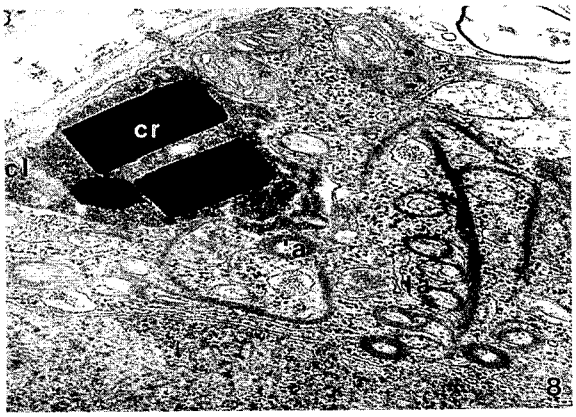


Fig. 7. Several vascular bundle parenchyma cells of SMV-resistant cultivar, Hwanggeum. Note one cell containing cylindrical potyviral inclusions (pinwheel) (p). Cellular organelles appear normal. Nucleus; (n), chloroplast; (cl), mitochondria; (m). Mag. 20000X.

Fig. 8. SMV resistant cultivar, Kwanggyo, showing various configurations of cylindrical inclusions, including laminated aggregates; (la) and tubules; (t). Note chloroplast; (cl) is filled with three large crystalline inclusions; (cr). Mag. 52000X.

(Figs. 9, 10), and other crystalline inclusions (Fig. 8) present in chloroplasts. Interestingly some vascular bundle cells of resistant cultivars contained sparse pinwheel inclusions (Fig. 7), indicating the occurrence of SMV infection with little viral replication. The process of necrosis of single cells (Fig .9) must have occurred quickly, since cells adjacent to the necrotic cells often showed no evidence of cellular degradation.

Necrotic reactions of cells infected with virus were considered to be a resistant reaction, *i.e.* so called hypersensitive reaction which leads to cellular death, and therefore blocks the systemic spread of the virus (5). However, our results from soybean-SMV interactions indicated that lethal responses failed to block the systemic spread of the virus. Virus particles, and inclusion bodies were observed in necrotic cells, and other cells adjacent to these necrotic cells indicating that viral replication and penetration occurred in these areas (Figs. 9 & 10)

Our results suggest that a signal derived from the infection of the host virulent strain to root cells might trigger redundant metabolic activities leading to cellular death, but the resistant response might be not so rapid to block the systemic spreads of virus to healthy cells.

It may be worthwhile to determine what biochemical components the host cell produces to trigger this redundant metabolic activity. This information can aid in determining the soybean genotype and SMV strain interactions.

Further research on this subject should also include ultrastructural studies involving sampling from systemically infected areas away from the initial infection site.

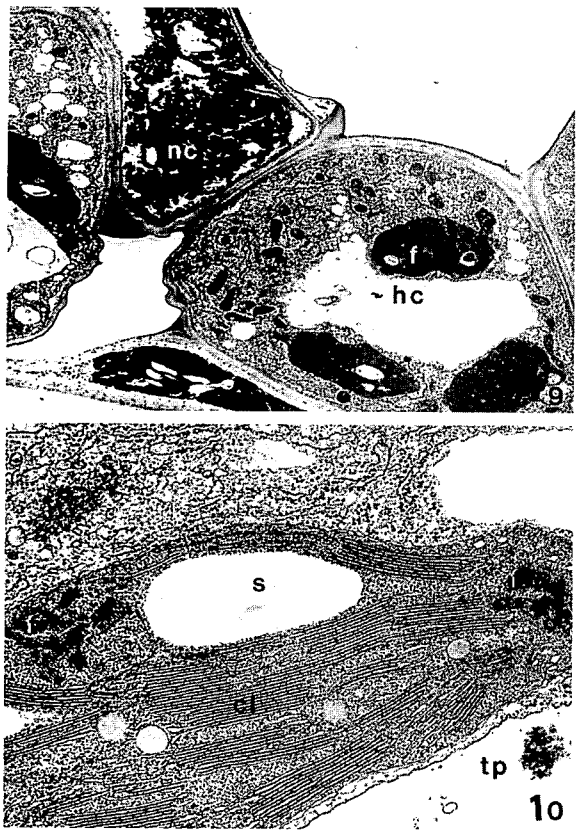


Fig. 9. SMV-resistant cultivar, Hwanggeum. Section containing healthy appearing cells (hc) adjacent to a necrotic cell(nc), indicating a hypersensitive-like cellular response. Note chloroplast in healthy looking cell contains if phytoferritin crystals; (f). Mag. 6. 6000X

Fig. 10. SMV-resistant cultivar, Hwanggeum. Higher magnification showing detail of phytoferritin crystals (f) contained in chloroplast (cl). Cell wall (cw), mitochondrion (m), tonoplast (tp), starch grain(s). Mag. 40000X.

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요 약

콩 모자이크 바이러스에 저항성이 높은 콩품종 광교와 황금콩을 콩 모자이크 바이러스 병독계통(SMV-G5H)(4)으로 접종한 결과 접종한 제일 본엽과 상위의 삼엽에 괴저병징이 나타났다. 병징이 나타나기 직전에 시료를 선택하여 전자현미경으로 조사한 결과 바이러스입자를 거의 관찰할 수 없었으며 포티바이러스군의 독특한 풍차형의 봉입체도 거의 관찰할 수 없었다. 이러한 현상은 바이러스감염에 의해 과민반응을 보이는 일반적인 과민반응의 괴저 병반 발생과는 다르게 바이러스의 전신감염을 억제하지 못하므로 콩모자이크바이러스 저항성 품종의 기작으로 주목된다.

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