

**Figure S1.** **Amplicons produced by the mitochondrial primer sets 1**. An agarose gel showing the amplicons produced by the mitochondrial primer sets 1 on *C. fimbriata*, *C. manginecans*, and three potential hybrid isolates from a cross between the two species. The hybrids show two amplicons which corresponded to the 1800 bp fragment from *C. fimbriata* and the 500 bp fragment of *C. manginecans*.

A picture containing graphical user interface

Description automatically generated

**Figure S2**. **Electropherograms from sequencing the amplicons using the mitochondrial sequencing primer**. Amplicons generated with mitochondrial primer set 1 yielded distinctly different sequencing profiles for the two parents and their hybrid progeny. The sequence of C. fimbriata has a 9 bp insertion whereas this sequence is absent in C. eucalypticola. There is a clear presence of two sequencing profiles in the region of the 9 bp insert in the hybrid progeny of this cross, indicative of the presence of both parent’s mitochondrial DNA.

**Table S1**. RFLP results showing the parental origin of individual spore drops for three self-fertile interspecific crosses generated during the first round of crosses.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Round 1 | | | C. fimbriata x C. eucalypticola | |  |  |  | C. fimbriata x C. manginecans | |  |  |  | C. eucalypticola x C. manginecans | |
| Spore drop | | | Marker 2 origin | Marker 3 origin | Spore drop | | | Marker 1 origin | Marker 3 origin | Spore drop | | | Marker 1 origin | Marker 2 origin |
| fe | 1 | A1 | F\* | F | fm | 1 | A1 | M | M | em | 1 | A1 | M | M |
| fe | 1 | A2 | E | E | fm | 1 | A2 | F | F | em | 1 | A2 | E | E |
| fe | 1 | A | H | H | fm | 1 | A | F | F | em | 1 | A | M | M |
| fe | 1 | B | H | H | fm | 1 | B | F | F | em | 1 | B | E | E |
| fe | 1 | C | H | H | fm | 1 | C | F | F | em | 1 | C | E | E |
| fe | 1 | D | H | H | fm | 1 | D | F | H | em | 1 | D | E | E |
| fe | 1 | E | H | H | fm | 1 | E | F | F | em | 1 | E | E | E |
| fe | 2 | A | - | E | fm | 2 | A | F | F | em | 2 | A | E | - |
| fe | 2 | B | H | F | fm | 2 | B | F | F | em | 2 | B | - | E |
| fe | 2 | C | H | E | fm | 2 | C | F | F | em | 2 | C | E | E |
| fe | 2 | D | H | E | fm | 2 | D | F | F | em | 2 | D | E | E |
| fe | 2 | E | F | F | fm | 2 | E | F | F | em | 2 | E | E | E |
| fe | 3 | A | H | H | fm | 3 | A | F | F | em | 3 | A | E | E |
| fe | 3 | B | F | F | fm | 3 | B | F | F | em | 3 | B | E | E |
| fe | 3 | C | F | F | fm | 3 | C | F | F | em | 3 | C | E | E |
| fe | 3 | D | F | F | fm | 3 | D | F | F | em | 3 | D | E | E |
| fe | 3 | E | H | H | fm | 3 | E | F | F | em | 3 | E | E | E |
| fe | 4 | A | E | E | fm | 4 | A | F | F | em | 4 | A | E | E |
| fe | 4 | B | H | F | fm | 4 | B | F | F | em | 4 | B | E | E |
| fe | 4 | C | E | E | fm | 4 | C | F | F | em | 4 | C | E | E |
| fe | 4 | D | - | E | fm | 4 | D | - | - | em | 4 | D | E | E |
| fe | 4 | E | E | E | fm | 4 | E | F | F | em | 4 | E | E | E |
| fe | 5 | A | H | H | fm | 5 | A | F | F | em | 5 | A | E | E |
| fe | 5 | B | H | H | fm | 5 | B | F | F | em | 5 | B | E | E |
| fe | 5 | C | H | H | fm | 5 | C | M | M | em | 5 | C | E | E |
| fe | 5 | D | H | H | fm | 5 | D | F | H | em | 5 | D | E | E |
| fe | 5 | E | H | H | fm | 5 | E | F | H | em | 5 | E | E | E |

