Research Article

Reproductive abnormalities in interspecific crosses involving *Gossypium hirsutum* and *Gossypium arboreum*

S.Saravanan, K. Koodalingam and T.S. Raveendran

Abstract :
A successful cross fertilization involving two different species implies that a chain of reproductive process takes place unhampered after pollination. The coordination and interaction between genes or gene complexes in the pollen parent and matching gene or gene complexes in the pistil parent should be hormonious. Understanding of pollen tube growth and pollen attrition is revealed from the study of pollen-pistil interaction and for understanding the function of components specifically accumulated in the transmitting tissue cell wall and intercellular matrix that may interact with pollen tube. The role of the pistil in inhibiting incompatible pollen is well known and has been extensively investigated. Apart from arrest of the pollen tube at different levels, several abnormalities like twisting and bulging of the pollen tube, knot formation in pollen tube, lateral enlargement of pollen tube and growth of pollen tube in opposite direction were noticed. In the present study, an attempt was made to study the pollen and pistil traits between parents and crosses of three cultivated American cotton and six diploid desi cotton. Palynological features were interpreted to determine the extent of incompatibility in triploids generated.
Key words:
Interspecific cross, palynology, incompatibility

Introduction

Gossypium is a rich and economically important genus comprising about 40 species of which four are commercially cultivated for cotton lint and seed. Among the linted species, two are old world Asiatic diploid cottons with somatic chromosome number 26 (2n=26), namely *Gossypium arboreum* L. and *Gossypium herbaceum* L. The other two are new world allotetraploid cotton species, the American cotton, *Gossypium hirsutum* L. and the Egyptian and sea Island cottons, *Gossypium barbadense* L. with 2n=52 chromosomes. Intra and interspecific hybridization with cultivated species of cotton has lead to improvement in productivity, earliness, ginning per cent, fibre qualities and resistance to pests and diseases. The successful utilization of the wild species in breeding programme is limited by the existence of either pre-fertilization or post fertilization barriers in wide crosses. The role of the pistil in inhibiting incompatible pollen is well known and has been extensively investigated (De Nettancourt, 1977; Heslop-Harrison, 1978; Linskens, 1983). Understanding of pollen tube growth and pollen attrition are revealed from the study of pollen-pistil interaction and for understanding the function of components specifically accumulated in the transmitting tissue cell wall and intercellular matrix that may interact with pollen tube (Graif *et al.,* 2001). Hence, an attempt was made to determine the causes for the incompatibility in interspecific crosses of *Gossypium*.

Centre for Plant Breeding and Genetics
Tamil Nadu Agricultural University
Coimbatore-3

Materials and methods

Self pollinated pistils of six diploid cotton genotypes (K 10, K 11, PA 255, DLSA 8 DL8A 16, and TKA 9410), three tetraploids (MCU 5, Sahana and MCU 12) and cross pollinated pistils of direct combination of above parents were collected at 2, 4, 6, 8, 10, 12, 24 and 36 hours after pollination and fixed in Carnoy's fluid (6:3:1) (ethanol, chloroform, acetic acid) at 4 -10°C for 24 hours. Pollen suspension prepared by gentle crushing of about anthers to

dehisce was utilized to investigate the extent of pollen fertility. The *in vivo* studies on pistils were carried out by aniline blue fluorescence technique described by Sitch (1990). However, the procedures need to be modified in respect of softening period, strength of NaOH and K_3 PO_4 and concentration of aniline blue by conducting a preliminary experiment.

