Rapid Diagnosis of Plant Virus Disease by TEM

Microwave Assisted Identification of Plant Virus Infection

Plant sample preparation for transmission electron microscopy (TEM) is very time consuming which has limited its application for the diagnosis of plant virus diseases. With the help of microwave irradiation sample preparation time can be drastically reduced to a few hours. Thus, it is now possible to visualize ultrastructural alterations induced by viruses and to clearly identify the virus through cytohistochemical immunogold labelling in plant leaves in less than one day by TEM.

Introduction

The rapid and unambiguous diagnosis of plant virus diseases is of great importance for agriculture and scientific investigations in plant pathology in order to identify the virus and to limit the spread of the disease. Ultrastructural and immunohistological investigations using transmission electron microscopy (TEM) are often applied for the detection and identification of viral agents and of ultrastructural changes induced in the host. As the ultrastructural features and the size of the virus are specific for each disease it can be diagnosed reliably by using such methods. Nevertheless, as conventional plant sample preparations for ultrastructural and immunohistological investigations can take several days they are not well suited for the rapid diagnosis of plant virus diseases [1]. Microwave assisted tissue processing can help to drastically reduce sample preparation time for TEM investigations with similar or even better ultrastructural preservation than that obtained with conventional sample preparation at room temperature [1,2,3]. The reduction of sample preparation time is caused by dielectric heating. Whereas conventional heating starts at the specimen surface microwave irradiation induces a rise of temperature inside the whole sample. The increase in temperature can then enhance and accelerate the diffusion of reagents, protein cross-linking and the polymerization of the resin [4,5]. The objective of this study was to develop protocols that enable the rapid ultrastructural and immunohistochemical diagnosis of plant virus diseases by using microwave assisted plant sample preparation for TEM.
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The application of microwave irradiation reduced sample preparation time of tobacco mosaic virus (TMV) infected tobacco leaves for ultrastructural and cytohistochemical investigations from about 75 hours each to 136 min and 89 min, respectively (table 1).

After ultrathin cutting and contrasting of the samples which took a total of about 40 minutes for one sample typical TMV-induced ultrastructural alterations such as the accumulation of virions in parallel form in the cytosol could be observed in the TEM (fig. 1). No visual differences were observed in the ultrastructural preservation of TMV-induced alterations between samples prepared with the help of microwave irradiation (fig. 1A) and samples prepared conventionally at room temperature (fig. 1B). Negative staining which was performed with the sap of the remaining leaves revealed typical rod shaped virions with an average length and width of 280 nm and 17 nm (inset in fig. 1B). The observed ultrastructural alterations and size of TMV-particles were in accordance to the ultrastructural properties and size range reported for these virions in the literature after TMV-infected leaves had been prepared conventionally by chemical fixation and embedded at room temperature [6,7].

Cytohistochemical immunogold labelling of TMV was performed by treating sections with a primary antibody against TMV-coat protein and a secondary gold conjugated antibody (10 nm gold particles) and took about 100 minutes [for details see 8]. Gold particles bound to TMV-coat protein could be found in the areas where TMV accumulated in parallel form within the cytosol (fig. 2). Gold particle density was higher in samples prepared with the help of microwave irradiation (fig. 2A) when compared to samples prepared conventionally at room temperature (fig. 2B). These results indicate that microwave assisted rapid sample preparation yields higher antigenicity of the sample when compared to conventionally prepared samples. Improved antigenicity of samples after microwave fixation has also been observed in other tissues [9,10] indicating the positive effects of microwave irradiation on the ultrastructural preservation of biological samples.

Conclusion

The results of this study clearly demonstrate that microwave assisted plant sample preparation is well suited for the rapid diagnosis of plant virus diseases. With this method it is possible to identify ultrastructural alterations induced by viruses in less than 4 hours and to perform cytohistochemical immunogold labeling of virus coat protein in about 4 hours (tab. 1). Negative staining of viral particles in the sap of
the remaining leaves with phosphotungstic acid or uranyllic acid takes about 15 minutes [1] and can be performed during sample preparation which is performed automatically by the tissue processor [1,8]. Thus, with these methods it is possible to visualize the ultrastructural alterations induced by viral pathogens and to clearly identify the viral agent through cytohistochemical immunogold labelling in plant leaves in less than one day by TEM. As the application of these protocols is not limited to plant tissue microwave assisted sample preparation could also be applied on human and animal tissue for the rapid diagnosis of diseases by TEM.

References

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