

## ANTHESIS AND POLLINATION IN BENGAL GRAM (*CICER ARIETINUM*)

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A study of the time of flower opening and anther dehiscence is a necessary pre-requisite in any scheme of plant breeding where artificial hybridisation is contemplated. As the mechanism controlling these phases of floral development are influenced by latitude and season, it is essential that separate data are collected on this aspect at each place of investigation. In Bengal gram (*Cicer arietinum*) Howards and Khan (1915) have recorded their observations under the conditions obtaining at Pusa. Since no information under South Indian conditions was available, detailed studies were undertaken at Coimbatore during 1931—32.

Bengal gram is usually sown in the Coimbatore District about the middle of November, and is in flowers between the second week of January and the first week of February. A pattern of its flowering curve is given in Chart I. It may be mentioned here that this crop belongs to the category of plants, the flowers of which open and close for two successive days.

**Anthesis.** Groups of flower buds were marked a day prior to their opening on 4 days in 1931, and 3 days in 1932 in a bulk rainfed crop grown on the black soil during the cold weather, and the time of their opening and closing were observed carefully every half an hour on two consecutive days during each period except on 9—1—1931 when the second opening and closing was not observed. Apart from these, two sets of flower buds were studied at hourly intervals in a crop raised for another purpose on red loamy soil under irrigated conditions during the summer of 1932. The data obtained on all these days are tabulated into hourly class intervals and given in Table I-(a) and (b).

When the figures relating to the time of opening of flowers are examined it is seen that:—

1. Some of the buds under observation did not open at all (even for the first time) and the proportion of such (cleistogamous) flowers was greater in summer crop. For instance in January 1932 the percentage of cleistogamous flowers ranged from 8 to 12, while it was between 32 to 42 in June and July.

2. Among the buds that bloomed on the first day some did not open on the second day. The ratio of such flowers increased with the

Table I-(a). Flower opening and closing in B. Gram in 1931.

Hours.	First opening and closing.				Second opening and closing.			
	15-1-1931		31-1-1931		1-2-1931		28-2-1931	
	Percentage of flowers Opened	Percentage of flowers Closed.	Percentage of flowers Opened	Percentage of flowers Closed.	Percentage of flowers Opened	Percentage of flowers Closed.	Percentage of flowers Opened	Percentage of flowers Closed.
9 A. M.	22	76*	27	84	4	22	5	2
10 "	36	79	31	79	1	22	5	2
11 "	8	79	9	84	...	2	5	2
12 Noon.	5	79	9	84	...	9	5	2
1 P. M.	6	79	8	84	...	5	0	2
2 "	...	79	...	84	...	5	0	2
3 "	...	79	...	84	...	1	3	2
4 "	...	79	...	84	...	...	...	2
5 "	64	79	...	84	...	...	...	2
6 "	12	79	...	84	...	...	...	2
7 "	...	79	...	84	...	...	...	2
Total.	77	76*	84	84	22	22	5	2

Percentage of Cleistogamous flowers. 23

One damaged.

Table I-(b). Flower Opening and Closing in B. Gram 1932.

Hours.	First opening and closing.										Second opening and closing.														
	4-1-1932					11-1-1932					22-1-1932					23-6-1932					2-7-1932				
	Open- ed	Clos- ed	Open- ed	Clos- ed	Percentage of flowers	Open- ed	Clos- ed	Open- ed	Clos- ed	Percentage of flowers	Open- ed	Clos- ed	Open- ed	Clos- ed	Percentage of flowers	Open- ed	Clos- ed	Open- ed	Clos- ed	Percentage of flowers	Open- ed	Clos- ed	Open- ed	Clos- ed	Percentage of flowers
9 A.M.	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
10 "	38	..	40	..	64	18	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
11 "	25	..	32	..	6	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
12 Noon	15	..	13	..	2	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
1 P.M.	7	..	1	..	2	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
2 "	2	..	3	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
3 "	1	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
4 "	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
5 "	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
6 "	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
7 "	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Total.	88	87*	90	90	92	88**	68	68	68	88	58	58	58	58	58	58	58	58	58	58	58	58	58	58	58
Percentage of Cleistogamous flowers.	12	..	10	..	8	..	32	..	..	42	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..

\* One damaged. \*\* 4 damaged.

advance in season. In 1931 the percentage of flowers that did not open for the second time on the 16th January, on the 1st February and on the 28th February were 72, 94, 97 respectively.

