POLLINATION STUDIES AFTER TRANSGENEIC PRODUCTION - A SEARCH FOR RECOMBINATION IN NECTARILESS GENOTYPE

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Abstract

Previous compatible and incompatible lines had a tufted-seed expression. Fuzzy seededness occurred when n1 common in cultivars was introduced by le1 n1 recombination. The fuzzy-seed incompatible version had 2le1 Le2dav Ne2 requiring recombination of Le2dav ne2 if nectariless cultivars were developed. In the F_2 population that fixed le1n1 was the heterozygous Le2dav Ne2 - / le2 - ne2 -. Selfing provided F_3 segregating Ne2 - to ne2ne2 that were tested for presence of Le2dav ne2. Failing to detect the recombinant at the 5cM frequency our effort was repeated for F_4, F_5, and F_6. But Le2dav ne2/ le2ne2 was not detected; suggesting the probability that recombination was larger than 0.4 and less than 2 cM. That there was genetic cause for nonrecovery could not be excluded.

A fixed line of the F_3 having 2 Le2dav-Ne2- was introduced into a tufted, compatible, pollen-sterile nectary-free stock for genetic studies. These F_2 verified Ne2 Ms9 recombination greater than 6 cM and the persistence of Le2dav Ne2. Information also indicated that the event introducing n1 was exceptional. The resulting 2gl2le1 * n1 ne1 Ms8: 2 gl3 le2 * ne2 Ms9 also upgraded the background by furnishing segments that breeders had selected in the development of nectariless and glandless.

For detecting the presence of Le2dav in pollen fertile plants using pollen of cultivars a simulation method was examined. It was highly correlated with emasculation and pollination by hand and in turn was similar to pollen vectoring by bees. Estimates of cross pollination in fields of Bt cultivars indicated a high level of ambient pollen. Simulation suggested either an advantage of application of pollen to tip of stigma or an efficacy of first pollination.

One Le2dav ne2 ms9 / lew ne2 ms9 recombinant was indicated by embryonic lethality. Its pollen-sterile offspring with embryonic lethality were confirmed.

Introduction

A genetic investigation of morphological markers by Rhyne and Menzel (1986) indicated the order Bw1 Gl2 n1 Ne1 Ms8, here indicating genes of cultivars. Later investigation of le1 and le2 recombination by Rhyne and Carter (1992a&b) and the Samora et al. (1994) formalization indicated loci near Le1 with differing amounts of recombination. Gl2 and the centromere having frequent recombination with Le1 were detected in double recombination (Samora et al. 1994). Infrequent recombination with Le1 for n1, lf, and Ne1 was reported by Rhyne and Carter (1992a). Rhyne (1996) reported a lack of recombination for Le1 n1 but usage of le1 N1 had produced recombinant le1n1. This effort should permit an incompatible phenotype for nectariless cultivars, because of the parallelism of genes of le2 and genes of the le1 group (Samora et al). Even so, no recombination of the Le2dav gene for incompatibility with ne2 was detected by Rhyne (1996). The purpose of this report is to validate unit le1 n1 in nectariless cotton and show efforts to obtain Le2dav ne2 recombination. Showing a relationship between vectoring of pollen in cultivars and a simulated procedure estimated open pollination in cotton fields receiving no insecticide during white flower production.

Materials and Methods

The cotton Winter Nursery reported a nectariless phenotype in a small F_2 population. This phenotype must indicate genotype 2 le1n1ne1-; 2 le2-ne2- and the probable genotype 2 le1 n1 ne1 -; le2-ne2- / Le2dav- Ne2 Ms9 among sibs in the F_2. Each was recognized in F_3. The F_4, F_5, and F_6 populations segregating Ne2- to nene in monomeric manner with presumed linkage of Ne2 and Le2dav were investigated. A homozygous 2le1n1 ne1-; 2 Le2dav-Ne2 of the F_3 was crossed on genetic stock 2le1- ne1 ms8; 2 le2-ne2 ms9 to produce monomeric segregation of Ne2 and indicate the number of Msx genes. The Le2dav pollen parent was observed when isolated in fields during 1996, 1997 and 1998. The presence of pollen sterile population in >96, 97, and 98 refined effort for detecting Le2dav - ne2 / le2-ne2.

