UNEXPECTED SEGREGATION INVOLVING A TEXAS MARKER STOCK

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Abstract

A gene for tolerance to herbicide was assigned to chromosome 1 using a Texas marker stock having the morphological markers L_1^L, lp_1 and Lc_x. This dominant marker was independent of the leaf shape marker on the short arm and 33.5 ± 9.2 cM from cotyledonary marker Lp_1 that is on the long arm. The lc_x for white lint was associated with Lp_1 of the tolerant parent such that $lc_x lc_x$ was common to plants unharmed by herbicide. The Lc_x for brown lint was independent of dominant leaf shape, but *circa* 30 ± 10 cM from the gene for tolerance. The undisturbed Lp_1 - L_1^L segregation for normal chromosome 1 reported in Endrizzi and Stein (1975) became a disturbed one in the present study. This disturbance could be attributed to foreign DNA. Since Lc_x has not been assigned in a formal manner to chromosome 1 and the three other brown linked markers have been placed on separate chromosomes, the Lc_4 is deduced for presence on chromosome 1. The lp_2 of chromosome 15 is part of a linkage group with multiple alleles for the green lint. The symbol for the dominant tolerance is pending instructions.

Introduction

In the course of investigating an association of Le_2^{dav} with Ne_2, the possibility developed that some recombination of Le_2^{dav} with ne had been overlooked (Rhyne and Tietjen, 1997). Therefore we evaluated current Le_2^{dav} cultivars also. When tester Le_2^{dav} Ne_2 female was pollinated with a new herbicide tolerant '5096' no offspring were produced. The apparent failure was paucity of pollen. We tested this on a stink-bug susceptible T stock that netted some seed. Most seed were faulty because of injury by the insects. A single F_1 plant failed to retain many selfed capsules (these seeds were introduced into the maintenance nursery) and the susceptible Texas marker stock T became pollen sterile in the drought. Fall rains allowed open pollination on the F_1. The open pollinated seed were planted in a field of cultivar that was sprayed with Round-up herbicide. The F_2 segregated, indicating herbicide tolerance, and various markers of the susceptible T parent were expressed also. However, the marker dd (lp_1 $lp_1lp_2lp_2$) was not detected. Its absence might be attributed to an inability to recognize the marker, to a depauperate result of herbicide, or even to a parallel to our study of foreign Le_2 DNA failure to recombine with ne in chromosome 26 (Rhyne and Tietjen, 1997). The Texas marker stock T as female was crossed again with herbicide tolerant and its F_2 examined for expression of the markers of the female parent. Meanwhile, we confirmed that Paymaster 2820 and a Sure Grow conventional produced seedling lethals with Le_2^{dav}, possibly indicating that our estimate of $Le_2^{dav}ne_2$ recombination was low.

The current study was a further evaluation of the absence of dd in the F_2.

Materials and Method

Texas T stock was crossed as female with the tolerant (H-) which had none of these useful markers. The T stock was also crossed with a 'conventional' (hh) to obtain an F_2 population for comparative purposes. The Texas marker stock T was contributed by Dr. R. J. Kohel for investigations of segregates in Le_2^{dav} harboring genotypes. According to work of J. E. Endrizzi *et al.* (1985), its markers were on chromosome 1 and chromosome 15. One marker was not listed, and its presence on these chromosomes was not documented in the later literature.

Reprinted from the *Proceedings of the Beltwide Cotton Conference*
Volume 1:426-429 (2001)
National Cotton Council, Memphis TN

An early planting study plot was established in a field in which "the old reliable Round-up 5096" was planted. Planting was in April. A severe drought occurred and no irrigation was applied in this field. Texas T marker stock, F_1s, as well as the F_2s were planted.
A second study plot was established in a field in which "New Round-up Ready" was planted. This planting was in June and followed the harvest of green beans, thus the field had good soil moisture. This field was irrigated during the growing season. The larger remnant of the F_2 was spaced out. A small sample of conventional (hh) was planted for a check population. The F_2 of T and conventional c was not included in this plot

In both study plot fields, herbicide application, defoliation, and picking were timely but in the hands of the experienced farmer. Classification counts for genetic analysis were made before and after herbicide application.

Results

Examination of the parents, the F_1s, and the literature (eg. Endrizzi, *et al.*, 1985) indicated that the Texas stock (T) had markers A, C, and d; the tolerant (t) had a, c, and D. The A (L_1^L) is a leaf character, the H is the expression brought on by herbicide, C (Lc_x) is a lint expression, and D (Lp_x) is the normal cotyledon expression. Numerical scores of F_2 indicate dominance for A, but discrete values for phenotypes AA, Aa, and aa. The C is conventionally classed as dominant, but numerical values show discrete CC, Cc, and cc classes. Thus Cc is closer to cc, HH and Hh are similar and hh is outright kill and/or damaged but slow recovery depending on the seasonal time of application. The dd is a distinct (from normal) cotyledonary character, but it can be tediously verified in floral buds the day prior to and on the day of early flower opening. These features of markers facilitated the genetic analysis.

