Microwave assisted rapid plant TEM sample preparation

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The preparation of plant samples with conventional methods (e.g. fixation and embedding at room temperature) and cryofixation with following freeze substitution for transmission electron microscopy (TEM) can be very time and labor consuming. The reasons therefore are that most substances are rather slow in infiltrating the specimen (especially at temperatures well below freezing during freeze substitution) and that the adequate infiltration and polymerization of the resin can take several days [1]. Therefore sample preparation with both methods takes several days which makes them unsuitable for diagnostic purposes, for the rapid evaluation of the physiological state of a sample or for the rapid testing of the effectiveness of a fixation and embedding protocol. Microwave fixation and embedding can help to drastically decrease fixation (as low as 6 seconds) and embedding time (5 hours or less) of tissues for TEM investigations [2-5]. This massive reduction of TEM sample preparation time can be attributed to dielectric heating induced by microwave irradiation which causes a temperature rise inside the whole sample whereas conventional heating starts at the specimen surface. The increase in temperature can then enhance and accelerate the diffusion of reagents, protein cross-linking and the polymerization of the resin [2, 4, 6]. Even though the positive effects of microwave irradiation for TEM sample preparation are well known for many years now, its practical use in plant sciences is still very limited for plant tissues.

In the present study we present a standard protocol (fixation, buffer washes, dehydration, infiltration and polymerization) for rapid plant sample preparation with the use of an automated microwave tissue processor. By using different leaf materials (*Arabidopsis thaliana*, *Nicotiana tabacum*, *Picea abies*) we demonstrate that it is possible to reduce sample preparation time from 3 days (conventional fixation and embedding) to about 5 hours without negative effects on the quality of the resin embedded blocks and the ultrastructure of the samples (Figure 1). With computer supported image analysis we were additionally able to show that there are no changes in the dimensions of membranes and also of the space they enclose as they remained statistically unchanged in one and the same plant sample processed either with the conventional method or the microwave assisted protocol (Table 1).

In summary, the present study gives a thorough comparison of the ultrastructural preservation of plant samples after conventional and microwave assisted TEM-sample preparation. Advantages (and disadvantages) of the here presented microwave assisted sample preparation method in comparison to conventional chemical fixation and cryofixation with freeze substitution as well as future perspectives on possible applications of this method in different fields of life sciences (e.g. for plant diseases diagnosis) will be discussed.

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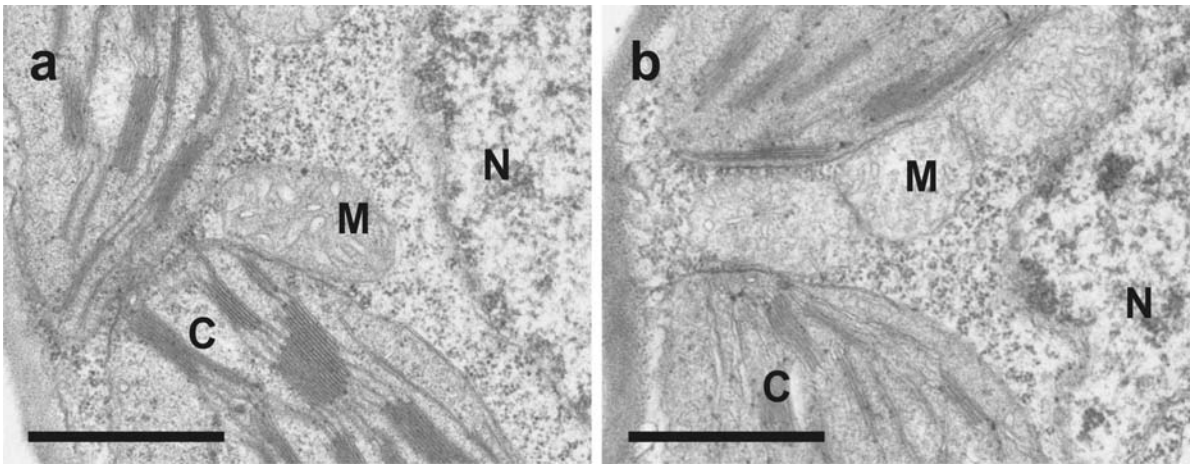

Figure 1. Transmission electron micrographs showing the ultrastructure of leaf mesophyll cells of *Nicotiana tabacum* plants after conventional (a) and microwave-assisted (b) sample preparation. Bars=1µm. Images show chloroplasts (C), mitochondria (M), nuclei (N) and a dense cytosol. No obvious differences in the ultrastructural preservation can be observed.

Relative thickness of membranes (in nm)						
Sample Preparation	Plasma membrane Mean (Stderror)^{abc}	Nuclear membrane Mean (Stderror)^{abc}	Thylakoid membrane Mean (Stderror)^{abc}	Perinuclear space Mean (Stderror)^{abc}	IAS Mean (Stderror)^{abc}	IES Mean (Stderror)^{abc}
Arabidopsis						
Conventional	10.7 (0.1)^{a}	7.4 (0.4)^{a}	4.4 (0.1)^{a}	18.9 (1.2)^{a}	9.7 (0.1)^{a}	0.5 (0.03)^{a}
Microwave	11.2 (0.1)^{a}	7.8 (0.5)^{a}	4.8 (0.2)^{a}	19.0 (1.4)^{a}	9.3 (0.2)^{a}	0.6 (0.02)^{a}
Nicotiana						
Conventional	10.7 (0.1)^{a}	7.5 (0.4)^{a}	5.3 (0.2)^{bc}	18.1 (0.8)^{a}	10.3 (0.1)^{ab}	0.5 (0.04)^{a}
Microwave	10.9 (0.1)^{a}	8.0 (0.2)^{a}	5.6 (0.1)^{b}	17.7 (1.1)^{a}	10.8 (0.3)^{b}	0.5 (0.02)^{a}
Picea						
Conventional	10.7 (0.1)^{a}	7.4 (0.3)^{a}	4.9 (0.2)^{ac}	18.3 (1.3)^{a}	11.1 (0.5)^{b}	0.5 (0.03)^{a}
Microwave	11.3 (0.1)^{a}	7.5 (0.6)^{a}	4.8 (0.1)^{a}	18.0 (0.9)^{a}	10.9 (0.3)^{b}	0.5 (0.03)^{a}

Table 1: Values are means with standard errors (parenthesis) and document the thickness of different membranes, perinuclear, intra- and interthylakoidal spaces. Plant leaves (*Arabidopsis thaliana*, *Nicotiana tabacum* and *Picea abies*) were prepared for TEM with a conventional and a microwave assisted protocol. Significant differences among samples of one cell structure are indicated by different lowercase letters. $P<0.05$ was regarded significant analyzed with Kruskal-Wallis test followed by post hoc comparison according to Conover. $n>100$ for each measurement. IAS= Intrathylakoidal space; IES= Interthylakoidal space.