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Gene Arrangement in the Duplicate Linkage Groups V and IX: Nectariless, Glandless, and Withering Bract in Cotton[1]

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ABSTRACT

Genetic investigations of Upland cotton, *Gossypium hirsutum* L., have established that the genes controlling the expression of nectariless (ne_1, ne_2), glandless (gl_2, gl_3), and withering bract (bw_1, bw_2) are at duplicate loci in homeologous chromosomes. Three-point linkage tests in these homeologous linkage groups indicate that the gene order was probably Gl_2-Bw_1-Ne_1 in the A genome and Bw_2-Gl_3-Ne_2 in the D genome. However, recombination percentages were not significantly different between Bw_1-Ne_1 and Gl_2-Ne_1 in the A genome, and between Bw_2-Ne_2 in the D genome.

Additional index words: **Three-point linkage tests, Recombination, *Gossypium hirsutum* L.**

THE absence of pigment glands in the aerial portion of the cotton plant (*Gossypium hirsutum* L.) is conditioned by two recessive genes, gl_2 and gl_3 (McMichael, 1960). Lee (1962) studied expression of the gl_2 and gl_3 loci in cotyledons and proposed that additional loci modified the density of gland expression. However, when gl_2 and gl_3 are homozygous recessive, the plant is devoid of pigment glands throughout its life-cycle.

Rhyne (1962) reported the association of gl_2 and withering bract (bw_1) to establish Linkage Group V in cotton. Withering bract was known only as a simple recessive mutant in *G. hirsutum*, but Lee (1965) and Rhyne (1965) were able to transfer normal alleles into *G. hirsutum* from *G. raimondii* Ulbr. and *G. thurberi* Tod., respectively, both D-genome species.

The introduction of the normal Bw_2 allele into *G. hirsutum* from the D genome established that the withering bract expression is conditioned by duplicate loci; however, all *G. hirsutum* lines tested have the recessive bw_2 allele. Rhyne (1965) found Gl_2-Bw_1 associated with the A genome. These findings demonstrate that the duplicate loci are associated with homeologous chromosomes. Linkage Group V (gl_2-bw_1) is in the A genome and Linkage Group IX (gl_3-bw_2) is in the D genome (Kohel, 1972).

Holder (1967) revealed that the genes ne_1 and ne_2 are linked to gl_2 and gl_3, respectively. The nectariless genes (ne_1 ne_1, ne_2 ne_2) were transferred from *G. tomentosum* Nutt. ex Seem., and in the homozygous recessive condition remove leaf and extrafloral nectaries from the cotton plant (Meyer and Meyer, 1961). Holder (1967) found gl_2 and ne_1 associated with 32.79% recombination, and gl_3 and ne_2 associated with 38.89% recombination.

The linkage of gl_2 and bw_1 has been reported to be between 13 and 17% recombination (Rhyne, 1962). Rhyne (1965) found less recombination (2 to 3%) between gl_3 and bw_2 when he used Gl_3^{thu}-Bw_2^{thu} alleles transferred from *G. thurberi*. Lee (1972) found similar results (2.4% recombination) when he used Gl_3^{rai}-Bw_2^{rai} alleles from *G. raimondii*; however, when he used the introduced alleles in repulsion, he obtained a recombination values of 20.2%.

In this paper, I report the synthesis of the multiple recessive tester line (gl_2 bw_1 ne_1, gl_3 bw_2 ne_2), and its use in three-point linkage tests of Linkage Groups V and IX.

MATERIALS AND METHODS

To conduct the three-point linkage tests in each of these linkage groups, a tester line, homozygous recessive for glandless, nectariless, and withering bract was synthesized. The parental lines used to synthesize the tester line and used to test the individual linkage groups were gl_2 bw_1 Ne_1, Gl_3^{rai} Bw_2^{rai} Ne_2^{rai} (obtained from J. A. Lee); and Gl_2 Bw_1 Ne_1, gl_3 bw_2 ne_2 and gl_2 bw_1 ne_1, gl_3 bw_2 Ne_2 (obtained from J. N. Jenkins).

