

HYBRIDIZATION OF NEW AUSTRALIAN *GOSSYPIMUM* SPECIES (SECTION *GRANDICALYX*) WITH CULTIVATED TETRAPLOID COTTON

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Abstract

There are at least seventeen indigenous wild cotton species in Australia, including 12 species (K genome) primarily distributed in the Kimberley area. The introgression of any useful features from the Kimberley cottons ($2n=2x=26$, KK) requires interspecific hybridization. We found that the Kimberley cottons had high pollen compatibility with cultivated tetraploids ($2n=4x=52$, AADD) when the tetraploids were used as female. The pollination between AD as female and K produced almost the same boll-set as intraspecific crossing, while the reciprocal crosses were unsuccessful. The hybrid bolls were smaller, had smaller seed and less seed per boll than the selfed bolls from the maternal parent plants. The hybrid seeds also germinated slowly and had lower germination percentage. The result demonstrated that the Kimberley cottons have pollen-stigma compatible factor(s) with AD, but that some postzygotic barriers exist. The variation in crossability varied with female parents and Kimberley species, with MDH-94 being more compatible than STV 453 with the Kimberley cottons. STV 453 x *G. marchantii* hybrid seed did not germinate and STV 453 x *G. rotundifolium* (NWA-22) hybrids died before emergence, indicating lethal factor(s) may exist in some Kimberley cotton accessions. Currently, we have obtained all interspecific hybrids between AD and seventeen Australian *Gossypium* and three crosses have been advanced to the BC2 generation.

Introduction

Genus *Gossypium* comprises about 50 taxons (Fryxell, 1992; Stewart, 1995), with a world wide distribution excluding Europe. There are at least seventeen species in Australia, including seven new species recognized recently as a result of extensive collection and observations. Those are *G. enthyle*, *G. exiguum*, *G. londonderriense*, *G. marchantii*, *G. nobile*, *G. rotundifolium* (Fryxell et al., 1992) and a new species with the manuscript name of *G. anapoides*. Together with five other species (*G. costulatum*, *G. cunninghamii*, *G. pilosum*, *G. populifolium*, and *G. pulchellum*), they form the section *Grandicalyx* of subgenus *Sturtia*. These species, primarily distributed in the Kimberley region of Australia, have unique features such as herbaceous shoots with perennial rootstock, naked seed with an aril, white or pink flower, and recurved pedicel,

among others (Craven et al., 1995). They provide an important resource of germplasm for cotton improvement (Stewart, 1995). However, little work has been done concerning interspecific hybridizations and utilizations among Kimberley wild cottons and cultivated cottons.

Very easy crossability between tetraploid cottons (AD) as a female and Kimberley cottons was observed in this laboratory (Stewart, unpublished). The objectives of the present study are, (i) to investigate the crossability between tetraploids and Kimberley cottons in terms of boll-setting, seed quality and germination, and (ii) to update the current status of the interspecific hybridizations involving Kimberley cottons in our laboratory.

Materials and Methods

The interspecific hybridizations have been made over many years with different species and accessions (Table 1), but the main experiments involved in this report were conducted in 1995 and 1996.

Experiment 1: In July 1995, plants of Stoneville 453 (STV) in the field and greenhouse, and of MDH-94 (MDH) in the field were used as maternal parents to cross with different Kimberley cotton species. Five to ten pollinations were made for each cross, and 1-6 open bolls were harvested in October. After seed-cotton from each boll was weighed and ginned, seed number and mature seed number, seed weight, and lint percentage were measured. The germination test was conducted in February, 1996 in a growth chamber. Ten to fifteen seeds per cross were used. The seeds germinated were counted daily, until 20 days after the germination test started. The selfed seeds of female parents of the hybrids were included as references in the test. F1 seedlings were maintained in the greenhouse and used as both female and male parents to cross with tetraploids.

Experiment 2: Although we found high boll-setting following interspecific pollination in experiment 1, the precise number of hybridizations from each cross was not recorded. In the 1996 growing season, D8cms in Hartz 1330 background was used as female parent to cross with Kimberley cottons. The day before anthesis, flower buds were covered with bags to prevent out-crossing. Pollinations were made on August 19, and boll-setting was recorded on September 2. Hybrid bolls were harvested after frost.

Experiment 3: Kimberley cottons, maintained in the greenhouse, were used as female parents to cross with cultivated tetraploids. Pollinations and boll-set were also recorded.

