Abstract

Some plant cytoplasms express novel mitochondrial genes that cause male sterility. Nuclear genes that disrupt the accumulation of the corresponding mitochondrial gene products can restore fertility to such plants. The Texas (T) cytoplasm mitochondrial genome of maize expresses a novel protein, URF13, which is necessary for T cytoplasm–induced male sterility. Working in concert, functional alleles of two nuclear genes, *rf1* and *rf2*, can restore fertility to T cytoplasm plants. *Rf1* alleles, but not *Rf2* alleles, reduce the accumulation of URF13. Hence, *Rf2* differs from typical nuclear restorers in that it does not alter the accumulation of the mitochondrial protein necessary for T cytoplasm–induced male sterility. This study established that the *rf2* gene encodes a soluble protein that accumulates in the mitochondrial matrix. Three independent lines of evidence establish that the RF2 protein is an aldehyde dehydrogenase (ALDH). The finding that T cytoplasm plants that are homozygous for the *rf2-R213* allele are male sterile but accumulate normal amounts of RF2 protein that lacks normal mitochondrial (mt) ALDH activity provides strong evidence that *rf2*-encoded mtALDH activity is required to restore male fertility to T cytoplasm maize. Detailed genetic analyses have established that the *rf2* gene also is required for anther development in normal cytoplasm maize. Hence, it appears that the *rf2* gene was recruited recently to function as a nuclear restorer. ALDHs typically have very broad substrate specificities. Indeed, the RF2 protein is capable of oxidizing at least three aldehydes. Hence, the specific metabolic pathway(s) within which the *rf2*-encoded mtALDH acts remains to be discovered.

Introduction

Maternally inherited cytoplasmic male sterility (CMS) occurs in many plant species and is widely used to facilitate the production of hybrid seed because it eliminates the need for emasculation by hand. Mitochondrial defects account for all instances in which the nature of the lesion responsible for CMS has been identified (reviewed in Mackenzie et al., 1994; Schnable and Wise, 1998). In many species, the deleterious effects of these mitochondrial defects can be avoided or overcome by the action of nuclear genes, termed nuclear restorers. However, the specific mechanisms by which restoration can occur are only poorly understood.

The male-sterile Texas (T) cytoplasm (cms-T) was used to produce ~85% of U.S. hybrid maize seed until the 1970 epidemic of southern corn leaf blight (Ullstrup, 1972; Pring and Lonsdale, 1989). cms-T maize is highly sensitive to a host-selective toxin (T toxin) produced by race T of *Cochliobolus heterostrophus*, the causal organism of southern corn leaf blight (Hooker et al., 1970; Comstock and Scheffer, 1973; Yoder, 1973). The genomes of T cytoplasm mitochondria contain a unique mitochondrial gene, *urf13*, which encodes the URF13 protein. URF13 accumulates in the inner membrane of the mitochondria (Forde and Leaver, 1980; Dewey et al., 1986; Wise et al., 1987a; Hack et al., 1991; Korth et al., 1991; Levings and Siedow, 1992) and is believed to be responsible for both the sensitivity to T toxin and the male sterility of cms-T maize (reviewed in Levings, 1990, 1993; Wise et al., 1999). The URF13 protein accumulates in many tissues of cms-T maize plants. However, in the absence of T toxin, the only severely affected tissue is the tapetal cell layer of the anthers, which undergoes a

premature degeneration at the early microspore stage, resulting in pollen abortion (Warmke and Lee, 1977).

The combined action of two dominant alleles of two nuclear genes, *rf1* and *rf2*, restores the male fertility of cms-T maize (reviewed in Wise et al., 1999). Neither of these restorers alters the sensitivity of cms-T maize to T toxin. The function of the *rf1* gene in restoration relates to its ability to modify the expression of *urf13*, thereby reducing the accumulation of URF13 (Dewey et al., 1987; Kennell and Pring, 1989).

As a first step toward determining its function in fertility restoration, the *rf2* gene was cloned via transposon tagging (Cui et al., 1996). The *rf2*-encoded protein, RF2, contains a predicted mitochondrial targeting sequence and exhibits ~60% identity and 75% similarity to class II mammalian mitochondrial aldehyde dehydrogenases (mtALDHs). ALDHs are a family of $NAD(P)^+$-dependent enzymes that catalyze the oxidation of numerous aldehydes (reviewed in Lindahl, 1992; Yoshida et al., 1998). A large number of ALDHs have been characterized in mammals, yeast, insects, and bacteria (reviewed in Perozich et al., 1999). In mammals and yeast, the class I (a cytosolic form) and class II (a mitochondrial form) isozymes have been particularly well characterized (Lindahl, 1992; Wang et al., 1998). According to recently revised nomenclature (Vasiliou et al., 1999), the *rf2* gene is equivalent to ALDH2B1. Only a few studies of plant class II mtALDHs have been reported (Asker and Davies, 1985; Osakovskii et al., 1992; op den Camp and Kuhlemeier, 1997). Although plant betaine ALDHs have been subjected to fairly intensive study (Vojtechova et al., 1997), these enzymes are only distantly related to class II mtALDHs.

The primary functions of ALDHs are believed to be the detoxification of ethanol-derived acetaldehyde and the oxidization of aldehydes derived from biogenic polyamines (Lindahl and Petersen, 1991). On the basis of the presence of indoleacetaldehyde dehydrogenase activity in cell-free extracts from mung bean seedlings (Wightman and Cohen, 1968), it has been suggested that ALDHs may be involved in the production of the plant hormone indole-3-acetyl acetate (Marumo, 1986). However, this biochemical reaction also can be catalyzed by an aldehyde oxidase (Rajagopal, 1971).

Most maize inbred lines carry functional *Rf2* alleles, even though they have never been exposed to T cytoplasm. This suggests that the RF2 protein has an important physiological role other than restoring male fertility to plants that carry T cytoplasm (Schnable and Wise, 1994). In this report, we demonstrate that the RF2 protein is, as predicted (Cui et al., 1996), an mtALDH that accumulates in most organs.

Methods and Materials

The method used to test for a linkage between Rf2a and BAR was polymerase chain reaction (PCR). The reason PCR was used is because it was reproducible, relatively inexpensive, not time consuming and many samples could be loaded. The materials we used to test the experiment were as follows: 96 well format plates; multi-channel pipette; tips; PCR ice tray; genomic DNA; 10X buffer; 25mM $MgCl_2$; 2mM dNTP's; dH2O; primers; 1X Taq; 1% agarose gel; 1X TBE buffer; gel trays; PCR thermal cycler.

Results

Event	Herb. Resist. & Rf2a transgene	Herb. Resist. w/o transgene
19	94% (31/33)	6% (2/33)
23	95% (39/41)	5% (2/41)
17	100% (13/13)	0% (0/13)
26	100% (17/17)	0% (0/17)

Discussion

From the PCR results attained, we know that there was a strong correlation between size bands in BAR and Rf2a. The method we used to come to this conclusion is called scoring, which is a method of counting corresponding size bands with BAR and Rf2a using 96 well score sheets in correlation with the order of sensitive or resistant corn planted in the field. The data gathered suggests that corn plants resistant to herbicide contain the gene of interest 94-100% of the time.

Conclusion

The high percentages rates shown from the results lead us to believe that plants that are resistant to herbicide contain our gene of interest. This is significant because it allows researchers to know which plants contain the transgene of interest without the time consuming and tedious collecting of tissues sample, and DNA isolation to test for the transgene.