Glandless classification was based on that proposed by Lee (1962). Seedlings were scored for cotyledonary glanding in the greenhouse and then transplanted to the field. The relative classification of Lee's was effective, but individual populations of different origin varied with respect to their overall level of glandedness. Caution had to be used when classifying glandless segregation in these diverse populations. These observations support Lee's (1962) proposal of modifier loci that influence the level (density) of glandedness.

Scoring for nectariless was based on the Lafever-Holder classification (Holder, 1967). Plants were scored at the full flowering stage. Variation observed in nectariless expression between populations of different origins, similar to that found in scoring for glandedness, suggested that modifier loci influenced the level of nectariless expression.

Gene control of withering bract expression is that of completely recessive duplicate factors. No intermediate level of expression was noted.

Seeds of the material used in these tests were germinated in peat pellets in the greenhouse; at 3 weeks, they were transplanted to the field nursery. In the field, 20 plants were spaced 45 cm apart in rows spaced 1 m apart.

Chi-square analyses were used to measure goodness-of-fit for the segregations, and least-squares analyses were used to compute recombination values.

RESULTS AND DISCUSSION

The multiple recessive tester line (gl_2 bw_1 ne_1, gl_3 bw_2 ne_2) synthesized to facilitate these tests was not vigorous, which seemed to be a consequence of the expression of the withering bract mutant. Because the mutants in question were combined into a single line with coupling linkage, the lack of vigor made working with them difficult and influended the recovery and identification of mutant segregants. We saw a significant deficiency of homozygous bw_1 bw_2 plants (Table 1).

The gene order in Linkage Group V (gl_2 bw_1 ne_1) was tested with the standard TM-1 (Gl_2 Bw_1 Ne_1, Gl_3 bw_2 Ne_2) (Kohel, Richmond, and Lewis, 1970) as the

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Table 1. Segregation and analysis of alleles at the gl_2, bw_1, and ne_1 loci in the cotton cross [$Gl_2Bw_1Ne_1$; $Gl_3bw_2Ne_2$ × $gl_2bw_1ne_1$, $gl_3bw_2ne_2$] $gl_2bw_1ne_1$, $gl_3bw_2ne_2$.

	Segregation (No. of plants)									
Phenotypic class	1972a	1972b	1973a	1973b	1976a	1976b	1978	Summation		
$Gl_2Bw_1Ne_1$	33	24	23	13	18	22	36	159		
$Gl_2Bw_1ne_1$	29	13	14	9	17	12	16	110		
$Gl_2bw_1Ne_1$	1	4	2	2	3	1	2	15		
$Gl_2bw_1ne_1$	6	4	1	0	1	2	5	19		
$gl_2Bw_1Ne_1$	6	4	3	11	13	6	4	47		
$gl_2Bw_1ne_1$	17	6	3	8	5	4	0	43		
$gl_2bw_1Ne_1$	21	13	7	5	6	6	12	70		
$gl_2bw_1ne_1$	48	23	10	16	14	9	32	153		
	161	91	63	64	77	62	98	616		
Chi-square analysis										
Source of variation									Pooled	Heterogeneity
Gl_2	3.78	0.01	4.59*	4.00*	0.01	2.32	0.00	14.21	0.16	14.05
Ne_1	9.45*	0.01	0.78	0.06	0.12	1.03	1.02	12.47	1.88	10.59
Bw_1	0.50	0.10	8.40*	5.06*	10.92*	10.90*	0.37	36.25	16.89*	19.36*
Gl_2Ne_1	8.50*	5.31*	2.68	3.06	0.12	1.61	5.33*	27.66	24.96*	2.70
Gl_2Bw_1	63.36*	33.24*	32.14*	7.56*	14.14*	20.90*	58.94*	230.28	219.84*	10.44
Ne_1Bw_1	38.20*	3.97*	1.92	4.00*	2.92	4.13	14.73*	69.37	31.82*	38.05*
$Gl_2Ne_1Bw_1$	0.30	0.99	0.40	2.25	3.75	0.58	1.47	9.74	1.88	7.86
Recombination percentage										
Gl_2Bw_1	18.63	19.78	14.28	32.31	33.76	20.96	11.22		20.12 ± 1.62	
Bw_1Ne_1	42.24	39.56	41.26	37.81	40.25	37.09	30.61		38.63 ± 1.96	
Gl_2Ne_1	38.51	37.36	39.68	39.06	48.05	41.93	37.75		39.93 ± 1.97	

* Chi-square values are greater than those expected with P = 0.05.

