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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 chasmogmaous (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 × CCB 12) × Suvin) showed complete chasmogamous lines; however, Back cross (BC_1F_1) from P_2 (BC_2 (CCB 12 × Suvin) × 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. *barbadens*e 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 × CCB 12) × Suvin) showed complete chasmogamous lines; however back cross from P2 (BC2 (CCB 12 × Suvin) × 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 cleisotgamous 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 cleisotgamy 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 (Daniell, 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	$\chi 2$ value	*P* value
		Chasmogamous	Cleistogamous			
Suvin	55	55	-	1:0	0.0	0.0
CCB 12	55	-	55	1:0	0.0	0.0
F1 (Suvin x CCB 12)	40	40	-	1:0	0.0	0.0
F1 (CCB 12 x Suvin)	45	45	-	1:0	0.0	0.0
F2 (Suvin x CCB 12)	277	260	17	15:1	0.21	0.65
F2 (CCB 12 x Suvin)	268	250	18	15:1	0.37	0.51
BC1(Suvin x CCB 12) x Suvin	145	145	-	1:0	0.0	0.0
BC2 (Suvin x CCB 12) x CCB 12	126	96	30	3:1	0.10	0.75

enhance the understanding of clesitogamous 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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