

Scanning electron microscopy of rickettsia-like organisms in latent rosette-diseased *Beta vulgaris*

Rasterelektronenmikroskopische Untersuchungen über Rickettsien-ähnliche Bakterien in *Beta vulgaris*-Pflanzen mit Latenter Rosettenkrankheit

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Summary

In the phloem and xylem tissue of latent rosette-diseased beet plants, elongated rickettsia-like organisms of $0.8 - 4.0 \times 0.15 - 0.3 \mu\text{m}$ and coccoid forms of approximately $0.4 \mu\text{m}$ diameter were detected by scanning electron microscopy.

Key words: sugar beet; *Beta vulgaris*; latent rosette disease; rickettsia-like organisms; scanning electron microscopy

Zusammenfassung

Im Phloem- und Xylemgewebe von *Beta vulgaris*-Pflanzen mit „Latenter Rosettenkrankheit“ wurden rasterelektronenoptisch gestreckte Rickettsien-ähnliche Organismen von $0.8 - 4.0 \times 0.15 - 0.3 \mu\text{m}$ und coccoide Formen von ungefähr $0.4 \mu\text{m}$ Durchmesser nachgewiesen.

Stichwörter: Zuckerrübe; Latente Rosettenkrankheit; Rickettsien-ähnliche Organismen (RLO); Rasterelektronenmikroskopie

1 Introduction

True Rickettsiae are rod-shaped, coccoid and often pleomorphic micro-organisms, with typical bacterial cell walls and no flagellae. They are Gram-negative and multiply by binary fission inside living host cells (MOULDER 1974).

Rickettsia-like organisms (RLO's) are known to infect a broad range of host plants (HAKKAART 1980, HOPKINS 1977, MARAMOROSCH 1974, NIENHAUS 1976, RUMBOS 1978). Generally, RLO's are xylem- or phloem-invading micro-organisms, but some forms occur in both tissues or even in meristematic and young differentiating cells (NIENHAUS and SIKORA 1979).

The latent rosette disease of *Beta* spp. first described for the Federal Republic of Germany by SCHMUTTERER and LORRA (1976), was also found in the German Democratic Republic

(PROESELER 1982), and is transmitted by the leaf bug *Piesma quadratum* Fieb. (SCHMUTTERER 1976). The symptoms consist of asymmetric outgrowths of new developing leaves with an occasional marginal chlorosis; subsequently formed leaves are dwarfed and downward rolled, resulting in the formation of a typical rosette or witches' broom (GREEN and NIENHAUS 1980). Typical RLO's measuring $0.7 - 1.4 \times 0.15 - 0.2 \mu\text{m}$, usually with a wavy wall, were detected in infected leaf bugs and in infected beet plants (NIENHAUS and SCHMUTTERER 1976, STRAUSS-GREEN 1980).

The RLO's from beet have not yet been cultured in vitro. The diagnosis of the disease agents involved was mainly based on electron microscopy of the suspected etiological agent in the vascular bundles of diseased plants and in tissues of the insect vector *Piesma quadratum* (NIENHAUS and SCHMUTTERER 1976), on staining of RLO nucleic acids in the sieve tubes (GREEN and NIENHAUS 1980), and on positive reactions with specific antisera (GREEN and NIENHAUS 1981).

This paper reports the use of scanning electron microscopy (SEM) for a further understanding of the localization of the RLO's associated with the latent rosette disease of *Beta vulgaris* and for the study of the morphology and colony-formation of these organisms in situ.

2 Material and methods

2.1 RLO's

The RLO's were kept in *Beta vulgaris* host plants showing typical symptoms (Fig. 1). They were routinely transmitted to healthy plants with infected leaf bugs (*Piesma quadratum*).

2.2 Preparation of specimens for SEM

Transverse sections of leaf petioles and roots of healthy and diseased plants were prepared for SEM according to the method of SAMSON et. al. (1980). The specimens were studied in a Leitz EMR 1000 A scanning electron microscope at an acceleration voltage of 30 kV.

3 Results and discussion

Elongated RLO's of $0.8 - 4.0 \times 0.15 - 0.3 \mu\text{m}$ and coccoid forms of approximately $0.4 \mu\text{m}$ were detected singly or in small colonies in the phloem and xylem tissues of roots and leaf petioles of 2 to 6-week-old *Beta vulgaris* plants. These organisms were very numerous in the phloem tissue of roots. The most abundant colonization was detected in the phloem tissue of the petioles of young leaves of a one-year-old sugar beet at approximately 6 weeks after infection. Contrary to the observations of STRAUSS-GREEN (1980), the RLO's were also detected in the xylem tissue.

Elongated forms were predominant (Fig. 2, 3), but also coccoid and irregular forms were observed (Fig. 4, 5). In some cases, the coccoid forms showed germtube-like outgrowths (Fig. 6), and the elongated forms sometimes showed binary fission.

