Introduction

Sex determination in maize involves the formation of unisexual flowers (called florets in grasses) through the selective elimination of unnecessary floral organs. The basic unit of the maize inflorescence is the spikelet that consists of two leaf-like subtending glumes enclosing two florets (referred to as the primary and secondary florets). During spikelet maturation, each floret meristem produces one lemma, one palea, three lodicules, three stamens and one pistil primordium (Fig. 1A,B,D). After reaching a bisexual stage, the fate of a floret is decided by its location in either the tassel or ear, and its position within the spikelet. In the tassel, pistil primordia are eliminated from both the primary and secondary florets (Fig. 1A top, B,C); a cell death process associated with cellular vacuolation and the breakdown of free ribosomes and organelles (Cheng et al., 1983). Pistil elimination requires functions encoded by the tasselseed (ts) genes, specifically ts1 and ts2 (reviewed by Dellaporta and Calderon-Urrea, 1994; Irish, 1996). In ts1-Reference (ts1-R) or ts2-Reference (ts2-R) mutant plants, primary and secondary pistils in tassel spikelets fail to abort and stamen primordia are arrested, resulting in a sexual conversion of the tassel from staminate to pistillate. The ts2 gene has been cloned and shown to be expressed in subepidermal pistil cells of developing male florets in the tassel (DeLong et al., 1993). The putative TS2 protein encodes a protein with similarity to short-chain alcohol dehydrogenases with signature motifs of hydroxysteroid dehydrogenases.

A more complex pattern characterizes the fate of floral organs in the spikelets of the ear inflorescence. Most often, mature ear spikelets contain a solitary pistillate floret resulting from the arrest of all stamen primordia and the selective elimination of the secondary pistil primordium (Fig. 1A bottom, D,E). Consequently, the primary floret of ear spikelets usually contains the only functional pistil found in most lines of maize. The tasselseed pathway is also required for the process of pistil elimination in secondary florets of the ear spikelets. In the ts1-R and ts2-R mutant plants, secondary pistils fail to abort resulting in double pistillate florets in ear spikelets. The stamen arrest process in ear spikelets is not affected by mutations in ts1 or ts2.

Here we show that the elimination of pistils in maize is a developmentally staged cell death process associated with nuclear degeneration. Pistil cell death begins in subepidermal cells of the pistil primordia and requires ts2 activity. We also show that TS2 RNA accumulation is regulated by ts1, and that all pistil primordia express TS2 RNA but the functional primary pistils that develop in ear spikelets are protected from tasselseed-induced cell death by the action of the silkless1 gene. The combined action of ts1-ts2-sk1 accounts for the elimination of unnecessary floral organs.

Materials and Methods

Identification of ts2-R;sk1-R double mutant

Homozygous ts2-R plants were crossed as females to homozygous sk1-R males and F1 progeny self-pollinated. In subsequent generations, plants were sib-mated and the double mutant genotype was confirmed by the use of a co-dominant ts2-linked microsatellite.
located 1.5 kb downstream from the 3’ end of the ts2 gene. Primers to amplify the microsatellite are 5’TGACGGAGCTGGATCGCTTACAGC and 5’AGACGGGCAAGGATCGCAGCCG3’. The ts2 mutant plants were identified by the presence of a single 120 bp band and absence of the 100 bp fragment associated with the Ts2 allele from the W22 inbred (our unpublished results). These ts2 mutant plants were crossed with slk-R pollen; slk-R non-complementation in these testcross progeny indicated the ts2 mutant parent was also homozygous for the slk mutation. As previously reported (Irish et al., 1994; Jones, 1932), double mutant plants (ts2-R; slk-R) showed complete epistasis of ts2-R to slk-R in the ear and incomplete epistasis in the tassel (the distal portion of predominantly pistillate tassel remained staminate). This partial epistasis allowed selfing of these double mutant plants for two generations. In advanced generations, the partial epistasis phenotype was replaced by complete epistasis of ts2-R to slk-R in both tassel and ear.

