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Genetic Effects on the Timing of Le_2^{dav} Induced Necrosis of Cotton

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ABSTRACT

Cotton (*Gossypium* spp.) embryos carrying the Le_2^{dav} allele in combination with the Le_1 allele at the Le_1 locus and/or the Le_2 allele at the Le_2 locus undergo a hybrid lethality reaction, which causes progressive necrosis and death between germination up to 21 d postgermination. Timing of the necrosis is thought to be controlled by the cumulative dosage of alleles Le_1 and Le_2 interacting with Le_2^{dav}, but there have been no studies to identify the exact genetic relationship between the alleles Le_1, Le_2, and Le_2^{dav} in terms of timing of necrosis induced by the Le_2^{dav} complementary lethality system. The objectives of this study were to determine: (i) if the timing of necrosis is under genetic control of the loci Le_1 and Le_2, and (ii) if the mechanism of timing or mode of gene action is consistent across genotypes. Five cultivars ($Le_1Le_1Le_2Le_2$) were used as sources of Le_1 and Le_2 alleles. Seedlings from reciprocal crosses of (cultivar × $le_1le_1le_2le_2$) F_1 plants and an $le_1le_1Le_2^{dav}Le_2^{dav}$ tester were scored for the presence and timing of the lethal reaction. Frequency histograms were used to phenotypically group seedlings according to the time of death. Observed frequencies of the phenotypic groups were tested against frequencies expected for digenic segregation. Results indicated that increased dosage of alleles Le_1 and Le_2 with Le_2^{dav} hastens necrosis, but variation among cultivars indicated that Le_1-Le_2^{dav} and Le_2-Le_2^{dav} interactions may not always be distinctly different. Three possible explanations for these differences are: (i) that additional loci are involved, (ii) that allelic action is modified by background genotypic differences, and/or (iii) that the Le_1 and Le_2 loci are polymorphic.

A BETTER UNDERSTANDING of the natures and mechanisms of hybrid lethality and sublethality systems is important because they are widespread among plant families, they entail unusual biological and genetic phenomena, and they have potential usefulness in the genetic improvement of crop plants. Intriguing biological questions arise for example, when one ponders their origin (Lee, 1981a), potential roles in evolution (Dobzhansky, 1941), modes of gene action (Lee, 1981a; Stelly and Rooney, 1989), and molecular and biochemical basis. Previous research alluded to below has made it possible to begin a fairly detailed analysis of one such system, the Le_2^{dav} complementary lethality system described by Lee (1981a).

The American diploid species *Gossypium davidsonii* Kell. ($2n = 2x = 26$, D_3), hybridizes with both cultivated tetraploid cottons, *G. hirsutum* and *G. barbadense*, but all hybrid seedlings become necrotic by the third week after germination (Webber, 1939). This response is caused by the intralocus and interlocus interactions of Le_2^{dav}, the D_3 complementary hybrid lethality system factor, with alleles at the Le_1 and/or Le_2 loci (Lee, 1981a). Little is known about the hybrid lethality system other than its basic genetics (Table 1). The biochemical basis of the lethal reaction is unknown, but Phillips (1977) found it to be temperature conditional. Stelly and Rooney (1989) inferred from phenotypes of chimeric plants that necrosis is due to intracellular events and involves no diffusible substances. Ultrastructural analysis revealed that mitochondria are the first organelles to degenerate during the lethal reaction (Phillips and Reid, 1975). The Le_2^{dav} lethality system has been incorporated into a few glandless *G. hirsutum* lines to preclude contamination from outcrossing with the glanded types, as proposed by Lee (1981b). Stelly et al. (1988) proposed the use of the Le_2^{dav} lethality system as part of a scheme to biologically produce large numbers of doubled haploid progeny, i.e., to produce pure lines cheaply and quickly.

