Theoretical and Applied Genetics 46, 281-287 (1975)

Genetic Analysis of the Duplicate Loci, Cluster and Short Branch in *Gossypium hirsutum* L.*

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Summary. Six generations, namely P_1, P_2, F_1, F_2, B_1 and B_2, of five different crosses involving diverse parents, H14 (Local standard), Res H141 (Reselection of H141), 419/49 (Punjab), 5143C (Ceylon) and Banda-I (Africa) on one hand and PRS-72 a cluster type strain on the other, were studied to gain understanding of the genetics of short fruiting branch and cluster boll bearing in upland cotton, *Gossypium hirsutum* L. Observations were recorded on cluster vs. noncluster normal plants in the first and second filial and B_1 and B_2 generations. The segregation ratios of cluster: normal boll bearing in the F_2 and B_2 (test cross) progenies confirmed that the character is monogenic recessive ($cl_1 cl_1$) in inheritance. Though the penetrance of the gene which controlled cluster boll bearing was complete, its expression varied. As many as 15 different types of 'cluster' have been described. Length of cluster bearing sympods varied. On average the sympod lengths in PRS-72 and the cluster type F_2 and B_2 were 2.13 and 4.54 cm, respectively, suggesting influence of specific modifiers evolved in the cluster donor parent and the different genetic backgrounds. It was suggested that there is a dosage effect of modifiers and interaction of homeologous duplicate allele ($Cl_2 Cl_2$) in governing the length of the cluster bearing sympods.

Introduction

The elongated fruiting branch (sympodium) in cultivated cottons generally is multinoded and bears only one boll at a node. The rare occurrence of an excessively shortened sympodium bearing two or more bolls at the terminal points has been reported by several workers. Thadani (1923), Neely (1942), Butany and Singh (1963), and Santhanam and Krishnamurthy (1966) in *Gossypium hirsutum*, and Patel et al. (1947) in *G. herbaceum* race *weightianum*, reported the simple monogenic recessive inheritance of short fruiting branch. Kearney (1930) in *G. barbadense* and Harland (1939) reported that this character is incompletely dominant over the normal multinoded branch type.

Since *G. hirsutum* is an amphidiploid it is expected to have many duplicate loci. The short fruiting branch habit is also reported to be governed by duplicate genes, but its inheritance is not fully understood. Bhat and Desai (1956), studying the F_1 of two short branch cluster mutants, found that the F_1 was normal and they concluded that the cluster and short branch characters were governed by complementary loci. Dalton (1966) reported that fruiting branch structure in New World Cotton is controlled by duplicate loci, cluster (Cl_1) in the D genome and short branch (Cl_2) in the A genome, but the duplicate factor has not been satisfactorily explained by any known model. Cluster (cl_1) is a recessive mutant in *G. hirsutum* and short branch (cl_2) is a recessive segregation within species with their duplicate nature being discovered only when the two species are crossed (*G. hirsutum* × *G. barbadense*). The phenotypic expression of the cluster and short branch is only moderately similar (i.e. reduction of the fruiting branch) on their respective species background. However, when cross transferences are made, the action of the mutant allele is similar, with the observed phenotypic differences of the mutants governed by the species background. When the recessive mutants are crossed, they behave as complementaries restoring the normal fruiting branch habit. The normal allele of one locus cannot mask the mutant allele of the opposite locus and this difference in masking ability is, as yet, not clearly understood. Dalton studied the expression of this character in interspecific hybrid populations of varying degrees of introgression of the gene complex of the two species and explained the differing expression of this character by the interpretation which he referred to as "dosage and interaction".

Understanding the inheritance of the short fruiting branch is important as this gene is being exploited to evolve efficient plant types in cotton. The studies reported here were designed to further examine the genetic nature of duplicate loci, cluster boll bearing and short fruiting branch, with special reference to penetrance, expressivity and stability in different genetic backgrounds for different generations of intra-*hirsutum* crosses.

* Part of the thesis submitted to the Punjab Agricultural University in partial fulfilment of requirements for the degree of Doctor of Philosophy (Genetics) by the senior author.

