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Scientific Notes

Rice Ragged Stunt Virus (*Oryzavirus*) Possesses an Outer Shell and A-spikes

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ABSTRACT

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Rice ragged stunt virus (RRSV) (*Oryzavirus*) particles prepared from extracts of fixative-treated infected leaf tissues were analyzed by electron microscopy. Complete double-shelled particles about 75 to 80 nm in diameter with extruding A-spikes were observed by phosphotungstic acid staining in extracts of infected leaf when tissues were fixed with either 2 % glutaraldehyde or a mixture of 1 % acrolein and 2.5 % glutaraldehyde prior to extraction. A-spikes, about 10 to 12 nm wide and 8 nm long, were attached to the outer end of the B-spikes. Frequently, subviral particles, 62 to 66 nm in diameter, with an inner shell and the B-spikes which were 25 to 27 nm wide and 10-13 nm long were observed in specimen negatively stained with phosphotungstic acid. The lack of outer capsid layer was previously reported for RRSV and was once considered as a distinct morphological criterion that separates the virus from other 10-segmented double-stranded RNA plant reoviruses. Current investigations

revealed that the RRSV possessed an outer shell and A-spikes similar to those reported for members of the virus in genus *Fijivirus*.

(Key words : electron microscopy, *Fijivirus*, *Oryzavirus*,rice ragged stunt virus, virion morphology)

Rice ragged stunt disease was first found in Indonesia in 1976[11], and later shown to be widespread in countries throughout Asia where rice is grown[5,14]. The disease is caused by rice ragged stunt virus (RRSV) and transmitted by the brown planthopper *Nilaparvata lugens* Stal in a persistent manner[3,11,14]. Symptoms induced by the virus in plants of Japonica-type rice include stunting with twisted and serrated dark green leaves. Whitish, spindle-shaped enations may develop on the lower surface of leaf blades and inner surface of leaf sheaths of infected plants on some Indica-type varieties.

The genome of RRSV consists of 10 double-stranded RNA (dsRNA) segments [7,9,13,18]. SDS-PAGE analysis of purified virus preparations revealed a protein profile with five major polypeptides and two minor ones[7,9]. These features are similar to the basic properties reported for a fijivirus[8,13]. There are, however, differences among investigators regarding to their observations on the particle morphology of RRSV [6,9,13,15]. Particles of about 50 nm, an equivalent to the nucleoprotein core of other plant reoviruses, were found in purified preparations of RRSV. No outer capsid layer (the outer shell) and spikes were seen [9,15,16]. In thin sections of viruliferous insect cells, normal particles of 70 nm in diameter were frequently observed[6,10]. These micrographs, nevertheless, failed to show the presence of a distinctive two layer structure for the particles. In this paper, we present evidence that shows the presence of an outer shell and the A-spikes on RRSV particles in leaf dip preparations.

A field-collected isolate of RRSV was maintained in a screen-house on the Japonica-type Tainung 67 or the Indica-type Taichung native 1 (TN1) rice plants by serial inoculations using viruliferous planthoppers *N. lugens* raised in insectaries[2,3]. For electron microscopy, young rice seedlings were inoculated with viruliferous insects. When the inoculated plants showed symptoms, pieces of diseased leaf tissues were fixed in 2 % glutaraldehyde (GA) in 0.1 M phosphate, pH 7.0, or in a mixture of 1 % acrolein and 2.5 % GA in 0.2 M cadodylate buffer (50 ml of 0.2 M cacodylate buffer, pH 7.2; 4 ml of 25 % acrolein ; 10 ml of 25 % GA in water, and 36 ml of distilled water) in an ice bath overnight. Slices of infected leaf tissues were triturated in drops of sterile water.

One drop of the crude sap and one drop of 0.1 % bacitracin were mixed on a parafilm membrane. Grids with carbon-stabilized formvar membranes were floated on top of the mixed liquid.After one minute, the liquid was removed with a filter paper and the specimen stained with 2 % neutral phosphotungstic acid (PTA), or 2 % uranyl acetate (UA), pH 4.2. The stained specimens were examined under a JEOL

200X electron microscope at 80 KV.

Subviral particles with an inner shell, about 55~60 nm in diameter, and some viral cores, about 50 nm in diameter, were frequently observed in dip preparations from RRSV-infected tissues when stained in PTA (Fig. 1A). Filaments, about 10 nm wide extending from these particles varied in lengths (Fig.1A). The subviral particles retained some B-spikes (arrows), which measured 10~12 nm in length and 20~22 nm in width when stained in UA (Fig. 1B) and 10~13 nm in length and 25~27 nm in width when stained in PTA (Fig. 1 C, D).

