



# Optimization of intra- and interspecific breeding of ornamental *Hibiscus* species

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# Outline

- Background
- Hypotheses
- Methods
- Experimental setup
- Results
- Next steps



# *Hibiscus rosa-sinensis*



- Popular, but with declining sales numbers
- High production costs
- High level of competition from other countries

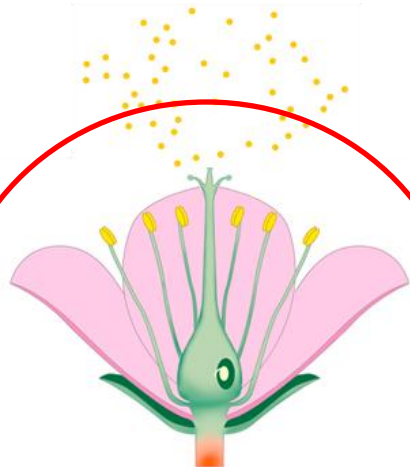
Solution: differentiate the product by overcoming breeding barriers

# Challenges

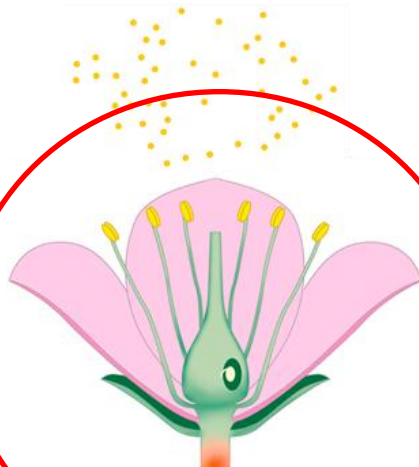
- *H. rosa-sinensis* has a low or absent seed set due to a complex genetic background
- Breeding is slow and untargeted and some cultivars represent “a dead end”
- The primary gene pool consists of only one species



# Methods for overcoming pre-fertilization barriers



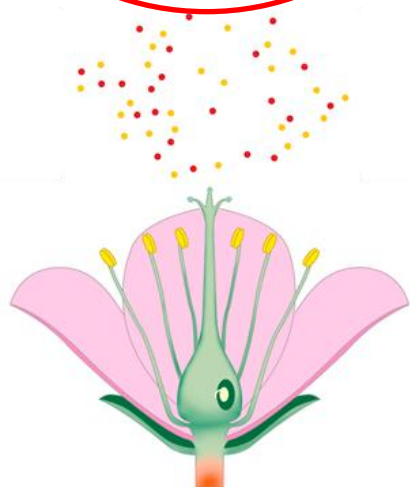
Timing of pollination



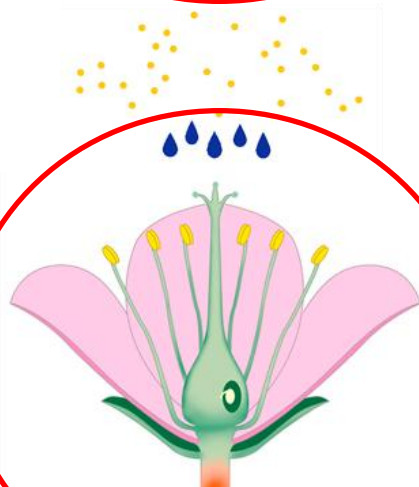
Cut style



Style grafting



Mentor pollination



Chemical application



Changed environmental conditions

# Hypotheses

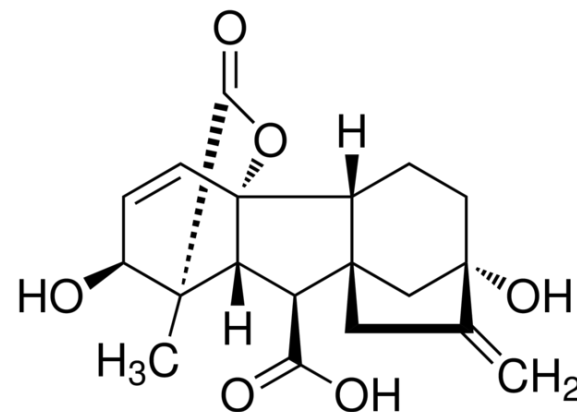
- Application of  $GA_3$  will increase pollen germination and the number of pollen tubes reaching the end of the style.
- An increased number of pollen tubes reaching the end of the style will increase the seed set.