Materials and Methods

The material consisted of 26 progenies; $5P_1$, $1P_2$ and 5 each of F_1, F_2, B_1 and B_2, derived from crossing the P_2 with $5P_1$s, described as below:

P_1s = the first parent of the crosses, i.e. H14 (local standard)
Res H141 (Reselection of H141)
419/49 (Introduced from Punjab)
5143C (Introduced from Ceylon)
Banda-I (Introduced from Africa)

P_2 = the second parent of the crosses, i.e. PRS-72 (Introduced from U.S.S.R.).

Though the P_1s differ in most of their characters their growth and fruiting habit is almost the same. All bear multinoded fruiting branches appearing either on the monopods or directly on the main stem. The P_2 has a strikingly different growth and fruiting habit. It (PRS-72) is erect growing, short-statured, and bears more than one boll on extremely shortened sympods (cluster bearing) as if the bolls are borne directly on the main stem. This variety is highly susceptible to jassids attack and is thus poorly adapted to north Indian conditions. However, it is very early maturing and its bolls are bold.

The other four generations are $F_1 = (P_1 \times P_2)$, $F_2 = (F_1$ selfed), $B_1 = (P_1 \times F_1)$ and B_2 (test cross) $= P_2 \times F_1$. F_1 was used as pollen parent so as to avoid selfing.

Individual plants in F_1 and the segregating generations, F_2, B_1 and B_2, were checked for fruiting habit. The plants which had two or more bolls on uni-noded sympods were classified as 'cluster', otherwise 'noncluster normal'. Among the cluster types further expression as varying lengths of the sympods with or without extra axillary buds was recorded. The segregation ratios in F_2 and backcross generations were tested for gooodness of fit on the expected Mendelian ratios of 3 : 1 in F_2 and 1 : 1 in test crosses (B_2) by chi-square.

Results

Fruiting Branches

Normally, *hirsutum* cottons are indeterminate in their growth habit and bear monopodial or sympodial or monopodial/sympodial branches. The different varieties possess specific branching patterns. The sympodial varieties are those which bear multinodal fruiting branches on their main stem in a descending order, giving a pyramidal shape to the plant. The monopodial varieties give vegetative branches which in turn bear fruiting branches. The mono/sympodial are those which bear both type of branches on the same plant. All the varieties, except PRS-72, involved in the present study were of the mono/sympodial type. However the proportion of monopodial to sympodial branches varied from variety to variety.

The growth habit of the cluster type parent, PRS-72, was quite different. The PRS-72 plants were dwarf and produced excessively reduced sympodial branches on the main stem. The sympods always terminated in two or more bolls. These plants had determinant growth habit. The internodes were very short (1.86 cm) and thus gave a compact stature to the plant. There was complete suppression of the monopodial branches. PRS-72 plants were highly susceptible to insect pests, particularly to jassids attack and angular leaf spot disease, under the north Indian conditions and thus were never able to express their full morphological vigour and yield potential. The leaves were most severely attacked by jassids and thus dried and dropped. Since the leaves, which are the photosynthetic unit, were badly damaged, the plants would be physiologically unbalanced, thus reducing the fruiting points and number of flowers. Even if few bolls were set they remained underweight. The main morphologically contrasting characters of the two sets of parents are summarized below:

Variety height	Plant height (cm)	No. of sympods per plant	No. of monopods per plant	No. of branches per plant	Sympodial length (cm)	Internodal length (cm)
H14	106.83	18.77	5.28	24.05	16.30	2.70
ResH141	102.02	26.69	2.92	29.61	14.44	2.79
419/49	77.32	21.60	3.93	25.53	9.41	2.39
5143C	96.46	23.84	3.50	27.34	15.77	2.88
Banda-I	91.78	19.05	6.29	25.34	17.46	2.67
PRS-72	52.35	19.29	-	19.29	2.13	1.86