In the farmer's field of "5096 Round-up" that was sprayed with the herbicide, plants were killed. His cultivar had no damaged plants, but the plants of the T marker stock were killed in the April planting. The F_1 T x t was not damaged. The F_2 segregated as unharmed plants or outright kill. If no herbicide was applied, then there was no expression in susceptible plants (hh). In the June planting, the remainder of the F_2 of Table 1 reacted differently from the April planting. In this field, the "Round-up" cultivar was a newer version and no damaged plants occurred. Susceptible plants had an array of damage ranging from outright kill to burning of leaves , delayed fruiting, and later boll maturity. Table 1 shows the information. A statistical test verified the ratios. A leaf shape ratio 1:2:1 and the H- to hh ratio of 3:1 support the conclusion that dominant H is independent of A. Neither the susceptible T marker stock nor the companion F_2 T x conventional were planted in the June experiment. The June sample of conventional reacted in similar fashion to the hh of Table 1, damaged, delayed, poor recovery.

The segregation seen in Table 2 is for the tolerant H class of plants in each experiment and marker C. Chi square for independence is P 0.01. Using the classification of discrete CC, Cc, and cc and the non separation of HH from H-, the calculation is H linkage with c *circa* 30±10 cM.

In Table 3 the cotyledonary feature for dd was detected prior to herbicide application. Normal D- were counted and the dd marked. It is obvious that the H- to hh ratio is 3:1. The 230 normal D- versus 13 dd plants with typical dd phenotype segregate as 15:1. The good fit to each ratio allows a test of coupling phase linkage and this estimate is 33.5±9.2 cM. In the nursery that has not received any herbicide, the test cross of the limited previous F_2 population, those with D- by the T parent as male showed families with all D- to 0 dd, 1 D- to 1 dd, and 3 D- to 1 dd ratios. Unsprayed, their H- presence is not known.

We find no report of C and D association. Our F_2 of T versus conventional was killed by herbicide in the April test. We found no place to plant its remaining seed as the nursery and most fields were under drought. The critical test of plants in Table 4, where C and dd are in repulsion phase, requires more than 13 dd plants to detect a close association. When we had cc of t parent, we had D_x- by linkage and by its duplication D_y-.

The Table 5 records segregation of monomerics C and A. If C were not on the same chromosome as A, then we should expect independence. Even if C were distal to D_1 on the chromosome, we should expect independence. However, AA or CC plants are the smallest number as if H were a disturbing foreign DNA unit.

Table 6 at bottom, grouped in conventional fashion, has 156 A-D-, 5 A-dd, 52 aaD-, and 4 aadd. The H presence has an interference directly in the loss of dd plants and perhaps elsewhere. The AD recombination / BC ratio is 624/260 and begs explanations.

Discussion

When the original F_2 of T x t was subjected to herbicide application, the expectation was for 50 to 75 percent H- plants. Similarly, A- could be nearer 50 percent and many Cc but few CC plants. But dd should not be absent. The D- would be increased by D (with aa and cc), but a few dd must be present even if t were $D_1D_1D_2D_2$. The original F_2 had been derived from late season outcrossing. However, our lesson from $Le_2^{dav}Ne_2(Ms_9)$ persistence (Rhyne and Tietjen, 1999) suggested the possibility that D_x and H traveled together. The unlikely probability for such was the long and short arms of chromosome arms of chromosome 1 versus the other 50 long and short arms of T tester. We repeated T x t F_2 and managed but a small population. If a 15 to 1 dd ratio of genes in repulsion phase should be the standard, then we had a population that limited our information. Table 1 indicated A and H were not both in short arm of chromosome 1 since they were acting independently. Table 2 indicated the cc of the t parent was traveling with H. This CC of T might be one of several altered lint markers found in cotton.

Table 3 indicates H and D_x of D_1D_2 are associated. The dd is hardly a marker to employ in field experiments. Its abnormal anther feature and anthers that fail to dehisce render it prone to outcrossing. Verification by crossing with T stock, as we did to determine that t was $D_1D_1D_2D_2$, in the earlier F_2, still required herbicide application to discriminate D_1 from D_2.

Table 4 indicates that dd traveled with C for 9 scoreable of 13 dd plants had C- phenotype. D_x and H provided the cc that is a feature of this tolerant cultivar.