Overhead irrigation interfering with emasculation and pollination efforts in 1995 and the scarcity of bee vectoring necessitated a simulation of insect vectoring of pollen. The F_3 and later populations extended linearally in the field, a single row among rows of cultivar. Two-three anthers were plucked from a flower of the cultivar and touched to the tip of the stigma of the test population, an individual plant of donor for the individual of the test population. Emasculation was also practiced and in the absence of simulation and emasculation, open pollination was necessary.

Simulation was practiced each date on the homozyous incompatible parent and on the segregating F_x such that Ne2- and all nene plants were challenged. Bee activity was considerable after 1995.

Cross-sector cutting of dried seed in the field indicated embryonic lethality by green-tan discolored tissue. For

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Le2davLe2dav genotype ten embryonic lethal require one lock for an emasculated flower had 7-9 seed and a portion of a second lock. Simulation required two or more. The Le2dav le2 plant required ten embryonic lethals or ten viable and remainder lethal for emasculation; and 2-3 locks for a simulation effort. Open pollination of Le2davle2 required 10 embryonic lethal, selfs, and viable un colored cross-pollinated embryos. The nene boll must show ten cross pollinations and ten self and no embryonic lethals. Uncut seed when planted had 0 emergence from emasculated Le2dav Le2dav or only self from simulation and open pollination. Le2dav le had three phenotypes in planted seed. The nene phenotype was 100% viable, showing two phenotypes.

The 1995 population (Rhyne, 1996) indicated recombinant Le2dav ne to be less than 6 cM. The expectation for a combined population showing recombination at 4 cM requires 100 nene plants that had been challenged. Challenge of Ne2- plants for presence of Le2dav was a control operation, which indicated the Le2dav - / Le1Le2 interaction resulted in embryonic lethality and lack of germination in planted seed.

Results and Explanation

The heterozygous genotype Le2davNe2 Ms9 / le2 ne2- segregated in the ratio 3 Ne2 to 1 nene in F_3 and thereafter. A presence of Le2dav was indicated, Table 1, by challenge of Ne2- plants with cultivar pollen. All plants had pollen fertility and fuzzy seed. This implied for Table 1 that n1 ne1 Ms8 of cultivar was involved in recombinant le1 n1ne1 - or le2-ne2 Ms9 was received from the cultivar or both. With le1 and le2 both gl2 and gl3 of the donor compatible became homozygous. Thus the challenged nene plants that lacked embryonic lethality were glanded viable. The Ne2Ne2 plants had no glanded viable. The Ne2 ne2 lants had both embryonic lethal and glanded viable. In the F_3 and F_6 1 Ne2 ne2 le2le2 was indicated by absence of embryonic lethality. No Le2dav ne2 le2 ne2 recombinant was detected in these populations.

The results in Table 2 are the lele genotype in nene plants and presence of Le2dav in Ne2- plants. Even if the cultivar background had been changed, the Le2dav and lele parental markers were the same. The challenging cultivars differ. The le2dav Ne2 remained linked. Embryonic lethality occurred in the Le2dav /Le1 Le2 interaction. The common F_3 lines at S & H locations were homozygous Ne2Ne2 or nene. The cultivar at H produced embryonic lethals, glanded and gland-free. Planted seed of S from open pollination showed gland-free that remained viable, glanded that quickly died, and nonemergent. The 21 F_3 at S location were challenged by the same cultivar, and by the cultivar that was the standard of Table 1. These had a common embryonic lethal when Le2dav was present. Combining 65 lele of Table 2 and more than 123 of table 1 indicate that Le2dav ne / le ne was not detected as embryonic lethal. Many nene plants had been advanced to nene populations and all had emergent seedlings that remained viable. Recombination at 2c M frequency should have generated 8 of 188 plants that would test as Le2dav ne2 showing embryonic lethality. The estimate is larger than 0 and less than 2 CM.

