植物硬组织切片番红固绿染色实验报告

一、实验器材及试剂

1、 实验器材

名称	厂家	型号
烤箱	天津市莱玻瑞仪器设备有限公司	GFL-230
载玻片	Servicebio	
正置光学显微镜	日本尼康	Nikon Eclipse E100
成像系统	日本尼康	Nikon DS-U3

2、 主要实验试剂

试剂名称	厂家	货号
无水乙醇	国药集团化学试剂有限公司	100092683
二甲苯	国药集团化学试剂有限公司	10023418
番红固绿(植物)染液	Servicebio	G1031
中性树胶	国药集团化学试剂有限公司	10004160
乙二醇乙醚乙酸酯	麦克林	E808814-2.5L

二、实验步骤

1、切片脱塑至水：依次将切片乙二醇乙醚乙酸酯I 6 h，37℃，乙二醇乙醚乙酸酯II过夜，37℃，乙二醇乙醚乙酸酯III室温 10-15 min，乙二醇乙醚乙酸酯IV室温 10-15 min，100%I乙醇 10 min，100%II乙醇 10 min，95%乙醇 10 min，90%乙醇 10 min，80%乙醇 10 min，自来水洗。

2、番红染色：切片入番红染液中染色 3-5 s，自来水稍洗，洗去多余染料即可。

3、脱色：切片依次入 50%、70%、80%梯度酒精中各 3-8 s。

4、固绿染色：切片入固绿染液中染色 4-6 s，无水乙醇三缸脱水。

5、透明封片：切片入干净的二甲苯透明5 min，中性树胶封片。

6、显微镜镜检，图像采集分析。

三、结果判读：

木质化的细胞壁呈红色，纤维素细胞壁呈绿色。

四、注意事项：

1、番红在水中易褪色，水洗时速度要快，将多余染液洗去即可，不能让番红褪色。

2、染色后的片子应该形态结构清晰，没有多余的固绿染液残留。

Hard Plant Safranin o-Fast Green (Hard Plant) Stain Report

1. Experimental equipment and reagents

1.1 Equipment

Brand name	Manufactures	Model
Oven	Tianjin Labotery instrument equipment Co. Ltd	GFL-230
Microslide	Servicebio	
Optical Microscopy	NIKON	Nikon Eclipse E100
Imaging system	NIKON	Nikon DS-U3

1.2 Reagent

Reagent	Manufactures	Cat
Ethanol absolute	Sinopharm Chemical Reagent Co.,Ltd	100092683
Xylenes	Sinopharm Chemical Reagent Co.,Ltd	100023418
Safranin O-Fast Green staining solution(plant)	Servicebio	G1031
Neutral balsam	Sinopharm Chemical Reagent Co.,Ltd	10004160
Ethylene glycol ethyl ether acetate	Macklin	E808814-2.5L

2. Experiment procedure

2.1 Remove the embedding agent and rehydration

Put sections into ethylene glycol ethyl ether acetate I 6 h at 37 ℃ and ethylene ether acetate II overnight at 37 ℃. Then put them into ethylene glycol ethyl ether acetate III 10-15 min minutes at room temperature and ethylene glycol ethyl ether acetateIV 10-15 min minutes at room temperature. The sections were rehydrated in 100%I% - 100%II - 95% - 90% - 80% alcohol. Each step takes anywhere for 10 minutes. Finally, rinse with running water.

2.2 Safranin O staining

Put sections into safranin O staining solution for 15-30 s and three cylinders of anhydrous ethanol for rapid dehydration.

2.3 Decoloration

Put sections into three cylinders of 50%, 70%, 80% alcohol respectively for 3-8 s. The concentrations of alcohol are 50,70 and 80 percent.

2.4 Fast Green staining

Put sections into fast green staining solution for 4-6 s and three cylinders of anhydrous ethanol for 5 s in turn.

2.5 Transparent and mount

Put sections into three cylinders of xylene for 5 min. Finally, mount the tissue section with neutral balsam.

2.6 Microscope observation and collection and analysis images.

3. Result determination

The cytoderm which has lignified appear red and the color of cellulose cell wall is green.

4. Matter need attention

1. Wash quickly after stain safranin O, because it is easy to fade in water.