3. Though a few commenced opening on the first days at about 9 A. M., the time of most active blooming was between 9 to 10 A. M. The laggards however kept on till 3 P. M. The buds developing in summer under irrigated conditions started opening at the same time as in cold months, but the rate of opening in the course of the day was very much slower, reaching its peak at 2 P. M.

4. On the second day, the opening was much earlier and less drawn out in distribution. Anthesis started at 8 A. M. and by 11 A. M. it was complete.

When these observations were compared with those recorded at Pusa, it is noticed that no mention of cleistogamy is made at the latter place except a small note made in the body of the Table IV, on page 220-(4) to the effect that two flowers (out of the sixteen under observation) did not open. Very probably this feature is not very much pronounced there. The difference in behaviour if present, may be due to varietal differences. At Coimbatore most of the North Indian types dry up when they are in flowers, while the local types complete their life cycle rapidly.

The occurrence of higher percentage of cleistogamous flowers in the summer crop needs an explanation. Henslow (1888), and Coulter, Barnes, and Cowles (1932), mention that fall or rise of temperature and illumination, poor nutrition, changes in turgor pressure, and parasites may induce cleistogamy which might also involve changes in structure, form and setting. The data on the setting of pods show that it was as good as in chasmogamous group. (Table II).

Table II.  
*Percentages of setting.*

Date of observation.	Open flowers.			Cleistogamous flowers		
	No. observed.	No. set.	Percent of setting.	No. observed.	No. set.	Percent of setting.
9-1-1931	70	44	63	20	16	80
15-1-1931	70	50	71	19	13	68
27-2-1931	61	37	61	14	7	50
4-1-1932	80	69	86	11	9	82
Total.	281	200	71	64	45	70

A scrutiny of the hourly figures relating to temperature, humidity, number of hours of sunshine, and total atmometric evaporation on these days, point out that they bear no relation to the phenomenon observed, Table III.

Table III. Air Temperature °F.

Date.	Hours.												
	6 A.M.	7 A.M.	8 A.M.	9 A.M.	10 A.M.	11 A.M.	12 N.	1 P.M.	2 P.M.	3 P.M.	4 P.M.	5 P.M.	6 P.M.
9-1-1931	67	67	68	69	74	78	79	80	81	82	82	82	79
15-1-1931	71	71	71	72	73	75	76	77	78	80	80	80	80
31-1-1931	72	72	72	72	72	74	75	79	80	81	81	81	81
27-2-1931	69	69	70	76	80	83	85	87	87	88	88	88	88
4-1-1932	68	69	70	74	76	78	80	80	82	81	80	79	76
11-1-1932	62	68	70	74	77	80	81	82	83	83	82	80	78
22-1-1932	70	70	70	72	74	75	80	82	83	83	84	83	82
23-6-1932	73	76	79	81	83	84	84	84	84	84	80	78	77
2-7-1932	68	72	74	78	80	83	85	87	87	87	83	80	77

Further if alterations in temperature are responsible for the higher percentage of cleistogamous flowers observed in the summer crop, one would expect a consistently higher or lower proportion in the latter part of the flowering period when the days get warmer. But a study of distribution of unopened flowers during the entire flowering period (vide chart I) shows that it is not so. The peaks noted on the 7th, 13th, 16th, 17th, 20th and 23rd January are not associated with any rise or fall in atmospheric temperature prevailing on these days. The only possible explanation left is the difference in the nutrition between the two crops studied. The soil on which the off-seasonal crop was grown and the water used for irrigation were slightly alkaline, while the seasonal crop was raised on more or less neutral soil under rainfed conditions. The summer crop was very much poorer in growth than that raised in the cold weather. The tardiness observed in the opening of the chasmogamous flowers produced in summer plots (Table I-b) tends to support this possibility. It is stated by Coulter, Barnes and Cowles that want of available food in the embryonic regions might bring about alterations in the time of opening and closing of flowers in spite of favourable temperature and illumination.

If the figures of the closing of the flowers are compared, (Tables I-(a) and (b) it is seen that:—

1. The march of closing is more gradual and less rapid. Although flowers in 1932 commenced the first closing earlier than in 1931, largest number closed nearly at the same time in both years viz, between 4 and 5 P. M.

2. The second closing starts very much earlier than the first. In 1931 the latter did not begin before 4 P. M. while in the former flowers closed as early as 11 A. M.

3. The daily scheme of closing in the summer crop is however similar to that obtaining in the crop grown in the normal season. This feature forms a direct contrast with its behaviour during opening.

The data collected at Pusa on this point show that the closing occurs at about the same time on both the days.

The results of the duration of flowering point out (Table IV) that the flowers remain open for a shorter time during the hot months

and also on the second day, and that the duration of flowering on the second day is not influenced by its performance on the first day.