Line 1 of Table 3 is from Rhyne (1996) showing Ne2 Ms9 tightly linked. A gene for tufting was segregating independently of Ne2 Ms9. The F_2 of 96 investigate 3 phenotypes. For presence of Le2dav the monomeric repulsion phase rendered the middle two phenotypes (line 1) homozygous for testing, although no recombination was expected to be detected for region Ne2 Ms9. Region Le2dav Ne2 / le2 ne2 remained in coupling. Tufting was homozygous. The two dimeric F_2 in coupling phase (lines 3 and 4) received homozygous pollen fertile Le2dav Ne2 of Table 1 for the 2le2 ne2 ms9 of Table 3. One pollen sterile was tufted and the other fuzzy. When Le2dav ne2 recombination occurred the nene ms9ms9 should indicate it. Thus after 100 plants had been saved the remainder of the F_2 population was reduced to nene plants. The NRS2 F_2 conforms to a tight Ne2 Ms9 linkage and the other with its large error is not informative. SRS 3 appeared to be the better upgraded and one of its nene ms nems became the ovule parent for upgraded parent of the 98 dimeric F_2. The >98 F_2 repeated Ne2 Ms9 recombination. Ms8 is confirmed by procedure to have been coupled as le1 n1 ne1 Ms8 when cultivar Stoneville 907 donored the ne (ne1 Ms8) unit.

The yellow pollen Le2davNe2Ms9 / le ne ms of Table 3 line 1, challenged cream pollen 907 derivative 2 Le1n1ne1 ms8 Le2 ne2 ms9. Embryos with Le2dav would be eliminated, le ne ms parental retained, recombinants le Ne2 Ms9 at 5 cM and lene Ms9 at 6 cM. All viable with Ms9 had yellow pollen.

Table 4 contains the challenge of monominic populations of Table 3 seeking Le2dav ne ms recombinant. The population of the no bee 1995 was by simulation. Pollen of adjacent cultivar was applied to msms flowers. The two with Ne2- had 50% embryonic lethal indicating genotype Le2davNe2 ms9 / le2 ne2 ms9. Also cultivar pollen on 21 nenemsms had no lethals and the 3 nene Ms8 had no lethals. The 1996 population in repulsion phase conformed to the msms parental having 2 Ne2Ne2 ms9ms9 genotype by showing 13

plants with 100% embryonic lethality. Two indicated genotype Le2dav Ne2 ms9 / le ne2 ms9 and 1 of ambiguous genotype. The parental nene conformed to genotype lele nene Ms9 -. The F_1 was partially sampled having 26 with circa 50% embryonic lethal. No Le2dav ne / le ne was detected.

Table 5 has the challenge of 1996 SRS 3. Dimeric NRS 2 of 1996 was challenged and its sizeable population omitted, to permit the 1998 at the lower portion of Table 5. Ne2 Ms9 plants were challenged in part showing parental genotype with total embryonic lethality and 20 with the typical F_1 and 2 with no embryonic lethality (probable genotype le Ne2 Ms9 / le ne ms). Two Ne ms ms with 50% embryonic were rigorously tested, by cut seed and planting. Genotype Le2 Ne2 ms9 le2 ne2 ms9 as in Table 4. Emphasis was on the nene plants where by linkage the msms class would be larger than 1/4 of the nene class. Two samples were performed: the 63 earlier group lacked embryonic lethals and 11 had been stripped by boll-worm. Recovering plants were tested later: 50 had no embryonic lethality, 4 had green bolls and 1 had embryonic lethals.

The upgraded 1998 population was rigorously tested by emasculation and challenged by cultivar used in 1991 and 1997, by simulation using current cultivar. The 30 nene plants had no embryonic lethality. The 2 Ne2 msms conform to genotype Le2davNe2 ms9 le2 ne2ms9. Two of the Ne2 Ms9 had no embryonic lethals remaining for verification of genotype.

The detection of Le2dav ne2 ms9 / le2 ne2 ms9 in over 200 nene plants was indicated by 1 plant. This plant was frosted and had several nearly mature bolls. It was stubbed and its slow recovery carried its flowering again to early summer. After several challenges by selected parents it died. The challenges were handled in 1998. If Le2dav ne2 ms9 / le2ne2 ms9 its challenge by le ne Ms9 / le ne ms9 should provide ms9ms9 plants that 50% should show embryonic lethality with the standard cultivar. Several male steriles performed as the stubbed parental. However tufting of seed reappeared.