# Application of gibberellic acid 3

- Application of GA<sub>3</sub> can improve the physiological environment for pollen germination, increase pollen tube elongation and promote seed set and seed development
- GA<sub>3</sub> was applied one hour before pollination in the concentrations:

- 25 mg/L
- 50 mg/L
- 100 mg/L

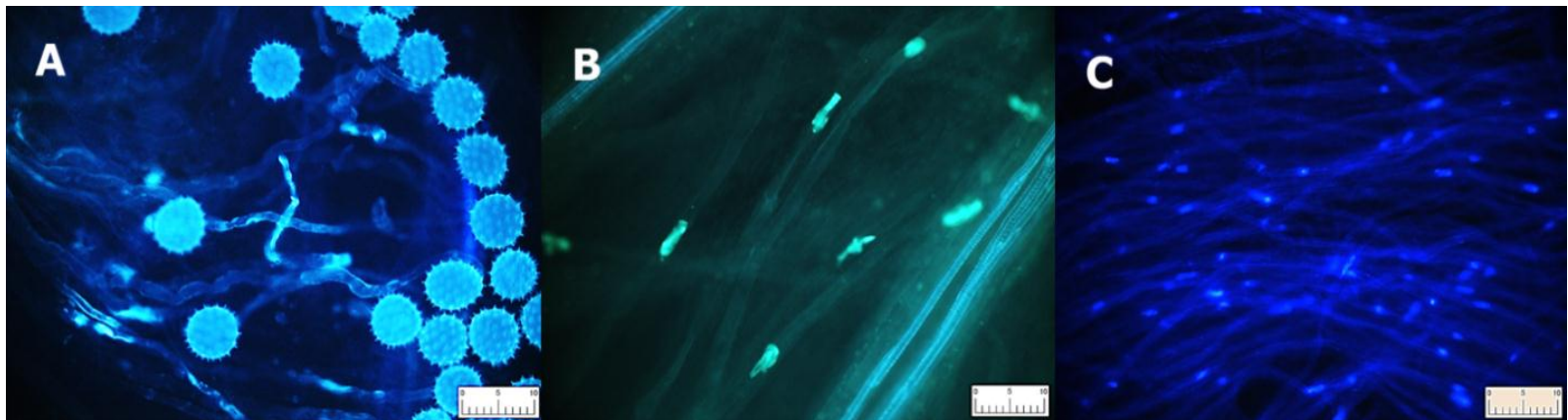




# Observation methods



- Dissected
- Stained with aniline blue
- Observed under UV-light



Seeds harvested at maturity and counted



# Plant material *H. rosa-sinensis*



'Adonicus' (Ad)



'Adonicus Pearl' (AP)



'Arionicus' (Ar)

# Plant material other *Hibiscus*



*H. Trionum* (tri)



*H. Moscheutos* 'Disco Belle' (mos)

# Experimental setup

♀	Ad		Ar		AP			tri		mos	
♂	Ad	Ar	Ar	Ad	AP	tri	mos	tri	AP	mos	AP

25 mg/L GA<sub>3</sub>

50 mg/L GA<sub>3</sub>

100 mg/L GA<sub>3</sub>

~10 pistils harvested and dissected

10-50 flowers left for observation of seed set



# Results of intraspecific crossings

Cross	Conc. of GA3	Pollen tube growth	Seed set
AdxAd	25 mg/L		+ 66.7%
	50 mg/L	+ 48.3%	
	100 mg/L	+ 38.5%/23.3%	+ 83.3%/66.7%
AdxAr	25 mg/L	+ 8.3%	+ 10%
	50 mg/L		+ 10 %
	100 mg/L	+ 3.3%/0%	+ 40%/0%
ArxAr	25 mg/L		None
	50 mg/L		None
	100 mg/L	+ 0%/10%	None
ArxAd	25 mg/L	+ 50%	None
	50 mg/L	+ 20%	None
	100 mg/L	+ 28.6%/0%	None

# Results of interspecific crossings

Cross	Conc. of GA3	Germination	Pollen tube growth
trixtri	25 mg/L	-	+ 50%
	50 mg/L	-	+ 50%
	100 mg/L	-	+ 25%
APxAP	25 mg/L	+ 4.3%	
	50 mg/L	+ 14.3%	
	100 mg/L	+ 14.3%	+ 17%
trixAP	25 mg/L	+ 23.3%	+ 11.1%
	50 mg/L		+ 100%
	100 mg/L		+ 66.7%
APxtri	25 mg/L	-	+ 10%
	50 mg/L	-	
	100 mg/L	-	