Table 1. Expected genotypes and phenotypes of progeny from the testcross ($Le_1Le_1Le_2Le_2 \times le_1le_1le_2le_2$) × $le_1le_1Le_2^{dav}Le_2^{dav}$, according to genotypes at the Le_1 and Le_2 loci.†

Genotype	Phenotype	Expected genotypic frequency
$le_1le_1le_2Le_2^{dav}$	Viable	0.25
$Le_1le_1le_2Le_2^{dav}$	Necrotic (semiquick death)	0.25
$le_1le_1Le_2Le_2^{dav}$	Necrotic (slow death)	0.25
$Le_1le_1Le_2Le_2^{dav}$	Necrotic (quick death)	0.25

† Alleles at the Le_1 locus include le_1 and Le_1; alleles at the Le_2 locus include le_2, Le_2, and Le_2^{dav}. (Based on Lee, 1981a).

Lee (1981a) proposed that the timing of necrosis was under genetic control. Increasing the number of Le_1 and Le_2 alleles interacting with the Le_2^{dav} allele decreased the length of time between germination and lethality, indicating a dosage effect. Furthermore, one of the two loci, Le_1 or Le_2, was found to exhibit a stronger interaction with Le_2^{dav} than the other. The Le_1 allele was found to exhibit the stronger interaction, based on linkage analysis of the Le_1 and Le_2 loci with the Gl_2 and Gl_3 loci, respectively (Lee, 1982). When 52 *G. hirsutum* cultivars were tested for allelic frequencies at the Le_1 and Le_2 loci, results generally supported Lee's results and inferences, but phenotypic classes were not as clear-cut as described by Lee, and phenotypes varied widely among cultivars (Rooney and Stelly, 1989).

To examine dosage effects and allelic differences more closely, we have screened larger families than we used previously, albeit involving fewer cultivars. We also wished to test our previous observation that marked differences can exist among cultivars in terms of the intensity and speed of the lethality reaction.

MATERIALS AND METHODS

Five *G. hirsutum* cultivars were selected randomly from 52 previously identified as $Le_1Le_1Le_2Le_2$ (Rooney and Stelly, 1989). In the summer of 1987 the cultivars were hybridized with a $le_1le_1le_2le_2$ line. In 1988, F_1 seed from each of the five cultivars (cultivar-F_1 seed) were planted in Jiffy-7 peat pellets and 20 cultivar-F_1 seedlings were transplanted from a greenhouse to the cotton cytogenetics research field in College Station, TX. Forty seedlings of a $le_1le_1Le_2^{dav}Le_2^{dav}$ line were transplanted to the same field. The cultivar-F_1 seed-

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lings were reciprocally crossed to the le_1le_1 Le_2^{dav}, Le_2^{dav} line to produce ca. 200 testcross seed per reciprocal cross segregating at the Le_1 and Le_2 loci. Reciprocal crosses were made to test if timing was affected by cytoplasmic inheritance or maternal effects. Seed were harvested, ginned, and stored by cross at room temperature until used in the seedling screening.

Testcross seed were evaluated for the lethal reaction in a greenhouse during September 1988. Earlier studies indicated some seed may germinate but fail to emerge due to early expression of the lethal reaction. In order to avoid classifying these seed as inviable due to other causes, the seed coat of each seed was removed and each seed was rated for symptoms of the lethal reaction prior to germination (Rooney and Stelly, 1989). Seed were planted by boll, and emergence dates for seedlings were marked daily with color-coded toothpicks. Emergence was defined as the time when the hypocotyl broke the soil surface. Necrosis was defined as the time when growth ceased and the seedling had lost vigor and healthy green color. Daily ratings for the lethal reaction were made for each seedling relative to cultivar parentage and day of emergence. Throughout the evaluation, which lasted 22 d, the seedlings were watered well, treated with fungicide, and maintained at a relatively constant temperature, 28 °C. Statistical analysis and graphics were made using SYSTAT univariate statistical analysis and SYGRAPH graphics software. Chi-square values were calculated without the Yates correction factor.