Discussion and Conclusions

Breeding lines available at the beginning had le1 and or le2 in combination with genes for tufting. Particularly le1 and tufting were coupled with a very low recombination rate. Failing to obtain le1 n1 by recombination with Le1 n1 of cultivar Stoneville 907 ne, Rhyne (1996) proposed the le1 N1 ne1 ms8 genetic stock be used versus Le1n1 ne1 Ms8 of the cultivar. This procedure generated le1 n1 - / le1 tufted Ne1 Ms8 and le2 - ne2 -- / Le2dav - Ne2 Ms9 and selfing should produce a nectariless genotype among other possibilities. Rhyne (1996) identified the nectariless and its companion Le2dav - Ne2 Ms9 / le2 - ne2 -, both having 2n1. Because le2 - ne2 had been developed from le2 - Ne2, recombinant Le2dav - ne2 - should also be obtained. The populations from the 907 donor failed to generate a Le2dav-ne2 recombinant that had embryonic lethality when challenged. F_3 through F_6 populations and upgraded kindred populations failed to harbor Le2dav- ne2-. More than 185 nene plants were challenged with 7.8 expected at Le2dav 2 cM ne2 frequency. Nondetection having P less than 1 and larger than 0.4% was a matter of a larger nene population. If not, a genetic cause such as Le2dav ne2 not expressing as an embryonic lethal or even its not occurring must be sought.

Nonappearance of tufting and pollen sterility in these populations of Table 1 - 3 indicated a concomitant change, possibly le1 n1 ne1 Ms8 and le2-ne2 Ms9 of donor 907 nene. So pollen-fertile, fuzzy-seeded Le2dav - Ne2 was introduced into tufted pollen sterile nectar-free primitive lines. The monomeric () Ledav - Ne2 Ms9 was identified and the dimeric F_2 (Table 3 - 6) indicated n1ne1 Ms8 and ne2 Ms9. Challenge of plants, particularly nene phenotypes, generated 1 possible Le2dav - ne2 ms9 in over 200 nene genotype. Using Le2dav - Ne2 Ms9 / le2-ne2 ms9 as pollen parent from Table 3 on recessive testor indicated recombinants le2 ne2 Ms9 and le2 Ne2 Ms9, for Le2dav would be eliminated. But Le2dav ne2ms9 should be possible if le2 - ne2 ms9 were the female. The solitary Le2dav - ne2 ms9 / le2 - ne2 ms9 of the 1996 population was verified in 1998 as a copy of the genotype.

Samora et al. (1994) showed double recombination involving gl2 le1 centromere, Rhyne et al. (1986) proposed N1 ne1 ms8 and identified N1 ne1 Ms8 to be a double recombinant but restricted data to F_2 identifying duplicate Ne1 Ms8. M. Y. Menzel (personal communication) indicated the Bw1 gl2 le1 * N1 ne2 ms8 was a single recombination and a significant event for cotton cytogenetics (Endrizzi et al., 1985). If not fortuitous, Gl3 le2 - ne2 ms9 occurred concomitantly. Similarly Bw1 gl2 le * tufted n1 ms8 and gl2le2 - ne2 ms9 occurred ne1 concomitantly. It is parent of the monomer of Table 3. A third singularity is the gl2 le1 * n1 ne1 ms8 gl2 le2 * ne2 Ms9 that occurred in the parentage of the present study.

That Le2dav Ne2 remains as an original contribution of D3 diploid can be suggested. Even if the Ms9 had another source for the Ne2 ms9ms9 occurred repeatedly in F_2 of the present study. The information is not precise, however, that

Ne2 ms9 recombination increased with upgrading of genetic background. J.A. Lee (1972) showed (Bw2 Gl3) rai tightly coupled in the distal end of chromosome 26, yet Bw2 gl3 recombined at a rate many times greater. le2, in the same arm of chromosome 26, recombines with ne2 at a frequency higher than Le2dav Ne2 herein. This genetic approach noting shifts in recombination value, conform to the phenomenon discussed by Endrizzi et al. (1985). Recombination in the proximal Le1N1 Lf Ne1 is greater than in the homoeologues Le2 (-) ne1 when Le2dav Ne2 is present. As formalized in Samora et al. (1994), values should be comparable. The le1n1 occurs at a value (Rhyne, 1996) larger than Le2dav Ne2 of the present study.

