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PARTHENOGENESIS IN COTTON

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The production of a cross between the American and Asiatic cottons, has been receiving the attention of various breeders in the world, and there was a time when there was no subject on which greater diversity of opinion existed than the possibility of this cross within the genus. Major Trevor Clarke (1870) held that the hybridization between these was not possible, while Gammie (1903) is mentioned as having successfully crossed *G. hirsutum* and *G. roseum*, *G. hirsutum* and *G. obtusifolium*, and probably these crosses are now lost. Zaitzev (1925) of Turkishtan station produced hybrids between *G. hirsutum* and *G. herbaceum*, but unfortunately the first generation did not produce any fruit due to sterility of pollen, although the plants produced a large number of flowers. Denham (1924) in his cytological studies in cotton, offers an explanation to this unsolved difficulty, and ventures a solution by way of finding out a diploid mutant of the Indian cotton. It will not be out of place to quote a few lines of his work.

The abrupt separation of the genus *Gossypium* into two groups, in which the chromosome number is twice that of the other, is in itself a matter of great interest. It is known that interspecific hybrids can be made with comparative ease in either of the groups, but as far as can be ascertained, no plant breeder has yet been successful in crossing an American or Egyptian cotton with a true Indian cotton. (It has been stated that this cross has been made in Russia, but no reference can be made to it in the available literature and it is probable, that the cross has now been lost.) The fact of the difference may perhaps open a further possibility for the

plant breeder who wishes to make the cross between Indian and American cottons, if it can be assumed that there is any hope of finding a diploid mutant of an Indian cotton (which would probably reveal itself as suitable for cytological examination by its abnormal size), it might be reasonably expected to prove a fertile parent for the experiment.' The research is highly interesting and educative at this period, setting down the impossibility as due to the inherent differences in chromosome number. In spite of the obstacles presented by way of the chromosome difference, Zaitzev claims to have succeeded in achieving what has been considered hitherto impossible, and he attributes his success to a large extent to the favourable conditions. He claims that the failures and sheddings are due to premature rupture of the style, consequent to the closing and twisting of the corolla, coupled with the slow and retarded growth of the pollen tubes in an altogether strange medium. He is of opinion that the differences are surmountable and may be made to result in the production of viable hybrid seeds, if only the technique of artificial crossing is a little modified.

In the Cotton Breeding station at Coimbatore definite attempts were made from the year 1922 to 1925 to get a cross, if possible, between *G. hirsutum* (Strain No. 1) and *G. indicum* (Strain No. 14). Early flowers were tackled, and in a majority of cases *G. indicum* was the ovule parent. The reciprocal cross was never successful and the flowers exhibited a tendency to shed a few days after artificial pollination. This might be due to the slow growth of the pollen tubes of *G. indicum* in *G. hirsutum* stigma and thereby failing to supply the necessary stimulus in the early stage, or it may be that *G. indicum* pollen failed to supply the full complement of twenty-six chromosomes possessed by the *G. hirsutum* ovule, resulting in poor endosperm development, and failure to unite with the egg cell, followed by the early abscission. The following method was adopted during these three years. The castrations were done on the day before flowering in the evenings, or in the mornings at about 7 A.M. on the day of flowering, but in either case the buds remained unopened at the time of emasculation. The *indicum* flowers open at about 9 A.M. in the morning, and simultaneously with the opening out of the corolla, the anthers begin to dehisce. The petals were completely removed by scissoring of just over the base of the ovary without in any way injuring the ovary or the stigma. The filaments were then cut off, taking care not to rupture the stigma or anthers, and in fact every precaution was taken to exclude the chances of pollen, being deposited on the stigma. The bud was then completely covered up, to prevent insect or wind pollination. One or two flowers of the male parent were also covered up to prevent the deposition of foreign pollen. On the day of opening of the flower at about 9 A.M., the pollen from the male parent was dusted on to the emasculated buds after carefully examining the stigmatic surface for any stray pollen and the flowers were completely covered up without letting an ingress to insects. The tissue bags were allowed to remain on the plants for about three or four days.

