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Studies on Some Barriers to Hybridisation in the four Diploid Species of Gossypium

by
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Introduction: For the successful exploitation of interspecific hybridisation in cotton improvement, the need to understand the barriers to species hybridisation is a vital prerequisite (Sikka 1957). Of the several "Reproductive isolating mechanism" (Stebbins 1958), studies were undertaken on (i) Time and nature of anthesis, (ii) Germination and growth of pollen and (iii) Effect of normal and bud pollination method on seed set, that are likely to encounter free crossing between four diploid species of *Gossypium*, at the Cotton Breeding Station, Coimbatore, during 1958–60 and the results are reported here.

Materials and Methods: A linted cultivar, K6 belonging to *Gossypium arboreum* L. was used as ovule parent. Three wild diploid species, belonging to non linted group and possessing divergent genomes and distinct geographical distribution viz., *G.anomalum* L., *G.raimondii* and *G stocksii* were used as pollen parent (Table 1).

TABLE 1. *Particulars of Four Species studied*

Species	Section	Genome classification	Geographical distribution	Economic and fundamental attributes
G. arboreum	Herbacea	A_2	Penninsular India	Annual, linted Cultivar
G. anomalum	Anomala	B_1	Africa	Perennial, non linted wild species induces fibre fineness. Possesses resistance to Jassid and Blackarm
G. raimondii	Klotzschiana	D_5	South America	Perennial, non linted wild species induces fibre density and strength possess resistance to Boll worm. Considered to be the progenitor of new World Cotton
G. stocksiana	Stocksiana	E_1	Indo Arabia	Perennial non linted drought resistant

The temperature and humidity, at the time of anthesis, were recorded with the help of whirling hygrometer and referring to Physchometric tables.

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For estimating pollen fertility, acetocarmine glycerol mixture was used. The stained and non stained pollen grains were counted and the percentage of staind grains was reckoned for fertility.

The hybridisation technique of Doak (1935) was adopted for normal and bud pollination methods. In normal method, pollination was done in the forenoon on the flowers emasculated previous evening. In bud pollination, the dusting of pollen was effected in the afternoon. One hundred crosses were effected in each combination.

To trace out the pollen tube growth, the method of Iyengar (1937) was suitably modified on the lines reported by Sanyal (1958) on jute. In this technique, the stigma, that was transferred to 1% cotton blue three hours after pollination, was put in a test tube containing fresh lactophenol and boiled for one to three minutes, till it became transparent. It was cooled and placed on a clean slide with lactophenol as a medium and gently pressed with a cover slip to spread the tissue and the germination of pollen grains was examined under low power. The length of pollen tube was measured with ocular micrometer. The entry of pollen tube into ovule was studied by the method of Iyengar (1937).

Results: The peak period of flowering, the time of anthesis, the prevalent temperature and humidity at the time of anthesis of four species studied are furnished in Table 2 given below. The particulars of pollen fertility and pollen size are also given.

TABLE 2. *Particulars of flowering and anthesis*

	Peak flowering period	Anthesis month	Time A.M.	Mean		% of pollen fertility	Mean pollen diameter in μ
				Temperature °F	Humidity %		
G. arboreum	Jan. to Mar.	Nov. 58	10.45-11.00	84	78-80	95.5	98.0 ± 0.94
		Jan. 59	10.30-10.45	85	75-78		
		Feb. 59	10.00-10.15	85	62-68		
G. anomalum	Jan. to middle of February	Nov. 58	9.00- 9.15	82	84-85	93.7	93.9 ± 0.99
		Jan. 59	8.45- 9.00	82	85-88		
		Feb. 59	8.30- 8.45	82	75-80		
*G. raimondii**	Jan. middle to April	Nov. 58	6.30- 7.00	73	86-92	90.0	94.1 ± 1.07
		Jan. 59	6.15- 6.30	73	90-92		
		Feb. 59	6.00- 6.15	73	78-86		
G. stocksii	Dec. to middle of February	Nov. 58	10.00-10.15	83	80-86	93.7	94.1 ± 0.69
		Jan. 59	9.45-10.00	83	83-85		
		Feb. 59	9.30- 9.45	83	73-78		

* The anther dehiscence advanced flower opening by 30 mts

In vivo germination of pollen, the percentage of pollen and seed set by normal and bud pollination methods and the percentage of germination of hybrid seeds are presented in Table 3. The bud pollination method was adopted to increase the percentage of bolls and seed set in crosses as previously reported by Loden *et. al.* (1950).