RESULTS AND DISCUSSION

The five cultivars were $Le_1Le_1Le_2Le_2$ (Rooney and Stelly, 1989), so according to Lee (1981a) the testcross progeny from cultivar-F_1 plants mated with the $le_1le_1Le_2^{dav}Le_2^{dav}$ tester were expected to segregate 1:1:1:1 for the genotypes and corresponding phenotypes noted in Table 1, and to exhibit a phenotypic ratio of 3:1, inviable:viable. Distinctiveness of the inviable genotypes according to the timing of necrosis was expected to depend largely on: (i) differences between Le_1 and Le_2, and (ii) whether or not zygotic dosages of Le_1 and Le_2 would affect their interactions with Le_2^{dav}, and thus, the rapidity of death. Accordingly, we envisioned four models of gene action and corresponding phenotypic expectations:

1. If allelic differences and dosage effects were absent, testcross progenies should segregate 3:1, inviable:viable, without perceptible subclasses for necrotic timing among inviable progenies;
2. If allelic differences were absent, but dosage ef-

Table 2. Testcross segregation data in cotton from reciprocal crosses of ($Le_1Le_1Le_2Le_2$ × $le_1le_1le_2le_2$) × $le_1le_1Le_2^{dav}Le_2^{dav}$, chi-square values for expected segregation ratios, homogeneity, and sums. Classes I, II, III, IV denote the timing-occurrence of necrosis: pre-emergence to 2d, 3d to 8d, 8d or more, and viable seedlings, respectively.

Source	Observed class totals					χ^2 values for segregation ratios			
	I	II	III	IV	Sum	1:2:1	1:1:1:1	3:1	2:1:1
	no.								
× Empire WR-61†	39	95	27	46	207	7.09**	51.77**	0.85	21.46**
Empire WR-61	35	76	28	37	176	5.86*	32.04**	1.49	12.94**
Empire WR-61 Sum	74	171	55	83	383	12.95	83.77	2.34	34.40
Total χ^2						12.85**	83.12**	2.26	33.99**
Homogeneity (1 df)						0.10	0.65	0.08	0.41
× Kasch†	32	80	46	53	211	12.14**	23.09**	0.00	1.27
Kasch	42	55	48	51	196	1.33	1.83	0.11	0.11
Kasch Sum	74	135	94	104	407	13.47	24.92	0.11	1.38
Total χ^2						10.82**	19.08**	0.06	0.79
Homogeneity (1 df)						2.65	5.84*	0.05	0.59
× MissDel†	49	45	49	61	204	2.67	2.83	2.61	2.67
MissDel	42	69	41	43	195	3.21	11.25**	0.90	3.78*
MissDel Sum	91	114	90	104	399	5.88	14.08	3.52	6.45
Total χ^2						1.05	3.93*	0.24	1.29
Homogeneity (1 df)						4.83*	10.15**	3.28	5.16*
×Wilds 15†	63	121	0	45	229	3.58	131.44**	3.50	102.06**
Wilds 15	62	89	0	47	198	4.30*	84.30**	0.17	76.94**
Wilds15 Sum	125	210	0	92	427	7.88	215.74	3.67	179.00
Total χ^2						5.22*	211.77**	2.72	177.93**
Homogeneity (1 df)						2.66	3.97*	0.95	1.07
× Acala†‡	55	117	11	45	228	4.32*	102.88**	3.37	69.16**
Acala	47	98	0	58	203	1.46	96.07**	1.38	70.43**
Acala Sum	102	215	11	103	431	5.78	198.95	4.75	139.59
Total χ^2						1.04	194.16**	0.28	134.89**
Homogeneity (1 df)						4.74*	4.79*	4.47*	4.70*
Variety Sum	466	845	250	486	2047	30.98	512.06	5.56	348.89
Total χ^2						10.38**	356.27**	1.73	215.93**
Homogeneity (4 df)						20.60*	155.79*	3.83	132.96*

* P (χ^2 1df > 3.84) < 0.05.
** P(χ^2 1df > 6.64) < 0.01.
† × cultivar indicates that the cultivar was used as the pollinator parent.
‡ Acala represents the cultivar Acala no. 111 Rogers.

