植物组织半薄切片甲苯胺蓝染色实验报告

一、实验器材及试剂

1、 实验器材

名称	厂家	型号
半薄切片机	Leica	Leica HistoCore Nanocut R
组织切片钻石刀	Daitome	Histo45°
载玻片	Wanwu	G6004

2、 主要实验试剂

试剂	厂家	货号
电镜固定液	Wanwu	G1102
无水乙醇	国药集团化学试剂有限公司	100092183
丙酮	国药集团化学试剂有限公司	10000418
812 包埋剂	SPI	90529-77-4
甲苯胺蓝染液	Wanwu	G1032
锇酸	Ted Pella Inc	

二、半薄切片染色步骤

1、取材固定：新鲜组织确定取材部位，尽量减小牵拉、挫伤与挤压等机械损伤，1-3min 内取样，取样组织 1mm³ 大小。取材前可提前准备装有电镜固定液的培养皿，将小组织块离体取下后立即投入培养皿内，用手术刀在培养皿的固定液中进行切割成 1mm³ 的小组织块。再将切割好的小组织块转移至装有新的电镜固定液的 EP 管内继续固定，并用真空泵抽气直至沉底，室温放置 2h 后 4℃固定保存及运输。0.1M 磷酸缓冲液 PB（PH7.4）漂洗 3 次，每次 15min。

2、**后固定：**0.1M 磷酸缓冲液 PB（PH7.4）配制的 1%锇酸避光室温固定 7h。0.1M 磷酸缓冲液 PB（PH7.4）漂洗 3 次，每次 15min。

3、室温脱水：组织依次入 30%-50%-70%-80%-95%-100%-100%酒精上行脱水，每次 1h。无水乙醇：丙酮=3：1 0.5h，无水乙醇：丙酮=1：1 0.5h，无水乙醇：丙酮=1：3 0.5h，丙酮 1h。

4、渗透包埋：丙酮：812 包埋剂=3：1 37℃ 2-4h， 丙酮：812 包埋剂=1：1 37℃渗透过夜，丙酮：812 包埋剂=1：3 37℃ 2-4h，纯 812 包埋剂 37℃ 5-8h。将纯 812 包埋剂倒入包埋板，将样品插入包埋板后 37℃烤箱过夜**包埋：**60℃烤箱聚合 48h。

5、聚合：包埋板放于 60℃烤箱聚合 48h，取出树脂块备用。

6、切片：半薄切片机切片，厚度 1.5μm，组织片贴附于载玻片上。

7、染色：将甲苯胺蓝染液放在 60°烤箱加热 1h，将片子放入染液中染 2min，水洗，95%酒精分化，在光镜下控制颜色，烤干，中性树胶封片。

Semi-thin section and Toluidine blue staining report for plant tissue specimen

1 Apparatus and reagents

1.1 Major apparatus

Name	Producer	Model
Semi-thin slicer	Leica	Leica HistoCore Nanocut R
Diamond slicer	Daitome	Ultra 45°
Microscope slides	Wanwu	G6004

1.2 Major reagents

Name	Producer	Code
Fixative for TEM	Wanwu	G1102
Ethanol	Sinaopharm Group Chemical Reagent Co. LTD	100092183
Aceton	Sinaopharm Group Chemical Reagent Co. LTD	10000418
EMBed 812	SPI	90529-77-4
Toluidine blue dye	Wanwu	G1032
OsO_4	Ted Pella Inc	

2 Procedure

2.1 **Harvest tissue block and fixation**: Targeted fresh tissues should be selected to minimize mechanical damage such as pulling, contusion and extrusion. Use a sharp blade to cut and harvest fresh tissue blocks quickly within 1-3 minutes. The size of tissue block should be no more than 1 mm^3. Before sampling, petri dishes with fixative for TEM should be prepared in advance, small tissue blocks could be removed from animal body and immediately put into petri dishes, and then cut into small size of 1 mm^3 in the fixative. The 1mm^3 tissue blocks were transferred into an EP tube with fresh TEM fixative for further fixation, meanwhile, keep vacuum extraction until the samples sink to the bottom. The samples fixed for 2h at room temperature and then fixed at 4℃ for preservation and transportation. And then wash the tissues using 0.1 M PB (pH 7.4) for 3 times, 15 min each.

2.2 **Post-fix:** Tissues avoid light post fixed with 1% OsO_4 in 0.1 M PB (pH 7.4) for 7 h at room temperature. After remove OsO_4, the tissues are rinsed in 0.1 M PB (pH 7.4) for 3 times, 15

min each.

2.3 Dehydrate at room temperature as followed:

30% ethanol for 1h;

50% ethanol for 1h;

70% ethanol for 1h;

80% ethanol for 1h;

95% ethanol for 1h;

100% ethanol for 1h;

100% ethanol for 1h;

ethanol: Acetone=3:1for 0.5 h;

ethanol: Acetone=1:1for 0.5 h;

ethanol: Acetone=1:3for 0.5 h;

Pure acetone for 1h.

2.4 Resin penetration and embedding as followed:

Acetone：EMBed 812=3:1for 2-4 h at 37℃;

Acetone：EMBed 812=1:1 overnight at 37℃;

Acetone：EMBed 812=1:3 for 2-4 h at 37℃;

Pure EMBed 812 for 5-8 h at 37℃;

Pour the pure EMBed 812 into the embedding models and insert the tissues into the pure EMBed 812, and then keep in 37℃ oven overnight.

2.5 Polymerization: The embedding models with resin and samples were moved into 65℃ oven to polymerize for more than 48h. And then the resin blocks were taken out from the embedding models for standby application at room temperature.

2.6 Semi-thin section: The resin blocks were cut to 1.5μm thin on the semi-thin slicer, and the tissues were fished out onto the microscope slides.

2.7 Toluidine blue staining: Keep toluidine blue dye solution in an oven at 60° for 1h, and then stain the slides in the dye solution for 2min. Wash the slides with running water, differentiate with 95% alcohol, and control the color under a light microscope. Keep the slides in the oven to dry , and then cover the slides with neutral resin.