Intergeneric Pollen Tube Growth in *Poaceae* Utilizing the *iap* Allele in *Sorghum bicolor*

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Introduction

Genetic improvement of crops is achieved by increasing the available genetic variation within a species (Sharma, 1995). Inbred development and ensuing hybrid production has been practiced in *Sorghum,* through selective breeding, to exploit genetic variation. Interspecific hybridization of *Sorghum bicolor* L. with divergent sorghum species has recently produced diversity not previously seen within *Sorghum*. Increased diversity has potential to improve desirable agronomic traits. Inhibition of pollen tube growth is a major barrier to interspecific and intergeneric hybridization in sorghum (Hodnett et al., 2005). Through germplasm screening Price et al. (2006) identified a specific line that lacks this barrier trait which was later named *iap* (Inhibition of Alien Pollen). Successful introgression of *iap* as well as *ms3* genetic male sterility into a S. bicolor line by Kuhlman et al. (2010) led to the development of Tx3361. The objectives of this study were to make intergeneric crosses using Tx3361 (*iap,iap*) and ATx623 (*Iap*, ___) with species belonging to different genera within Poaceae to determine the relative effectiveness of *iap* in allowing the foreign pollen to germinate and grow into sorghum pistils.

Materials & Methods

•Multiple accessions of *Pennisetum ciliare* (L.) Link*, Pennisetum glaucum* (L.) R. Br., *Sorghastrum nutans* (L.) Nash, *Miscanthus floridulus* (Labill.) Warb. ex K. Schum. & Lauterb., *Miscanthus sinensis* Andersson, *Zea* mays L., and *Zea mays* subs. *mexicana* (Schrad.) H. H. Iltis were used as pollen donors. *S. bicolor* accessions Tx3361 and ATx623 were used as female parents.
•Initial pollinations were made in a greenhouse using Tx3361 as the female parent.
•Tx3361 and ATx623 x *S. bicolor* crosses served as controls for the experiment.
• Pollinated florets were harvested 24 hrs post pollination (controls were harvested 1 hr post pollination) and fixed in a 3:1 (95% ethanol: glacial acetic acid) fixative.
•Pistils were excised from the florets and placed in NaOH, stained with aniline blue, and analyzed using fluorescent microscopy to determine pollen germination and pollen tube growth.
• Accessions showing pollen tube growth into the ovary of Tx3361 were crossed onto ATx623 and analyzed for comparison.
•Statistical analysis was performed using JMP 8 statistical software.

Results

•Tx3361 had a 97% germination rate and 6.71% of the tubes grew into the ovary when pollinated with *S. bicolor*.
• ATx623 had a germination rate of 96% and 6.45% of the tubes grew into the ovary when pollinated with *S. bicolor*.
•When Tx3361 was pollinated using six *Zea* accessions, pollen germination was more than 90% in four of the accessions.
• Four accessions within *Zea* showed pollen tube growth into the ovary of Tx3361.
• Of the four *Zea* accessions crossed onto ATx623, one had pollen tube growth into the stigma axis and three had germination rates below 10%.
• When both *Pennisetum ciliare* cultivars were crossed onto Tx3361, more than 3% of the pollen tube grew into the ovary.
• When both *P. ciliare* cultivars were crossed onto ATx623, none of the pollen tubes grew into the pistil.
• Of the four *Miscanthus* accessions crossed onto Tx3361, all had germination rates over 80% and 3% of the tubes of three accessions grew into the style.
• No *Miscanthus* accessions crossed onto ATx623 showed pollen tube growth into the stigma axis.

Summary

Data from this study show that in comparison to ATx623, presence of the *iap* allele in Tx3361 greatly reduces the reproductive barriers within *S. bicolor*. The rate and location of pollen tube inhibition varies not only between species but also within species. Location of pollen tube inhibition appears to be of lesser importance than the degree of inhibition within most accessions. The reproductive barriers observed do not remain consistent throughout each genus or each species. The mere presence of pollen grains on stigmas or growth of pollen tubes within the pistil does not ensure survival at the next level of observation. Further studies are required to determine the overall ability of the *iap* allele in allowing fertilization of *S. bicolor* by foreign species. Due to the reproductive barrier inconsistencies within each species, it is most appropriate to test more accessions within a species. If fertilization occurs utilizing the *iap* allele, it may be necessary to perform embryo rescue to recover successful hybrid progeny.

