



Digenic inheritance of cleistogamous flowering type in Egyptian cotton (*Gossypium barbadense* L.)

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ABSTRACT : Cleistogamy in *Gossypium barbadense* L. is controlled by digenic inheritance. Chasmogamous flower is dominant over cleistogamous flowers in cotton. However, full cleistogamy expression depends on the environment in many plants. However, an accession of CCB12 showed full cleistogamy over the season and environment. Its inheritance was studied by crossing cleistogamy line with chasmogamous line. Cross between chasmogamous (Suvin) and cleistogamous (CCB12) produced chasmogamous flowers only and same the case with the reciprocal crosses. However, the F_2 showed a segregation of 15:1 for chasmogamous to cleistogamous ratio indicates, involve of double recessive genes. Back cross (BC_1) from P_1 (BC_1F_1) (BC_1 (Suvin \times CCB 12) \times Suvin) showed complete chasmogamous lines; however, Back cross (BC_1F_1) from P_2 (BC_2 (CCB 12 \times Suvin) \times CCB 12) expressed 3:1 ratio for chasmogamous to cleistogamy lines. In future, these cleistogamous lines can be transferred to non-cleistogamous genotypes for maintaining the genetic structure and useful in maintenance, this would reduce the hectic labor-intensive selfing.

Keywords: Chasmogamous, cleistogamy, cotton, inheritance

Cleistogamy is a form of reproductive mechanism, which promotes self-pollination. It is a condition in which the flower remains closed even after anthesis, such a closed flower ensures there is no pollen outcrossing from outside. Cleistogamy the term first used by Kuhn in 1867 to denote the bud-like flower in plants, cleistogamy literally means closed marriage, while chasmogamy is open marriage in which the flower part is open and which facilitates outcrossing. Darwin (1877) observed cleistogamy in *Viola*, *Oxalis*, and *Impatiens* species. This phenomenon is noticed in 59 families of 228 genera in 693 species of angiosperm. Lord (1981) reported the occurrence of cleistogamy in 56 families of 287 species. Investigations indicated that the evolution of cleistogamy in taxa may be influenced by the presence of heterogeneous environments, inbreeding depression, geitonogamy and differential seed dispersal, as well as by various ecological factors and plant size (Zhang *et al.*, 2017). Cotton belongs to the genus *Gossypium* of Malvaceae family characteristics of producing

chasmogamous flower. Bees and winds are the responsible for chances of out crossing which accounts up to 0.53 to 15.36 per cent of cross-pollination, which makes cotton as often cross-pollination category. Out crossing is a major problem in germplasm and varietal maintenance. Since every time genetic purity of a line get contaminated with outcross pollens as a results condition of homozygosity of alleles get converted into heterozygosity resulting in heterogeneous population. In few germplasm in *G. barbadense* lines possess the cleistogamy nature of flowers (Mukhiddinov and Abzalov, 1995; Mukhiddinov, 2010). Self-pollination is favored in many plant species where out crossing is hindered by the shortage of pollination mechanisms like pollinators and harsh environment for free pollen flow. In such cases, plants possess plasticity to produce cleistogamous flowers as a backup mechanism to survive the reproduction. Cleistogamous flowers produces self fertilized seeds by preventing outcross and generally aid in fixing locally adapted gene complexes of any species. Genes can

be preserved and fixed in an effective mechanism. It would be effective to transfer the cleistogamous trait into non cleistogamous lines to maintain the genetic composition. Segregating generation of F_2 derived from a cross between *G. hirsutum* and *G. barbadense* revealed that two recessive genes (*cg1 cg2*) were responsible for cleistogamous inheritance (Hau *et al.*, 1980). In lieu of the above background, Khattab *et al.*, 1982 observed recessive genes responsible for cleistogamous flowering in a BC2 population from a cross of *G. hirsutum* and *G. barbadense*. Zhang (1992 and 2002) identified stable cleistogamous lines from various segregating populations. The purpose of this study was to investigate the inheritance of cleistogamy in *G. barbadense* and would be used to transfer the trait of cleistogamy into non cleistogamy lines.

