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UNEXPECTED SEGREGATION INVOLVING ROUNDUP TOLERANCE IN TEXAS MARKER STOCK

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Abstract

In the course of investigations involving the association of incompatible and nectariless (Rhyne & Tietjen 1999) data suggesting the location of herbicide tolerance on chromosome one was acquired. This paper describes further investigations of the original population and that discussed by Rhyne 2001. The results indicate that herbicide tolerance is apparently associated with normal palisade and white lint. Only one abnormal palisade, brown-linted individual was homozygous herbicide tolerant. Unexpectedly, individuals showing variegation (as named by Kohel, pers comm 2001) were recovered and investigated. The data indicate that sterility is involved with exceptional inheritance when herbicide tolerant genes are present in TM marker stock. Furthermore, the data clearly indicate that in field situations insect-mediated outcrossing is considerable and should not be ignored.

Introduction

Texas multiple marker stock has three marker loci: laciniate leaf, abnormal palisade, and light brown lint. The first two have been assigned to chromosome one (Endrizzi & Stein, 1975). Light brown lint and herbicide tolerance have not yet been assigned to a chromosome (Endrizzi, Turcotte, and Kohel, 1985).

Although the light brown lint of Texas multiple marker has been conventionally treated as a dominant, it is possible to consistently identify the heterozygotes and the trait has been scored here as brown (Lc_xLc_x) tan (Lc_xlc) and white (lclc). Similarly, individuals heterozygous Ll are clearly distinguishable from either homozygote. In this paper the resulting leaf shape is called lobed.

The original cross was Texas multiple ovule parent: LL lp_1 lp_2 Lc_x ht x herbicide tolerant-5690 cultivar pollen parent. A companion cross was $Le_2{}^{dav}$ nectariless with herbicide tolerant which also produced a single F1, which was phenotypically wildtype. The plant was selfed and produced approximately 1000 seed.

The Texas marker cross produced but a single offspring, with lobed leaves, a normal palisade and light brown lint. The low fertility was at least partly the result of drought conditions. Some flowers of the sole F_1 were self pollinated, and some were test cross pollinated; after September rain the plant also produced 150 seeds as the result of open pollination.

The 150 open pollinated seed of the first cross and 300 selfed seed of the second cross were planted in the field and challenged with herbicide application at standard times and rates to verify their herbicide parentage. The plants were scored for palisade character in the seedling stage (before herbicide application). Leaf shape and lint color were necessarily scored after herbicide application.

Cross 1 and Progeny

Selfing the single Cross 1 F1 resulted in only 4 F_2 plants, which had lobed leaves with normal palisade and very light brown lint. They showed pollen sterility as in Texas marker stock. Backcrossing in both directions resulted in no progeny in either case.

Test-cross pollination resulted in 20 plants, whose phenotypes are described in Table 1. Note that the 10 individuals with normal palisade were scored brown if any lint color was detectable, although later work led to the regular scoring of heterozygotes as tan. There was a noticeable number of aborted seeds (motes).

This test cross shows the presence of lp_1 lp_2 which was absent in the herbicide-treated open pollinated F_2 population. It was also detected in the offspring of the plants produced by selfing the Cross 1 F1 as described below.

Plant 1 of the Cross 1 selfed F2 was backcrossed to the Texas marker and self pollinated; it was also allowed to open pollinate and the resulting offspring were herbicide treated. The backcross progeny segregated one to one for abnormal palisade (11 Lp _ to 11 lp_1 lp_2). All had laciniate leaves and brown lint; there were a total of 28 seeds but only 22 viable plants. When Plant 1 was selfed it produced 5 F3 segregating 2 Lp _ to 3 lp_1 lp_2. Neither the backcross offspring nor the selfed offspring were treated with herbicide due to the low number of seed produced by those crosses..

The open pollinated offspring of Plant 1 segregated 19 Lp_ to 1 lp_1lp_2 when herbicide treated. This represents the first occurrence of abnormal palisade in a herbicide treated population. The seed of the abnormal palisade individual was sent to Kohel for confirmation as abnormal palisade and was therefore not available for further analysis.

The 3 abnormal palisade F3 from the "selfing" of plant 1, (labelled A, B, and C for convenience) were verified as to herbicide phenotype by planting approximately 200 seed from each, 100 each in a split-plot design with herbicide application as the treatment variable. We present data from the herbicide treatment only.

Four-fifths of the plants treated with herbicide were killed (Table 2). These data indicate that the ovule parent was homozygous for ht and that the surviving plants must have been cross-pollinated, which also explains the dearth of LL individuals. This is consistent with the high pollen sterility of the lp_1 lp_2 phenotypes. Anther-flecking and non-dehiscent anthers are clearly visible in Picture 2 above.

