FIELD EVALUATION OF COTTON TRANSFORMED FOR TOLERANCE TO IMIDAZOLINONE HERBICIDES

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Abstract

Genes encoding acetohydroxyacid synthase were isolated from cotton, engineered to express an imidazolinone-resistant form of the enzyme and reintroduced into cotton utilizing an Agrobacterium mediated transformation system. Herbicide tolerant plants were regenerated and outcrossed with various breeding lines. Field trials were conducted with F2 progeny of a cross with El Dorado Acala to assess productivity (yield), fiber properties and herbicide tolerance versus the recurrent parent.

Introduction

The imidazolinones (such as Scepter™ and Cadre™) manufactured by American Cyanamid represent a class of environmentally safe herbicides that effectively control broadleafed weeds. Many problem weed species in cotton production including nightshades, annual morning-glory, pigweed, nutgrass, and lambsquarters could be controlled were tolerance to imidalolinones to be transferred into cotton. An imidazolinone herbicide used in conjunction with a grass herbicide such as Prowl could conceivably provide season-long weed control with a single application.

Imidazolinones are known to act by inhibiting the activity of acetohydroxyacid synthase (AHAS), the first enzyme specific to the biosynthesis of the essential branched chain amino acids valine, leucine and isoleucine. In order to develop cotton with tolerance to imidazolinones, the cotton AHAS gene family was isolated and characterized. A point mutation conferring tolerance specifically to imidazolinone herbicides was introduced into a constitutively expressed member of the AHAS gene family. This native gene, driven by its homologous promoter, was then reintroduced into cotton experimental line AW4, a Coker 312 derivative using an Agrobacterium transformation system. Regenerates were outcrossed to an Acala parent and then outcrossed again to El Dorado Acala, a San Joaquin Valley Acala variety with superior spinning properties and good productivity. A bulk F2 of this cross was evaluated in the field in 1996 for herbicide tolerance, productivity and fiber properties. Answers to three primary questions were sought from this field trial: 1) to what extent is progress being made in achieving suitable productivity in the transgenic breeding lines we are working with; 2) how do fiber qualities of the transgenic lines compare with the SJV Acala standard (Maxxa); and 3) can suitable levels of herbicide tolerance be obtained with the mutated form of the endogenous cotton AHAS gene driven by its native promoter.

Materials and Methods

The field test was conducted at a location in King's County, California during the 1996 season. Plots were 4 rows, 60' long, with three replications in a randomized block design. A 40" row spacing was used. The transgenic line, identified as AC1, was compared versus AW4 (the regenerable parent line), El Dorado Acala (one of the recurrent parents in the ongoing breeding program), and Acala Maxxa (the current San Joaquin Valley Cotton Board standard). Imidazolinone herbicide rates were 0X, Cadre at 2X (125 ppb) and 3X (188 ppb) field rates, and Raptor at a 3X (125 ppb) field rate. Plants were sprayed 30 days post emergence at the 4-6 leaf stage. The middle two rows of each plot were harvested for seed cotton weights and each replicate was ginned separately to obtain lint yield. Fiber qualities were obtained from samples of each plot measured on individual instruments.

Results and Discussion

Lint yield

The lint yield performance of transgenic line AC1 was evaluated versus AW4 (the regenerable parent), El Dorado Acala (the Acala recurrent parent in the breeding program involving AC1), and Acala Maxxa (the current SJVCB standard) under conditions of no herbicide application. The data (see Table 1) show the lint yield of AC1 to be equal to Acala Maxxa, 13% greater than El Dorado, and 38% greater than AW4. AC1 is an F2, and a portion of the yield advantage is expected to be due to heterosis. This point will become clearer as F3 and F4 progeny rows are evaluated in coming seasons.

Fiber Properties

In order for a cotton variety to become eligible for production within the San Joaquin Valley, it's fiber properties must equal or exceed those of the current standard for the Valley, which presently is Acala Maxxa. The fiber properties of AC1 were compared with those of Acala Maxxa, El Dorado Acala and AW4. The data, presented in Table 2, show that good progress is being made in bringing the transgenic lines up to the Acala standard. All selections made from the AC1 F2 populations, and from later backcross generations, will be evaluated for fiber properties to ensure that this germplasm will have suitable fiber quality for production within the San Joaquin Valley.