Table 2. Segregation and analysis of alleles at the gl_3, bw_2 and ne_2 loci in the cotton cross [$bw_1gl_2Ne_1$, $Bw_2^{rai}Gl_3^{rai}Ne_2^{rai}$ × $bw_1gl_2ne_1$, $bw_2gl_3ne_2$] $bw_1gl_2ne_1$, $bw_2gl_3ne_2$.

	Segregation (No. of plants)					
Plenotypic class	1972	1973	1978	Summation		
$Bw_2Gl_3Ne_2$	57	23	35	115		
$Bw_2Gl_3Ne_2$	34	12	11	57		
$Bw_2gl_3Ne_2$	1	3	0	4		
$Bw_2gl_3ne_2$	1	2	1	4		
$bw_2Gl_3Ne_2$	2	2	1	5		
$bw_2Gl_3ne_2$	3	0	1	4		
$bw_2gl_3Ne_2$	33	12	14	59		
$bw_2gl_3ne_2$	47	25	34	106		
	178	79	97	354		
Chi-square analysis						
Source of variation					Pooled	Heterogeneity
Bw_2	0.36	0.01	0.09	0.46	0.10	0.36
Gl_3	1.10	0.32	0.11	1.53	0.18	1.35
Ne_2	0.36	0.01	0.09	0.46	0.41	0.05
Bw_2-Gl_3	151.10*	53.48*	85.37*	289.95	289.26*	0.69
Gl_3-Ne_2	7.28*	7.91*	20.88*	36.07	31.74*	4.33
Bw_2-Ne_2	8.11*	6.69*	19.06*	33.86	30.55*	3.31
Bw_2-Gl_3-Ne_2	0.56	0.32	0.26	1.14	0.28	0.86
Recombination percentage						
Bw_2-Gl_3	3.93	8.86	3.09		4.80 ± 1.13	
Gl_3-Ne_2	39.89	34.18	26.80		35.03 ± 2.54	
Bw_2-Ne_2	39.32	35.44	27.84		35.31 ± 2.54	

* Chi-square values are greater than those expected with P = 0.05.

donor of normal alleles in the test crosses. Seeds of the testcross populations were produced, and four populations were grown in 1972 and 1973 (Table 1). These populations clearly confirmed the linkage of gl_2 with bw_1, but the placement of ne_1 with respect to gl_2-bw_1 varied among the populations. Because I could not clearly distinguish the gene order from these populations, I grew additional testcross populations in 1976 and 1978. In the 616 plants classified in the four years, the probable gene order was Gl_2-Bw_1-Ne_1. The pooled estimated recombination between Gl_2 and Bw_1 was 20.12%, which compared favorably to the 13-17% observed by Rhyne (1962) in F_2 populations. The Gl_2-Ne_1 recombination was 39.93% compared to 32.79% measured by Holder (1967). The Bw_1-Ne_1 recombination estimate was 38.63% which places Bw_1 between Gl_2 and Ne_1. However, the latter two estimates were not significantly different from each other and caution should be used in interpreting these data on gene order.

Gene order in Linkage Group IX was studied with the Gl_3^{rai} Bw_2^{rai} Ne_2^{rai} alleles transferred into *G. hirsu-*

Table 3. Segregation and analysis of native *G. hirsutum* alleles at gl_3 and ne_2 loci n the cotton cross [$Gl_2Bw_1Ne_1$, $Gl_3bw_2Ne_2$ × $gl_2bw_1ne_1$, $gl_3bw_2ne_2$] $gl_2bw_1ne_1$, $gl_3bw_2ne_2$.