The following steps were followed for *in vivo* studies

1. The stigma and stylar pollinated pistils along with ovary were fixed in 6:3:1 (ethanol, chloroform, acetic acid) fixative at different periods of interval after pollination
2. The pistils were gently washed with distilled water for 3 to 4 times.
3. Pistils were macerated in 10N NaOH for 12 hours for enabling softening of pistils.
4. Pistils then thoroughly washed in 10N NaOH for 8 hours for diploids and 10 N NaOH for 10 hours in case of tetraploids for enabling softening of pistils
5. Pistils then thoroughly washed in distilled water and stained for 4 hours to 12 hours in 0.25 per cent aniline blue prepared in 0.10 N K_3 Po_4
6. The stained pistils were placed on a glass slide containing a drop of glycerol and covered with 23 x 30 mm cover slip and pressed gently.
7. The slides were observed under Nikon microphot-Fx microscope with fluorescence attachment, illuminated with 200w high pressure UV lamp. The observations were taken with B (380-490nm) and or BG (650nm) excitation filters in combination with BA 520 or BA 530 barrier filter. Colour photographs were taken with Kodak Gold 400 ASA film either with barrier filter (greenish yellow) or without barrier filter (blue background with bright white fluorescing pollen tube).

Results and discussion

To ascertain compatibility in wide crosses, pollen germination, pollen tube growth and fertilization are determined by fluorescence microscopy. In this technique, pistils are stained with water-soluble aniline blue and viewed under ultra violet illumination (Plate 1). Callose plugs present in the pollen tube fluoresce brightly. Pollen tube is therefore clearly distinguished from the stylar tissue and may be readily counted and quantified. Besides pollen germination on the stigma, fluorescence microscopy has proved very effective to estimate the penetration of the pollen tubes into the stigma also (Kho and Baer, 1968). Insufficient softening of pistils leaves the specimen hardy which makes difficult to observe the pollen tube growth due to differential refraction. Excessive softening causes the breakage of pistils even at a gentle pressing rendering the observation of pollen tube difficult. The softening of the pistils of diploid parents was optimum at 12N and 10N NaOH for 6 and 8 hours duration respectively, while the tetraploids had 10N NaOH for 10 hours duration as optimum. The optimum staining and better fluroscence was obtained when 0.25 per cent aniline blue was dissolved in 0.1N K_3PO_4 and kept for 10 hours. Gunasekaran (1997) determined the optimum strength of NaoH, K_3PO_4 and concentration of aniline blue as 10N, 0.1N and 0.3 per cent respectively for cotton. The adhesion of pollen to the stigma, pollen hydration, pollen germination and initial growth on the stigmatic surface are most deciding factors for successful fertilization (Wheeler *et al.,* 2001). Hence, the study of the role of pollen tube growth and time required for germination of pollen on stigma to deliver the generative nuclei into embryosac becomes important.

In the present study, among the triploids, MCU 5 x PA 255 exhibited higher rate of pollen fertility (Table 1). Focusing on parents, MCU 12 (tetraploid) recorded higher pollen tube growth and rate of pollen tube growth at initial and final stages of its growth followed by MCU 5 (tetraploid), Sahana (tetraploid) and PA 255 (diploid). Among triploids, hybrids MCU 5 x PA 255 and MCU 12 x K11 exhibited higher pollen tube elongation and rate of pollen tube growth (Table 3). The rate of pollen tube growth in triploids was very slow as that of length of pollen tube when compared to parents. In the present study, the pollen tube of the tetraploid species when selfe pollinated took longer time to reach the respective ovule than that of self pollinated diploids, probably due to longer pistil length in tetraploid cottons (Table 2). The time taken for the pollen tube to reach the respective ovule in triploid cross was lesser than that required by the pollen to reach the ovule of its own upon selfing. Weather wax (1919) pointed out that time required for the entry of pollen tube into ovules was variable depending on the distance between pollen (on the stigma) and ovule as observed in maize and wheat. In triploids, the pollen tube growth rate was in increasing trend upto 10 hours after pollination and thereafter progressively in decreasing trend (Table 3). The slow rate of growth of pollen