Inheritance of Cluster

The mode of inheritance of cluster was studied in crosses between the strains which had noncluster normal bearing habit, viz., H14, ResH141, 419/49, 5143C and Banda-I, and the cluster bearing strain, PRS-72. The segregation ratios are presented in Table 1. The cluster character behaved as a recessive to the normal in F_1. The F_2 segregation data gave the ratio of 3 normal: 1 cluster (χ^2 P value = 0.20-0.50). The test cross (B_2) ratio, i.e. backcrossing the F_1s to the cluster parent, PRS-72, was 1 normal: 1 cluster (χ^2P value = 0.10-0.20). These segre-

Table 1. Inheritance of cluster character

Cross	Number of plants with normal bearing	cluster bearing	Total number plants
F_2(H114 × PRS-72)	100	41	141
F_2(ResH141 × PRS-72)	61	33	94
F_2(419/49 × PRS-72)	49	22	71
F_2(5143C × PRS-72)	73	15	88
F_2(Banda-I × PRS-72)	77	17	94
F_2(C.T.I. × PRS-72)*	2512	792	3304
Total:	2872	920	3792
χ^2: 1.1027			
Value of P (monohybrid 3 : 1 segregation) = 0.20-0.50 (good fit)			
BC(H114 × PRS-72) × PRS-72	85	53	138
BC(ResH141 × PRS-72) × PRS-72	20	22	42
BC(419/49 × PRS-72) × PRS-72	28	26	54
BC(5143C × PRS-72) × PRS-72	27	18	45
BC(Banda-I × PRS-72) × PRS-72	26	40	66
Total:	186	159	345
χ^2: 2.1130			
Value of P (monohybrid 1 : 1 segregation) = 0.10-0.20 (good fit)			

* This cross was used only for scoring the segregation.

gation data suggest that the cluster character is monogenic recessive in its inheritance.

Clustering Pattern

A great variation in the pattern of expression of the cluster boll bearing habit was observed in the cluster type plants scored in F_2 and backcross generations. Variations were noted in the number of bolls in a cluster and in the way they were arranged on the sympodia. On this basis as many as 15 different types of "cluster" were recorded (Fig. 1) and described under:

1. Uninodal, cluster of two bolls with a common bract
2. Same, but, in separate bracts
3. Binodal, cluster of three bolls, 1 + 2
4. Binodal, cluster of four bolls, 2 + 2
5. Quadrinodal, cluster of five bolls, 1 + 1 + 1 + 2
6. Uninodal, cluster of three bolls with a common bract
7. Same, but, each boll with a separate bract
8. Same, but sparse
9. Binodal, cluster of three bolls, 1 + 3
10. Binodal, cluster of five bolls, 2 + 3, two bolls with common bract
11. Binodal, cluster of four bolls, 2 + 2 with two extra axillary bolls
12. Binodal, cluster of five bolls, 2 + 3
13. Uninodal, cluster of five bolls very close to one another
14. Trinodal, cluster of five bolls, 2 + 1 + 2
15. Binodal, cluster of six bolls, one node had two stalks, (1 + 3) + 2

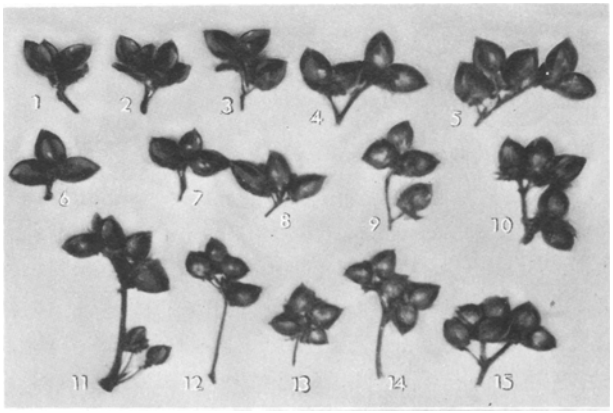

Fig. 1. Fifteen different clusters of bolls scored in F_2 and backcross generations of crosses between cluster (PRS-72) and normal parents