Table 3 showed that 23 plants were killed by the herbicide application. Of the 62 herbicide tolerant plants 1 could not be scored for variegation or lint color. These data from nursery grown plants suggest that pollen sterility is very high and under these conditions cross pollination is routine. We confirm previous results (Rhyne 2001) showing that herbicide resistance is associated with lc, so that in herbicide treated populations there is an excess of white and tan lint.

Progeny of the Test Cross F2 (Table 1).
Only one of the 10 asterisked individuals in Table 1 produced offspring as the result of self pollination. Note that this individual was originally classed as having brown lint, but was in fact tan (Lc_x lc) The resulting progeny were herbicide treated.

In Table 4, note the low number of plants, the lack of brown linted progeny and the low number of tan at the same time the leaf marker and herbicide resistance show the expected proportions.

100% of the selfed offspring of the abnormal leaf palisade individual from Table 1 scored as having tan lint (ie Lc_x lc) and laciniate leaf shape (LL) were killed by herbicide. The same individual was crossed to Ht ht recurrent. The test cross progeny segregated 10 Ht_ to 4 ht ht., ie statistically 1 to 1. All the offspring had lobed leaves (Ll). Two of the 10 herbicide tolerant plants showed a new phenotype corresponding to variegation sensu Kohel (personal communication, 2001). These plants both had white lint.

The seeds produced by these two variegated individuals were field planted. The resulting offspring are described in tables 5 and 6. Due to the low number of seeds produced by the first variegated individual, no herbicide treatment was applied. Note absence of abnormal leaf palisade (Table 5). The condition described as mosaic is not the same as the parental variegation; the mosaic shows green, yellow and white on every leaf as opposed to variegation which is not present in all leaves. It lives but is sterile (never flowers). The mosaicism is not visible in the cotyledons. A similar variegation with lethality has been shown to be due to a transposon (Palmer, Burzlaff & Shoemakeer, 2000; Imsande et al., 2001)

The data again strongly suggest that these plants are male sterile and all the offspring are cross pollinated, even under field conditions (Table 6a and 6b). These results are not consistent with the low level of cross pollination reported in the literature (Umbeck et al., 1991). In this population we have not recovered a brown-linted abnormal palisade plant showing herbicide tolerance.

Cross 2 and Progeny
Texas multiple marker stock was crossed to the herbicide tolerant F2 offspring from Cross 2, because of the low number of offspring in many generations derived from cross 1. Populations were challenged with herbicide retaining seed from each population. Some were killed in their entirety; some segregated as in Table 8.

Remnant seed from the entirely susceptible populations were grown in the absence of herbicide. Data presented in Table 7 shows that there is an excess of white in the normal palisade individuals but this is not statistically significant. This data confirms the independence of the three marker loci.

In Table 8, the nine individuals showing abnormal palisade were tested for herbicide genotype, as were several of the plants with normal palisade. Only one of the nine proved to be homozygous for herbicide tolerance; as indicated by 100 % survivorship of its offspring under field (herbicide) conditions. It had laciniate leaves and brown lint and was thus homozygous at all marker loci as well. Most of its progeny must have been the result of outcrossing with the 5690 cultivar planted as a crop in the rest of the field because 57 of 75 had normal palisade, lobed leaves and tan lint. In fact the lint of these progeny was barely recognizable as tan except by direct comparison with true white lint. Four more offspring must also have been the result of outcrossing, but to sister plants carrying marker alleles (one abnormal palisade, brown lint but lobed leaves, two normal palisade but brown lint and laciniate leaves and one normal palisade and lobed leaves but brown lint) Only fourteen of the seventy five offspring were identical to the parent. This represents the maximum level of selfing, which could be lower if some of these are also the result of outcrossing to sibs.

All other plants of the population described in Table 8 surrounded by cultivar 5690 showed similar high outcrossing, but herbicide susceptibility.

Conclusions

These genetic investigations have been hindered by problems with sterility, which arises from several sources. First of all, the crosses were carried out during four consecutive seasons of severe drought. Secondly, the abnormal palisade genotype is associated with pollen sterility. Finally, herbicide application is associated with abortion of early flowers even in herbicide tolerant plants. The degree of boll loss is influenced by the timing of herbicide application, with the greatest loss due to herbicide applied after the initiation of flowering. Because of the delay between the initiation of flowers and their appearance, farmers may think it safe to apply the herbicide, when in fact after leaf three herbicide application will result in loss of the early flowers.