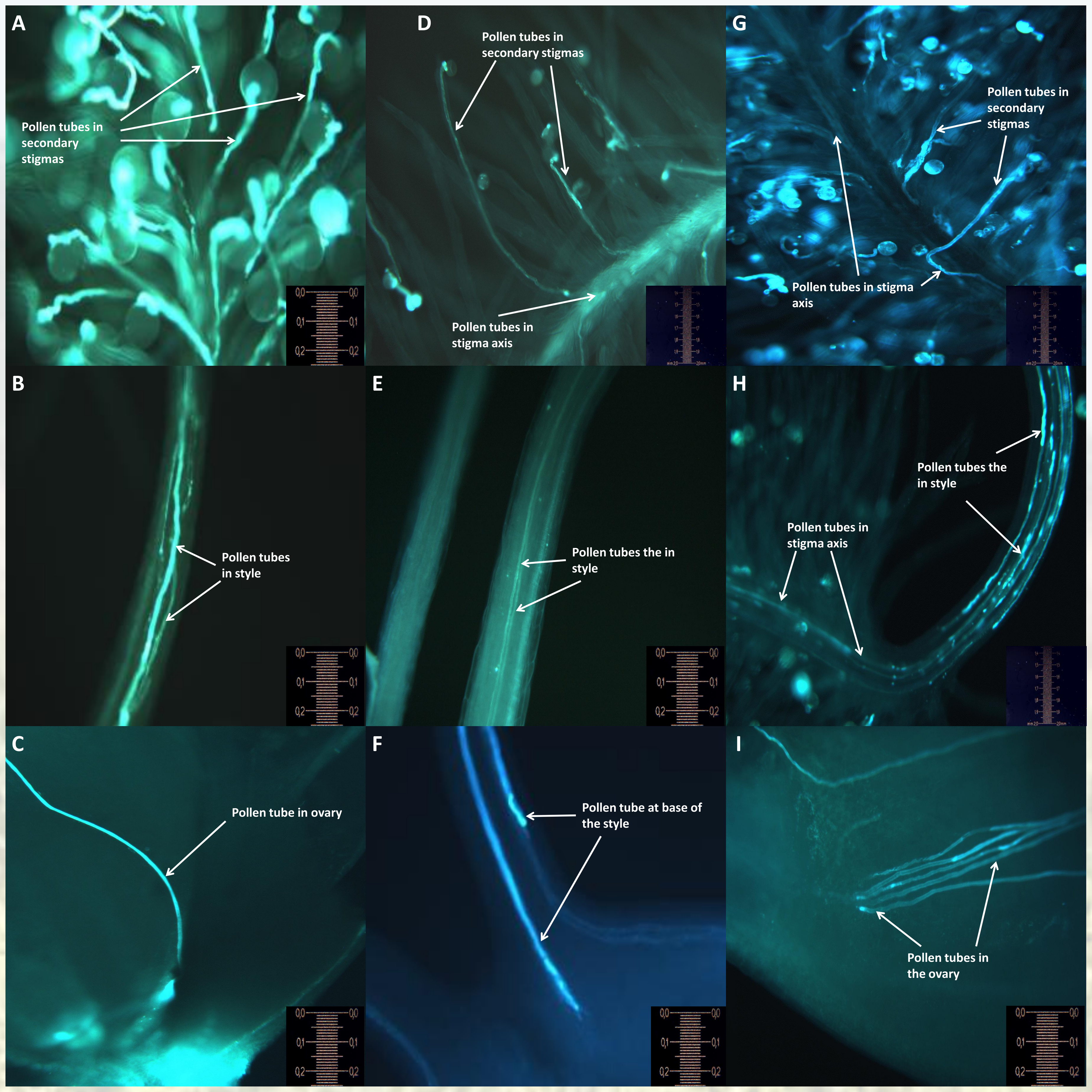

Fig. 1
Pollen tube growth of intergeneric crosses in *S. bicolor* Tx3361 pistils. (A) *Zea mays* var. Kandy Korn pollen grains that germinated and pollen tubes that have grown into secondary stigma branches. (B) Pollen tubes of *Zea mays* var. Kandy Korn that grew down the style. (C) Pollen tubes of *Zea mays* var. Kandy Korn that have grown into and toward the base of the ovary. (D) Germinated pollen grains of *Miscanthus floridulus* and pollen tubes that grew into the secondary stigma branches and down the stigma axis. (E) *Miscanthus floridulus* pollen tubes that have grown into the style. (F) Pollen tubes of *Miscanthus floridulus* that have reached the base of the style but did not enter the ovary. (G) *Pennisetum ciliare* pollen grains that have germinated and pollen tubes that grew into secondary stigma branches and the stigma axis. (H) Pollen tubes of *Pennisetum ciliare* that have grown down the style. (I) Pollen tubes of *Pennisetum ciliare* that grew into and toward the base of the ovary. Micrometer readings are in millimeters.

Table 1. Means and standard deviations of pollen germination and pollen tube growth of *S. bicolor* (*iap,iap*) and *S. bicolor* (*Iap,*__) x *Poaceae* species. Letters within columns indicate significant differences between groups based on LSD, *P* < .05. Significant differences were calculated in groups utilizing the same female parent.

Cross	Pistils Observed	Pollen Grains Observed	Pollen Germination	Pollen tube growth to: Stigma Branches	Stigma Axis	Style	Ovary
	no.		%				
S. bicolor (*iap,iap*) crosses							
Tx3361 x S. bicolor (control)	12	487	97.81 ± 3.75 ab	83.49 ± 5.91 a	32.22b ± 21.34 ab	20.22 ± 17.81 a	6.71 ± 13.93 ab
Tx3361 x Kandy Korn	26	>1572	92.93 ± 4.06 abc	52.6 ± 5.09 ef	9.21 ± 2.35 def	3.42 ± 1.33 d	1.92 ± 1.27 bc
