

Identification of SNPs that completely co-segregate (100%) with male sterility trait in an Arabica plant naturally devoid of pollen grains

Delphine Mieulet^{1,2}, Benoît Bertrand³, James A. Holzwarth⁴, Solenn Pruvost⁴, Norvin Gutierrez⁵, Laurence Bellanger⁶, Victoria Berry⁶, Lison Marie⁷, Jean-Luc Verdeil^{1,2}, Eduardo Beche⁶, Rayan Awada⁷, Lucile Toniutti^{1,2}, Jean-Christophe Breitler⁷, Juan Carlos Herrera⁶, Fabrizio Arigoni⁸, Patrick Descombes⁴ and Maud Lepelley⁶

¹CIRAD, AGAP institute, Avenue Agropolis, 34398 Montpellier, France. ²University of Montpellier, CIRAD-INRAE-Institut Agro-University of Montpellier, Montpellier, France. ³Former CIRAD Researcher, Montpellier, France. ⁴Société des Produits Nestlé SA, Nestlé Research, Lausanne, Switzerland. ⁵Fondation NiciFrance, Monaco, Nicaragua. ⁶Société des Produits Nestlé SA, Nestlé Research, Tours, France. ⁷CIRAD, UMR DIADE, Montpellier, France. ⁸Former Nestlé Research, Lausanne, Switzerland.

Introduction

Using male-sterile (MS) plants for Arabica makes it possible to offer farmers F1 hybrid seeds at a reduced cost compared to those produced by vegetative propagation.

In 1992, four MS plants, including the most recent addition, MS01⁽¹⁾, were identified in the genetic resources collection at CATIE in Costa Rica. In order to study the genetic basis of the MS01 plant's male sterility, an F2 population of 400 plants was created by self-pollinating a single MS01xIAPAR59 F1 plant, which was then established in Nicaragua (Fig. 1).

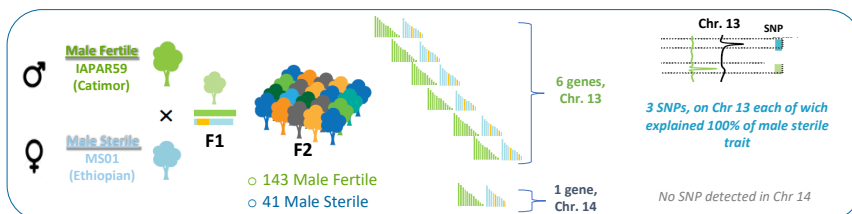


Figure 1: Schematic representation of the contrasted parents and F2 segregant population for the male sterile trait. Targeted sequencing of seven genes for the identification of SNPs associated with male sterility.

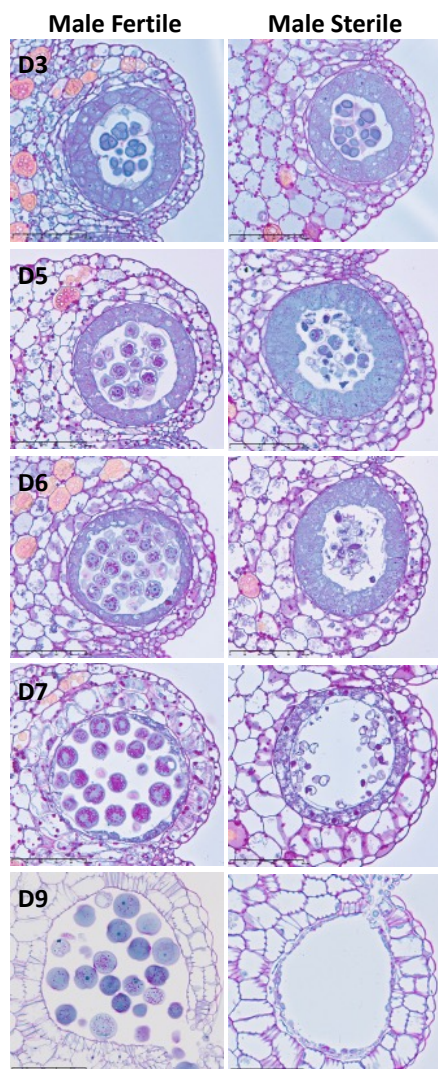


Figure 2: Observation of male gametogenesis in MS01 compared to the control. Cross sections of the anther in Male fertile (MF) and MS01 (MS), during nine days (D) from flower initiation (D1) to anthesis (D9). Stained with naphthol blue (proteins, blue) and Schiff's reagent (carbohydrates, pink). Scale bar, 100 µm

Materials and methods

- Phenotyping was conducted by microscopic evaluation of presence or absence of pollen
- Targeted sequencing, followed by mapping and variant calling, was applied to identify variants
- Marker-trait association was performed
- A histological and transcriptomics kinetics study was conducted during microgametogenesis in MS and MF parents

Results

Previous studies have identified quantitative trait loci (QTLs) on chromosome 13 (Chr.)⁽²⁾ and Chr. 14 (unpublished).

Seven candidate genes, annotated as potentially involved in MS trait based on sequence similarity analysis with model plants were identified in this QTLs. Those seven genes were target sequenced ~250x coverage in the parents and the 184 F2 plants. Within this population, 22% of the plants (41 out of 184) inherited MS (Fig. 1).

Subsequent variant calling and marker-trait association analysis identified three SNPs, each of which explained 100% of the phenotype, which is remarkable (Fig. 1). These three SNPs are located in a gene on Chr. 13 that is annotated as a being a transcription factor (LOB domain-containing protein 4-related)⁽³⁾. However, the SNP positions lie outside the annotated coding regions, suggesting that they may not directly affect the function of the protein. A transcriptomic analysis comparing microsporogenesis in MS and male fertile (MF) plants revealed a temporal correlation in the expression of these seven genes (data not shown).

To compare the morphological differences associated with MS-SNPs to those observed in MF, anther cross-sections were examined from floral initiation to anthesis over a period of nine days (Fig. 2). No significant difference was observed between the two phenotypes until the end of meiosis on day three (D3). The number of tetrads was equivalent for both the MS and the MF plants (D3 Fig. 2). By day five (D5), there were fewer free microspores in the MS plants than in the MF plants. Microsporogenesis continued normally in the MF between D5 and D7, but microspore degradation was observed in the MS during this period. This resulted in empty pollen lodges by days eight (D8) and nine (D9) in the MS, explaining the total absence of pollen grains in the MS01 plant in D9 (Fig. 2).

Conclusion

Identifying a single nucleotide polymorphism (SNP) that fully co-segregates with the male sterility trait in the CIR-MS01 plant allows for the creation of a straightforward screening tool that can be easily integrated into breeding programs. This tool can then be used to establish Arabica seed gardens under natural pollination, thereby facilitating the efficient and economical production of hybrid seeds.

References:

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3. Salojärvi, J., et al. (2024). The genome and population genomics of allopolyploid Coffea arabica reveal the diversification history of modern coffee cultivars. *Nature genetics*, 56(4), 721-731.