MATERIALS AND METHODS

One chasmogamous line (Suvin) and another cleistogamous line (CCB 12 which is a mutant from the *Gossypium barbadense* intra cross SS-2 (Suvin x Giza-45)) were taken as a parent for production of hybrid crosses to study the inheritance pattern of cleistogamy in tropical *G. barbadense* genotypes. The following crosses were produced from 2017-2018 at ICAR-Central Institute for Cotton Research, Regional Station, Coimbatore.

- (1) F_1 and F_2 paired reciprocal hybrids: F_1 : (a) $P_1 \times P_2$ and (b) $P_2 \times P_1$.
- (2) Backcross hybrids: BC_1 : $(P_1 \times P_2) \times P_1$ and BC_2 : $(P_1 \times P_2) \times P_2$.

P_1 : Suvin is a chasmogamous flower, P_2 : CCB 12 is a cleistogamous line

The crop was raised in normal agronomic conditions with spacing of 90 x 60 cm. All the plants in the parental and segregating generations were observed for flowering behavior from anthesis to boll formation. The results were tested for goodness of fit to postulated ratios using the chi-square test.

RESULTS AND DISCUSSION

In cotton plant, once it begins to bloom it is called as "flowering." *Gossypium barbadense* cotton typically flowers for about 6-7 weeks. Once blooms are onset, the stage of cotton development is discussed in terms of weeks of bloom. Cotton square is actually a flower bud; three bracts surround the flower bud in a pyramid-like shape. *G. barbadense* cotton plant produces perfect flowers, meaning the flower contains both male and female organs. The first square is typically visible on node 5 to 6 about 37-39 days after planting. Anthesis or a flower bloom occurs approximately 24 days after the first square appears. Flowering is important to cotton production because pollinated flowers produce cotton bolls. The bloom process takes several days and bloom age can be estimated by the bloom characteristics. On the day a flower opens, it is yellow in color. Pollination of that flower usually occurs within a few hours after the yellow flower opens. However, in case of cleistogamous type, the



Fig.1 Cleistogamous line CCB12 is a mutant from the *Gossypium barbadense* intra cross SS-2 (Suvin x Giza-45)

flower bud remains closed and it never opens at all. Once the anthesis over, the flower color turns into pink and is dried. This dried corolla remains intact until the boll develops, often we can see the cap like scar on the tip of the boll (Fig 1).

The cross between P_1 (chasmogamous) suvin variety crossed with P_2 (cleistogamous) (CCB 12) and F_1 hybrid was produced which showed complete chasmogamous flower types only (Table 1). Reciprocal cross between both parents also showed chasmogamous type, which evident that cleistogamy was completely recessive when compared with chasmogamous line. Subsequent F_2 segregation gave the segregating ratio of 15:1 for each contrasting character (260 chasmogamous and 17 cleistogamous) from $P_1 \times P_2$ and 250 chasmogamous and 18 cleistogamous from $P_2 \times P_1$. It evinced the double recessive nature of cleistogamy inheritance. Back cross from P_1 (BC1 (Suvin \times CCB 12) \times Suvin) showed complete chasmogamous lines; however back cross from P_2 (BC2 (CCB 12 \times Suvin) \times CCB 12) expressed 3:1 ratio for chasmogamous to cleistogamy lines. Cleistogamy is an adaptation to ensure seed production under adverse conditions. The cleistogamous plant appears to sacrifice long-term genetic fitness in preventing out crossing, in order to ensure seed set.

The mechanism of cleistogamy and effects of ecological factors on cleistogamous expression studies are being carried out widely in different plant species. A considerable range (1 to 81 %) for natural out-crossing in cotton has been reported, with most reports citing more than 10 per cent (Meredith and Bridge, 1973). This not

only leads to rapid genetic deterioration of released cultivars and genetic stocks, but it also influences the efficiency of crop-breeding procedures since selfing of early generation selections is expensive and difficult. Cotton with cleistogamous flowers would reduce the natural out-crossing to an extent that the problem of genetic contamination could be solved. Because of the ability of the cleistogamy character to maintain the genetic purity of genotypes, the trait appears to be extremely useful. Once the pattern of cleistogamy inheritance in *G. barbadense* studied well; it can be transferred to other *Gossypium* species (Manivannan, 2020).