Tx3361 x Tx732 mays	17	>1404	87.93 ± 3.48 bcd	69.92 ± 5.83 abc	12.68 ± 2.00 de	2.97 ± 0.51 d	0.76 ± 0.28 c
Tx3361 x *Z. mexicana*-1	19	>2850	†100 ± 0.00 a	†66.67 ± 0.00 c	†6.67 ± 0.00 efgh	1.54 ± 0.8 d	0.07 ± 0.21 c
Tx3361 x *Z. mexicana*-3	24	>2900	97.49 ± 0.80 ab	60.01 ± 1.95 cde	1.25 ± 0.31 gh	0.02 ± 0.02 d	0.02 ± 0.02 c
Tx3361 x M.floridulus-1	13	1366	84.56 ± 4.62 cd	67.79 ± 5.55 bc	37.83 ± 9.48 a	4.88 ± 3.11 cd	0.00 ± 0.00 c
Tx3361 x M.sinensis-1	11	>1570	99.09 ± 3.01 ab	67.35 ± 2.29 bcd	7.35 ± 2.29 defgh	6.14 ± 0.92 cd	0.00 ± 0.00 c
Tx3361 x M.sinensis-2	22	645	81.00 ± 9.68 d	46.16 ± 9.89 fg	23.71 ± 8.03 bc	14.49 ± 9.54 ab	0.00 ± 0.00 c
Tx3361 x Buffel Common	14	>1800	†100 ± 0.00 a	63.67 ± 8.10 cde	†6.67 ± 0.00 defgh	3.95 ± 3.23 cd	3.00 ± 2.78 abc
Tx3361 x Buffel Frio	52	346	81.58 ± 24.30 d	37.14 ± 35.84 gh	20.30 ± 29.82 c	8.45 ± 18.78 c	6.04 ± 18.78 a
S. bicolor (IAP,___) crosses							
ATx623 x S. bicolor (control)	7	260	96.43 ± 3.57 a	88.38 ± 7.53 a	30.13 ± 9.91 a	18.14 ± 4.18 a	6.45± 5.99 a
ATx623 x Kandy Korn	4	5	0.00 ± 0.00 e	0.00 ± 0.00 c	0.00 ± 0.00 bc	0.00 ± 0.00 b	0.00 ± 0.00 b
ATx623 x Tx732 mays	10	15	45.00 ± 15.72 bc	15.00 ± 10.67 b	5.00 ± 5.00 b	0.00 ± 0.00 b	0.00 ± 0.00 b
ATx623 x *Z. mexicana*-1	15	27	7.7 ± 20.7 e	0.00 ± 0.00 c	0.00 ± 0.00 c	0.00 ± 0.00 b	0.00 ± 0.00 b
ATx623 x *Z. mexicana*-3	23	77	0.43 ± 0.43 e	0.00 ± 0.00 c	0.00 ± 0.00 c	0.00 ± 0.00 b	0.00 ± 0.00 b
ATx623 x M.floridulus-1	6	10	16.67 ± 25.81 de	0.00 ± 0.00 c	0.00 ± 0.00 c	0.00 ± 0.00 b	0.00 ± 0.00 b
ATx623 x M.sinensis-1	22	128	31.59 ± 24.04 cd	0.25 ± 0.25 c	0.00 ± 0.00 c	0.00 ± 0.00 b	0.00 ± 0.00 b
ATx623 x M.sinensis-2	21	285	51.33 ± 20.47 b	1.83 ± 3.27 c	0.00 ± 0.00 c	0.00 ± 0.00 b	0.00 ± 0.00 b
ATx623 x Buffel Common	8	19	41.67 ± 11.78 e	0.00 ± 0.00 c	0.00 ± 0.00 c	0.00 ± 0.00 b	0.00 ± 0.00 b
ATx623 x Buffel Frio	17	74	6.73 ± 14.50 e	0.00 ± 0.00 c	0.00 ± 0.00 c	0.00 ± 0.00 b	0.00 ± 0.00 b