Dual infections of plants with RLO's and mycoplasma-like organisms (MLO's) are known (GRUNEWALDT-STÖCKER and NIENHAUS 1977, DAVIS et. al. 1982). Therefore, the possibility that the coccoid forms in our preparations are MLO's cannot be excluded. The true nature of these coccoid forms may be elucidated by transmission electron microscopical (TEM) studies of latent rosette-diseased plant material treated with specific antisera.

Most of the RLO's observed in latent rosette-diseased beet plants had smooth walls. In some cases, however, wavy walls were observed (Fig. 7), comparable to the wavy or rippled cell walls observed by TEM studies of these RLO's in both *Beta vulgaris* and *Piesma quadratum* (STRAUSS-GREEN 1980). GREEN and NIENHAUS (1980) suggested that this rippling is an artifact due to the fixation process. It is necessary to study the walls of other RLO's as well with more sophisticated EM techniques.

Diseased beet plants show remarkable cell wall thickenings in the phloem tissue of the petioles (Fig. 8). But in healthy plants, where RLO's are absent, cell wall thickenings were not found. Comparable wall thickenings are frequently found in MLO-diseased plants (GIUNCHEDI et. al. 1982).

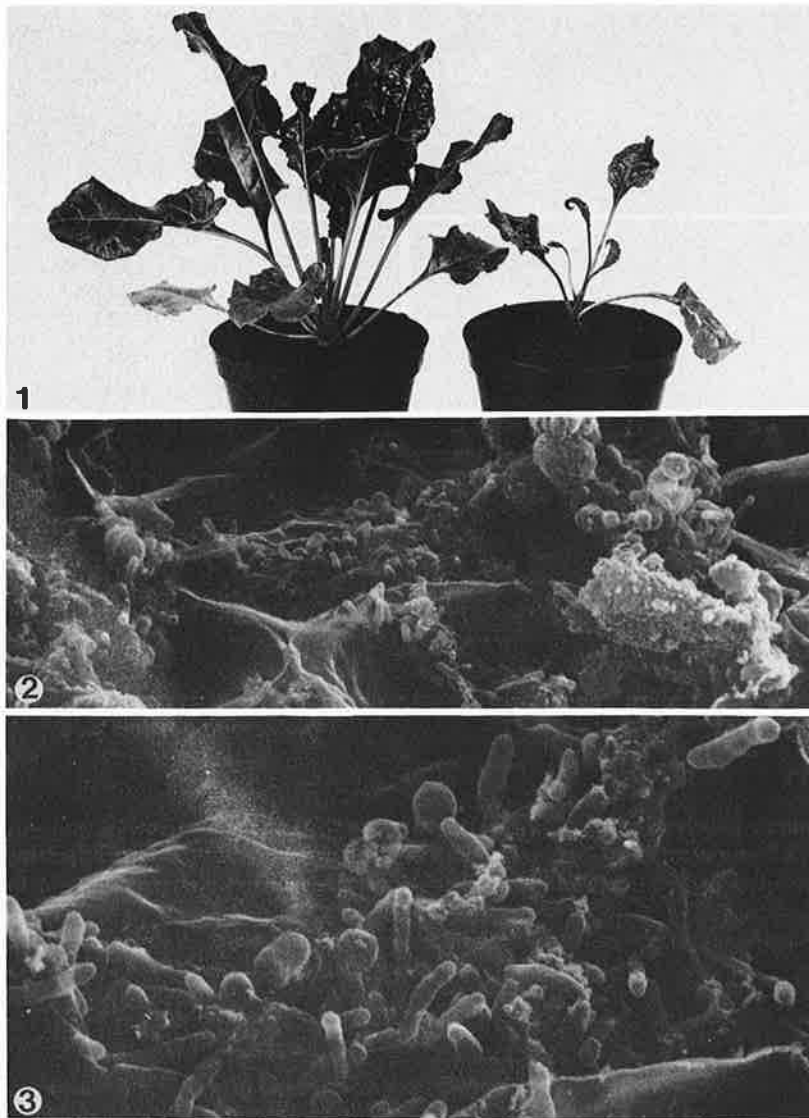
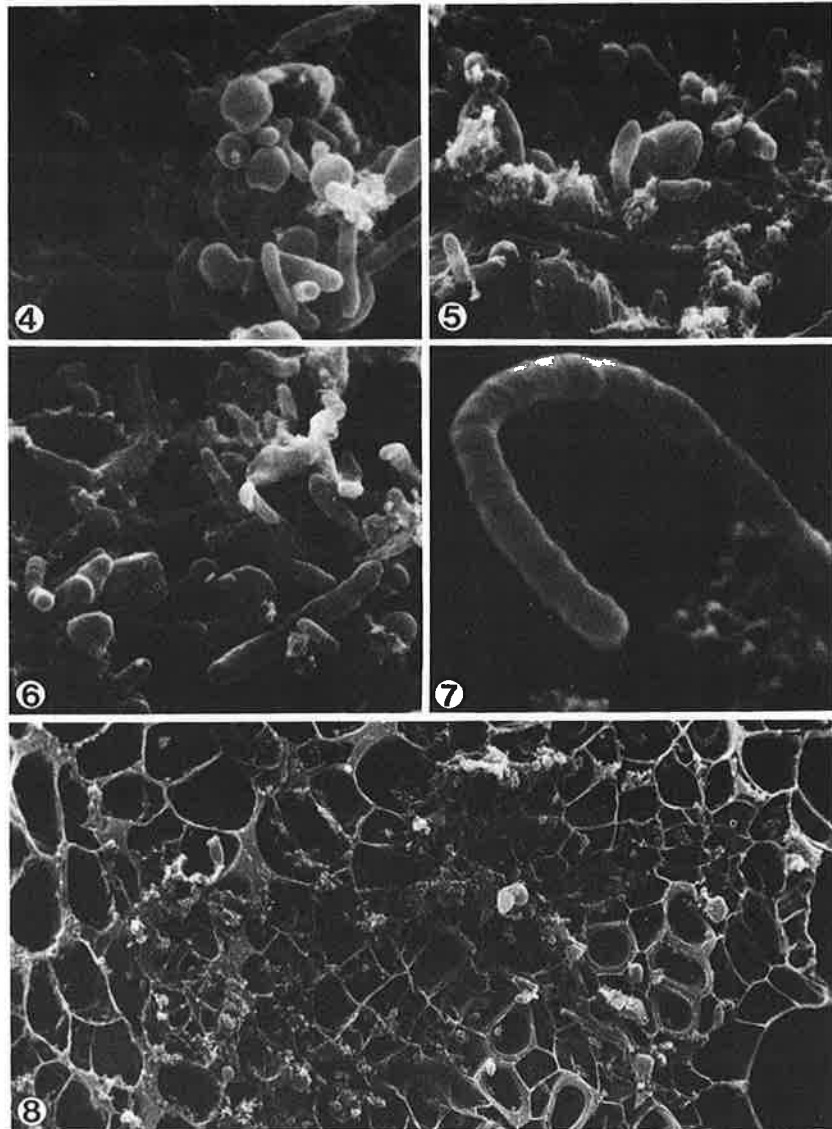