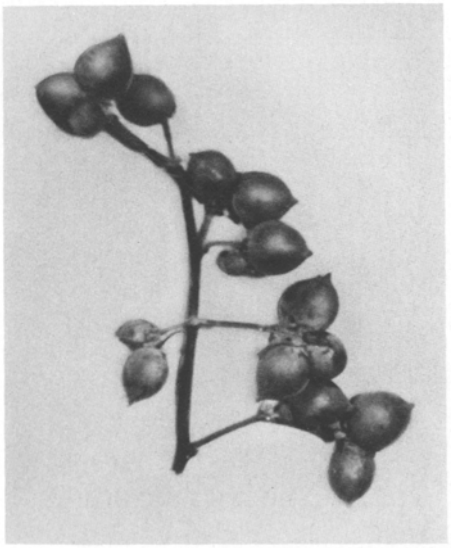

Fig.2. An F_2 (H14 × PRS-72) heavy bearing cluster plant - bolls equally developed in the clusters

The node number per sympod varied from 1 to 4. Always the sympods terminated in more than one boll, usually in two. The nodes were very close. Generally the bolls were arranged in twos to a cluster. The sparsely borne bolls were usually larger than those in a close cluster. Even in one single cluster type plant, there were different grades of cluster-bearing. However, all cluster-type plants were dwarfer and usually had thick leathery and broad dark green leaves.

In the segregating generations different combinations of these clustering patterns were observed. In no case were cluster-bearing segregates as miserable looking as the PRS-72 parent. A complete array of plant types, tall to dwarf, long to short sympods, bushy or spreading, resistant to susceptible, early to late, with varying combination, was isolated. Many desirable cluster-bearing plants were recovered. These plants were short ($2\frac{1}{2}$ to $3\frac{1}{2}$ feet), with a high number of bolls which were arranged in clusters of 2 to 3 and enclosed by enlarged bracts. The sympods were extremely reduced, giving the bolls the appearance of being borne directly on the main stem. The bolls were borne from the very lowest portion of the plants. Some of these plants were free from diseases and pests. Sometimes, such cluster-bearing plants were much taller (about 5 feet), and their stems were thick and sturdy, but the bolls were not borne so much from the lower part of the plant. Usually, all the bolls of a cluster were not equally bold, but rarely some plants were isolated which had their bolls equally developed (Fig.2). Within the dwarf cluster types the internode lengths varied. Plants with extremely short internodes gave bunch type bearing, while some plants had internodes a little longer which is considered to be highly desirable for boll setting and boll development.

Rarely some plants were observed which had monopodial branches, which bore bolls in clusters on elongated or shortened sympods. Cluster-bearing on elongated sympods is desirable for giving bolder bolls and cleaner picking. Usually the cluster type segregants did not combine the insect pest resistance of the resistant parents and were severely attacked by jassids, thus yielding less. Also, bearing was gappy. Plants similar to noncluster normal bearing parents were recovered in plenty. Among such plants, short plants were isolated which attained a height of about 3' to $3\frac{1}{2}$', with short multinodal sympodia arising in ascending order starting from the lower portion of the plant (about 6" above the ground), and closely spaced nodes. Bolls borne on these plants were bolder than in either of the parents. Two crosses, namely H14 × PRS-72 and 5143C × PRS-72, threw such desirable recombinants at higher frequencies than did other crosses.

Penetrance and Expressivity of the Cluster Gene

As described above, penetrance of the gene which controlled cluster boll bearing habit was complete. In the homozygous condition, for which the symbol may be cl_1cl_1, the sympodium terminated in a cluster of two or more bolls. The differing pattern of expression of this character has already been described above. Since the F_1 was as normal as the normal bearing parents, the homozygous dominant and heterozygous combinations, $Cl_1\ Cl_1$ and $Cl_1\ cl_1$ gave noncluster normal bearing habit.