About sixteen crosses were tried in the year 1922-23 and over forty crosses in 1924-25, all of these with *G. indicum* as the mother parent. Every one of the attempts pointed towards success in the early stages, since they exhibited increase in the size of the fruit from the third or the

fourth day, and shedding commenced only after the eighth day. The shedding was very pronounced after the twentieth day in both years, the susceptibility being at its maximum between twenty and thirty days. A surmise that a critical period is existent at or about the twenty-second day after flowering is not improbable, if we give due attention to the closeness of agreement in the two different years, conducted under different conditions. The true nature of changes that take place in the inner mechanism of such a boll is not known, and it is impossible to venture a hypothesis without a critical cytological study.

The bolls that were crossed, were picked up after shedding and examined. All the external evidences of fertilization were there, as the formation of lint on seeds, development of ovules into seeds, etc. The seeds were normal in size though the interior was not completely filled up, and the testa alone was well formed without the full development of the embryo. It might be tentatively put forth that the initial necessary stimulus required by the *G. indicum* flower, for the growth of the ovary, was supplied to a large measure by the pollen of *G. hirsutum*, and that the other changes taking place in the inner mechanism of the boll later on, when the growth period was over, retarded the formation of viable hybrid seeds followed by an abscission of bolls in a majority of instances.

The swelling up of the ovary continued to the time of shedding and a perceptible fall in size was noticed to occur a day or two before the shedding. To find out how closely it agreed with the growth of normal bolls, a series of measurements were taken, on a number of bolls whose dates of flowering closely approximated to the flowering time of crossed flowers. In the tables 1 and 2, the results are presented for the two years. The age of boll, average measurement of shed bolls, and average measurements of normal bolls are given. It was surprising to note the closeness of agreement between the two measurements in the two years. The crossed bolls appeared to be bigger in the majority of instances, and this might be due to the initial bigger ovary to start with as the difference was noticed even at the beginning. The bolls attained their maximum size between twenty-one to twenty-three days and thereafter remain fairly steady till about the time of bursting when a little shrinkage was noticed. This shrinkage was more pronounced in crosses. The increments in size of boll (Plates I and II) showed that the rate of growth fell down, after three weeks and the abrupt descending curve as the boll matured, indicated the sudden cessation of growth, shrinkage indicative of premature opening, and the inevitable abscission. This type of curve is characteristic of the crosses in both the years, and it is possible that the stimulus for parthenocarpy is very much in excess and in advance of the tendency for parthenogenesis. It is also likely that the dehydration commences in the carpel after attaining the maximum growth in about three weeks in the absence of the usual balance of growth between the boll contents and the covering of a normal boll. Although the measurements indicated the closeness between normal and the crossed bolls, the flatness and a formation of carpellary grooves foreshadowing early opening, pointed towards the partial or total lack of development of the ovules.

Although every one of the attempts proved successful, inasmuch as all of them developed into bolls of varying ages, the percentage of mature bolls capable of giving seeds was very low. As many as nine bolls were got in

1922-23, and 18 bolls in 1924-25, but many of them gave immature seeds. Four bolls out of 18 in 1924-25, gave few good well-formed seeds with lint, and presented healthy normal external characters. The stray instances of good boll production recorded, naturally threw a halo of doubt on the achievements, if they could be styled as such. This might be attributed to errors of technique, but it must not be accepted off-hand, considering the necessary precautions taken in the matter of bagging, etc. If any foreign pollen had been transferred, it must have been during the few seconds of dusting with hirsutum pollen. In the absence of any accidental errors of technique it must be a cross or the outcome of parthenogenesis.

In angiosperms, parthenogenesis is not a common occurrence although a few genera do exhibit it. With the available literature, it was not possible to mention the name of any writer, who has drawn the attention to this phenomenon in cotton; and under the present circumstances it seemed worth while to investigate the problem in a systematic way at least to dispense with one phase of the possibility.