	Segregation (No. of plants)								
Phenotypic class	1972a	1972b	1973a	1973b	1976a	1976b	Summation		
Gl_2Ne_2	29	29	19	21	27	24	149		
Gl_2ne_2	51	22	12	20	23	8	136		
gl_2Ne_2	32	16	16	10	14	18	106		
gl_2ne_2	49	24	16	13	16	15	133		
	161	91	63	64	80	65	524		
Chi-square analysis									
Source of variation								Pooled	Heterogeneity
Gl_2	0.01	1.33	0.02	5.06*	5.00*	0.02	11.44	4.04*	7.40
Ne_2	9.44*	0.01	0.78	0.06	0.05	5.55*	15.89	0.37	15.52*
Gl_2Ne_2	0.16	2.47	0.78	0.25	0.45	2.60	6.71	3.05	3.66
Recombination percentage									
Gl_2Ne_2	51.55	41.75	44.44	46.87	46.25	40.00		46.18 ± 2.18	

* hi-square values are greater than those expected with P = 0.05.

tum from *G. raimondii* by J. A. Lee. The total distance between the markers in this linkage group was reduced compared to that in its homeologue (Table 2). Recombination between Bw_2 and Gl_3 was 4.80%. This value compares to 2 to 3% for coupling linkage observed by Lee (1972) and Rhyne (1965). As in the case of the homeologous linkage group, Ne_2 is not readily placed with respect to Bw_2 and Gl_3. The pooled estimate identified the gene order as Bw_2-Gl_3-Ne_2, but the recombination values between Ne_2 and Gl_3 or Ne_2 and Bw_2 were not significantly different.

The use of the introduced chromosome segment from *G. raimondii* reduced the amount of crossing over. Bw_2^{rai} and Gl_3^{rai} and their associated chromosome segment were known to originate from *G. raimondii,* but there was no way to monitor the segment to the Ne_2 locus because both *G. hirsutum* and *G. raimondii* carried the normal alleles. The pooled estimate of the recombination between Gl_3 and Ne_2 in *G. hirsutum* was 46.18% (Table 3). Holder (1967) established 38.89% and Rhyne and Rhyne (1972) estimated 40%, compared to 35.03% in the introduced segment. The total recombination in the introduced segment was reduced, as C. L. Rhyne (personal communication) verified for Gl_3^{thu}-Ne_2^{thu}. However, the reduction was not sufficient to significantly enhance the discrimination of the position of Ne_2 with respect to Gl_3 and Bw_2.

The results of these tests place the gene order in Linkage Group V as Gl_2-Bw_1-Ne_1 and in Linkage Group IX as Bw_2-Gl_3-Ne_2. The reduced recombination between Gl_3 and Bw_2 and the different gene order in Linkage Group IX compared to its homeologue suggest a possible inverted segment. However, Lee (1972) has shown that the Gl_3-Bw_2 recombination was increased when repulsion linkage of the introduced alleles was used. Rhyne (1962) also observed similar behavior in recombination of the Gl_3^{thu}-Bw_2^{thu} alleles from *G. thurberi.* It has not been established, however, whether this phenomenon was caused by reduced recombination due to an inverted segment in *G. hirsutum* or to foreign chromatin per se. The size of the discrepancy is such that one would expect observable sterility and imbalance in gametic frequency associated with an inversion.

Additional marker loci are needed to aid in establishing gene order and total length of these linkage groups. Turcotte and Feaster (1979) established the association of *Male-sterile-11* and members of Linkage Group XIII with Linkage Group V. Similar homeologues for Linkage Group IX have yet to be identified, although Rhyne and Rhyne (1972) identified an indehiscent anther mutant linked with nectariless with the gene order Gl_3-40-ne_2-16-*ms*. As additional associations are identified, they will provide a means to establish the relationship between these homeologous chromosomes.

Cytogenetic research may be useful in resolving the unanswered questions regarding these homeologous chromosomes. J. E. Endrizzi (personal communication) has identified chromosomes 12 and 26 as associated with Linkage Groups V and IX, respectively. Appropriate translocation or telosome stocks, once identified, can be used to establish the gene order and recombination values of the linkage groups.

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