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Herbicide Tolerance

Tolerance in cotton to imidazolinone herbicides could represent a significant new tool to assist growers in controlling problem weed species. Furthermore, imidazolinones, which are extensively used in soybean production, carry an 18 month label restriction for growers who would like to follow their soybean crop with cotton. Cotton tolerant to imidazolinones could potentially remove that plant back restriction. In the line evaluated in this study, a single cotton AHAS gene was isolated, mutated to produce an imidazolinone-resistant form of the enzyme, and then reintroduced into cotton. Hence, the data are relevant not only to the issue of developing tolerance to imidazolinones, but also to the more general case of engineering cotton gene systems which are multigenic in organization.

The data from the herbicide trial are presented in Table 3. El Dorado, the recurrent parent in use with AC1, is highly sensitive to the imidazolinone Cadre™. Indeed, there was not a single boll formed in any of the three reps sprayed with Cadre™. Although AC1 exhibited tolerance to both Cadre™ and Raptor™, AC1 was clearly more tolerant to Raptor™. AC1 sprayed with Raptor™ at the 3X rate yielded 72% of unsprayed AC1and 82% of the recurrent parent. AC1 sprayed with Cadre™ at 3X yielded 52% of the unsprayed AC1 and 59% of the recurrent parent.

Summary

This is the first field test of cotton transformed specifically for tolerance to imidazolinone herbicides. The transgenic line carries a single reintroduced cotton AHAS gene mutated to express a herbicide-resistant form of the enzyme. The gene is driven by its native AHAS promoter. The transgenic line AC1 (an F2) is being backcrossed with Acala germplasm including El Dorado Acala as a recurrent parent. AC1 outyielded El Dorado in this test by 11.4% in the absence of herbicide, indicating good productivity and adaptation. The fiber properties of AC1 are still below the Acala standard, but improved over the regenerable AW4 parent. AC1 is a segregating population of plants including 25% homozygous nulls, 50% heterozygous for the tolerance gene, and 25% which are homozygous for imidazolinone tolerance. Given the extreme sensitivity of the nulls in the population to imidazolinones, it appears that progeny selections homozygous for the imidazolinone tolerance gene may have sufficient tolerance for a 3X application of Raptor, if not Cadre. Homozygous lines developed from AC1 may have suitable tolerance to support removal of the 18 month plant back restriction for cotton following Scepter use on soybeans, thereby providing growers with another tool for weed control in their crop production practices.

References

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Table 1. Lint yield in the absence of herbicide

Entry	Lint per Acre	Gin Turnout	Gin Loss
AC1	1216	29.9	10.3
MAXXA	1205	32.2	11.6
El Dorado	1077	29.6	10.6
AW4	881	27.4	11.1
LSD (0.05)	144	1.4	0.7
CV%	10.1	3.2	4.4

Table 2. Fiber analysis

Entry	SL25	UR	T1	E1	MIC
AC1-0X	1.18	47.1	22.7	5.9	4.57
MAXXA-0X	1.20	47.4	24.7	5.6	4.12
El Dorado-0X	1.19	48.2	24.2	6.1	4.19
AW4-0X	1.16	48.3	22.1	5.5	5.16
AC1-Raptor 3X	1.20	46.7	22.6	6.4	4.15
AC1-Cadre 2X	1.21	47.1	22.7	6.1	3.99
AC1-Cadre 3X	1.20	47.0	22.3	6.4	3.93
LSD (0.05)	0.02	2.0	1.7	0.5	0.4
CV%	1.0	2.4	4.1	4.7	5.5

Table 3. Lint yield in the presence of herbicide

Entry	Lint per Acre	% of Check	% of Recurrent
AC1-0X	1216	100	112.9
El Dorado-0X	1077	88.6	100
AC1-Raptor 3X	862	70.9	80
AC1-Cadre 2X	654	53.8	60.7
AC1-Cadre 3X	637	52.4	59.1
El D-Cadre 3X	0	0	0
LSD (0.05)	144		
CV%	10.1		