† (*) indicates maximum reliable pollen tube count was reached for all observations.
‡ (>) indicates maximum reliable pollen grain count was reached for one or more observations.

Table 2. Genus and species identification of plant material used as male and female parents. Reference codes indicate designation given to species during the experiment used to cross reference cultivars. ARS-TAMU refers to the USDA-Agricultural Research Service-Texas A&M University at College Station, Texas.

Genus	Species	Cultivar / accession	Reference Code	Source Location
Pennisetum	*ciliare*	Frio	Buffel Frio	ARS-TAMU Forage Breeding Program
Pennisetum	*ciliare*	Common	Buffel Common	ARS-TAMU Forage Breeding Program
Pennisetum	*glaucum*	PI 286837	P. glaucum-1	National Plant Germplasm Program
Pennisetum	*glaucum*	PI 164410	P. glaucum-2	National Plant Germplasm Program
Sorghastrum	*nutans*	PI 476279	Indian 476279	ARS-TAMU Forage Breeding Program
Sorghastrum	*nutans*	PI 47699	Indian 47699	ARS-TAMU Forage Breeding Program
Miscanthus	*floridulus*	PI 230189	M. floridulus-1	National Plant Germplasm Program
Miscanthus	*sinensis*	PI 295764	M. sinensis-1	National Plant Germplasm Program
Miscanthus	*sinensis*	CANE 9233	M. sinensis-2	National Plant Germplasm Program
Miscanthus	*sinensis*	PI 294602	M. sinensis-4	National Plant Germplasm Program
Zea	*mays*	Kandy Korn	Kandy Korn	Commercial Hybrid Release
Zea	*mays*	Silver Queen	Silver Queen	Commercial Hybrid Release
Zea	*mays*	Tender Treat	Tender Treat	Commercial Hybrid Release
Zea	*mays*	Tx-732	Tx732 mays	TAMU Corn Breeding Program
Zea	*mexicana*	PI 566677	Z. mexicana-3	National Plant Germplasm Program
Zea	*mexicana*	PI 566673	Z. mexicana-1	National Plant Germplasm Program
Sorghum	*bicolor*	Tx3361	Tx3361	TAMU Sorghum Breeding Program
Sorghum	*bicolor*	ATx623	ATx623	TAMU Sorghum Breeding Program

References

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