

Application Note

Leica EM AMW –

Nicotiana tabacum (L.) cv. Samsun nn

Courtesy of: Mag. Dr. Bernd Zechmann and Univ. Prof. Dr. Günther Zellnig
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Living up to Life

Leica
MICROSYSTEMS

Leica EM AMW Application Note

Nicotiana tabacum

Nicotiana tabacum (L.) cv. Samsun nn

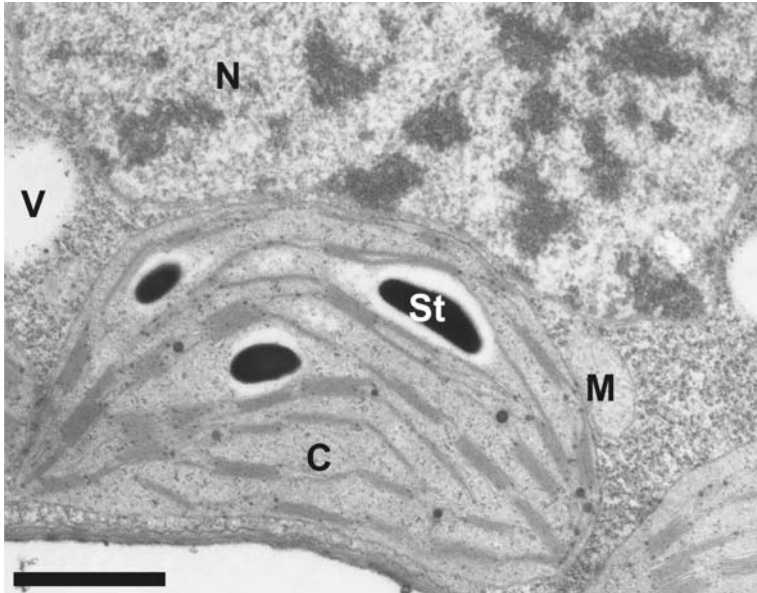
Plants were cultivated in growth chambers at constant conditions of day/night temperature 24/20°C, illumination 250 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and photoperiod 16/8 h light/dark. Plants were grown in pots with soil and were well watered. Seven weeks after germination the youngest fully developed leaves (about 8cm long and 5cm in width) were harvested two hours after the onset of daylight and prepared for electron microscopy with the Leica EM AMW. Samples were taken from the middle of the leaves close to the middle vein. Small sections of leaves (1mm²) were cut with a razor blade on a modelling wax plate in a drop of 3% glutaraldehyde in 0.06M Sørensen phosphate buffer at pH 7.2. Sections were then evacuated with a water jet vacuum pump for a maximum of 10 seconds in a vial filled with the above described fixative solution. Subsequently, the specimens were transferred into small baskets with a mesh width of about 200 μm . These baskets were then stacked on top of each other and transferred into the mono-mode chamber of the Leica EM AMW which already contained a vial filled with the above mentioned fixative solution. Microwave fixation was then started about 2 minutes after the cutting of the samples by starting the previously programmed protocol.

Sample preparation for transmission electron microscopy (TEM) was performed in order to develop a standard protocol that would reduce sample preparation time for TEM-investigations. Therefore the overall and fine structure of leaf cells prepared with the Leica EM AMW were compared with leaf cells that were prepared with a conventional fixation protocol at room temperature. Additionally, the diameter of membranes from different cell compartments (chloroplasts, nuclei and plasmamembrane) was determined by using quantitative computer supported transmission electron microscopy.

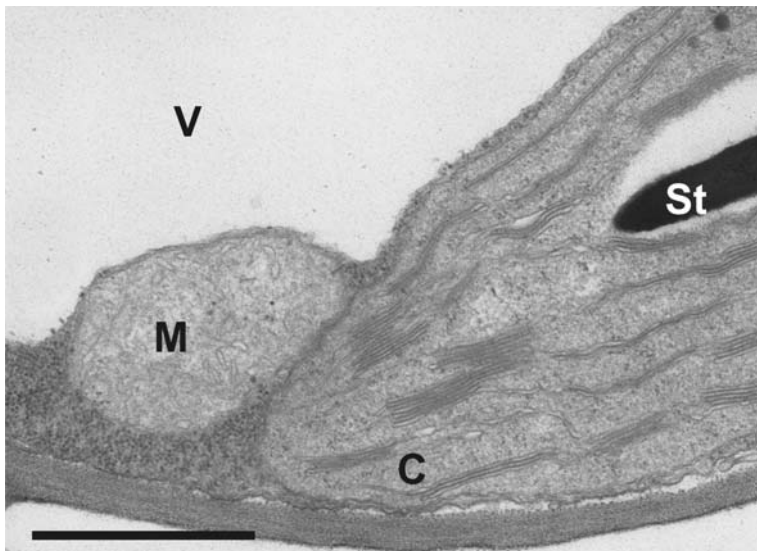
Sample: *Nicotiana tabacum* (L.) cv. Samsun nn

Processing						
vial	step	time	max. temp.(°C)	reagent	mode	max. power (W)
1	1	00:02:00	37	Buffer + glutar aldehyde	Cont.	15
1	2	00:02:00	20	Buffer + glutar aldehyde	Cont.	0
1	3	00:02:00	37	Buffer + glutar aldehyde	Cont.	15
1	4	00:02:00	20	Buffer + glutar aldehyde	Cont.	0
2	1	00:00:40	37	Buffer	Slope	20
3	1	00:00:40	37	Buffer	Pulse	15
4	1	00:00:40	37	Buffer	Slope	20
5	1	00:12:00	37	Buffer + OsO4	Cont.	15
6	1	00:01:00	37	Buffer	Cont.	15
7	1	00:01:00	37	Buffer	Cont.	15
8	1	00:01:00	37	Buffer	Cont.	15
9	1	00:01:00	37	Acetone 50%	Slope	20
10	1	00:01:00	37	Acetone 75%	Slope	20
11	1	00:01:00	37	Acetone 90%	Slope	20
12	1	00:02:00	37	Acetone	Slope	20
13	1	00:02:00	37	Acetone	Slope	20
14	1	00:03:00	37	Resin 3:1	Cont.	10
15	1	00:03:00	40	Resin 1:1	Cont.	10
16	1	00:03:00	45	Resin 1:3	Cont.	10
17	1	00:03:00	50	Epon	Cont.	12
18	1	00:03:00	50	Epon	Cont.	12
19	1	00:03:00	50	Epon	Cont.	12
Total time		00:49:20				

Polymerisation						
vial	step	time	max. temp.(°C)	reagent	mode	max. power (W)
1	1	00:05:00	65	Epon	Slope	30
1	2	00:05:00	75	Epon	Slope	30
1	3	00:15:00	85	Epon	Slope	30
1	4	02:35:00	85	Epon	Cont.	30
Total time		03:00:00				



Overview



Detail

C = Chloroplast
 ST = Containing starch
 M = Mitochondria
 N = Nucleus
 V = Vacuoles

Scale Bar = 1 μ m

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