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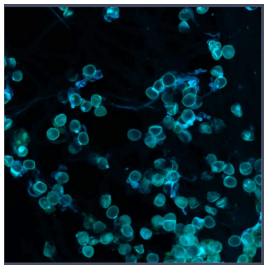
Pollen germination on wheat stigmas



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Marina Millán Blánquez[1]

[1]John Innes Centre

Marina Millán Blánquez
John Innes Centre

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Protocol status: Working
We use this protocol and it's working

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Abstract

Aniline blue staining of pollen tube growth on the wheat stigma and through the style 4 h and 30 min after pollination.

Hand pollination

1 Please refer to wheat-training.com for detailed explanations on how to perform hand pollinations. Link to pdf: https://www.wheat-training.com/wp-content/uploads/Wheat_growth/pdfs/How-to-cross-wheat-pdf.pdf

Carpel dissection and fixation

2 Using a pair of tweezers, dissect carpels 4.5 h after pollination to allow sufficient time for pollen tube
emergence.

3 Store samples in a fixative solution of 95% ethanol and absolute acetic acid (75% v/v) and kept at 4 °C until sample preparation for fluorescence microscopy.

Aniline blue staining of pollinated stigmas

4 On the day of sampling, prepare a solution of 0.1% aniline blue in 0.1 M K_3PO_4. You can prepare a stock solution of 1% aniline blue dissolved in 1x PBS. The stock solution should be kept in the fridge at
4 °C. Use tin foil to avoid exposure to light.

5 Wash fixed samples three times for 5 minutes in sterile water and transferred to 0.1% aniline blue
solution and kept overnight at 4 °C. Use tin foil to avoid exposure to light.

6 Without washing the samples, dissect out the ovary using a sharp razor blade. Try not to damage the stigma. Leave the remaining stigmatic tissue to dry at 45 °C in a hot plate for a few minutes until most of the aniline blue solution is evaporated. Cover the hot plate with an opaque lid to avoid exposure to light.

7 Use Vectashield (catalogue No. H-1000-1, 2BSCIENTIFIC LTD) as an antifade mounting media to
preserve fluorescence.

8 Happy microscopy.