tube during the initial period was probably brought about by lack of coordination between the pollen and pistil proteins. In the present study, the rate of growth of pollen tube in selfed tetraploid and diploid plants differ significantly. However, when the diploid parent's pollen was used for pollination in tetraploid pistils, only one cross MCU 5 x PA 255 exhibited significantly higher rate of pollen tube growth than other crosses (Table 3). Though more food reserves present in the pollen of the tetraploid x diploid crosses, such reserves could not provide good growth due to the presence of inhibitory substances responsible for cross compatibility. Liu *et al.*(1992) observed differential rate of pollen tube growth in *Gossypium hirsutum* x *Gossypium arboreum* crosses. However, Venkatasubramanian (1953) reported that the rate of growth of pollen tube in the style was uniform throughout in rice pistils. In the present study, significant differences for pollen tube length and rate of growth were observed in selected entries of selfed parents and triploids and the results are akin with the findings of Sarkar and Paria (1980) in maize. Pollen attrition value was found to be significantly lower in tetraploid as compared with diploid parents (Table 4). The entry, MCU 5 x PA 255, recorded significantly lower value for stylar attrition. Ascher (1984) reported quantitative variation in pollen tube attrition after compatible crosses in petunia. From the present investigation, it was inferred that the number of pollen tubes reaching the base of the style is dependent upon the source of the pollen and appears to be a decelerating function of the number of pollen tubes present in the stigma. The severe reduction in the number of pollen tubes as they grew in the style depending on the initial number of pollen grains deposited. In the study, pollination was profuse and more than adequate. However, the present reduction in the number of pollen tube in style was independent of the initial load of pollen grains in stigma suggesting that genetic interactions played a major role in the regulation of pollen tube attrition (Table 5). Similar cases also studied by Kahn and De Mason (1986), Herrero (1992) and Plitmann (1993). Ockendon and Gates (1975) reported that bottleneck for pollen tube attrition seems to be in the upper portion of the style and few others stop growing between mid style and ovary as in *Brassica*.The extent of ovules with pollen tube and seed set were low in case of triploids when compared to parents. This indicated the existence of pollen tube – ovary or ovule interaction. The variation between the pollen of paternal parent and ovule of maternal parent influence the seed set. The breakage of pollen tube in the middle stages of pollen tube growth passing through the stylar region observed in the present study(Plate 4). Apart from arrest of the pollen tube at different levels, several abnormalities were noticed in the present investigation. The abnormalities include twisting and bulging of the pollen tube, knot formation in pollen tube, lateral enlargement of pollen tube and growth of pollen tube in opposite direction (Plate 2 and 3). A similar type of malformation in pollen tube was observed in Rhododendron (Williams *et al*., 1982), maize (Manickam, 1996) and cotton (Gunasekaran, 1997).

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Table 1. Status of pollen fertility in parents and triploid hybrids

Entries	MCU 5	Sahana	MCU 12	PA 255	K 10	K 11	DLSA 8	DLSA 16	TKA 9410
MCU 5	92.73	-	-	28.43*	14.26	16.16	20.23	22.43*	18.43
Sahana	-	93.42	-	12.11	14.82	11.84	16.04	20.86	19.41
MCU 12	-	-	94.23	28.40*	20.17	17.83	21.47	12.84	19.37
				Parents	Triploids				
			SE_d	:	1.33		1.13		
			CD (0.05)	:	3.06		2.38		

Table 2. Details of length of pollen tube in parents under *in vivo* conditions

Parents	Length of pollen tube (microns) minutes after pollen deposition							
	2	4	6	8	10	12	24	28
Tetraploid								
MCU 5	4085^{a}	7160^{b}	11127^{a}	15320^{a}	16951^{a}	18120^{a}	18650^{ab}	18921^{ab}
MCU 12	4182^{a}	7351^{a}	11360^{a}	15850^{a}	17283^{a}	18950^{a}	19126^{a}	19823^{a}
Sahana	3860^{a}	6361^{ab}	10971^{ab}	14748^{a}	16820^{a}	17230^{ab}	18620^{ab}	18828^{ab}
Diploids								
K 10	2640^{bcd}	4360^{c}	8920^{c}	10850^{bc}	13120^{bc}	14860^{bc}	15280^{cd}	15341^{cd}
K 11	2425^{cd}	4272^{c}	8630^{c}	9104^{cd}	10323^{d}	11892de	12851^{d}	-
DLSA 8	3125^{b}	6285^{b}	9350^{bc}	10280^{bcd}	12085^{cd}	13860^{cde}	14930^{cd}	-
DLSA 16	2120^{d}	4148^{c}	5895^{d}	8328^{d}	10131^{d}	11320^{e}	12385^{d}	-
PA 255	2980^{bc}	4890^{c}	9410^{bc}	11828^{b}	14840^{ab}	15200^{bc}	15951^{bc}	16583^{bc}
TKA 9410	2285^{d}	4235^{c}	6135^{d}	9584^{bcd}	12620^{bcd}	14238^{cd}	15163^{cd}	-
SE	264.08	442.94	668.65	949.57	932.51	877.19	824.02	839.33
CD (5%)	610.02	1023.18	1544.59	2193.50	2154.09	2026.32	1903.48	1938.86