Cleistogamous flowers help in reproduction with minimal energy and resource expenditure. It also maximizes the chance of reproduction, which is an important factor where the agents of pollination are scarce. Cleistogamy is a model system which used to study the evolution of diverse floral morphologies and reproduction strategies and breeding systems, which illustrate the importance of floral biology in applied breeding (Stebbin, 1974; Lord, 1981). Cleistogamous plants could also be important for better control of genetically modified lines of agriculturally important crops (Danicell, 2002). For these reasons, a better understanding of the genetic control of cleistogamy is necessary. In case of controlling of spread of foreign pollens especially in case of genetically modified crops, where cotton is leading in world acreage in terms of transgene, cleistogamy is an ideal system to control the pollen spread. Further molecular studies would

Table 1. Inheritance of cleistogamous flowering in *G. barbadense*

Type	Number of plants	Phenotypic class		Ratio	χ^2 value	Pvalue
		Chasmogamous	Cleistogamous			
Suvin	55	55	-	1:0	0.0	0.0
CCB 12	55	-	55	1:0	0.0	0.0
F_1 (Suvin \times CCB 12)	40	40	-	1:0	0.0	0.0
F_1 (CCB 12 \times Suvin)	45	45	-	1:0	0.0	0.0
F_2 (Suvin \times CCB 12)	277	260	17	15:1	0.21	0.65
F_2 (CCB 12 \times Suvin)	268	250	18	15:1	0.37	0.51
BC1 (Suvin \times CCB 12) \times Suvin	145	145	-	1:0	0.0	0.0
BC2 (Suvin \times CCB 12) \times CCB 12	126	96	30	3:1	0.10	0.75

enhance the understanding of cleistogamous mechanism (Kumar *et al.*, 2021). Therefore, in view of above cited all these reasons, cleistogamy in cotton has to be studied well for further utilization of this phenomenon.

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An approach for developing compactness measurement indices in cotton (*Gossypium* spp.)

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Cotton, the best options for rainfed regions would be early-maturing short duration straight varieties, resistant to sucking pests, dwarf statured, zero-monopodial, which are amenable for high-density planting at populations of 1,00,000 per acre or more. High density planting system has been suggested as an alternative strategy instead of conventional one to increase yield as reported by Darawsheh *et al.* (2009). Higher productivity in Brazil was achieved through development of compact sympodial varieties suited for high density planting (Kranthi 2012). Machine picking ultimately warrants high density planting system with compact genotypes for its suitability. Compact plant and HDPS are need of the hours for Indian situation (Venugopalan *et al.* 2013) hence; there is a need to develop proper indices to measure compactness in plant and to screen available genotypes. A more compact plant structure (Reddy *et al.* 1990) improves light penetration in the canopy. Compact plant type with zero monopodia and short sympodia is suitable for high density planting.

In cotton, main stem has an erect, indeterminate monopodial growth habit. Sympodial branches bear fruit directly, so they are called fruiting branches (Oosterhuis 2001). Under high density planting system (HDPS) encourages formation of sympodial branches. The length of sympodial branch is maximum at base of the plant and decreased proportionately towards top of the plant. Sympodial branches on the main stem are located in a spiral order, angled along the main stem. Proportionate decreasing and spiral order of sympodia could make conical morphoform for cotton plant above the ground. Cotton is planted in rectangular geometry by higher row to row spacing with less plant to plant spacing for need of easy intercultural operation and other management. Thus influences the sympodial length, higher length by perpendicular to row direction and short branches are formed adjacent to row

direction; which make elliptical in shape by area occupied by the plant.

