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

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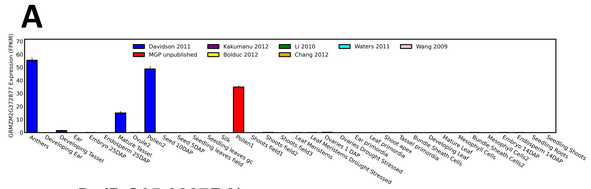
Abstract

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 (*delegen14*) included in a 65-kb deletion associated with the rice no-pollen mutant (*Osnop*) (Jiang 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 or pollen tube development, will also be presented.

A



B

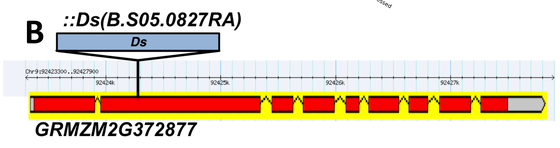
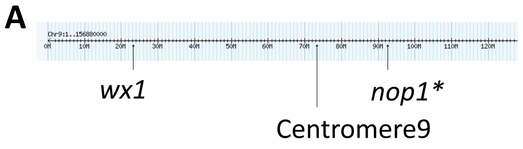


Figure 1. High expression in pollen suggested that a *Ds* insertion in *GRMZM2G372877* (*nop1) would be associated with a male gametophyte-specific defect.** A. Transcripts are detected by RNA-seq (as displayed via qTeller) only in male flower and pollen samples. B. A *Ds* insertion from the Brutnell/Vollbrecht 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.emble.de) suggests a role in signal transduction and/or membrane-associated function. C2 domains target proteins to membranes in a Ca^{++}-dependent manner. The GRAM domain can bind phosphoinositides (phospholipid signaling molecules). The newly described VASt domain has no known function.

A



B

$\frac{Wx1^+\text{-}nop1^*::Ds}{wx1\text{-}Nop1^+\text{-WT}} \times \frac{wx1}{wx1}$

	Progenies			
	Parental Type		Recombinant Type	
	Wx^+ - *nop1*::Ds*	*wx* - *Nop1*-wt*	*wx* - *nop1*::Ds*	Wx^+ - *Nop1*-wt*
Family 1	27	21	11	6
Family 2	4	9	0	2
Family 3	7	2	2	3
Total	**38**	**32**	**13**	**11**

Recombination Frequency	**24/94 = 25.5%**

Female

(A) $\frac{Wx1^+\text{-}nop1^*::Ds}{wx1\text{-}Nop1^+\text{-WT}} \times \frac{wx1}{wx1}$

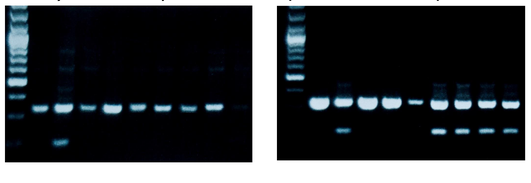
(B) $\frac{nop1^*::Ds}{Nop1^+\text{-WT}} \times \frac{Nop1^+\text{-WT}}{Nop1^+\text{-WT}}$

Male

(C) $\frac{wx1}{wx1} \times \frac{Wx1^+\text{-}nop1^*::Ds}{wx1\text{-}Nop1^+\text{-WT}}$

(D) $\frac{Nop1^+\text{-WT}}{Nop1^+\text{-WT}} \times \frac{nop1^*::Ds}{Nop1^+\text{-WT}}$

Figure 4. The *Ds* insertion in *nop1 is associated with a male-specific transmission defect, and severity of the defect depends on pollen load.** A, B. *Ds* mutation shows mendelian ratios when transmitted through the female. C. Effects of varied pollination on ears of corn show the degree of kernel reduction with less pollen. D. Male *wx1* kernel count display transmission defect in $Wx1^+$ that decreases when less pollen is used during pollination. E. *nop1** male progenies display a strong transmission defect in *wx1* that decreases when less pollen is used during pollination.



C

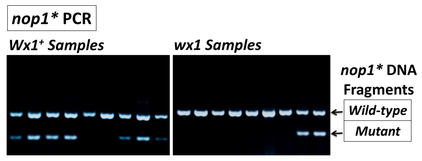


Figure 3. *nop1 is 25.5 map units away from *Wx1*.** A. Map of chromosome 9 shows placement of *nop1** and *waxy* approximately 70 million base pairs apart. Centromere9 decreases recombination frequency. B. Progenies of *nop1** and *wx1* heterozygote females crossed by wild-type males. C. PCR gels of wild-type and *wx1* samples from the same family show linkage.

Reciprocal Cross Data

A ***nop1**** **Female Progenies**

Experiment	Het *nop1**	Homo WT	Proportion Het *nop1**
1	9 (50%)	9 (50%)	0.5
2	13 (47%)	15 (53%)	0.464
3	10 (37%)	17 (63%)	0.37
4	18 (58%)	13 (42%)	0.581

B ***wx1 (nop1*)* Female Progenies**

Experiment	$Wx1^+$ *(nop1*)*	*wx1*	*Proportion* $Wx1^+$ *(nop1*)*
1	80	88	0.476
2	73	62	0.541
3	123	131	0.484

Cochran-Mantel-Hanzel Test
***wx1 (nop1*)* Male Progenies**

C

Experiment		$Wx1^+$ *(nop1*)*	*wx1*	Proportion $Wx1^+$ *(nop1*)*
1	Hvy	116	245	0.321
	Sps	9	14	0.391
2	Hvy	68	118	0.366
	Sps	5	3	0.625
3	Hvy	109	195	0.359
	Sps	7	3	0.700

Chi-Square:	4.506
d.f.:	1
P-value:	0.034

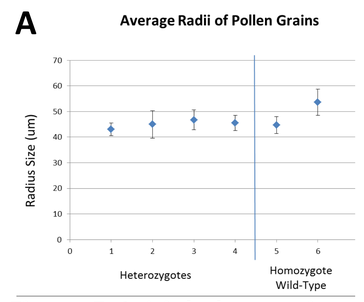
Cochran-Mantel-Hanzel Test
nop1* **Male Progenies**

D

Experiment		Het *nop1**	Homo WT	Proportion Het *nop1**
1	Hvy	1	49	0.020
	Sps	4	29	0.121
2	Hvy	4	64	0.059
	Sps	4	17	0.190
3	Hvy	7	46	0.132
	Sps	5	4	0.556
4	Hvy	3	29	0.094
	Sps	1	4	0.200

Chi-Square:	12.847
d.f.:	1
P-value:	0.000338

A



B

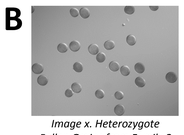


Image x. Heterozygote Pollen Grains from Family 2

C

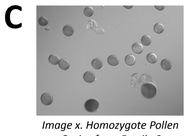


Image x. Homozygote Pollen Grains from Family 6

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 that 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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