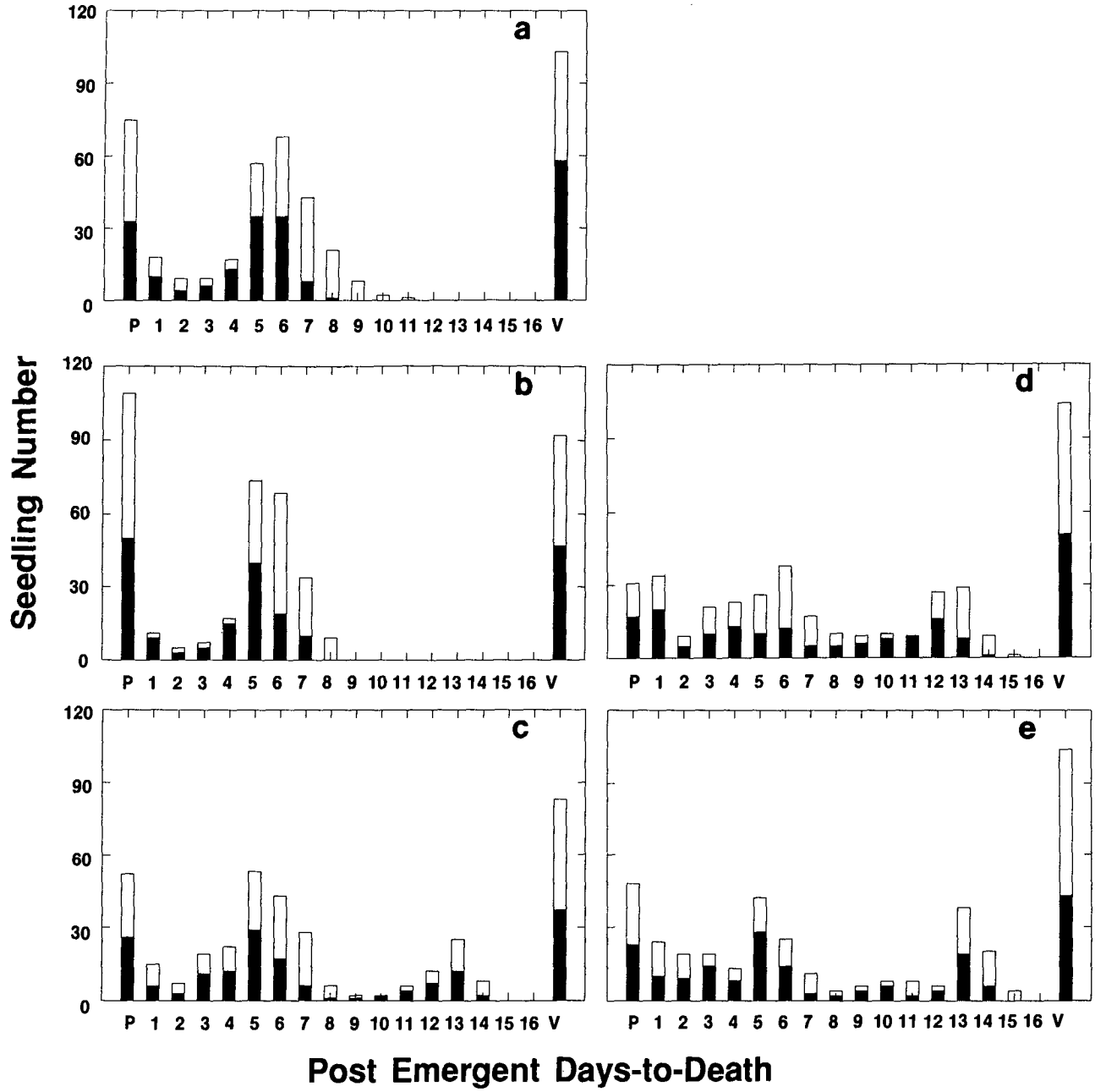

Fig. 1. Frequency histograms showing incidence and timing of necrosis among seedlings from testcross seed, [(cultivar × le_1le_1 le_2le_2) × $le_1le_2Le_2^{dav}Le_2^{dav}$], and reciprocal crosses for five cotton cultivars. The light and dark sections of the bars represent the cultivar F_1 as the female and male parents, respectively. a = Acala No. 111 Rogers, b = Wilds 15, c = Empire WR-61, d = Kasch, and e = Missdel. P = seed which became necrotic prior to emergence, V = viable seedlings. Distributions a and b are trimodal, whereas distributions c, d, and e are tetramodal.

fects existed, testcross progenies should segregate 1:2:1 (quick death:slow death:viable);

3. If allelic differences and dosage effects existed, testcross progenies should segregate 1:1:1:1 (quick death:semiquick death:slow death:viable); and
4. If allelic differences existed but dosage effects were absent, testcross progenies should segregate 2:1:1 or 1:2:1 (quick death:slow death:viable), depending on the relative epistatic strengths of the alleles. If the allele causing semiquick death was epistatic, testcross progeny should segregate in a 2:1:1 ratio, whereas if the allele causing slow death was epistatic, testcross progenies should segregate in a 1:2:1 ratio.

Based on Lee's proposal (1981a) we distinguished four phenotypic classes from graphed data. He described the four classes as: (i) moribund seed or extremely fast death, (ii) necrosis within 3 or 4 d, (iii) normal germination and survival for 1 wk or more, and (iv) viable. We described the four classes as (i) nongermination or death at or before 2 d, (ii) death at or before 8d, (iii) death after 8 d, and (iv) viable.

