Crossing Brachypodium

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Comments

The major improvements in this update are:
-emasculate 1-2 days prior to pollination
-use pollen as soon as possible after anthers dehisce
We have used this updated method several times with good success; between 40-50% of the ~60 crosses attempted have produced seeds. Note that this protocol spreads the crossing procedure over 2 or more days.

This method is based on the fact that immature anthers will dehisce shortly after removal from the plant. This provides a lot of fresh pollen for crossing. We use a dissecting microscope so it is very obvious what stage everything is at and if you successfully pollinated the flowers.

Materials

- Dissecting microscope
- fine tweezers (Ted Pella Inc. Cat# 5622)
- microscope slides
- small scissors (Spring-type micro scissors work great for this. Ted Pella Inc. Cat # 1346(straight blade) or #1347(curved blade))
- (optional) Stand-mounted or visor-type magnifying glasses. These are useful for picking out flowers at the right stage
-(optional) glassine shoot bag (Lawson #214)

Growth conditions and timing

We have successfully used plants grown in a growth chamber with 20 hr days and a greenhouse at 16 hour days. However, all of the crosses using this updated method have been made with growth chamber grown plants. It is very important to have plants at the proper stage for crossing. We primarily use the first few large tillers that are produced by a plant. Each plant will only be at the proper stage for a few days. For a pot of 10 plants there may be flowers at the proper stage for about a week. After that, you can use some of the later secondary tillers. When crossing different ecotypes it is probably a good idea to make several plantings separated by a few days to make sure you have plants at the right stage at the same time.

For plants grown in a growth chamber under 20 hr days removing anthers for pollen collection works best in the morning (we've been crossing at 9 am, lights go on in GC at 4 am.), as this is when the highest percentage of anthers will dehisce. We have notice that anthers open best under warmer temperature and occasionally incubate anthers on a glass

slide for ~5 minutes at 37° C to speed up dehiscence. High temperatures can kill pollen, but we have obtained fertile pollen with this brief 37° incubation.

When making crosses we have had the greatest success by emasculating the female parent one day and then pollinating within the next two days. This ensures that the stigma is fully developed and receptive to the pollen. See Figure 1 for emasculation procedure. After emasculation, be sure that the palea and lemma are tightly closed so that the stigma doesn't dry out. If the flower doesn't stay closed, we use a small piece of masking tape at the tip of the flower to secure it shut until pollination. Typically, we emasculate up to 40 flowers in one day (this takes approx 1-2 hours, depending on your skill and ease of finding flowers at the correct stage), although I don't always get enough pollen to pollinate every flower. We use masking tape to hold the tiller in place while working under the dissecting microscope.