Seven plants were selected in a plot of *G. indicum* (Strain No. 14) sown in the month of October, in lines 3' x 3' apart. The castration or the decapitation was done on two sets of plants (1) in which all bolls and flowers were removed previous to the day of experiment since it has been definitely shown by Mason (1922), that the presence of bolls on the plants inhibits the development of latter bolls increasing the tendency to shed and (2) in which the normal conditions were allowed to prevail. Flowers were tackled in the first and the second weeks of March, when flowers were produced in large numbers and this period corresponded to the period of maximum production. The decapitations were done in the following manner. By mere emasculation, it was thought that the danger of pollen sticking on to the stigma lurked, in spite of the many precautions taken, and with a view to eliminate this error, the whole staminal column with the stigma was cut just over the ovary after removing the corolla. Such a procedure would cause an acute shock to the flower, resulting in increased shedding, but we would be treading on surer grounds if we were to succeed in getting a boll. All the treated flowers were dated, numbered, and covered by tissue paper bags, which were removed only on the fourth day after flowering.

Over 700 flowers were handled in the year 1924-25, and a shedding record was maintained and wherever and whenever possible, the cause of shedding was given. To collect the shed bolls the plants were shaken early in the morning, and such of them that shed, were collected and examined. Some of them shed due to the weight of paper bags, rough and violent handling, insect attack or wind, while some others got dried up in the plant and would not fall even after violent shaking. These bolls were not included. In almost all the treated buds the ovary began swelling up to fifth or the sixth day when growth appeared to stop, followed by contraction, shrivelling, and shedding and sometimes abscission occurred even when the bolls were quite healthy. Unfortunately no measurements were taken this year, to find out whether the growth keeps pace with the normal ones, and as such the results are the results of eye judgments. In 1922-23 about 25 flowers were castrated but they were not covered. The measurements indicated the increase in growth till the fourth or fifth day, and decrease in size after that in the majority of cases. The swelling up

perhaps indicates a tendency towards fruit formation but these seedless fruits are thrown out before they are many days old, due perhaps to the lack of initial stimulus. Whether these exhibit a tendency towards parthenocarpy is again doubtful.

The shedding began by about the third day and proceeded with increasing rate up to the seventh day or the eighth day when a decline was noticed ending abruptly by the seventeenth day. Comparing the interval of shedding percentage in the two series, treated and untreated, there seems to be a difference of one day as regards the mean day of shedding. It might be that treated plants retained the bolls longer than the other, but it must be sufficiently corroborated before hazarding the statement. The mean interval for treatment 1 is $8.67, \pm 0.155$ while in the other it is 7.766 ± 0.18 . The difference by the probable error of the difference, works out to 4.79 times, giving high odds in favour of the series 1, or in other words the difference is statistically significant. A certain number of flowers were dated and shedding noted. The mean of normal bolls was at 11.00 days, as against 21.62 days of the crosses. The decapitated flowers were the first-shed, the normal coming second in order of merit, while the crosses were retained the longest. This point with the percentage of success and agreement of measurements taken in conjunction with the continued growth ovary till the twenty-second day or thereabouts, once more suggests the evidence of stimulus by the *G. hirsutum* pollen, favouring in most cases a tendency akin to the parthenocarpy with the production of phenospermic seeds in the majority of instances, and resulting in about five to ten per cent in the production of good, well-formed seeds. Of the 700 attempts that were made, only seven developed into bolls, and were picked after the lapse of the normal growing period. They appeared healthy, but were smaller than normal bolls. Out of the seven bolls, only four gave good seeds with lint, while the rest contained immature or hollow seeds with little or no lint on them. The percentage is as low as two but it goes to indicate that the crosses obtained may be the result of parthenogenesis.

In 1925-26 further attempts were made to corroborate the behaviour in previous years, and it was deemed worth while to include another set of experiments into the group. In addition to the usual castrations and bagging, a number of flowers were dusted with N. 14 pollen after decapitation and covered, to know whether the stray pollen if deposited, during the short interval of few seconds between cutting and covering, may not be the cause of such development of boll. Out of 100 attempts made, only two exhibited tendencies of growth, but finally shed. This set at rest a probable plea that the growth of bolls might have resulted from the deposition of pollen by agencies not forecasted for in our adoption of a set of precautions to exclude them.