Segregation for lethality among testcross progenies

Table 3. Expected genotypic frequencies and interaction dosages of Le_1 and Le_2 genes with Le_2^{dav} in endosperm from reciprocal testcrosses of $Le_1le_1Le_2le_2$ with $le_1le_1Le_2^{dav}Le_2^{dav}$. Classes I, II, III, and IV denote the timing/occurrence of necrosis: pre-emergence to 2d, 3d to 4d, 8d or more, and viable seedlings, respectively.

Endosperm genotype	Expected genotypic frequency	*Le* dosage (I)†	Le_2^{dav} dosage (II)	Interaction dosage (I × II)	Embryo genotype§
		$Le_1le_1Le_2le_2 \times le_1le_1Le_2^{dav}Le_2^{dav}$			
$Le_1Le_1le_1Le_2Le_2Le_2^{dav}$	0.25	4	1	4	A
$Le_1Le_1le_1le_2le_2Le_2^{dav}$	0.25	2	1	2	B
$le_1le_1le_1Le_2Le_2Le_2^{dav}$	0.25	2	1	2	C
$le_1le_1le_1le_2le_2Le_2^{dav}$	0.25	0	1	0	D
		$le_1le_1Le_2^{dav}Le_2^{dav} \times Le_1le_1Le_2le_2$			
$Le_1le_1le_1Le_2Le_2^{dav}Le_2^{dav}$	0.25	2	2	4	A
$Le_1le_1le_1le_2Le_2^{dav}Le_2^{dav}$	0.25	1	2	2	B
$le_1le_1le_1Le_2Le_2^{dav}Le_2^{dav}$	0.25	1	2	2	C
$le_1le_1le_1le_2Le_2^{dav}Le_2^{dav}$	0.25	0	2	0	D

† *Le* dosage includes all dominant alleles at the Le_1 and Le_2 loci.
‡ Interaction dosage assumes a multiplicative relationship between the alleles at the Le_1 and Le_2 loci.
§ A = $Le_1le_1Le_2Le_2^{dav}$; B = $Le_1le_1le_2Le_2^{dav}$; C = $le_1le_1Le_2Le_2^{dav}$; and D = $le_1le_1le_2Le_2^{dav}$.

