Map-Based Cloning of a Male Sterile Mutant Gene, *csmd1*, in Maize

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Abstract

callose, somatic, and microspore defect 1 (csmd1) mutant exhibits normal anther development throughout meiosis, unlike many male sterile mutants, which are interrupted during or before meiosis. In order to map the specific location of this gene, we employed map-based cloning techniques to narrow down the region of interest. Using PCR with leaf samples from fertile and sterile plants, we examined the number of recombinants found in sterile samples to locate the position of the gene on chromosome 10. Based on our results, we have narrowed down the region between positions 123,814,426bp and 127,576,070bp to be where the gene resides. This location contradicts previous estimates, but our trials indicate that this is a more accurate region.

Introduction

The male reproductive organs in maize are contained within the tassels at the apex of the plant (see Fig. 1). Composed of approximately twenty branches, the tassels house the anthers which, following dehiscence, release the pollen.

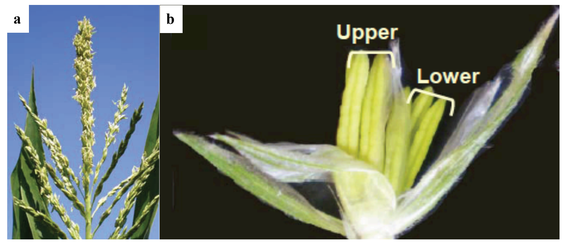

Fig. 1 Tassel and spikelet of maize. Fully mature tassel extends above the height of the plant (**a**). Each branch of the tassel contains spikelets, containing the upper and lower florets, which each hold three anthers (**b**). Image reproduced from Nan et al. (2011)

Unlike other male sterile mutant genes, which interfere with pre-meiotic anther development or disrupt meiosis, the *csmd1* mutant has normal anther development in comparison to their wild-type counterparts. As seen in Fig. 2, male sterile tassels are similar in length and have approximately the same number of branches in comparison to fertile tassels (**a** and **b**). However, they do not possess exposed anthers of fertile plants (**c** and **d**). Still, anther length is nearly identical between the two plants (**e** and **f**). Other phenotypic traits of the *csmd1* mutant include increased levels of callose around meiocytes during prophase I, additional anticlinal divisions and reduced elongation of epidermal cells after meiosis, and normal anther elongation. Even with pre-meiotic irregularities, meiosis continues without interruption. However, *csmd1* microspores cease growth during the uninucleate stage as the nucleoli degrade and the cytoplasm vacuolizes. While male reproductive development in maize is complex and specific, the excess callose buildup does not induce meiotic arrest and the failure of pollen to develop does not inhibit anther growth, which gives the *csmd1* mutant this particular phenotype.

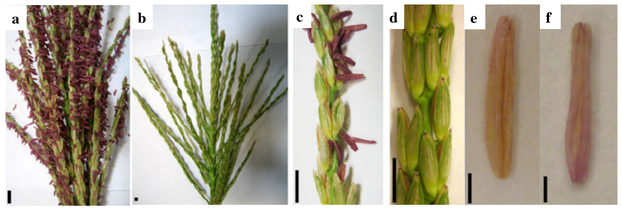

Fig. 2 Comparison of fertile tassel (**a**), spikelet (**c**), and anther (**e**), with *csmd1* mutant (**b, d,** and **f**). Scale bars: 10 mm (**a, b, c,** and **d**); 1 mm (**e** and **f**). Image reproduced from Wang et al. (2011)

csmd1 was originally named *mei775* due to beliefs that meiotic arrest was responsible for male sterility. However, Dongxue Wang's observation of pre-meiotic abnormalities proved that meiosis was not the cause of sterility, and thusly renamed the mutant *csmd1*. Previously, Wang projected the location of the *csmd1* gene to be between position 122,774,351bp and 123,814,426bp on chromosome 10. Our goal was to narrow down the specific location, whether that be within this projected range or out of it. The overall purpose of investigating *csmd1* and male sterility is to ensure the proper crossing of ear parents with an inbred line in order to promote hybrid seed production and vigor. Given the prominence and value of maize as a crop, it is important to understand these mechanisms in order to improve future corn cultivation. Using manual tassel removal as a means of preventing pollen maturation and spread is too cost-ineffective a method, and employing a singular type of cytoplasmic sterility can be susceptible to pathogen attack. Thus, researching other options of male sterility will ensure a more stable method of maize production and will benefit future developments in maize research.

Methods

We worked with three families of maize plants which had a 3:1 segregation of fertile to male sterile – ZLL 287, 288, and 289. These *csmd1* mutant families trace back to a crossing of a female ear, homozygous with *csmd1*, with the pollen from a plant of a wild type inbred line. (see Fig. 3). Inbred lines are homozygous fertile and are maintained each generation through self-pollination. For ZLL 287 the inbred line was A632, for ZLL 288 the inbred line was W23, and for ZLL 289 the inbred line was A619. This cross created a completely heterozygous population and through self-pollination, we obtained our current families with a 3:1 segregation of fertile to male sterile. This is broken down into 1:2:1 of homozygous wild type to heterozygous to homozygous mutant.