Means followed by a common letter are not significantly different at 5 per cent by DMRT

Table 3. Details of length of pollen tube in tetraploid x diploid crosses under *in vivo* conditions

Parents	Length of pollen tube (microns) hours after pollination						
	2	4	6	8	10	12	24
MCU 5 X K 10	385^{a-d}	1584^{a}	3310^{cde}	6981^{a-e}	9641^{abc}	10312^{ab}	12579^{ab}
MCU 5 X K11	360 $^{a-d}$	994^{cde}	2854^{e}	7154 $^{a-e}$	10314^{a}	11147^{ab}	12612^{ab}
MCU 5 X PA 255	435^{a}	1634^{a}	5014^{a}	8106^{a}	10549^{a}	12067^{a}	13562^{a}
MCU 5 X DLSA8	384 $^{a-d}$	1014 ^{cde}	3014^{e}	6918 $^{a-e}$	8642^{abc}	9615^{b}	-
MCU 5 X DLSA 16	401 $^{a-d}$	1608a	3204de	6273^{d-g}	8841^{abc}	9948^{ab}	11684^{ab}
MCU 5 X TKA 9410	420^{abc}	1415^{ab}	3251^{cde}	7421 $^{a-e}$	9234^{abc}	10042^{ab}	12281^{ab}
Sahana X K 10	365 $^{a-d}$	882de	2784^{e}	7006 $^{a-e}$	9186^{abc}	10085^{ab}	11681^{ab}
Sahana X K11	340^{cd}	912de	4357^{b}	7681^{a-d}	10114^{ab}	11542^{ab}	12863^{ab}
Sahana X PA 255	414^{abc}	1221^{bc}	3818^{bcd}	5104^{fg}	8921^{abc}	9841^{b}	11742^{ab}
Sahana X DLSA8	381 $^{a-d}$	1594^{a}	4152^{b}	7821^{abc}	9731^{abc}	10218^{ab}	13476^{a}
Sahana X DLSA 16	331^{d}	812^{e}	2016^{c}	4982^{g}	7981^{c}	9461^{b}	-
Sahana X TKA 9410	394 $^{a-d}$	1438^{ab}	3081^{e}	6521^{b-e}	9061^{abc}	10073^{ab}	11817^{ab}
MCU 12 X K 10	342^{cd}	1386^{ab}	3128^{e}	6408^{c-f}	9014^{abc}	10025^{ab}	11564^{ab}
MCU 12 X K11	430^{ab}	1482^{a}	3860^{bc}	7901^{ab}	9842^{abc}	10742^{ab}	13287^{ab}
MCU 12 X PA 255	332^{bcd}	974 ^{cde}	2801^{e}	6047^{efg}	9305^{abc}	10164^{ab}	12304^{ab}
MCU 12 X DLSA8	371 $^{a-d}$	899de	2804^{e}	6143^{efg}	8963^{abc}	9961^{ab}	11701^{ab}
MCU 12 X DLSA 16	396 $^{a-d}$	1213^{bc}	3110^{e}	6112^{efg}	8273^{bc}	9563^{b}	-
MCU 12 X TKA 9410	404 $^{a-d}$	1124 ^{cd}	3184de	7262^{a-b}	9940^{abc}	10681^{ab}	12584^{ab}
SE	7.34	68.00	163.56	211.56	162.76	163.23	162.19
CD (5%)	15.34	142.13	341.84	442.15	340.18	341.16	338.97