Some outcrossing might be expected in significantly pollen sterile plants. However these results suggest that regardless of genotype, outcrossing occurs consistently in field environments and cannot be ignored.

Unexpected segregation for lint color indicates some self sterility in heterozygous individuals (Table 4). In addition, lint color and herbicide tolerance are apparently linked such that herbicide tolerance is associated with white lint.

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Cross 1. Texas multiple x herbicide tolerant. Open pollinated F2.

	Expected segregation	**Observed segregation**
Laciniate leaf L to entire l	3:1	3:1
Green palisade (Lp) to abnormal (lp_1 lp_2)	15:1 or 3:1	All green (Lp _)
Brown lint (Lc_x) to white lint (lc)	3:1	Excess white lint
Herbicide tolerant (Ht) to susceptible (ht)	3:1	3:1

Cross 2. Incompatible nectariless X herbicide tolerant. Selfed F2.

	Expected segregation	**Observed segregation**
Incompatible (Le_2^{dav}) to compatible (le_1 le_2)	All compatible	All compatible
Nectaried (Ne_1Ne_2) to nectariless (ne_1ne_2)	15:1	15:1
Herbicide tolerant (Ht) to susceptible (ht)	3:1	3:1

Table 1. Cross 1(TM) Test cross (equivalent to F2). No herbicide. Leaf shape: 7 LL 11Ll 2 ll

	Lint color	Brown	Tan	White	
	lp_1 lp_2	2	1	0	3
Palisade	Lp_	10*		7	17

Table 2. Offspring of Plant A Genotype LL Lclc

Herbicide tolerance	Palisade	Leaf Shape	Lint Color
81 ht ht			
19 Ht_	17 Lp _	1LL	1 Lc_x Lc_x
		16 Ll	9 Lc_x lc
			7 lc lc
	2 lp_1 lp_2	1LL	1 Lc_x lc
		1 Ll	1 lc lc

Table 3. Offspring of Plant C Genotype Ll Lclc.

Herbicide tolerance	Palisade	Leaf Shape	Lint Color
23 ht ht			
		3 LL	
	52 Lp _	29 Ll	
		20 ll	
			note 0 Lc_xLc_x
		6 Ll	4 Lc_xlc
62 Ht _	9 lp_1 lp_2		2 lc lc
		3 ll	1 Lc_xlc_x
			1 lc lc

Table 4. Offspring resulting from selfing of a testcross F2.

Herbicide tolerance	Palisade	Leaf Shape	Lint Color
16 ht ht			
	34 Lp _	8 LL	0 Lc_xLc_x
		18 Ll	12 Lc_x lc
34 Ht_	0 lp_1 lp_2	8 ll	22 lc lc

Table 5. Open pollinated offspring of variegated individual 1.

Palisade	Cotyledon color	Leaf Shape	Lint Color
33 Lp _	21 green	1 LL	1 lc lc
		5 Ll	5 lc lc
		1 Ll	1 Lc_x lc
		13 ll	13 lc lc
		1 ll mosaic	no flower
	12 yellow (lethal)		
0 lp_1 lp_2			

Table 6a. Open-pollinated offspring of variegated individual 2. Herbicide treated.

Herbicide Tolerance	Palisade	Leaf Shape	Lint Color
ht ht			
85 Ht_	85 Lp _	2 LL	2 lc lc
		42 Ll	3 Lc_x lc
			38 lc lc
			1 no bolls
		40 ll	1Lc_x lc
			36 lc lc
		1 mosaic	no flower
			2 no bolls
	0 lp_1 lp_2		

Table 6b. Open pollinated offspring of variegated individual 2. No herbicide.

Palisade Variegation	Leaf Shape	Lint Color
44 Lp _	5 LL	1 Lc lc
		4 lc lc
	16 Ll	1 Lc lc
		15 lc lc
	23 ll	1Lc lc
		21 lc lc
	1 mosaic *	no boll
0 lp_1 lp_2		

Table 7. TM X Cross 2 derived ht ht No herbicide Leaf shape: 25 LL: 45 Ll : 25 ll

	Brown	Tan	White	
lp_1 lp_2	2	4	3	9
LP_	19	37	28	84

Table 8. TM X Cross 2 derived Ht. Herbicide treated (Data presented at Cotton Conference 2001.) Leaf shape segregated 50 LL: 86 Ll : 43 ll in the three quarters of the plants that survived.

	Brown	Tan	White	
lp_1 lp_2	2	7	0	9
Lp_	33	83	63	179

P of 1:2:1 for lint color is < 0.002.