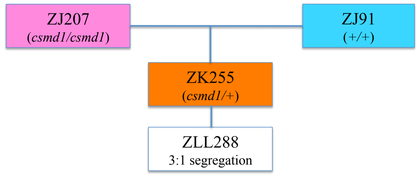

Fig. 3 Two generations of genetic heritage for ZLL 288.

Through screening our families – ZLL 287, ZLL 288, and ZLL289 – we obtained a nearly 3:1 ration with 203 fertile plants and 75 male sterile. We distinguished between fertile and male sterile plants based on phenotypes. Fertile plants have tassels with exerted anthers that visibly shed pollen while male sterile plants have tassels with no exposed anthers (see Fig. 2 **a** and **b**)

In our method of map-based cloning, we focused on the number of recombinants near the area of interest, which indicates the direction of the location of the gene. As illustrated in Fig. 4, approaching the gene on the chromosome, there is a decreasing probability of finding recombinants made during meiosis in contrast with moving away from the gene's location, where there is greater area for recombination to occur. Thus, by choosing primers on particular regions near the area of interest and screening the samples through PCR to find recombinants, we can infer the direction in which the gene resides and narrow down its location.

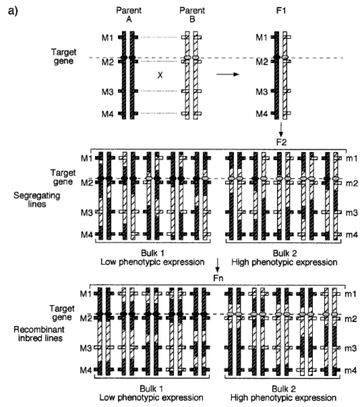

Fig. 4 Schematic representation of bulk segregant analysis (BSA). M1, M2, M3, and M4 indicate markers located on a hypothetical chromosome containing a gene of interest. Moving farther away from the target gene shows an increase in probability for recombinants. Image reproduced from Quarrie et al. (1999)

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Results

After an original screening of family 288 with twelve primers, ranging in position from 121,417,101bp to 127,571,627bp on chromosome 10, we found seven markers that identified polymorphisms between fertile and sterile samples. Further testing those on all twenty sterile plants in family 288 produced recombinants on plant 67 identified by two primers, IDP6827 and IDP620. Because the recombinant was on the same plant, we concluded that we could not have passed the gene within that region and further testing of more primers was needed to find the location of the gene. We moved on to screen family 287, which had thirty-three sterile plants, and found recombinants with primers IDP6827 and IDP7775. With IDP6827 we identified recombinants on plants 19 and 58, and with IDP7775 we identified recombinants on plants 22 and 58 (see Fig. 5).

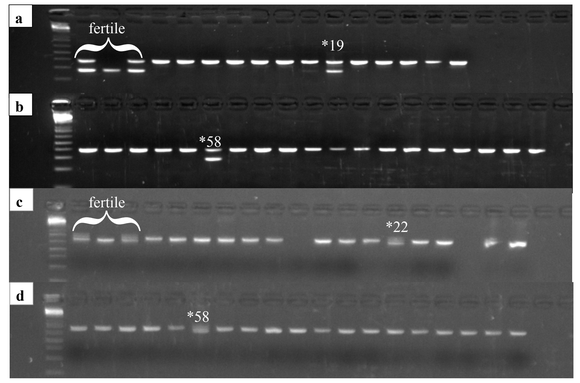

Fig. 5 Gel images under UV transillumination of PCR results with ZLL 287. Recombinants were found on plants 19 and 58 (**a** and **b**); and on plants 22 and 58 (**c** and **d**). Sample 58 was determined to be field sterile – fertile in its genotype but phenotypically sterile.

Conclusion

Because of the continuing presence of a recombinant on plant 58, we determined that it is a field sterile, meaning that it has the genetic DNA of a fertile plant, but is physically expressed as sterile. Thus, we did not include it in the analysis of our results. However, the loss of a recombinant on 19 and gain of 22 indicates that *csmd1* lies within the region between these two markers (see Fig. 6). This area extends from position 123,814,426bp to 127,576,070bp on chromosome 10, which is beyond the region Dongxue Wang predicted. Given the results of our tests, we conclude that this is the new area of interest.

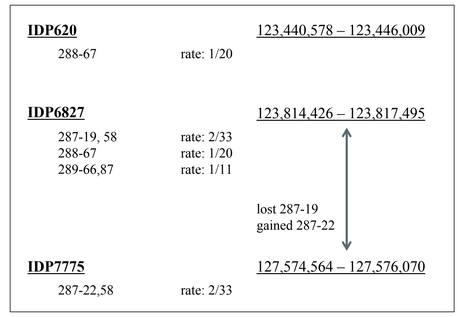

Fig. 6 Map of primer locations and recombination rates for each family.

Although we have made progress in identifying the region wherein resides *csmd1*, this area is approximately 3.7Mbp. Our future work will focus on narrowing down this region through further work with PCR and gene model analysis in order to obtain a more specific area for the location of the gene.

Acknowledgements

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