Biometric observation on 100 plants were made in three compact genotypes (CICR CSH 19-2, PAU 1 and CICR RS 2013) planted at 75 cm × 30 cm at Central Institute for Cotton Research, Coimbatore during 2018 in summer cotton at 90 DAS revealed that the sympodia with highest mean length was observed in 3/4th node then sympodial length was proportionately decreased towards top of the plant. The results confirmed that proportionate decreasing and spiral order of sympodia could make conical morphoform for cotton plants. Genotypes were planted at 75 cm row to row distance with 30 cm of plant to plant spacing. The sympodial length is varying with direction especially opposite and adjacent to row direction. The results observed highest length of 17.9 and 16.3 cm with both side opposite to row direction. The adjacent to row direction observed the length of 13.8 and 13.7 cm in both side of the plants. Spiral order of sympodia could make conical morphoform for cotton plants. However, Cotton is planted in rectangular geometry commonly by higher row to row spacing (75 cm) with less plant to plant spacing (30 cm) for want of easy intercultural operation and other management. Which influenced the growth behavior resulted in variation in length of sympodia; thus ultimately resulted as elliptical cone morphoform for cotton plants with elliptical base.

Compactness measured by different approaches includes sympodial length (cm), plant height (cm), sympodial length per plant height, plant height per sympodial length, area occupied by individual plant (cm²) and total volume of the plant (cm³). Measurement on sympodial length (cm) or plant height (cm) alone does not provide correct picture of land area occupied by the plant or total volume of the plant, which is essential for measuring compactness. The ratio of sympodial length (cm) per plant height (cm) did not provide correct pictures of compactness in all situations. Compact plant may not be productive always. Hence necessity arises to work out efficacy of compactness. The two indexes are proposed here. The compactness efficiency index CEI 1 (area) (mg/cm²) was measured by kapas yield (mg) of plant divided by area occupied by plant (cm²). The compactness

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efficiency index CEI 2 (volume) (mg/cm^3) was measured by kapas yield (mg) of plant divided by total volume of the plant (cm^3).

$$\text{Compactness Efficiency Index CEI 1 (area)} = \frac{\text{Kapas yield (mg) of plant}}{\text{Area occupied by plant (cm}^2\text{)}}$$

$$\text{Compactness Efficiency Index CEI 2 (Volume)} = \frac{\text{Kapas yield (mg) of plant}}{\text{Total volume of the plant (cm}^3\text{)}}$$

The area occupied by plant is calculated by using formulae of elliptical base

$$\text{Area A} = \pi \times R \times r$$

The volume of the individual plant was calculated by using elliptical cone formulae

$$V(\text{cm}^3) = (\pi \times R \times r \times h)/3$$

where R, mean of highest sympodial length (cm) measured in both side of the plant opposite to row direction; r, mean of highest sympodial length (cm) measured in both side of the plant adjacent to row direction; h, height of the plant.

The data of Bt varietal evaluation trial (25 genotypes) conducted at Central Institute for Cotton Research, Coimbatore during 2017 was used to calculate land area occupied by individual plant to identify compact genotype (Table 1). The genotype NSBT 108 (101.3), OUAT Bt 2 (125.6), PAU 1 (129.5), CICR-K 34007 (142.0), CICR-F1861 (154.2) and NSBT (154.6) were identified as compact genotypes, which needs less land area (cm^2) for cultivation.