**Table IV.** *Duration of flower opening. Frequency distribution.*

Duration in hours.	First Opening.								Second Opening.								
	9-1-31	15-1-31	31-1-31	27-2-31	4-1-32	11-1-32	22-1-32	23-6-32	2-7-32	16-1-31	1-2-31	28-2-31	5-1-32	12-1-32	23-1-32	24-6-32	3-7-32
1	::	::	::	::	::	::	::	6	2	::	::	::	::	::	::	::	::
2	::	::	1	4	2	2	::	32	14	5	1	1	3	1	::	::	::
3	2	1	12	4	4	2	::	12	22	6	1	::	3	1	::	::	::
4	7	6	5	4	6	7	4	8	6	5	2	::	4	4	::	::	::
5	7	2	7	13	13	9	12	6	6	5	2	::	5	6	2	::	::
6	27	11	28	27	16	31	12	4	8	1	1	1	7	::	::	::	::
7	28	36	14	10	21	19	26	::	::	::	::	3	3	::	::	::	::
8	6	23	16	11	19	11	20	::	::	::	::	2	2	::	::	::	::
9	::	::	::	::	6	8	8	::	::	::	::	::	1	::	::	::	::
10	::	::	::	::	::	1	6	::	::	::	::	::	::	::	::	::	::
Average hours.	6.2	6.8	5.9	5.8	6.4	6.4	7.1	2.8	3.5	3.6	4.2	4.0	5.0	4.3	5.0	5.0	...

**Anther dehiscence and pollination.** It has been observed that on the day of flower opening the pollination is complete by the time flowers unfurl their petals. Examinations of buds one day previous to the anthesis have shown that the process had already started by 8 A.M. A large number of buds due to open two days hence were then labelled and examined in lots of fifteen or more, at intervals of two hours from 2 P. M. onwards, on the 20th January 1931 for anther dehiscence, and on the 4th, 13th, and 23rd January 1932 for both anther dehiscence and for pollination. The data obtained are furnished in Table V.

**Table V.** *Dehiscence of anthers in Bengal Gram.*

Hours of observa- tion.	20-1-1931	4-1-1932		13-1-1932		23-1-1932	
	% of buds with de- hisced anthers.	% of buds with de- hisced anthers.	% of buds with pollinated stigma.	% of buds with de- hisced anthers.	% of buds with pollinated stigma.	% of buds with de- hisced anthers.	% of buds with pollinated stigma.
4 P.M.	8	...	...	...	...	...	...
6 "	23	...	...	...	...	4	...
8 "	31	13	...	8	...	25	...
10 "	36	28	...	25	...	38	10
12 "	45	39	4	32	12	65	12
2 A.M.	48	42	4	34	14	86	13
4 "	80	42	4	40	23	91	20
6 "	100	46	21	61	44	92	32
8 "	100	69	35	65	52	98	42
10 "	...	84	43	72	70	100	52
12 Noon	...	100	76	100	92	100	67
2 P.M.	...	100	96	100	100	...	100
4 "	...	...	100	...	...	...	...

It is seen that the anthers commenced to dehisce more than 40 hours prior to the flower opening. In 1931, they started at 4 P. M. and by 6 A. M. on the next day all had liberated their pollen, while in 1932 the first anther opened between 4 and 6 P. M. only, and the last did not do so till 12 noon of the next day. The active period of opening was between 2 A. M. and 12 noon according to the variations in the phenological conditions.

The stigma, however, got pollinated for the first time at 12 P. M. in the night but the majority showed abundance of pollen grains between 8 A. M. and 2 P. M. on the succeeding day (i. e.) a day previous to the opening of the flower. It may be mentioned here that Pusa types 6 and 8 dehisced their anthers 12 hours later than the local varieties, while Type 1 showed very low percentage of dehisced anthers.

It is reported at Pusa that bees visit flowers in large numbers and a certain percentage of cross pollination occurs in nature. As far as the observations made at Coimbatore go, self pollination seems to be the invariable rule. Few insects are found visiting the flowers. Besides, no hybrid plant has ever been found so far in the several populations raised. The small number of aberrant plants found in them proved to be mutants. These indicate definitely that cross pollination is practically absent at Coimbatore. A few experiments conducted on this aspect fully confirmed the above inference. In one case 50 flowers were emasculated and left unpollinated. No pods resulted from any of these. In another set, 46 flowers were emasculated two days previously and pollinated on the day of flowering with the pollen secured from flowers opening on that day. Only one pod developed. This result might point out that conditions were not favourable for natural cross pollination on the day of flower opening. It could not be said that the pollen grains used in the second experiments would have lost their viability as they were more than 24 hours old by the time the anthesis occurred. For, germination tests made in vitro in culture media made up of 2% agar and 20% of cane sugar showed that on the first day of flower opening 96% of pollen grains were yet viable (Table VI) and it was only at the time of second day opening, the germination capacity fell down to 13%.

