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DESCRIPTION OF A MUTANT WITH LOW GOSSYPOL; POTENTIAL FOR USE IN COMMERCIALIZING COTTON SEED FOR HUMAN CONSUMPTION

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Abstract

The presence of the toxic terpene gossypol limits the value of cottonseed as animal food, including humans. FDA regulations in effect since 1980 set a limit of 450 ppm of free gossypol in products for human consumption, although limits for animals can be much higher. Because gossypol and related sesterterpenoids (heliocides) are critical for insect resistance, a project was initiated to eliminate gossypol from seed but not from other tissues. Plants transformed to express an antisense version of δ-cadinene synthase, the first enzyme unique to biosynthesis of gossypol, were regenerated and self-pollinated in order to recover plants homozygous for the insert. Plants with the antisense construct produced seed with significantly reduced levels of gossypol but surprisingly, the progeny that produced seed with the lowest level of gossypol did not include an antisense insert. Second and third generation seed from this apparent somaclonal variant maintained a 50% reduction in seed gossypol, but total heliocides in leaf tissues were reduced only 30% from the parental cultivar. The decrease in terpenoid products was not associated with a decrease in number or size of glands. Crosses have been made between the variant and commercial varieties to determine if the low seed gossypol trait is simply inherited.

Introduction

Cottonseed contains highly nutritious protein and high quality oil, but the additional presence of gossypol at levels toxic to humans and non-ruminants greatly lowers the food value of whole seed. Gossypol and chemically related derivatives are found in lysigenous glands in the seed and foliar tissues (Bell and Stipanovic, 1977). Glandless cotton free of gossypol has been developed by incorporation of two recessive genes *(gl1gl1, gl2gl2*) (Mcmichael, 1960) but is not of great commercial value because the plants are disease susceptible and subject to insect damage. Advances in plant biochemistry and biotechnology over the past decade presented a new opportunity to reduce levels of gossypol in the seed without eliminating the essential downstream heliocides in foliar tissues. The discovery that δ-cadinene is the first unique compound in the branch of the terpene metabolism that leads to gossypol synthesis (Benedict et al., 1995; Davis and Essenberg, 1995) and cloning of a δ-cadinene synthase gene (Chen et al., 1995) were critical. Once a cotton seed-specific promoter (Song et al., 2000) had also been cloned, it became possible to create constructs that when transformed into the cotton genome should express antisense δ-cadinene synthase during cottonseed development to limit expression of the normal gene in a tissue specific manner. Such plants have been made and shown to have somewhat reduced levels of seed gossypol (Martin et al., 2003). Here we describe an unexpected product of this research; the recovery of a low seed-gossypol progeny from a transformed plant that contains no trace of the construct used for transformation and selection. Progeny from this "mutant" plant have shown stable transmission of the low gossypol trait over 2 generations despite having apparently normal glands. Although the progeny also have reduced levels of leaf heliocides, the reduction is only 30% compared to 70% reduction in leaf gossypol.

Materials and Methods

Complete details for the construction of the antisense *cdn1-C1* plasmids used for T-DNA mediated transformation of Coker 312 callu*s* have been described previously (Martin et al., 2003). The transformation construct used in this study included both the *nptII* gene (kanmycin resistance) and an antisense version of *cdn1*-C1, each driven by CaMV35S promoters. Protocols used to extract and quantify gossypol and its precursors from individual seeds as well as the methods for quantifying leaf heliocides are found in the same publication.

Results and Discussion

Plants regenerated from callus following growth on kanaycin-containing medium were verified to have incorporated the T-DNA cassette into genomic DNA via Southern hybridization. Since these plants would be hemizygous for one or more inserts, each regenerated (R_0) plant was self-pollinated, and the resulting seeds were grown for further analysis. As expected, R_1 plants that included the antisense version of *cdn1*-C1 produced seed with lower levels of gossypol than controls (Martin et al., 2003) Likewise, all but one of the progeny that lacked antisense *cdn1-C1* produced near normal levels of seed gossypol. Unexpectedly, the R_1 plant that produced R_2 seed with the lowest level of gossypol lacked any trace of the transformation insert; it lacked DNA from a T-DNA border, the *nptII* gene and the antisense *cdn-C1*. When this plant was self pollinated, the progeny also produced seed with levels of seed gossypol that averaged about 50% of that found in regenerated control plants (Table 1). The same reduction was found in the next generation of seed. Levels of leaf heliocides were also reduced in the

progeny of the variant plant, but only by 30% compared to controls (Table 2). In both cases, the "mutant" variant produced levels of terpenoids that are less than those of the plants that included the antisense construct.

The unexpected recovery of a plant following transformation that does not include any trace of the transformation and yet has significantly reduced levels of seed gossypol has several implications for the ability to produce consumable cottonseed. Although the level in seeds is still above the level considered safe for human consumption, the reduction has been stable over 3 generations, so may be useful in breeding. Crosses have been made to commercial cultivars to test ability to transmit the trait. If the trait can be transferred and does not increase the ratio of (-) versus (+) gossypol, the amount of cottonseed used for example in dairy rations could increase substantially.

References

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Table 1. Average amounts of Gossypol (mg/g) in seeds of control, variant and antisense seed

Source	# plants x # of seed	[Gossypol] (mg/g)
R_2 Progeny of variant plant	8x6	7.2
R_3 progeny of R_2 variants	13x6	6.7
R_2 progeny of antisense plants	10x6	9.9
Progeny of non-antisense R_1s	2x6	11.2
Coker 312	21x1	13.3

Table 2. Concentration of leaf gossypol and combined heliocides H_1-H_4

Source	# of plants	total # leaves	[Gossypol] (mg/g)	[H1-H4] (mg/g)
R_2 Progeny of variant plant	8	27	0.043	1.191
R_2 progeny of antisense plants	4	15	0.043	0.934
Progeny of regenerated control	5	15	0.138	1.715
Coker 312	1	3	0.117	2.142