Male Gametophyte Specific Expression Helps Identify A Conserved Gene Associated with Increased Pollen Fitness

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Abstract

GRMZM2G372877 was identified as a gene with potential function in the male gametophyte based on its strong expression in mature pollen relative to other maize tissues (Chettoor et al. 2014). Identification of a Ds insertion mutation in this gene from the Brutnell/Vollbrecht collection provided further support for this hypothesis, as initial data indicated the insertion was associated with a male-specific transmission defect. In this study, we confirmed the location of GRMZM2G372877 on chromosome 9, approximately 25 map units away from wx1. We used linkage of the Ds insertion to Wx1+, as well as PCR genotyping, to follow up on the initial results, confirming a male-specific transmission defect from mutant heterozygotes. Because the severity of the transmission defect varied with different crosses (2% to 13%), we tested the idea that the defect decreased pollen fitness when in competition with wild-type pollen. Consistent with this idea, we found that male transmission of the mutation increases in frequency when less pollen is applied to the silk (12% to 43%). Based on DNA sequence, we found GRMZM2G372877 was orthologous to a gene (dedecon14) included in a 65-kb deletion associated with the rice no-pollen mutant (Gong et al. 2005), suggesting a conserved function for this gene in pollen. We have tentatively named the gene nop1*, and it encodes a protein with C2 and GRAM domains that are predicted to interact with calcium and phosphoinositides, respectively. Results from microscopy experiments, to visualize specific cellular defects, and to help better determine the function for this gene in pollen, and pollen tube development, will also be presented.

Figure 1. High expression in pollen suggested that a Ds insertion in GRMZM2G372877 (nop1*) would be associated with a male gametophyte-specific defect. A. Transcripts are detected by RNA-seq (as displayed via qTeller) only in male flower and pollen samples. B. A Ds insertion from the Brutnell/Vollbrecht collection is predicted to truncate the encoded protein at amino acid 288 (of 1141 total amino acids).

Figure 2. The domain architecture of NOP1* (via SMART.embde.de) suggests a role in signal transduction and/or membrane-associated function. C2 domains target proteins to membranes in a Ca2+-dependent manner. The GRAM domain can bind phosphoinositides (phospholipid signaling molecules). The newly described VAST domain has no known function.

Figure 3. nop1* is 25.5 map units away from Wx1. A. Map of chromosome 9 shows placement of nop1* and waxy approximately 70 million base pairs apart. Centromere9 decreases recombination frequency. B. Progenies of nop1* and w1 heterozygote females crossed by wild-type males. C. PCR gels of wild-type and w1 samples from the same family show linkage.

Figure 5. Initial observations show no consistent differences in the average size of pollen from nop1*::Ds heterozygotes and wild-type siblings. At least 50 pollen grains were measured per sample (A). Images of pollen from a heterozygote (B) and a wild-type homozygote (C).

Conclusions

PCR genotyping and the linked wx marker were used to confirm a male-specific transmission defect associated with the nop1*::Ds mutation. Furthermore, we showed that the nop1*::Ds defect can be mitigated when less pollen is used in pollination, suggesting that the defect is due to decreased fitness in competition with wild-type. Initial observations suggest there are no obvious defects in morphology in nop1*::Ds pollen, motivating a detailed assessment of later stages of development, e.g., germination and growth of the pollen tube.

Future Directions

- Phenotypically characterize pollen germination and pollen tube growth in pollen from homozygous nop1*::Ds plants (growing in the greenhouse).
- Assess floral development in nop1*::Ds homozygotes.
- Recover revertants via Ds excision in active Ac lines, to confirm the causal nature of the nop1*::Ds mutation.

References


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