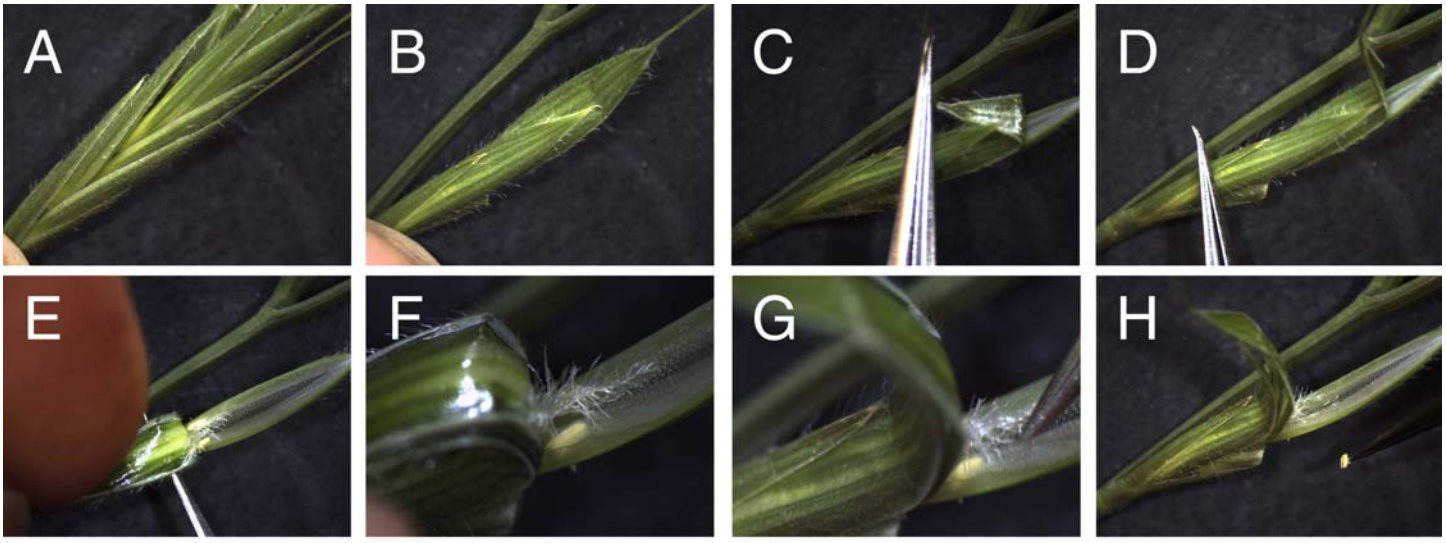

Figure 1. Prepare the female flower (Day 1).

Day 1: To identify flowers at the proper stage, pull the lemma back and look at the flower. You want flowers that are as mature as possible, but before the anthers dehisce. A stand-mounted magnifying glass or visor is useful for this but flowers that are already pollinated can be seen with the naked eye. (A) Proper stage inflorescence. (B) All flowers except the one targeted for crossing are removed either before emasculation (shown) or after emasculation (our preferred way). We use either the first or second flower on the inflorescence. (C) Fold back lemma. (D) To fold back lemma without damaging the flower you can place your forceps across the lemma where shown then pull back lemma while releasing any tension with the forceps. A damaged lemma will start to dry out within a day, which may result in a dried-out flower. Exposed flower shown in (E). (F) Flower ready for emasculation. (G) Remove anthers by inserting forceps in fold of palea

and pulling out anther. Take care not to puncture or damage palea or knock the gynoecium loose. Under our conditions the palea is necessary for normal seed development. (H) Anther comes out easily. Examine carefully to make sure no pollen has fallen out. Repeat on other side. Push lemma back over to close the emasculated flower until pollination.