Absence of bees in 1995 was advantageous to study of F_2 having male steriles (Table 3) and a hindrance for study of F_2 having pollen fertile (Table 1). In addition to emasculation of females and lack of bee vectoring a simulation of open pollination was used. A single locule of an emasculated female provided 7-9 embryonic lethal and 1-6 lethal in Le le genotype. Simulation that took 2-3 anthers of contiguous cultivar and touching the tip of the stigma of normal flower gave fewer lethals, although many times a single lock exhibited 7-9. Two-three locks usually detected 10 embryonic lethals in the simulation procedure. However some locks in simulation and in open pollination had only selfs and others had all lethals. Characterization of a genotype was the same whether by emasculation, simulation, and open pollination. In the 1995 study, characterization of nene plants was missed by emasculation and simulation, and open pollination was inadequate. Advances of individual plants to individual populations for 1996, and bee vectoring was exceptional, cutting seed of open pollination readily showed them to harbor lele. The 1997 and 1998 years repeated the 1996, for insecticide application during white flower was unnecessary. The observations recorded in Table 6 - are a measure of ambient pollen carried by bees to gland-free upgraded material. For example, the 25 plants of the 1997 Le2davLe2dav were used as control and simulation was done for comparison with simulation in segregating populations elsewhere. Cutting showed embryonic lethality was considerably above 50%. Flowers that were not used being open pollinated were collected. Cutting showed more than 50% embryonic lethals. Planting the sizeable amount of remnant seed showed gland-free, nectaried viable. Butting a sample of the remnant at planting showed more than 50% embryonic lethals.

The simulation procedure and bee vectoring were similar. Particularly a single lock with its 7-9 seed showing 100% embryonic lethality and the other 4 locules had 0 lethals. As Finker in his Crop Science 1954 papers and Stephens and Finkner evaluated, first pollination was highly successful even if the tip of stigma was a favorable location. Our examination of stigmas of open pollinated flowers generally found abundant pollen on each locule. Multiple visitation by bees had occurred; flowers of cultivar and test phenotypes had received abundant pollen in addition to pollen of their own anthers.

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Table 1. Challenged plants in segregating generations for presence of the Le_2^{dav} gene for embryonic lethality

	ne ne	Ne_2 ne_2	Ne_2Ne_2
F_3 1995	>30	>30*	4
F_4 1996	>30	>30	>3
F_5 1997	>30	>30	>3
F_6 1998	33	20*	3
Genotype	lele	$Le_2^{dav}Ne_2$ *llNe $_2$	$Le_2^{dav}Le_2^{dav}$

Table 2. Challenged plants in 1998 for presence of Le_2^{dav} from F_2 and F_3 having a related genetic background

Location		nene	Ne_2 ne_2	Ne_2Ne_2	
F_2	B	9	5	2	
	S	5	1	1	
		___	___	___	
F_3	S	25	17	4	
	H	25	11	1	
		___	___	___	
F_3line	S+H			1	
	S+H			1	
		65 lele	$34Le^{dav}_{le}$	9 $Le2^{dav}$	$Le2^{dav}$

Table 3. Pollen fertility and nectary expression in F_2 population, two in primitive and two in upgraded cultivar background

	Ne_2 Ms_9	Ne_2msms	nene Ms	nene msms	Explanation
95	70	2	3	21	5.3 " 2.4 cM
96 NRS 3	49	20	27	0	No recombinants
96 NRS 2	74	0	28	4	No recombinants
96 SRS 3	59	2	20	6	15.6 " 11.4 cM
98 707 3	70	2	24	6	15.7 " 10.3 cM

Table 4. Challenge of two monomeric F_2 for Le_2^{dav} setting recombinant $Le_e^{ddav}ne_2$

Genotype	Ne Ms_9	Ne msms	nene Ms_9	nene msms
$Le_2^{dav}Le_2^{dav}$		0	0	0
$Le_2^{dav}le_2$	20	2	0	0
le le		0	3	21
$Le_2^{dav}Le_2^{dav}$		13	0	0
Le_2^{dav} le	26	2	0	0
le le		1	22	0

Table 5. Challenge of two dimeric F_2 populations seeking Le_2^{dav}ne

Genotype	Ne_2Ms_9	Ne_2msms	$nenems_9$	nene msms
$Le_2^{dav}Le_2^{dav}$	10	0	0	0
Le_2^{dav}le	20	2	0	0&1*
le le	2	0	60&46	3&4*
stripped in SRS3			11&4	0
$Le_2^{dav}Le_2^{dav}{}_3$		0	0	0
Le_2^{dav}le	26	2	0	0
le le	2?	0	24	6

? 98-707 to be verified *later sample

Table 6. Outcrossing in Bt cotton field

	CUT	PLANTED	
1996	abundant	>40 a	
1997	47	54 a	
	>50	35 b	
1998	>50	75 a	
	58	-- b	
	53	-- c	c 25 plants

a=Le2davLe2dav b=Le2davle c=nene