Though the cluster-bearing plants had their bolls borne in one cluster of more than one boll on one sympodium, wide variation in the length of the boll bearing branches gave varying intensities of clustering, from extreme clustering (i.e., bearing right on the main stem) to sparse clustering (where the bolls are set away from the stem at varying lengths). Frequency distribution of sympodium length in the cluster-bearing parent (PRS-72) and the segregants recovered in F_2 and $B_2 = (P_1 \times P_2 = \text{PRS-72}) \times \times P_2$, of the five different crosses, are presented in Fig. 3 and 4, respectively. The average sympod length of P_1 was about seven times the average sympod length of P_2 (PRS-72), their average values being about 15 cm and 2 cm, respectively. Among the P_1s, 419/49 had the shortest sympods (9.41 cm). The sympods of F_1s were

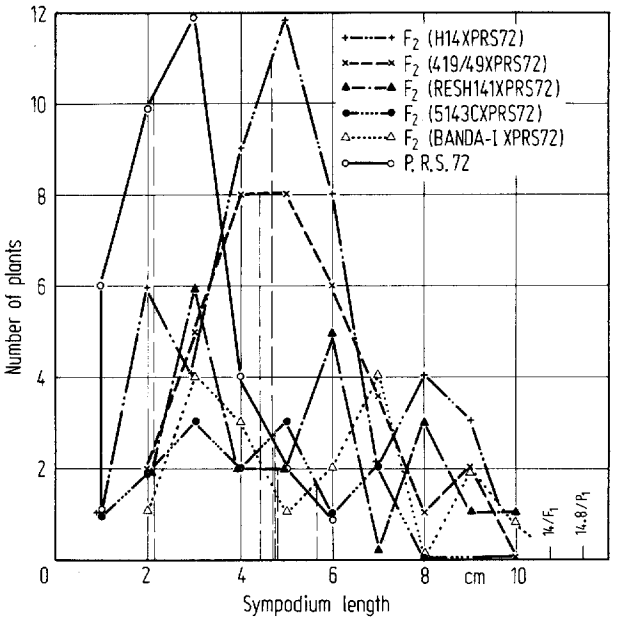

Fig. 3. Frequency distribution of sympodium length for PRS-72 and five cluster-bearing F_2 populations

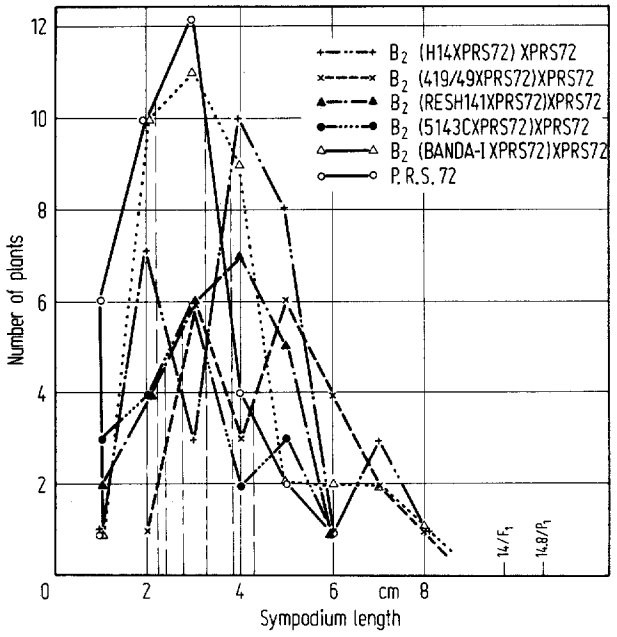

Fig.4. Frequency distribution of sympodium length for PRS-72 and five cluster-bearing backcross populations

almost as long as those in their normal parents (P_1s). This indicates that in the crosses studied, which had as one parent the cluster-bearing PRS-72 with extremely short sympods, longer sympods were dominant over the short sympods of the cluster parent. The situation might be different if the inheritance of sympod length was studied within the noncluster types.