Table 1 Compactness index and yield of different genotypes

Genotype	Ht (cm)	Sympodial length (R) opposite to row direction (cm)	Sympodial length (r) adjacent to row direction (cm)	Area (cm^2)	Volume (cm^3)	Index I	Index II	Yield (q/ha)
PAU1	42.8	9.2	4.6	129.5	1847.8	43.4	3.0	7.5
Rahuri 1	42.8	12.3	5.1	194.9	2780.4	41.6	2.9	10.8
OUAT Bt 2	47.8	8.5	4.8	125.6	2001.3	80.0	5.0	13.4
OUAT Bt 1	49.6	12.0	4.7	175.9	2909.0	58.4	3.5	13.7
CICR-Suraj	56.7	16.1	4.7	232.7	4394.8	51.6	2.7	16.0
CICR-Rajat	52.5	14.4	4.2	185.9	3255.5	64.9	3.7	16.1
CICR-SRI-5	48.9	12.4	5.2	198.7	3240.5	41.5	2.5	11.0
CICR-CSH 19-1	53.3	11.8	4.7	170.2	3022.3	39.7	2.2	9.0
CICR-CSH 19-2	43.8	11.4	4.5	159.1	2323.3	38.2	2.6	8.1
CICR-RS 2013	52.6	12.2	4.7	175.8	3081.7	45.7	2.6	10.7
CICR-F 1861	52.3	11.3	4.4	154.2	2690.3	58.8	3.4	12.1
CICR-K 34007	52.7	10.9	4.2	142.0	2492.6	48.6	2.8	9.2
CICR-SRI 1	54.5	15.7	4.8	232.5	4221.1	43.9	2.4	13.6
CICR-GH 5	48.7	15.4	4.8	228.1	3699.7	40.8	2.5	12.4
CICR-GH 8	49.0	10.1	5.1	158.7	2591.7	54.4	3.3	11.5
CICR-PKV 081	48.8	15.7	5.0	243.7	3964.1	39.4	2.4	12.8
Shakti	61.1	10.8	4.8	161.5	3291.5	66.9	3.3	14.4
CICR-CPT 1	55.9	16.0	5.1	251.4	4681.0	43.3	2.3	14.5
CICR-CPT 2	53.8	13.4	4.9	202.7	3634.9	63.3	3.5	17.1
CICR-CPT 3	62.9	13.3	5.0	207.4	4346.2	46.3	2.2	12.8
NSBT 145	57.6	11.2	4.5	154.6	2968.1	72.3	3.8	14.9
NSBT 306	65.7	16.3	4.7	235.6	5161.3	57.6	2.6	18.1
NSBT 207	47.9	12.6	4.6	180.3	2881.0	64.9	4.1	15.6
NSBT 108	51.3	7.0	4.7	101.3	1730.5	67.4	3.9	9.1
BG II check	31.5	12.2	5.0	189.3	1989.3	24.2	2.3	6.1
Mean	51.4	12.5	4.7	183.7	3168.0	51.9	3.0	12.4
SED	3.6	1.9	0.2	27.5	644.6	6.4	0.4	1.7
CD (5%)	7.2	3.8	0.4	55.4	1296.0	12.8	0.9	3.4
CV	8.4	18.1	5.7	18.2	24.1	19.1	32.0	20.9
S/NS	S	S	NS	S	S	S	S	S

The results on total volume of individual plant to identify compact genotype found that the genotype CICR-CSH 19-1 (3022.3) NSBT 145 (2968.1), OUAT Bt 1 (2909.0), NSBT 207 (2881.0), Rahuri 1 (2780.4), CICR-F 1861(2690.3), CICR- GH 8 (2591.7), CICR-K34007 (2492.6), CICR-CS19-2(2323.3), OUAT Bt 2(2001.3), PAU 1(1847.8), and NSBT 108 (1730.5) were identified as compact genotypes, which showed less plant volume (Table 1).

The compactness efficiency index CEI 1 (area) (mg/cm^2) used to identify compact and efficient genotype. The genotype OUAT Bt 2 (80.0), NSBT145 (72.3) and NSBT 108 (67.4) were identified as compact efficient genotypes, which needs less land with high performance. The compactness efficiency index CEI 2 (volume) (mg/cm^3) used to identify compact and efficient genotype with respect to volume of the plant. The genotype OUAT Bt 2 (5.0), NSBT 207 (4.1) and NSBT 108 (3.94) were identified as compact efficient genotypes with respect to volume of the plant. In agriculture, land is limited resource and there is no limitation to use vertical space for utilization of plants to increase productivity of crop. Hence better indices for compactness is area occupied by plant is calculated by using formulae of elliptical base $A (\text{cm}^2) = \pi \times R \times r$. The correlation matrix observed that high significant correlation is observed ($(R \times r) \times \sqrt{h}$) with area ($r = 0.954$) occupied and volume ($r = 0.979$) of the plant and this may be the better indicator for compactness.

SUMMARY

High density planting with compact genotypes proved, as high potential system of cotton cultivation. There is a need to develop proper indices to measure compactness in plant, is necessitated for screening of genotypes. Compactness measured using $((R \times r) \times \sqrt{h})$ was identified as an efficient method, based on that genotypes NSBT 108, PAU1, OUAT Bt 2, CICR- K 34007 and CICR-CSH 19-2 were identified as compact genotypes. Compact Efficiency Index (CEI 1) was suitable for measuring efficiency of compactness.

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