Results and Discussion

Boll-setting: Boll set in crosses between AD and Kimberley cottons can be seen in Table 2. In 1995, 5-10 pollinations were made for each cross involving six new Kimberley

cotton species, and 1-6 open bolls were obtained, indicating a high crossability between AD and Kimberley cottons when AD was used as female parent. In 1996, when 5-10 flowers from a *G. hirsutum* 'D8 cms' line were used to make crosses, 80% and 90% boll-set were observed for D8cms x *G. nobile* and *G. exiguum*, respectively. The hybridizations of D8cms x *G. pilosum* and D8cms x *G. populifolium* also gave 90% and 100% of boll-setting, compared to D8cms x Hartz 1330 with 100% of boll-set. This result indicated that there is very high pollen compatibility between AD (as female) and Kimberley cottons. The high compatibility is characteristic of all the Australian Kimberley cotton species when crossed as male with AD. Other data showed that hybridizations among Kimberley cotton species, and between Kimberley species and other Australian cotton species (all with $2n=2x=26$) were not difficult. Although *G. arboreum* and *G. thurberi* as female were relatively easily crossed with some Kimberley cottons (Stewart, unpublished), no successful crosses were made between *G. triphyllum*, *G. laxum*, *G. lobatum* and *G. aridum* as female, and Kimberley cottons in this experiment. However, when *G. trilobum* (a sister species of *G. thurberi*) was used as female to cross with Kimberley cottons, *G. nobile* (PI 499806) and *G. exiguum* (PI 499790 and PI 499794), hybrid bolls were set, but only *G. trilobum* x 499794 produced 13 mature seed out of 45 seed. Although it is without doubt that Kimberley cottons have cross-compatible factor(s), this result implied that the crossability varies with female species and Kimberley species.

When Kimberley cottons were used as female parents to cross with tetraploids, no bolls with viable seeds set (Table 2). The reciprocal hybrid embryos would have the same genomic composition ($3X=ADK$), but their endosperm would be different. When tetraploids were used as female, the hybrid endosperm had genomic composition AADDK ($5X$). The ratio of embryo genome number to endosperm genome number is 0.6, which is very close to the normal ratio (0.5) from intraspecific hybrids. On the other hand, when Kimberley cottons were used as female, the genomic composition of the hybrid endosperm is ADKK ($4X$), giving 0.75 for the ratio of embryo genomic number to the endosperm genomic number. The differences in chromosomal number and composition of reciprocal hybrids may result in failure in development of hybrid endosperm and its normal functions when Kimberley cottons are used as female.

Boll traits: The hybrid bolls had markedly different traits from maternal parents (Table 3). Hybrid bolls were much smaller in size ranging from 1.10-3.78 g, compared to maternal parents with 6.30 g of seed-cotton per boll. Hybrid bolls of STV x *G. rotundifolium* had the lowest boll weight, while MDH x *G. marchantii* had the largest boll among crosses. Maternal parent influenced hybrid boll size. The interspecific hybrid bolls from STV had smaller size with a mean of 1.88 g while those from MDH averaged 3.20 g,

70% higher than the former one. The same tendency was found for seed weight per boll and seed number per boll. Crosses with STV and MDH averaged 15.9 and 23.0 seeds per boll, respectively, which were 58.9 and 76.7% of their respective female parent. STV x *G. rotundifolium* had the lowest number of seed and mature seed (8.7 per boll). The other crosses with STV had around 14 mature seeds (data not shown). The two crosses with *G. enthyale* had very low seed number. STV x *G. rotundifolium* also had the smallest seed size among nine crosses. On average, STV crosses had 5.26 g seed index, and MDH crosses 7.73 g. This result indicated that there is variation in compatibility within upland cotton and Kimberley cottons, with MDH being more compatible than STV. Among Kimberley cottons, *G. rotundifolium* is the most incompatible to AD, while *G. exiguum*, *G. pilosum* (with STV), and *G. marchantii* (with MDH) are most compatible.

Interestingly, due to small seed and almost normal fiber development on hybrid ovules, an abnormally high lint percentage (e.g. 62.8% for STV x *G. marchantii*) was observed. There was an association between parents and their hybrid bolls in lint percent. STV hybrid bolls had an average lint percent of 55.1% (47.8% higher than STV), while the lint percent for MDH hybrids averaged 45.0% (31.5% higher than MDH).