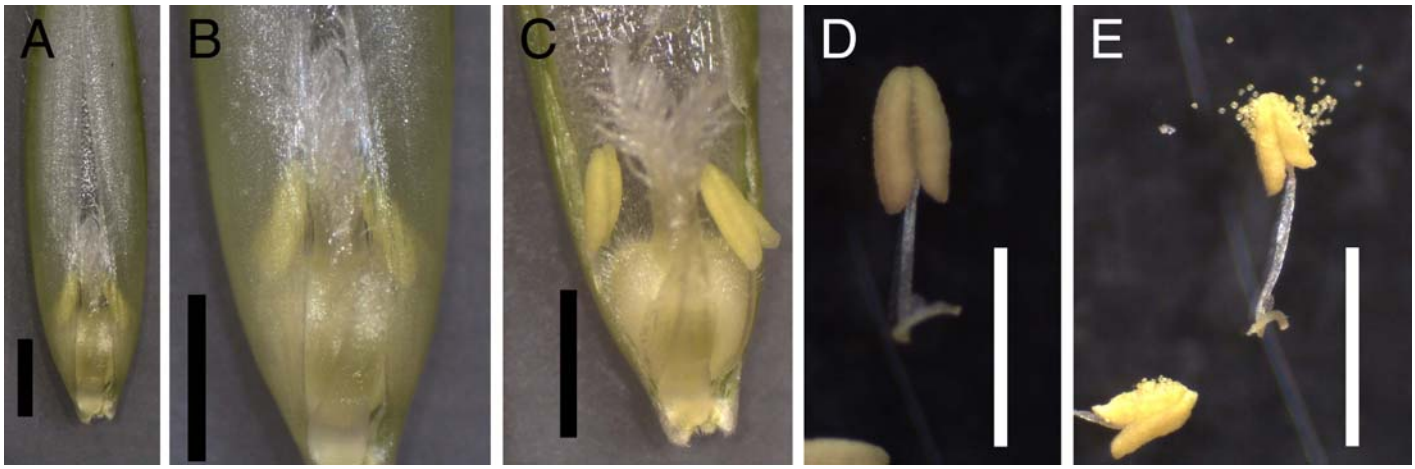

Fig. 2. Preparing pollen
Select flowers with very mature anthers. You can bend back the lemma with your hand to see the anthers. Remove the palea and flower by pulling the palea away from the lemma. (A and B) Proper stage flower. (C) same flower with top side of palea removed for a better view of the anthers. Note it is not necessary to remove the palea when removing anthers. (D) Ripe anthers on a microscope slide. Choose anthers that are between yellow to white, depending on the ecotype being crossed. Typically, anthers that have a slight shade of green do not dehisce. (E) Same anther 25 minutes later. Note that it has dehisced and shed over a hundred pollen grains. I typically pick out about 20 or more anthers per line and after 10-40 min some usually dehisce. The percentage varies from 0 to 50%. Use pollen as soon as possible because viability seems to decrease rapidly.* (A-E) Scale bar is 1 mm.

*Initial tests with fluorescein diacetate stain indicate that pollen viability begins to decline approximately 15 minutes after anther dehiscence.

Figure 3: Pollinating the emasculated flower
Day 2: (I) Previously emasculated flower. Mature stigmas are very feathery with many branched structures. (J) Carefully pick up dehisced anther with tweezers, trying not to spill any pollen still resting inside. Apply pollen from anther to the stigma by carefully brushing across the stigma. You can then use the anther as a brush to pick up any excess pollen on the slide and dab on stigma. You might also use a few fine brush bristles. (K-L) Stigma with pollen grains. (M) Carefully close palea over lemma. (N) Turn flower over and carefully push palea back into place. (O) Final pollinated flower.

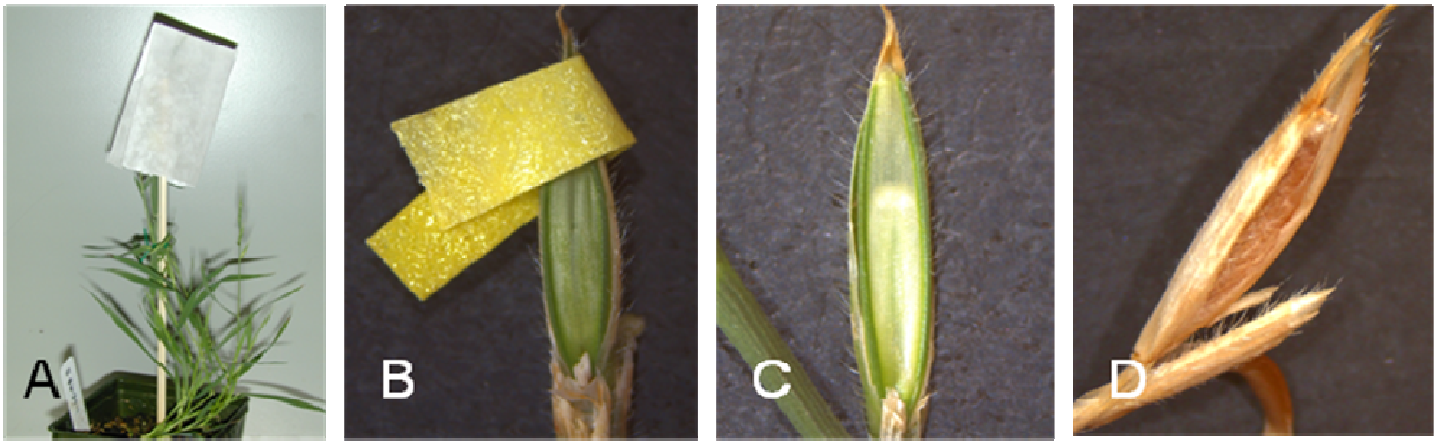

Figure 4: Flower after pollination
(A) After applying pollen to the flower, you can choose to either cover with a glassine shoot bag (Lawson #214) or not. We have observed good pollination rates with and without. (B) Again, be sure the palea and lemma are tightly closed, using tape if necessary. (C) After a few days you should be able to observe a developing endosperm through the palea for those flowers that were successfully pollinated. (D) Fully mature seed.

Good luck!

Notes:
Different *Brachypodium* lines seem to have different amounts of pollen/anther. Bd3-1 seems to generally have bigger anthers with much more pollen than Bd21-3.

Flower size seems to differ with different ecotypes, so we've based our crossing strategy on using the larger of the two flowers as the female parent to make emasculation easier to perform.