Table VI. *Pollen Germination. Average of 6 counts in plates.*

Date of observation.	First day flowers.			Second day flowers.			Remarks.
	Total grains.	No. of grains germinated.	% of germination.	Total grains.	No. of grains germinated.	% of germination.	
23--7--1932	66	63	95	71	10	14	* 4 readings only.
24--7--1932	73	68	93	81	9	11	
25--7--1932	83	79	95	78	11	14	
26--7--1932	85	83	98	51	6	12*	
30--7--1932	96	94	98	75	9	12	
Total.	403	387	96	356	45	13	

It is clear from the foregoing data that in Bengal gram the flowers are protandrous in nature and the pollination is complete in bud stage itself.

**Technique of Crossing.** Artificial crossing in this crop has been found to be very difficult on account of the smallness of buds and their sensitivity to manipulation. In spite of the great care taken during the process as well as in the selection for fertile parents, only 15 to 19 % flowers managed to set (Table VII).

**Table VII.**  
*Percentage of setting in Crosses.*

Date of crossing.	No of flowers crossed.	No of flowers set.	Percentage of setting.
30-12-1932 to 9-1-1933	570	108	19
10-1-1933 to 19-1-1933			
20-1-1933 to 29-1-1933	435	84	19
30-1-1933 to 11-2-1933			
	242	37	15
	283	48	17

The following method has been found to give very successful results at Coimbatore. Flower buds due to open two days later are selected and labelled. Such buds can be easily identified by their size after a little experience. In the evening between 2 and 6 P. M. the keel is gently pushed apart with a blunt needle and the anthers are carefully removed with a light upward jerk given at the point of attachment with filament, without pricking the anthers. A large number of flowers could be operated quite easily in this manner when one gets familiar with the work. It may, however, be pointed out that care has to be taken to see that the pedicels are handled least, and a few of the tagged flowers are left undisturbed for use as pollen parent later on. On the next morning pollen grains from these buds are removed and dusted between 9 and 11 A.M. on the stigmas of the buds emasculated on the previous evening. It has been found that emasculated buds left exposed overnight without any covering kept fresh on the next day. As insects are seldom found visiting the flowers, there is hardly any risk of cross pollination. Freshness of the pedicel on the subsequent days will indicate the success of crossing. Pods will be seen from these 5 or 6 days hence.

The above method differs from the one described by Khan and Akhtar (1934) in the non-removal of petals and in dispensing with the muslin bags. In fact tissue bags were used at first to cover the emasculated flowers, but they were abandoned when they were found to accelerate shedding. It has been experienced that crossing is more



successful if the earlier formed buds are selected for crossing. They are also easier to manipulate on account of their size and capacity to keep fresh for a longer time.

Pusa types differ among themselves with regard to their sensitiveness to crossing. T-6 and T-8 stand handling better. Types 11 and 13 give higher percentage of setting if they are used as pollen parents. Shaw and Khan (1931), Khan and Akhtar (1934), experienced the same drawback at Pusa in the last two types.

**Acknowledgement.** The writers' thanks are due to Mr. Devasirvatham for the help rendered in recording some of the observations, and to the Indian Central Cotton Committee under whose financial help the work was carried on, as an off-seasonal study in the Madras Herbaceum Scheme.

**Summary.** Observations made at Coimbatore on the time of opening and closing of flowers in Bengal gram (*Cicer arietinum*) disclosed that :—

- (a) Cleistogamy was present and its proportion seemed to be altered by differences in nutritional conditions in the soil.
- (b) All the flowers that opened on the first day did not open on the second day.
- (c) The time of active blooming was between 9 and 10 A. M. on the first day. In a summer crop, most of the flowers opened at 2 P. M.
- (d) The march of closing was more gradual and less rapid than that of opening.
- (e) The petals opened and closed much earlier on the second day of their opening.
- (f) The total period during which a flower remained open was less on the second day of opening and during hot months.

2. It was also found that the anther dehiscence and pollination occurred one day before the opening of the flowers, and there was hardly any cross pollination notwithstanding the fact that the pollen grains were viable at the time of flower opening.

3. The method of crossing that has been found successful is described.

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