In 1996, since the crosses were made late in the season, hybrid bolls were subjected to lower temperature during their development than in 1995. As a result, bolls did not open normally. Therefore, the mature hybrid seed was much less than the previous year. However, the crosses made within upland cotton at the same time matured more or less normally. This may indicate that hybrid embryo development needs higher temperature than that of upland cotton.

Germination: The results are shown in Table 4. All hybrid seeds germinated slower and had lower germination percentage than selfed maternal seeds. But, differences attributable to both paternal and maternal parents existed. The hybrid seeds from *G. enthyale* and *G. marchantii* with MDH had higher germination percentage than hybrids of these with STV, suggesting higher compatibility of MDH with Kimberley cottons. The result was consistent with the boll traits. Although germination percentage was positively correlated with boll size ($r=0.5799^*$, $P=0.10$), seed weight per boll ($r=0.5946^*$, $P=0.10$), seed number per boll ($r=0.4772$ ns), and seed index ($r=0.6041^{**}$, $P=0.05$), there was a discrepancy between these traits. For example, *G. marchantii* was one of the most crossable species with AD in terms of hybrid boll size and seed number per boll. However, its hybrid seed had the lowest seed index and germination percentage. STV x *G. pilosum*, STV x *G. exiguum*, MDH x *G. londonderriense* and MDH x *G. enthyale* had higher germination percentage, while STV x *G. marchantii*, STV x *G. enthyale* and MDH x *G. marchantii* had lower values. In fact, STV x *G. marchantii* hybrid seed

did not germinate. Although STV x *G. rotundifolium* seed germinated (40%), no seedlings were produced. An additional germination test was done on this cross. Four of 12 seed germinated, but again the germinated seedlings died before cotyledons unfolded. The symptoms resembled those of *Rhizoctonia solani* infestation. A brown necrotic lesion developed on the hypocotyls near the cotyledons, which blocked the cotyledons from unfolding. Perhaps complementary lethal factor(s) exist in this specific accession (NWA-22) of *G. rotundifolium*, which made it incompatible with AD. Fortunately, F1 plants of a hybrid between AD2 (57-4) x *G. rotundifolium* (499788) grew very well in the greenhouse.

The F1 hybrid plants grew very slowly, and had small cotyledons and slender stem.

The status of interspecific hybridizations involving Australian cottons: Presently, we have obtained all hybrids between tetraploids and Australian wild cotton species (Table 5). Three crosses have been advanced to the BC2 generation via a hexaploid pathway. Other crosses are triploid, awaiting chromosome doubling.

Conclusions

1. Hybrid bolls between AD and Kimberley cottons were very easy to obtain when AD was used as maternal parent, while the reciprocal crosses were unsuccessful.
2. The hybrid bolls had smaller size, smaller seed and less seed per boll than selfed bolls from the maternal parent plants, indicating both prezygotic and postzygotic barriers exist.
3. Hybrid seeds germinated slowly and had lower germination percentage than the maternal parents. One hybrid seed (STV x *G. marchantii*) did not germinate and one hybrid cross (STV x *G. rotundifolium*) died shortly after emergence. The results demonstrated that certain postzygotic barriers exist between AD and Kimberley cotton species.
4. The crossability varies in maternal parents and Kimberley wild cotton species.
5. All interspecific hybrids between AD and seventeen Australian *Gossypium* have been obtained.

References

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Table 1. Materials used.

Species	Genome	Accession
<i>G. enthyale</i>	K	NWA-60
<i>G. exiguum</i>	K	499769, 499770, NWA-49, NWA-58
<i>G. londonderriense</i>	K	SC-29
<i>G. marchantii</i>	K	NWA-6
<i>G. nobile</i>	K	499806
<i>G. rotundifolium</i>	K	499788, NWA-22
<i>G. anapoides</i>	K	NWA-24
<i>G. pilosum</i>	K	499785, 499786, 499803
<i>G. hirsutum</i>	AD1	D8cms, MDH94, Stoneville 453, Hartz 1330
<i>G. barbadense</i>	AD2	57-4, TX-110

Table 2. Interspecific boll set between section *Grandicalyx* species and tetraploid cottons.