The observed B-spikes of RRSV appeared to be broader at base when compared with most fujiviruses[1]. Negative staining with PTA (Fig.1A, C, D, E) usually resulted in larger-sized virion particles than those stained with UA (Fig. 1 B, F). Subviral particles with A-spikes that rested on the B-spikes were also observed (Fig. 1E) . UA-stained intact particles, 75~80 nm in diameter, with papilla-like (globular) A-spikes, 10~12 nm in width and 8 nm in length, were observed when infected tissues were fixed with a mixture of 1 % acrolein and 2.5 % GA prior to extraction (Fig. 1F).

Current grouping has placed RRSV in a newly established genus, *Oryzavirus*, formerly plant reovirus subgroup 3 (or Phytoreovirus III). RRSV is the type member of the genus[1,12,17]. The only other member is *Echinochloa* ragged stunt virus (ERSV), which infects *Echinochloa* but not the rice plant[2]. Both RRSV and ERSV are transmitted specifically by planthopper vectors in a persistent manner, RRSV *by N. lugens*, whereas, ERSV by *Sogatella longifurcifera* (Esai et Ishihara)[2,4,11]. The two oryzaviruses are serologically related[2]. They show similar genomic organizations and share many biological properties similar to those reported for *Fijivirus*[1,11,18]. Members of both *Fijivirus* and *Oryzavirus* contain 10 segmented dsRNA, whereas viruses in the genus *Phytoreovirus* contain 12 segmented dsRNA[1,2,7,12,13,18].

Grouping of plant reoviruses based on virion morphology is difficult and could be misleading. Although a larger size of virions previous observed in thin section of RRSV infected plant leaves or insect tissues suggest the presence of an outer structure[6,10,15], only the B-spiked subviral particles and the smooth spherical viral cores were commonly seen in dip preparations[2,6,7,10,13,15]. The lack of an outer capsid layer was once considered a major feature for distinguishing RRSV from members of *Fijivirus*[8,12,15,16,17]. The demonstration of an outer capsid layer and both A and B spikes in RRSV in current studies and in ERSV in a previous investigation[6] shows similar virion morphology described for the members in the genus *Fijivirus*[8,12].

Nucleotide sequence analysis has revealed conserved oligonucleotides at both termini for the dsRNA segments of each of the three genera of plant reoviruses. Members of same genus share the same sequence homology. Thus the terminal oligonucleotide sequence are (+) 5'-GGU/CA....U/CGAU-3' for the genus *Phytoreovirus* and (+) 5'-AAGUUUUU.....GUC-3' for the genus *Fijivirus*; whereas, the terminal oligonucleotide sequences for RRSV and ERSV are (+) 5'-GUAAA....GUGC-3' [19,20]. The terminal oligonucleotide sequ-