Fig. 1. *Beta vulgaris*; left: healthy plant; right: plant affected by latent rosette disease, showing malformed, dwarfed leaves.

Abb. 1. *Beta vulgaris*; links: gesunde Pflanze; rechts: von latenter Rosettenkrankheit befallene Pflanze mit deformierten, verzweigten Blättern.

Figs. 2,3. Elongated, coccoid and irregular forms of rickettsia-like organisms in the phloem tissue of a leaf petiole. Fig. 2: 5000x; Fig. 3: 15 000x.

Abb. 2,3. Stäbchenförmige, coccoide und pleomorphe Formen von Rickettsien-ähnlichen Organismen im Phloemgewebe eines Blattstiels.



- Figs. 4,5. Coccoid and irregular forms of RLO's in the phloem tissue of *Beta vulgaris*. Fig. 4: 12 000x; Fig. 5: 9 750x.
- Abb. 4,5. Coccoide und pleomorphe Formen von Rickettsien-ähnlichen Organismen im Phloemgewebe von *Beta vulgaris*.
- Fig. 6. Coccoid form of beet RLO showing a germtube-like outgrowth. 9 750x.
- Abb. 6. Coccoide Form von RLO aus Rüben mit Keimschlauch-ähnlichem Auswuchs.
- Fig. 7. Beet RLO showing a rippled or wavy wall. 31 500x.
- Abb. 7. RLO aus Rüben mit einer gerunzelten oder welligen Wand.
- Fig. 8. Transverse section of a leaf petiole of a diseased beet plant with cell wall thickenings in the phloem tissue. 825x.
- Abb. 8. Querschnitt eines Blattstiels einer erkrankten Rübe mit Zellwandverdickungen im Phloemgewebe.

KAO and DAMANN (1980) studied with SEM the in situ localization of a xylem-invading small *Corynebacterium*, which is considered to be the cause of the ratoon stunting disease of sugarcane. These workers observed swollen forms which resemble the irregular cells detected by us in latent rosette-diseased beet plants.

Our observations indicate that this SEM technique can be used as an easy tool for the localization and the in situ study of the morphology and colony-formation of RLO's and similar organisms in plants.

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