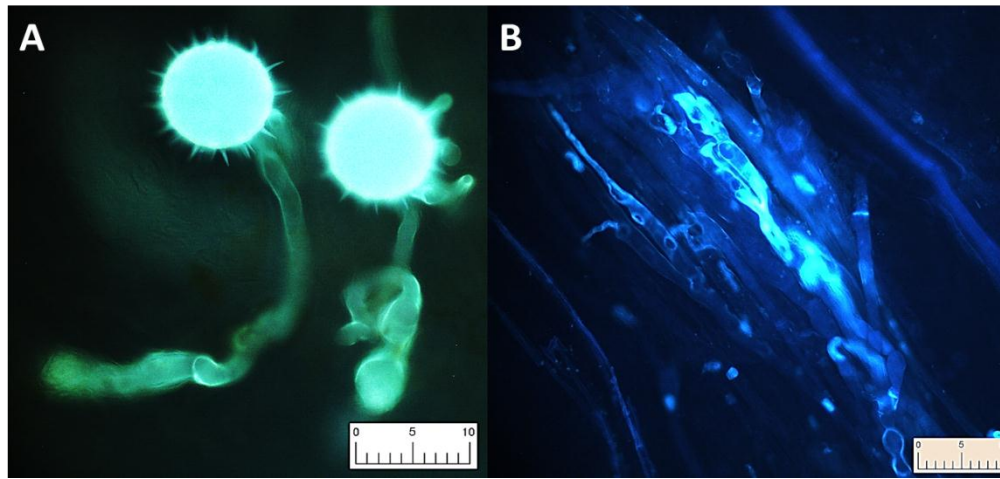
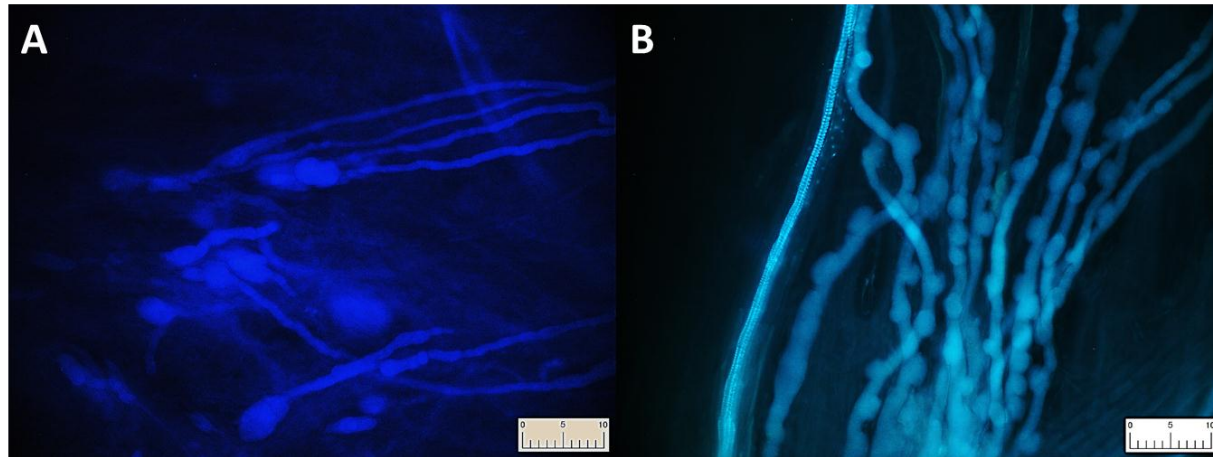
# Results of interspecific crosses

Cross	Conc. of GA3	Germination	Pollen tube growth
mosxmos	25 mg/L	+ 80%	+ 71.4%
	50 mg/L	+ 80%	+ 100%
	100 mg/L	+ 80%	+ 77.8%
mosxAP	25 mg/L	+ 16.7%	
	50 mg/L	+ 50%	
	100 mg/L	+ 50%	+ 50%
APxmos	25 mg/L	+ 20.4%	
	50 mg/L	+ 47.1%	+ 66.6% (33.3%)
	100 mg/L	+ 23.8%	+ 33.3%





# Observations





# Hypothesis 1

- Application of  $GA_3$  will increase pollen germination and the number of pollen tubes reaching the end of the style.

**Intraspecific crosses:** Confirmed.

**Interspecific crosses:** Yes, in the cross APxmos pollen tubes grew to the end of the style. In all other crosses germination and pollen tube growth was improved.



## Hypothesis 2

- An increased number of pollen tubes reaching the end of the style will increase the seed set.

**Intraspecific crosses:** Yes, but not when Ar is the pollen receiver

**Interspecific crosses:** No seed set was observed.



# Evaluation of methods

Methods only help some pre-fertilization problems

Part of an integrated approach

Chromosome numbers should be investigated

Different genotypes should be included

These experiments can serve as a basis for further investigations



# Next steps

- Investigations on optimization of dose and application of GA<sub>3</sub>
- Karyotyping and selection of species and cultivars, possibly chromosome doubling
- Selection of older cultivars with less chromosomes, or species in the origin of *H. rosa-sinensis*
- Bridge crossing





**Thank you for your attention!**