\* F indicates the banding pattern produced by C. fimbriata, E indicates the banding pattern for C. eucalypticola, M indicates the banding pattern for C. manginecans, and H stands for hybridization, meaning that the banding pattern observed was a combination of two individual parents. A1 and A2 were positive controls.

**Table S2.** RFLP results showing the parental origin of individual spore drops for three self-fertile interspecific crosses generated during the second round of crosses.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Round 2 | | | *C. fimbriata x C. eucalypticola* | |  |  |  | *C. fimbriata x C. manginecans* | |  |  |  | *C. eucalypticola x C. manginecans* | |
| Spore drop | | | Marker 2 origin | Marker 3 origin | Spore drop | | | Marker 1 origin | Marker 3 origin | Spore drop | | | Marker 1 origin | Marker 2 origin |
| fe | 1 | A1 | F | F | fm | 1 | A1 | F | F | em | 1 | A1 | M | M |
| fe | 1 | A2 | E | E | fm | 1 | A2 | M | M | em | 1 | A2 | E | E |
| fe | 1 | A | E | E | fm | 1 | A | F | F | em | 1 | A | M | M |
| fe | 1 | B | H | H | fm | 1 | B | - | F | em | 1 | B | M | M |
| fe | 1 | C | E | E | fm | 1 | C | F | F | em | 1 | C | M | M |
| fe | 1 | D | H | H | fm | 1 | D | F | F | em | 1 | D | M | M |
| fe | 1 | E | E | E | fm | 1 | E | F | F | em | 1 | E | M | M |
| fe | 2 | A | E | E | fm | 2 | A | F | F | em | 2 | A | M | M |
| fe | 2 | B | H | E | fm | 2 | B | F | F | em | 2 | B | E | - |
| fe | 2 | C | E | E | fm | 2 | C | F | F | em | 2 | C | E | E |
| fe | 2 | D | E | E | fm | 2 | D | F | F | em | 2 | D | E | E |
| fe | 2 | E | H | E | fm | 2 | E | F | F | em | 2 | E | E | - |
| fe | 3 | A | H | E | fm | 3 | A | F | F | em | 3 | A | E | E |
| fe | 3 | B | E | E | fm | 3 | B | H | F | em | 3 | B | E | E |
| fe | 3 | C | H | H | fm | 3 | C | H | F | em | 3 | C | E | E |
| fe | 3 | D | E | E | fm | 3 | D | H | F | em | 3 | D | E | E |
| fe | 3 | E | E | E | fm | 3 | E | F | - | em | 3 | E | E | - |
| fe | 4 | A | E | E | fm | 4 | A | F | F | em | 4 | A | E | E |
| fe | 4 | B | H | E | fm | 4 | B | F | F | em | 4 | B | E | E |
| fe | 4 | C | E | E | fm | 4 | C | F | F | em | 4 | C | E | E |
| fe | 4 | D | H | E | fm | 4 | D | F | F | em | 4 | D | E | E |
| fe | 4 | E | E | E | fm | 4 | E | H | F | em | 4 | E | E | E |
| fe | 5 | A | H | E | fm | 5 | A | F | F | em | 5 | A | E | E |
| fe | 5 | B | E | E | fm | 5 | B | H | F | em | 5 | B | E | E |
| fe | 5 | C | E | E | fm | 5 | C | F | F | em | 5 | C | E | E |
| fe | 5 | D | H | E | fm | 5 | D | F | F | em | 5 | D | E | E |
| fe | 5 | E | E | E | fm | 5 | E | F | F | em | 5 | E | E | E |