The same experiment in another modified form was undertaken. Here a number of flowers were treated and left uncovered giving thereby a chance for the agencies to play their role, but the result did not prove in any way conflicting to the previous statements. Out of the 300 attempts, seven bolls of normal age were obtained though reduced in size and the mean interval of shedding worked out to 8.89 days.

The pollen stimulus is sufficient to induce parthenocarpy and parthenogenesis, while the drastic decapitation probably supplies the necessary

stimulus although greatly reduced, for inducing parthenogenesis in a smaller percentage of instances. Another interesting feature is the development of one or two seeds only in the picked bolls. Comparing the crossed and emasculated lots, the degree of development attained by the boll and the boll contents are very much in advance in the former, and this is also indirectly shown by the mean shedding intervals. Cytological studies to find out the nature of changes in the ovule, and the conditions favouring such a development, will form the subject of a further enquiry.

SUMMARY

1. The pollen of *G. hirsutum* supplies the necessary initial stimulus for the growth of the ovary till about the nineteenth day, when the development of boll is almost over.

2. It is suggested that a crucial period is existent about the twentieth day.

3. The growth rates of the cross bolls are same as that of the normal, but there is an abrupt decline indicative of premature bursting.

4. A small percentage of parthenogenesis is present in *G. indicum*, and this seems to be rather corroborated by the identity of success in the two experiments.

5. Parthenocarpy is present but the seedless fruits are thrown out early. Probably the foreign pollen favours this, resulting in many cases in phenospermic seeds.

6. The few bolls that happen to be the outcome of parthenogenesis are much smaller in size, and contain only one or two seeds.

I wish to take this opportunity to thank Mr. R. C. Broadfoot, Cotton Specialist, Coimbatore, for the facilities given to me for carrying on the problem.

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TABLE I

1922-23

Age of boll in days	Averages			
	Length in mm.		Breadth in mm.	
	Normal boll	Shed boll	Normal boll	Shed boll
4	8.3	8.9	6.3	7.0
5	9.9	9.9	7.4	7.6
6	10.3	10.8	7.6	8.2
7	11.1	12.2	8.5	8.9
8	12.9	13.5	9.5	9.8
9	14.7	15.2	10.5	11.2
10	16.9	16.8	11.7	11.5
11	18.0	18.3	12.6	12.3
12	20.2	19.6	13.7	12.9
13	21.6	21.2	14.3	13.8
14	22.6	22.5	14.9	14.7
15	23.4	24.4	15.5	15.8
16	24.4	26.0	15.9	16.7
17	24.9	26.8	16.2	17.5
18	25.4	27.4	17.1	17.6
19	25.8	27.8	17.7	18.3
20	25.8	28.1	17.7	18.9
21	25.8	28.5	17.8	19.1
22	25.8	28.9	17.8	19.1
23	26.0	29.0	18.0	19.1
24	26.0	29.0	18.0	19.1
25	26.0	29.0	18.0	18.8
26	26.0	29.0	18.0	18.7
27	26.0	29.2	18.0	18.7
28	26.0	29.2	18.0	18.3
29	26.0	29.2	18.0	18.1
30	26.0	27.3	18.0	17.6
31	26.0	27.3	18.0	17.3
32	26.0	26.6	18.0	17.3
33	26.0	26.4	18.0	16.9
34	26.5	26.3	18.5	16.9
35	26.5	24.5	18.6	16.3
36	26.5	24.5	18.5	16.3
37	26.5	24.5	18.5	16.3
38	26.5	23.0	18.4	16.3