Cross	Pollination no.	Boll no.	Boll setting (%)
D8cms x <i>G. enthyale</i>	5	5	100
D8cms x <i>G. nobile</i>	5	4	80
D8cms x <i>G. exiguum</i>	10	9	90
D8cms x <i>G. populifolium</i>	5	4	80
D8cms x Hartz 1330	5	5	100
<i>G. pilosum</i> x STV	6	0	0
<i>G. nobile</i> x STV	14	0	0
<i>G. exiguum</i> x STV	7	0	0
<i>G. anapoides</i> x TX-110	4	0	0

Table 3. Boll characteristics for different interspecific pollinations.

Cross	Boll size	Seed wt	Seeds	Seed index	Lint
	g/boll	g/boll	no./boll	g	%
STV x <i>G. enthyale</i>	1.89	0.92	15.0	6.13	51.3
STV x <i>G. exiguum</i>	2.37	1.00	17.8	5.63	57.8
STV x <i>G. marchantii</i>	1.88	0.70	17.0	4.12	62.7
STV x <i>G. nobile</i>	1.92	0.80	13.3	6.04	58.3
STV x <i>G. pilosum</i>	2.13	1.62	18.3	5.59	52.1
STV x <i>G. rotundifolium</i>	1.10	0.57	14.0	4.07	48.2
STV selfed	6.33	3.97	27.0	14.70	37.3
MDH x <i>G. enthyale</i>	3.16	1.62	19.0	8.53	48.7
MDH x <i>G. londonderriense</i>	2.65	1.51	20.5	7.37	43.0
MDH x <i>G. marchantii</i>	3.78	2.15	29.5	7.29	43.1
MDH selfed	6.29	4.14	30.0	13.80	34.2

Table 4. Cumulative germination percentage (%) over time (days after sowing) in different hybrids.

Cross / parent	Days after sowing						
	3	5	7	10	12	14	20
STV x <i>G. enthyle</i>	10.0	10.0	10.0	20.0	20.0	20.0	20.0
STV x <i>G. exiguum</i>	0	6.7	13.3	53.3	53.3	53.3	53.3
STV x <i>G. marchantii</i>	0	0	0	0	0	0	0
STV x <i>G. nobile</i>	0	13.3	13.3	26.7	33.3	40.0	40.0
STV x <i>G. pilosum</i>	0	6.7	13.3	53.3	60.0	66.7	66.7
STV x <i>G. rotundifolium</i>	0	6.7	6.7	20.1	26.7	33.3	40.0
STV selfed	13.3	26.6	46.7	66.7	73.3	73.3	73.3
MDH x <i>G. enthyle</i>	0	10.0	10.0	30.0	40.0	40.0	60.0
MDH x <i>G. londonderriense</i>	6.7	13.4	20.1	26.8	53.6	53.6	66.7
MDH x <i>G. marchantii</i>	0	13.4	13.4	20.1	26.8	33.3	40.0
MDH selfed	0	20.0	30.0	60.0	60.0	70.0	70.0

Table 5. Current status of interspecific hybrids involving tetraploid cottons (AD) and Australian wild cottons.

Cross	Status	Backcrossing
AD x <i>G. enthyle</i>	F1 plants (3x)	Failed in getting BC1
AD x <i>G. exiguum</i>	F1 plants (3x)	Failed in getting BC1
AD x <i>G. londonderriense</i>	F1 plants (3x)	Failed in getting BC1
AD x <i>G. marchantii</i>	F1 plants (3x)	Failed in getting BC1
AD x <i>G. nobile</i>	F1 plants (3x)	Failed in getting BC1
AD x <i>G. rotundifolium</i>	F1 plants (3x)	Failed in getting BC1
AD x <i>G. anapoides</i>	F1 seed (3x)	
Other crosses		
AD x <i>G. costulatum</i>	BC2F1 plants	Very difficult to obtain BC2
AD x <i>G. cunninghamii</i>	F1 seed (3x)	
AD x <i>G. pilosum</i>	F1 plants (3x)	Failed in getting BC1
AD x <i>G. populifolium</i>	F1 seed (3x)	
AD x <i>G. pulchellum</i>	F1 seed (3x)	
AD x <i>G. australe</i>	F3 plants (6x)	
AD x <i>G. bickii</i>	BC2F3	Relatively easy to get BC2
AD x <i>G. nelsonii</i>	F1 plants (3x)	
AD x <i>G. robinsonii</i>	F1 seed (3x)	
AD x <i>G. sturtianum</i>	BC2 plants	Relatively easy to get BC2