GERMINATION OF BEAN POLLEN

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Germination of bean pollen is quite tricky and results are not always consistent. The cause of the inconsistency is not clear.

MEDIA

Use modified Brewbaker and Kwack (1963) media:

100 ppm H_3BO_3 50 % sucrose
300 ppm $Ca(NO_3)_2 \cdot 4H_2O$ pH = 6.5, (phosphate buffers A and B)
200 ppm $MgSO_4 \cdot 7H_2O$ use purest water available

Prepare 50 ml of 200 X stock solutions of each of the three salts:

1.0 g H_3BO_3 /50ml
3.0 g $Ca(NO_3)_2 \cdot 4H_2O$ /50 ml
2.0 g $MgSO_4 \cdot 7H_2O$ /50 ml

Prepare 100 ml of 0.1 M solutions of buffers A and B:

1.42 g Na_2HPO_4 /100 ml - Buffer A
1.36 g KH_2PO_4 /100 ml - Buffer B

To prepare 100 ml media:

0.5 ml of each of the stock solutions
50.0 g of sucrose
fill to 100 ml with the purest water available
pH to 6.5 with buffers A and B

Keep media refrigerated, and prepare new media periodically, or if contamination occurs. It is convenient to store a 1 ml sample of media in a microfuge tube for current use. Remove media from refrigerator and warm in water bath (37.5°C is fine) prior to collecting flowers.

FLOWER COLLECTION AND PREPARATION OF SLIDES

Use flowers from plants grown in a growth chamber, if available. Plants should be well watered and fertilized. Pick off old flowers and pods on a regular basis to maintain a constant supply of flowers. Collect flowers in petri plates lined with moistened filter paper just prior to preparing pollen slides.

Place a 5 µl drop of media in the hollow of a depression slide. Pick a well formed flower and hold its base in the left hand between thumb and forefinger with the wings pointing to the right. Pull the wing petal closest to you to the right and down with the right hand (Figure 1) and hold in place with the middle finger of the left hand. This will cause the stigma with its attached pollen load to protrude from the keel. With tweezers, gently break off and hold on to the stigma as far back as the stigma is accessible.

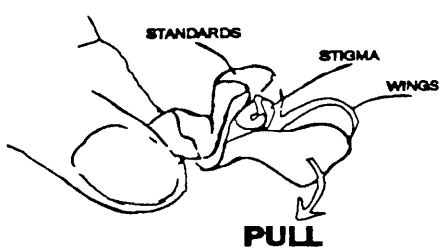

Figure 1. Hold flower in left hand. Pull down on closer wing petal to get stigma with its pollen load to protrude from keel.

Carefully touch the tip of the stigma to the drop, so that the pollen floats on top of the media. Avoid crush-

ing the stigma, since allowing substances from a crushed stigma to diffuse into the media reduces germination. Wipe off the tweezers. Gently swirl the pollen in the media with the tweezers to spread the drop out as flat as possible. This appears to aid in germination, possibly by allowing oxygen to rapidly diffuse into the media. Quickly cover the hollow of the depression slide with a regular glass slide and seal with a drop of distilled water applied to the edge where the slides meet. The same procedure can be repeated for the other hollow in the depression slide with a new flower.

POLLEN GERMINATION AND EVALUATION

Place the slide-sandwich upside-down (with the depression slide on top) in a petri plate lined with moist filter paper. Incubate at 20-25°C for approximately 4-6 hours. Pollen germination is fairly rapid at this temperature; germination should begin within 1 hour and may be completed in as little as 1.5 hours, depending on conditions. As pollen germination continues, additional pollen grains start to germinate, while existing pollen tubes increase in length. Bursting seems to occur only on pollen grains that have initiated at least very short pollen tubes and tends to increase with time. The ideal time for counting the percent germination will depend on conditions. However, as a guide, an incubation time of at least 4 hours is suggested; percent pollen germination can still be evaluated after more than 24 hours.

Pollen germination was scored using a microscope at 100 X magnification (Figure 2). In order to avoid bias due to differences in percent germination in different regions of the slide, scoring was started at one edge of the drop and all grains were counted in a straight line to the other edge. Counting up to 100 pollen grains is sufficient (no differences were observed when doing repeated counts of 100 grains on a number of slides). If necessary, view another path (perpendicular to the first) through the slide until 100 grains have been counted. Only pollen grains with tube lengths greater than the pollen diameter are considered to have germinated.

Figure 2. Pollen in 50% sucrose media. Some grains have germinated but tubes have burst.

Percent pollen germination may drop drastically if plants or flowers were stressed (plants allowed to dry out or flowers at high temperatures near lights). However, percent germination (mean = 70%) was not significantly affected by growth chamber, genotype, plant within growth chamber (same genotype), location of inflorescence on the plant, or position of flower on the inflorescence in a study in which a total of 107 slides were evaluated.

REFERENCES

Brewbaker, J. L. and B. H. Kwack. 1963. The essential role of calcium ion in pollen germination and pollen tube growth. Am. J. Bot. 50:859-865.