TABLE 2

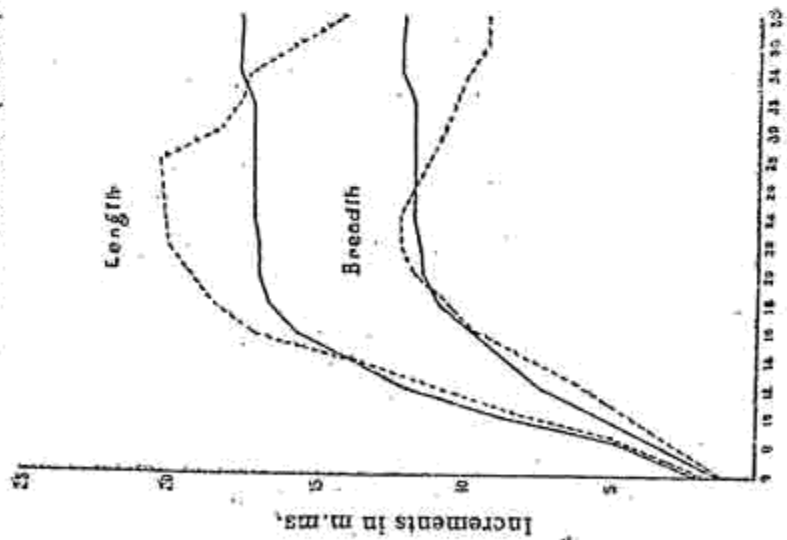
1924-25

Age of boll in days	Averages			
	Length in mm.		Breadth in mm.	
	Normal boll	Shed boll	Normal boll	Shed boll
4	8.0	10.2	6.0	7.8
5	...	11.3	...	8.5
6	...	11.3	...	8.7
7	10.0	13.1	7.3	9.3
8	11.7	13.8	7.7	9.8
9	12.8	15.1	8.0	10.5
10	12.8	16.8	8.8	11.3
11	14.3	18.1	9.3	12.0
12	15.7	19.8	10.7	12.9
13	17.5	21.9	11.0	14.0
14	18.5	22.7	12.3	15.2
15	21.3	24.7	13.3	15.6
16	22.8	26.0	14.3	16.8
17	23.0	26.6	15.0	17.3
18	24.3	26.9	15.0	17.7
19	25.3	27.3	16.0	17.9
20	25.3	27.8	16.3	18.0
21	25.3	27.8	16.3	18.0
22	25.3	27.8	16.3	18.0
23	26.0	27.8	16.3	18.1
24	26.0	27.8	16.3	18.1
25	26.0	27.8	16.3	18.1
26	26.0	27.8	16.3	18.3
27	26.0	27.8	16.3	18.3
28	26.0	27.0	17.0	18.0
29	26.0	27.0	17.0	18.0
30	26.0	27.0	17.0	18.0
31	26.0	27.0	17.0	18.0
32	26.0	26.0	17.0	18.0
33	26.7	26.0	17.0	18.0
34	27.0	26.0	17.0	18.0
35	27.0	26.0	17.0	17.0
36	27.0	26.0	17.0	17.0
37	27.0	25.0	17.0	16.0

Plate 1.

1922-23

— Normal bolls.
- - - Shed bolls (crosses).

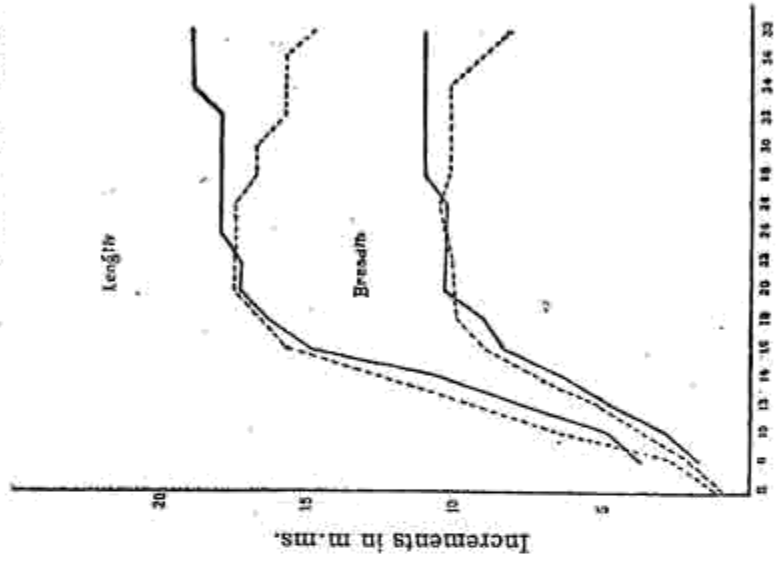


Age of boll in days.

Plate 2.

1924-25

— Normal bolls.
- - - Shed bolls (crosses).



Age of boll in days.