from each cultivar-F_1 fit that expected for a 3:1 (inviable:viable) ratio $P < 0.50$ ($X^2_{1d.f.} = 1.73$), confirming that all five cultivars were $Le_1Le_1Le_2Le_2$ and each F_1 was $Le_1le_1Le_2le_2$ (Table 2). Homogeneity of the 3:1 segregation ratio was generally good between reciprocal testcrosses within each cultivar-F_1, and among the summed reciprocal testcross data from the cultivar-F_1 plants. Reciprocal testcrosses showed no difference in timing of necrosis, indicating that maternal effects and cytoplasmic inheritance were absent. In contrast to typical situations, these reciprocal crosses were not expected to reveal endosperm effects. Endosperm effects on the timing of necrosis presumably would depend on the interaction dosages of Le_1 and Le_2 genes with Le_2^{dav}. If so, reciprocal testcross differences that could arise from typical endospermic gene expression would not be expected (Table 3). Furthermore, interaction dosages of the endosperm and embryo would be completely confounded (Table 3), whereas in a typical reciprocal cross test endosperm and embryo genotypes are not completely confounded.

Histograms revealed that the frequency of necrotic timing was variable among testcross families from the five cultivar-F_1's (Fig. 1a–e). Testcross families from three of the five cultivar-F_1's showed a tetramodal distribution (Fig. 1c–e), indicative of a 1:1:1:1 phenotypic ratio. Testcross families from the other two cultivar-F_1's showed a trimodal distribution (Fig. 1a–b), indicative of a 1:2:1 phenotypic ratio. Chi-square tests, however, revealed poor fits to the 2:1:1, 1:2:1, and 1:1:1:1 ratios expected under the various models for gene action (Table 2). The lack of homogeneity for 1:2:1, 2:1:1, and 1:1:1:1 segregation ratios from data summed for testcrosses from cultivar-F_1's was caused by the variation among the cultivar-F_1's. Recent data (Stelly, 1989) has revealed that differential viability can skew genetic segregation ratios at the *Le* loci.

Although chi-square values for 1:2:1, 2:1:1, and 1:1:1:1 segregation ratios fit poorly, the modalities of the distributions (Fig. 1a–e) indicated that the alleles (Le_1 and Le_2 affected the timing of necrosis. With reference to the four models mentioned previously, the two cultivars showing trimodal distributions fit Model 2 or 4, i.e., allelic differences *or* dosages influenced the timing of necrosis, dependent on the particular model. More importantly, the tetramodal distributions observed in the other three cultivars fit Model 3, indicating that allelic differences *and* dosages affected the timing of necrosis.

The tetramodal testcross distributions indicate that allelic differences and dosage both affect the timing of necrosis, so the trimodal distributions indicate that these effects are variable in different genotypes. Three plausible explanations are that: (i) additional loci are involved, (ii) the allelic interactions are modified by background genotypic and environmental differences, and (iii) Le_1 and Le_2 genes are polymorphic, as are many isozyme loci, and thereby vary in reaction intensity. Under the latter hypothesis, 1:2:1 distributions result from instances where Le_1 and Le_2 alleles are equally interactive with Le_2^{dav} and 1:1:1:1 distributions result from instances where Le_1 and Le_2 are unequally interactive with Le_2^{dav}.

Data show that allelic differences and dosage affect the timing of necrosis caused by the Le_2^{dav} hybrid lethality system, and that specific effects of these allelic differences and dosage effects vary among different *G. hirsutum* genotypes. Definitive research on the genetic control of the timing of necrosis, the relative intensity of Le_1–Le_2^{dav} vs. Le_2–Le_2^{dav} interactions, and locus polymorphisms may require development of isolines differing at the loci Le_1 and Le_2, or other manipulations that facilitate direct comparisons among *Le* alleles from different sources.

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