叶片真菌菌丝 WGA-PI 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
CO_2 恒温培养箱	SANYO	MCO-15AC
洁净工作台	苏净安泰	SW-CJ-1FD
载玻片	Servicebio	
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

2、 主要实验试剂

试剂名称	厂家	货号
卡诺固定液	Servicebio	G1120
氢氧化钾	国药	
WGA-AF488 储存液	Servicebio	
PBS 粉剂	Servicebio	G0002
PI 储存液	Servicebio	
抗荧光淬灭封片剂	Servicebio	G1401

二、实验步骤

1、染色液配制：

①、配制 0.2%Tween-PBS 溶液：0.2ml Tween-20 加入 100mlPBS 中溶解；

②、WGA 染色液：向 970ul 的 0.2%Tween-PBS 溶液中加入 10ul 的 WGA 储存液和 20ul 的 PI 储存液，充分混匀。(现配现用，注意避光)

2、固定：卡诺固定液固定样本（同时脱去叶绿素）

3、透明：将叶片转入 10%KOH 溶液，用封口膜将管口封严，防止管口崩开，85℃孵育一定时间（视情况而定，一般玉米叶片处理 4h，大麦叶片处理 2h）。

4、染色预处理：组织经 PBS 洗涤 4-5 次。

5、染色：向装有叶片的离心管中加入染色液，真空抽滤泵抽滤 4 次，每次 5min，期间在常压下间隔 5min。

6、拍照：叶片经 PBS 洗涤 2-3 次，抗荧光淬灭封片剂封片，于 4℃避光保存，荧光显微镜或者共聚焦显微镜拍照。

三、结果判读：

真菌菌丝呈绿色荧光，植物组织细胞壁呈红色荧光。

Leaf Fungal Hyphae WGA-PI Staining Experimental Report

1. Experimental equipment and reagents

1.1 Experimental equipment

Name	Manufactor	Model
CO_2 incubator	SANYO	MCO-15AC
Clean workbench	Su Jing An Tai	SW-CJ-1FD
Slides	Servicebio	
Cover glass	Jiangsu Shitai	10127105P-G
Orthostatic microscope	Nikon, JAPAN	Nikon Eclipse E100
Image system	NIKON, JAPAN	Nikon DS-U3

1.2 Main experimental reagents

Reagent name	Manufactor	Article number
Carnot fixative	Servicebio	G1120
Potassium hydroxide	Sinopharm Group Chemical Reagent Co. LTD	
WGA-AF488 storage solution	Servicebio	
PBS	Servicebio	G0002
PI stock solution	Servicebio	
Anti-fluorescence quenching sealed tablets	Servicebio	G1401

2. Experimental steps

2.1 Dye preparation: Add 10 μL WGA storage solution and 20 μL PI stock solution to 970 μL 0.2% Tween-PBS, and mix thoroughly.

2.2 Fix: Use Carnot fixative to fix sample (Remove chlorophyll at the same time).

2.3 Transfer the leaves into 10% KOH solution, and seal the tube with a parafilm to prevent the tube from collapsing, and incubate at 85℃ for a certain period of time (Generally speaking, corn leaves are treated for 4h, and the barley leaves are treated for 2h.).

2.4 Staining pretreatment: Tissues are washed 4 or 5 times with PBS.

2.5 Staining: Add dye solution to the centrifuge tube containing blades, and filter 4 times with vacuum filter where each time lasts for 5min, and each interval is 5min under normal pressure.

2.6 Photographing: The leaves were washed 2 or 3 times with PBS, and sealed with anti-fluorescence quenching to be stored at 4°C in the dark. And photographed with a fluorescence microscope or a confocal microscope.

3. The results were as follows

Fungal hyphae shows green fluorescence, cell walls of plant show red fluorescence.