Means followed by a common letter are not significantly different at 5 per cent by DMRT

Table 4. Details of pollen tube distribution (stigma and style) and stylar attrition in parents

Entry	Pollen tube at stigmatic surface	Pollen tube at base of style	Stylar attrition
MCU 5	279^{c}	241^{a}	13.65^{a}
MCU 12	410^{a}	306^{a}	25.37^{c}
Sahana	352^{b}	281^{b}	20.18^{b}
K 10	240^{c}	160^{c}	33.33^{d}
K 11	280^{c}	192^{c}	31.43^{d}
DLSA 8	360^{ab}	189^{c}	47.50^{e}
DLSA 16	235^{c}	176^{c}	25.13^{c}
PA 255	260^{c}	181^{c}	30.38^{cd}
TKA 9410	226^{c}	160^{c}	29.20^{cd}
SE	21.66	17.86	3.14
CD(5%)	48.95	40.37	7.09

Table 5. Details of pollen tube distribution (stigma and style) and stylar attrition in tetraploid x diploid pollination

Entry	Pollen tube at stigmatic surface	Pollen tube at base of Style	Stylar attrition
MCU 5 X K 10	230^{b-e}	125^{bcd}	45.65^{bc}
MCU 5 X K11	255^{abc}	138^{bc}	45.88^{bc}
MCU 5 X PA 255	290^{a}	206^{a}	28.98^{a}
MCU 5 X DLSA8	103^{h}	56^{e}	45.63^{bc}
MCU 5 X DLSA 16	110^{h}	67^{e}	39.09^{b}
MCU 5 X TKA 9410	210^{c-g}	110^{cd}	47.62^{bc}
Sahana X K 10	187^{d-g}	103^{d}	44.92^{bc}
Sahana X K11	235^{d-g}	136^{bc}	42.13^{bc}
Sahana X PA 255	165^{fg}	98^{d}	40.61^{bc}
Sahana X DLSA8	284^{b}	152^{b}	46.48^{bc}
Sahana X DLSA 16	102^{h}	56^{e}	45.10^{bc}
Sahana X TKA 9410	185^{d-g}	103^{d}	44.32^{bc}
MCU 12 X K 10	175^{efg}	101^{d}	42.29^{bc}
MCU 12 X K11	265^{abc}	147^{b}	44.53^{bc}
MCU 12 X PA 255	229^{b-e}	115^{cd}	49.78^{c}
MCU 12 X DLSA8	156^{gh}	95^{d}	39.10^{bc}
MCU 12 X DLSA 16	112^{h}	66^{e}	41.07^{bc}
MCU 12 X TKA 9410	216^{c-f}	114^{cd}	47.22^{bc}
SE	14.51	8.91	1.10
CD (5%)	30.46	18.70	2.30

Means followed by a common letter are not significantly different at 5 per cent by DMRT

Plate 1. In vivo Pollen germination

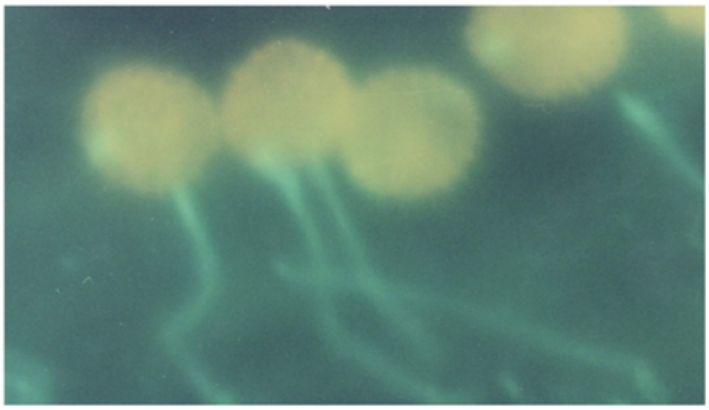

Plate 4. Breakage of pollen tube in stylar region