Pollen Fertility/Viability Assay Using FDA Staining

Xiyan Li*

Department of Genetics, Stanford University, Stanford, USA
*For correspondence: lixiyan@stanford.edu

[Abstract] Pollen grains can be fertile or sterile by nature. This method stains pollen grains for an enzyme as the vital indicator of membrane integrity. Only fertile grains fluoresce under microscopic examination.

Materials and Reagents

1. Fluorescein diacetate (FDA)
2. Excision: At the time of anthesis
3. Stain: Stock solution of FDA 2 mg FDA/ml of acetone (stored at -20 °C in an Eppendorf tube)
4. BK buffer S15 MOPS (see Recipes)
5. BK buffer S15 (see Recipes)

Equipment

1. Fluorescence microscope
2. Eppendorf tube

Procedure

1. Take 1 µl of the stock solution of FDA and add to 1 ml of the BK buffer S15 MOPS (pH 7.5).
   Note: The stock solution of FDA is very volatile-the FDA-buffer mixture will not keep for more than 2 h.
2. Mounting: Place a drop of the FDA-buffer mixture on a slide cleaned with alcohol and put a few pollen grains on the drop. Place a coverslip on top.
3. Observation: Observe under optical microscope in blue light (wavelength = 495 nm). The viable pollen grains show fluorescence (FCR^+).
   Remarks: The fluorescein diacetate, an apolar and non-fluorescent molecule, penetrates the pollen grain. Its hydrolysis by pollen esterases liberates fluorescein, a polar and

fluorescent molecule. When the properties of membrane permeability are intact, the fluorescein accumulates inside the pollen grain, which appears fluorescent in blue light. The FCR test brings to light the esterase activity and the membrane integrity of the pollen grains.

Recipes

1. BK buffer S15 MOPS (pH 7.5)

$Ca(NO_3)_2$ $4H_2O$ (MW 236)	30 mg/L (0.127 mM)
$MgSO_4$ $7H_2O$ (MW 246.5)	20 mg/L (0.081 mM)
KNO_3 (MW 101)	10 mg/L (0.1 mM)
Sucrose	15%
MOPS (MW209)	10 mM (pH 7.5)

   Stored at -20 °C in an Eppendorf tube

2. BK buffer S15 (50 ml)

100 mM MOPS (pH 7.5)	5 ml
Sucrose	7.5 g
$Ca(NO_3)_2$ (1 M)	6.35 µl
$MgSO_4$ (1 M)	4.05 µl
KNO_3 (1 M)	5 µl

References

1. Heslop-Harrison, J. and Heslop-Harrison, Y. (1970). Evaluation of pollen viability by enzymatically induced fluorescence; intracellular hydrolysis of fluorescein diacetate. *Stain Technol* 45(3): 115-120.