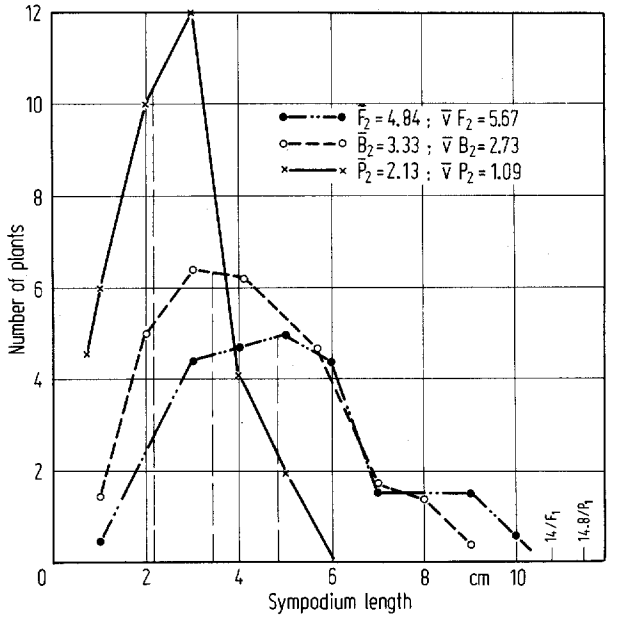

Fig.5. Frequency distribution of sympodium length for PRS-72 and cluster-bearing F_2 and B_2 populations

In none of the cluster type plants did sympod length come within the range of the noncluster parents, except in 419/49. The mean length of cluster boll-bearing branches increased from 2.13 cm in P_2 (PRS-72) to 3.33 cm in the $B_2(P_1s \times PRS\text{-}72) \times PRS\text{-}72$, and to 4.84 cm in the F_2s (Fig.5). This means that the reduction in sympod length of the cluster-bearing plants was brought about by the increased proportion of the gene complex from PRS-72. In other words, if one hundred per cent of the gene complex of PRS-72 was capable of giving 2.13 cm long sympods, the cluster-bearing F_2s, which had only 50 per cent of the PRS-72 gene complex, expected to have about 4.26 cm long sympods; similarly, the backcrosses, which had about 75 per cent of the PRS-72 gene complex, would be expected on average to produce cluster-bearing sympods measuring about 3.19 cm long. The observed average sympod length of the cluster-bearing plants of the B_2s and F_2s was very close to the expected value (O : E = 3.33 : 3.19 for B_2s and 4.84 : 4.26 for F_2s), so it seems that sympod length of cluster-bearing plants is controlled by the gene complex of the parent which donates the cluster-bearing habit.

The effect of genetic background of the noncluster parent on the sympodium length of the cluster-bearing segregates is also evident. The average sympod length of the different progenies within the two segregating generations, i.e., F_2 and B_2 varied, as given below:

Cross	Average sympod length (cm)	
	F_2	$B_2 = F_1 \times$ PRS-72
H14 × PRS-72	4.68	3.87
ResH141 × PRS-72	4.72	2.70
419/49 × PRS-72	4.42	3.34
5143C × PRS-72	4.66	2.44
Banda-I × PRS-72	5.72	3.25

The mean values of sympod length in the different crosses in the two segregating generations, as shown above, indicate that relative values were not consistent in the two generations, except for 5143C × PRS-72 which had shorter sympods both in the F_2 and B_2. Banda-I × PRS-72 had the longest sympods in F_2. The cross 419/49 × PRS-72, which had the shortest sympods in F_2, had the longest in the B_2.

The relative dispersion of the different crosses in the two segregating generations, i.e., F_2 and B_2, have been given in Figs. 3 and 4. The dispersion pattern indicates that the crosses of PRS-72 with H14, 419/49 and Banda-I had lower variances. A comparison of the mean variances of the P_2 (PRS-72), F_2 (VF_2) and B_2 (VB_2) may be made from Fig. 5. It seems that F_2 had about three times as much variance as PRS-72 while the B_2 falls between P_2 and F_2.

