胼胝质染色-苯胺蓝法实验报告

一、实验器材及试剂

1、实验器材

名称	厂家	型号
CO2 恒温培养箱	SANYO	MCO-15AC
洁净工作台	苏净安泰	SW-CJ-1FD
载玻片	Wanwu	
盖玻片	江苏世泰实验器材有限公司	10127105P-G
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
脱水机	DIAPATH	Donatello
包埋机	武汉俊杰电子有限公司	JB-P5
病理切片机	上海徕卡仪器有限公司	RM2016
冻台	武汉俊杰电子有限公司	JB-L5
组织摊片机	浙江省金华市科迪仪器设备有限公司	KD-P
烤箱	天津市莱玻瑞仪器设备有限公司	GFL-230

2、主要实验试剂

试剂名称	厂家	货号
洗涤液	Wanwu	
苯胺蓝染色液	Wanwu	
脱色液	Wanwu	
中性树胶	国药集团化学试剂有限公司	10004160

二、实验步骤

针对植物切片

1、切片制备：脱色后的叶片取材包埋切片后脱蜡至水，纯水洗涤三次。

2、切片染色：切片用洗涤液浸洗 3 次，甩干洗涤液，切入 0.01%苯胺蓝染色液中常温避光浸染 2 h。

3、封片：切片用洗涤液浸洗 3 次，吸干水分。滴加抗荧光淬灭封片液封片，盖盖玻片。

4、拍照：观察拍照，用荧光显微镜（DAPI 滤光片）观察胼胝质沉积，荧光易淬灭，应尽快拍照，注意避光，切片可短暂保存于 4℃。

针对叶片

1、脱色：新鲜的植物组织（幼嫩的叶片）用脱色液脱色处理。

2、染色：组织经洗涤液洗涤 3-5 次，滤纸吸干多余液，在 0.01%苯胺蓝染色液中常温避光漂染 2 h。

3、铺片及封片：组织经洗涤液漂洗 3-5 次，滤纸吸干多余液体并平铺于载波片上，滴加抗荧光封片剂，盖盖玻片并轻轻将组织压平不可有气泡。

4、拍照：观察拍照，用荧光显微镜（DAPI 滤光片）观察胼胝质沉积，荧光易淬灭，应尽快拍照，注意避光，切片可短暂保存于 4℃。

三、结果判读

1、胼胝质呈现亮蓝荧光广泛分布在筛管周围。

Aniline blue staining experimental report

1. Experimental equipment and reagents

1.1 Experimental equipment

Name	Manufactor	Model
CO2 incubator	SANYO	MCO-15AC
Clean Bench	Sujing Antai	SW-CJ-1FD
Slides	Wanwu	
Cover glass	Jiangsu Shitai Experimental Equipment Co.,Ltd	10127105P-G
ORTHO OPTICAL MICROSCOPE	NIKON, JAPAN	NIKON ECLIPSE E100
IMAGING SYSTEM	NIKON, JAPAN	NIKON DS-U3
Dehydrator	DIAPATH	Donatello
Embedding machine	Wuhan Junjie Electronics Co., Ltd	JB-P5
Pathology slicer	Shanghai Leica Instrument Co., Ltd	RM2016
Frozen platform	Wuhan Junjie Electronics Co., Ltd	JB-L5
Tissue spreader	Zhejiang Kehua Instrument Co., Ltd	KD-P
Oven	Tianjin Laibo Rui Instrument Equipment Co.,Ltd	GFL-230

1.2 Main experimental reagents

Reagent name	Manufactor	Article number
Washing liquid	Wanwu	
Aniline blue staining solution	Wanwu	
Decolorizing solution	Serbicebio	
Neutral resin	Sinopharm Group Chemical Reagent Co. LtD	**10004160**

2. Experimental

For plants

2.1 The decolorized leaves were taken, embedded and sliced, then dewaxed to water and washed with pure water for three times.

2.2 Wash the slides with detergent for 3 times, dry the detergent and put into 0.01% aniline blue dye,dip away from light at room temperature for 2 hours.

2.3 Seal:The slides were washed with detergent for 3 times to absorb dry water. Drop anti-fluorescence quenching sealing film liquid sealing sheet, cover glass slide .

2.4 Take photos: observe and take photos. Observe the deposition of callose with fluorescence microscope (DAPI filter). The fluorescence is easy to quench. Take photos as soon as possible and avoid light. The slices can be stored at 4 ℃ for a short time.

For blades

2.1.Decolorization: fresh plant tissues (young leaves) are decolorized with decolorizing solution.

2.2 Dyeing: the tissue was washed with washing solution for 3~5 times, the excess liquid was dried by filter paper, and bleached in 0.01% aniline blue dyeing solution at room temperature without light for 2 hours.

2.3 Spreading and sealing: the tissue is rinsed with washing solution for 3~5 times, the filter paper absorbs the excess liquid and lays it flat on the carrier, dripping with anti-fluorescent sealing agent, cover glass and gently flatten the tissue without air bubbles.

2.4 Take photos: observe and take photos. Observe the deposition of callose with fluorescence microscope (DAPI filter). The fluorescence is easy to quench. Take photos as soon as possible and avoid light. The slices can be stored at 4 ℃ for a short time.

3. The results were as follows

3.1 The callose showed bright blue fluorescence and was widely distributed around the sieve tub.