Table 5 indicates that the C of T stock is not close to A that is on the short arm of chromosome 1. Table 6 suggests that the well behaved A of T stock in the short arm is disturbed by H DNA in chromosome 1. A and D apparently showed no disturbance in euploid investigations (Endrizzi and Stein, 1975) that accompanied a placement of D_1 marker on chromosome 1. Because chromosome 15 is the carrier of green lint and Lp_2, and no known C (brown lint) counterparts, we must deduce that H is in the long arm of chromosome 1. A personal communication with Dr. R. J. Kohel is that this T stock had Lc_4. Three brown linted genes mark three linkage groups and three chromosomes (Endrizzi *et al*., 1985). The T stock had the A_2 leaf shape marker L_1^L transferred by the author and placed in the Endrizzi hands. Endrizzi and Stein (1975) indicated that L_1^L is on the short arm of chromosome 1 and the Lp_1 on the long arm proximal the centromere. Thus, lp_1 and Lc_4 are distributed on the long arm. The t stock has the common broad leaf l_1, D_1 H and lc_4. The length of H DNA is undetermined.

Summary

Absence of one of four morphological markers and altered segregation ratios in an F_2 that involved a Texas marker stock led to a repetition of the cross and growing the F_2 under field conditions. One portion of the F_2 growing under drought conditions had a herbicide expression more severe than the larger portion that was grown under irrigation after late planting. The previously absent marker was present in each portion segregating in the dimeric 15 to 1 ratio. That its absence in the earliest F_2 was due to an under sized population was discarded as an explanation when the leaf shape marker segregated in standard fashion as did the marker for herbicide damage. The L_1^L was 1:2:1 in H- and hh classes. The D- and dd classes reflected the emergent F_2 population. The depletion of the population by herbicide showed that linkage of H was coupled with D_x of the dimeric D_1D_2 in the tolerant parent.

The H marker was responsible for presence of white lint lc_x in excess. Lc_x in a depleted number was associated with herbicide damage hh gene type. The association using the 1:2:1 for Lc_x levels was *circa* 30±10 cM. The presence of H disturbed Lp_x segregation such that $L_1^L$of the short arm of chromosome 1 showed excess recombination with lp_1. In effect, L_1Lp_1 and l_1lp_1 became coupled although normally independent. When non-hirstum DNA with a marker gene was inserted, adjacent marked segments had increased recombination (Rhyne, 1958 and 1960, Lee, 1972). Inserted non-hirstum DNA of B_1 *Gossypium* marked with R_2^G interfered with LC_1 frequency on the chromosome now numbered 7 (Rhyne, 1951). The herbicide tolerant gene H is transgenic in donor “5096".

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Acknowledgements

For linear row space in cultivars, herbicide spraying - undergirded this research - courtesy of Roy F. Daniel Jr., Americus, Georgia 31709. For interest and support of members of the Department of Biology, Georgia Southwestern State University, Americus, Georgia 31709: Drs. Elisabeth Elder, Rebecca Freund, and Alicia Lesnikowska. Especially for critical input of manuscript by Emeritus Professor W. L. Tietjen, Jr.

Table 1. F, plants with A and H markers in April and June experiments

	AA	Aa	aa	//	total
April experiment					
H-	15	27	11	0	53
hh	0	0	0	20	20
June experiment					
H-	34	60	32	1*	126
hh	4	21	15	4	40

// column are damaged, non-recovering plants

* long lived undamaged seedling lacking cotyledonary bud

Table 2. F, plants with C and H markers in April and June experiments

	CC	Cc	cc	//	total
April experiment					
H-	10	25	17	1	53
June experiment					
H-	18	56	40	12	126
total	28	81	57		166

// damaged, late opening when defoliated

Table 3. F, plants with D and H markers in April and June experiments

	D-	dd	total
April experiment			
H-	51	2	53
hh	17	3	20
June experiment			
H-	121	5	126
hh	41	3	44
Total			
H-	172	7	179
hh	58	6	64

Table 4. F, plants with C and D markers in April and June experiments

	CC	Cc	cc	//	total
April experiment					
D-	10	24	17	1	52
dd	0	1	0	4	5
June experiment					
D-	23	59	46	34	162
dd	2	5	0	1	8
Total					
D-	33	83	63	35	214
dd	2	6	0	5	13

// damaged, late opening when defoliated

Table 5. F, plants with C and A markers in April and June experiments

		CC	Cc	cc	total
AA					
	April	4	8	4	16
	June	5	12	7	24
	total	9	20	11	40
Aa					
	April	4	13	9	26
	June	12	36	20	68
	total	16	49	29	94
aa					
	April	2	5	3	10
	June	14	21	10	45
	total	16	26	13	55
Total					
	April	10	26	16	52
	June	31	69	37	137
Grand total		41	95	53	189

Table 6. F, plants with D and A markers in April and June experiments

		DD	dd	//	total
AA					
	April	15	0		15
	June	36	1		37
	total	51	1		52
Aa					
	April	27	0		27
	June	78	4		82
	total	105	4		109
aa					
	April	9	2		11
	June	43	2		45
	total	52	4		56
Total					
	A-	156	5		161
	aa	52	4		56
Grand total		208	9	4	221

// these plants were identified as dd but were killed before scoring for other characters.