TABLE 3. *Pollen germination, boll setting and seed germination*

Nature of Cross	In vivo germination percent of pollen	% of boll set in		No. of seed obtained in		Percentage of seed germination
		Normal pollination	Bud pollination	Normal pollination	Bud pollination	
G. arboreum×*G. arboreum*	95	66	2	331	15	80
G. arboreum×*G. anomalum*	93	43	2	105	16	50
G. arboreum×*G. raimondii*	92	27	3	158	5	0
G. arboreum×*G. stocksii*	93	26	1	76	3	20

Discussion: All the four species viz., *G. arboreum*, *G. anomalum*, *G. raimondii* and *G. stocksii* showed synchronisation in flowering at the Cotton Breeding Station, Coimbatore. Anthesis was noted in the forenoons between 6-11 A. M ; *G. raimondii* commencing from 6-7 a. m.; *G. anomalum* from 8-30 a. m.; *G. stocksii* at 9-30 a.m. and the cultivated species from 10-00 a. m. onwards. The atmospheric temperature seems to play a greater part than relative humidity in controlling anthesis. This is evident from the fact that as the summer months advanced, the time of anthesis also started much earlier by about 30-45 minutes than the usual timings (Table 2). Dehiscence of anthers and opening of flowers took place more or less at the same time except in *G. raimondii* wherein anther dehiscence always preceded the opening of flowers by about 30 minutes. The fertility of pollen in all the four species was very high being above 90%. The size of grains of the cultivated species was significantly bigger than that of all the wild species. *In vivo* study of germination of pollen grains of the wild species on the cultivated species gave interesting results. There was no significant species differences in germination which ranged from 92-95% (Table 3). It was also seen that between normal and bud pollination methods, seed setting was normal and high in the former than in the later method. This finding is not in agreement with that of Loden *et. al.* (1950), probably due to varied environmental factors. However, the percentage of seed set varied from species to species even though the entry of pollen tube into the ovule was noted in all the four crosses. With *G. anamolum*, the percentage of setting was 45%, while with *G. raimondii* and *G. stocksii* it was 27 and 20% respectively. This reduction in seed set was found to be directly associated with the extent of divergence of the constituent

genomes of these species (Table 1). This conclusion was again reinforced by the extent of germination of the hybrid seeds obtained from these species crosses. With *G. anomalum* the percentage of germination was 50, while in the case of G. stocksii it was only 20%. In crosses between *G. arboreum* × *G. raimondii*, all the 158 seeds obtained failed to germinate. This is very similar to that of stephens (1944) who reported 0.5% viability of hybrid seeds in this species crosses. A critical examination of these seeds revealed that they were hollow, shrivelled and with dried up embryo. This indicates that breakdown of endosperm and lethality of embryo might have occurred very late after fertilization as recorded by Brink and Cooper (1947) and Weaver (1958) in species crosses between tetroploid and diploid cottons. Embryological studies of these crosses will throw more light on this aspect.

Summary: The time of anthesis of four diploid species of cotton at Cotton Breeding Station, Coimbatore is between 6-11 A. M. The percentage of germination of pollen grains of three wild sp. *G. anomalum.*, *G. raimondii* and *G. stocksii* on the stigma of the cultivated species *G. arboreum* was high and there was no significant differences between species. In all these cases, entry of pollen tubes into the ovules had been noted. However, the percentage of seed set and germination of hybrid seeds have clearly established the existence of differentiation of costituent genomes and their inter relationships between each other, which is in conformity with the previous findings. The hybrid lethality noticed in *G. arboreum* × *G. raimondii* is similar to that of somataplastic sterility, wherein break down of embryo and endosperm occur very late after fertilization. It is suggested that detailed analytical studies on these species crosses might throw more light in the barrier mechanism that are operating among these species.

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On Certain Aspects of Quality of Irrigation Water on the Cationic Equilibrium in Typical Soils of Tamil Nadu

by

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Under permanent irrigation agriculture, the quality of irrigation determines the equilibrium cationic composition of soil colloids. Much understanding has been made on the attainment of equilibrium conditions through the study of ion exchange properties of soil colloidal surface. The colloidal surfaces with counter ions behave like condensers, and electro-kinetic theory of double-layer on the surface was first developed by Helmotz (1881). To explain the exponential decrease on ionic concentration from surfaces towards the bulk of the liquids was further developed by Guoy (1910) and later by Chapman independently on the diffused double layer theory. Pauli and Valko (1929) used empirical hyperbolic equations to describe the ion exchange phenomenon. Gans (1913) and Kerr (1928) used Law of Mass action principles to study ion-exchange phenomenon. The kinetically derived formulae of Gapon (1937) and Jenny (1936) also follows the Law of Mass action.

The application of Donnan equilibrium was futher extended to diffuse layer and the Donnan and Guoy theories led to the same conclusions on ionic equilibrium and represented two different ways of approaching the problem. The electro-kinetic and electro-chemical potentials of the charged surfaces, arising from diffuse layer acting as Donnan membrane in creating the differential distribution of ions near the surface and the bulks of liquid phase, explain the physical and chemical properties of soil colloids and also determine the equilibrium conditions. Helmy (1964) has further described an exchange equation based on positive adsorption. Heald, Frere and Dewit (1965) studied the problem of ion exchange equilibrium with Guoy and Stern model. These equations are based on a model of equilibrium between ion-pairs at the surface and a diffuse Guoy layer.

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