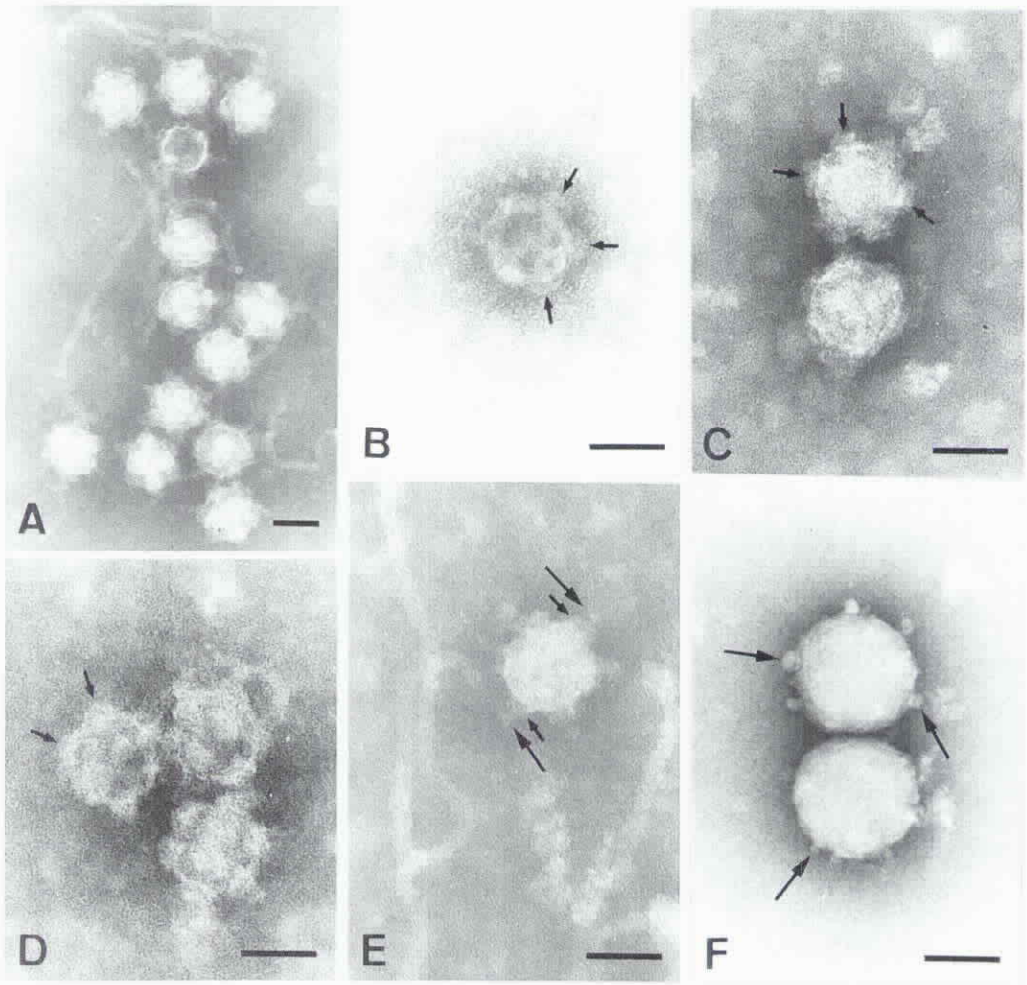

Fig.1. Electron micrographs of rice ragged stunt virus (RRSV) particles in leaf dips of infected plants. Infected leaf tissues were fixed with 2% glutaraldehyde (Panels A-E),or 1 % acrolein-2.5% glutaraldehyde (Panel F) before extraction. A. Subviral particles with B-spikes. Filamental structures extending from some of the B-spiked particles were also observed. B. A single B-spiked subparticle stained with 2% uranium acetate, pH 4.2. The B-spikes are indicated with short arrows. C and D. Subviral particles stained with 2% neutral phosphotungstic acid (PTA). The B-spikes are indicated with short arrows. E. A subviral particle stained in PTA. Note the A-spikes (shown with long arrows) attaching to the outer ends of the B-spikes (shown with short arrows). F. Complete virions of RRSV showing A-spikes (shown with long arrows) residing on the outer shells. Panels A, E, and F were from preparations derived from virus infected TN1 plants, whereas panels B, C, and D were from preparations made from virus infected Tainung 67 plants. Bars represent 100 nm.

ence information, so far analyzed, are genus specific, providing an additional reliable taxonomic criterion for plant reoviruses[17,19].

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摘　要

陳慶忠[1]、陳脈紀[2]、邱人璋[3]、徐惠迪[4]　1996　水稻皺縮矮化病毒具有外鞘及外鞘上之A突起。植保會刊　39: 383-388。([1]台中區農業改良場；[2]國立中興大學植病系；[3]國立中興大學遺傳工程中心；[4]美國農部農業研究中心花卉與觀賞樹木研究室)

水稻皺縮矮化病病葉組織，先以2％戊二醛或1％ acrolein 混合2.5％戊二醛之混合液於24℃固定逾夜，移出置蒸餾水中磨碎，其粗汁液與等量之0.1％ Bacitracin 混合，再經 PTA 陰染，置電子顯微鏡檢查，可觀察到直徑75～80nm 具雙層鞘蛋白及 A-spikes 突起之完整水稻皺縮矮化病毒 (Rice ragged stunt virus, RRSV)顆粒。A-spikes 呈乳頭狀，寬約10～12 nm，長約8 nm，附著於外層鞘蛋白上，其基部與內層之 B-spikes 相銜接。在電顯下亦經常觀察到僅含內層鞘蛋白而具有 B-spikes 之粒子，直徑約55～60 nm。B-spikes 基部寬約25～27 nm，長約10～13 nm。植物 Reoviruses 根據其形態、遺傳基因體片段數(dsRNA segments)及各片段末端核甘酸序列而區分爲三屬，即 *Phytoreovirus*、*Fijivirus* 及 *Oryzaviru*。RRSV 爲 *Oryzavirus* 屬之代表成員，稗草皺縮矮化病毒 (*Echinochloa* ragged stunt virus, ERSV)爲另一成員，二者之形態均與fijivirus 相似。過去筆者等報告指出形態完整之 ERSV 病毒顆粒具有雙層鞘蛋白及 A-spikes 等構造。而 RRSV 之外層鞘蛋白及 A-spikes 構造存在與否，則在文獻中多有爭議。本文首次提供明確之電顯證據，證明經過適當固定之 RRSV病毒顆粒具有雙層鞘蛋白及著生於外鞘上之 A-spikes。

(關鍵詞：電顯、植物病毒、病毒形態、水稻皺縮病毒、*Fijivirus*、*Oryzavirus*)