Discussion

The monogenic recessive inheritance of cluster boll bearing on shortened sympodia has been confirmed. It was observed that the pattern of clustering varied, depending upon the number of bolls in a cluster and length of the fruiting branch (sympod). All the recessive homozygous plants of the genotype $cl_1\,cl_1$ had their sympodia terminating in a cluster of bolls, but the length of the sympods varied from cross to cross and from generation to generation. Thus, while the penetrance of the "cluster" gene is complete, its expressivity in terms of the extreme shortening of the sympod, seen in the donor parent, is variable in the segregants. Therefore, the present study indicates that though the cluster bearing habit is controlled by a major gene, the length of the fruiting branch is governed by the genetic background or minor genes, the so-called modifiers.

The cytogenetic makeup of the amphidiploid New World cottons (*G. hirsutum* and *G. barbadense*) has great bearing on the expression of characters like cluster boll bearing. The fruiting branch structure in New World cotton is controlled by duplicate loci, cluster (Cl_1) in the D genome and short branch (Cl_2) in A genome. Cluster (cl_1) is a recessive mutant in *Gossypium hirsutum* and (cl_2) is a recessive mutant in *G. barbadense*. Both mutants follow a monohybrid segregation within the species with their duplicate nature discovered only when two species are crossed (Stephens, 1951, and Dalton, 1966). The genotypes of the two species for this character may be written as cl_1cl_1/Cl_2Cl_2 and Cl_1Cl_1/cl_2cl_2, for *G. hirsutum* and *G. barbadense*, respectively. The normal allele of one locus cannot mask the mutant allele of the opposite locus. In the present study, therefore, the cross was made between cl_1cl_1/Cl_2Cl_2 (cluster) × $Cl_1Cl_1/Cl_2^*Cl_2^*$ (non-cluster). For a segregant to be cluster-branched it must inherit its cl_1cl_1 from PRS-72, while the Cl_2 loci may exist in one of the three possible forms, viz., Cl_2Cl_2 or $Cl_2Cl_2^*$ or $Cl_2^*Cl_2^*$, in the proportion of 1:2:1. Of course no information is available on whether there may be any difference in the potentialities of Cl_2 and Cl_1^*. However, in the PRS-72 cluster donor parent the cl_1cl_1 is evolved and adjusted to the Cl_2Cl_2 condition and to its specific genetic background which gives extremely shortened uninoded cluster-bearing branches. It is possible that $Cl_2^*Cl^*{}_2$ and $Cl_2Cl_2^*$ may show differential interaction with cl_1cl_1 and thus the length of fruiting branch may differ compared with the cl_1cl_1/Cl_2Cl_2 combination. It was also observed that with an increasing proportion of PRS-72 gene complex in the cluster type segregants (B_2), the length of the sympod was proportionately reduced. It is also expected that with an increasing proportion of PRS-72 gene complex in the progenies through backcrossing to the cluster parent, the frequency of cl_1cl_1/Cl_2Cl_2 proportions will increase. Therefore, it is likely that, as well as the probable change in the strength of the duplicate Cl_2 gene, a change in the balance of modifiers is very important in changing the lengths of the boll-bearing branches. This suggests that there is a dosage effect of the modifiers and the interaction of Cl_2 locus with cl_1cl_1 genotype which govern the length of cluster bearing sympods. The present findings support the interpretation proposed as 'dosage and interaction' by Dalton (1966) to explain the behaviour of cluster and short fruiting branch habit of interspecific and intraspecific crosses of New World cottons.

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* To differentiate the material and paternal Cl_2 allele.

The fruiting branch lengths of the cluster segregants varied from cross to cross. This further suggests the importance of the quantitative background of genes with small individual effects. The genetic system of cluster-bearing, with one major gene associated with several modifiers, emphasizes the importance of the genetic background which may allow the optimum expression of the gene. In the present study the cross 419/49 × PRS-72, in which the P_1 had the shortest sympods among the normal types, did not necessarily have cluster segregants with the shortest fruiting branches. This suggests that the genes governing sympod length in the normal types have little to do with the length of the cluster-bearing sympods.

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Received January 27, 1975
Communicated by H. Stubbe

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