Cytological techniques

The formaldehyde tissue fixation, parafilm embedding and microtomy were essentially as described by Jackson (1991) except that Hemo-De (Fisher, Scientific Co.) was substituted for Histoclear (Agar Aids Ltd.), 8μm sections were used for all staining, and sections were dried overnight onto ProbeOn Plus microscope slides (Fisher Scientific Co.). Nuclear loss assays were performed by deparaffinizing and rehydrating tissue sections according to the method of Jackson (1991). Slides were then incubated in a 0.3 mM solution of 4’,6-diamidine-2-phenylindole dihydrochloride (DAPI; Boehringer Mannheim) for 5 minutes at room temperature. After incubations, slides were washed in PBS and mounted in 70% glycerol, 30% PBS.

Microscopy and photography

Sections were examined using a Zeiss Axioshot photomicroscope (Carl Zeiss, NY). Sections were observed and photographed on Ektachrome Elite 400 film, using UV light. The filter used for observations was D460/50 (31000M from Chroma Technology Corp.), 35 mm slides were digitized using a Polaroid SprintScan 35 slide scanner (Polaroid Corp.), Composites figures were generated and labeled using Adobe Photoshop 4.0 (Adobe Systems Incorporated).

Plasmid templates and riboprobes

Plasmid DNA was prepared using Qiagen 100 columns (Qiagen Inc.) following the manufacturer’s instructions. DNA templates for TS2 sense and antisense riboprobes were linearized by digesting plasmid pADL171 and pADL170 (DeLong et al., 1993), respectively, with EcoRI. In vitro transcription reactions using T7 or T3 RNA polymerase and 11-digoxigenin-dUTP (Boehringer Mannheim), riboprobe DNase treatment, and hydrolysis was according to the manufacturer’s instructions.

In situ hybridization

Tissue sections were pretreated for in situ hybridizations essentially as described by Jackson (1991). Sections were hybridized as previously described (Langdale, 1993) with the exception that the final concentration of riboprobe was adjusted to 10 ng/ml/kb and hybridization was performed at 62°C overnight. Procedures for hybridization, posthybridization incubations, and antibody detection were obtained from Bruce Veit (Massey University, New Zealand; personal communication). Nuclear degeneration assays were performed by incubating in a 75 mM solution of propidium iodide (Sigma Co.) for 10 minutes at 4°C. After incubations, slides were washed in PBS and mounted in 70% glycerol, 30% PBS. Sections were counterstained with Calcofluor and examined and documented using a Zeiss Axioshot photomicroscope (Carl Zeiss, NY). Sections were photographed on Ektachrome 160T film with simultaneous illumination under bright and UV-fluorescent light. Propidium iodide stained sections were observed and photographed using the filter D605/55 (31002M from Chroma Technology Corp.) and Ektachrome Elite 400 film.

RT-PCR analysis

Total RNA from tassels 15 mm in length was isolated using the TRIzol Reagent (Gibco BRL), according to the manufacturer’s instructions. RNA was treated with RNase-free DNase I (Boehringer Mannheim) for 20 minutes at 37°C. First strand cDNA synthesis was performed on 5 μg of total RNA by the RACE procedure (Frohman et al., 1988) modified as follows: 200 units of SuperScript II reverse transcriptase (Gibco BRL) were added to the first strand reaction mix and synthesis was carried out at 42°C for 2 hours. The reaction was diluted to a final volume of 100 μl with dH2O. 5 μl of the diluted reaction mix was used as template in a PCR amplification reaction containing an adapter primer (Frohman et al., 1988) and a TS2-specific primer (5’ATGCTCATCAACGCCTGGCG3’). The conditions for amplification were: 94°C, 2 minutes; then 30 cycles of 94°C, 30 seconds; 60°C, 30 seconds; 72°C, 30 seconds. The reaction was then extended at 72°C for 15 minutes. PCR products were resolved on a 1% agarose gel in TAE buffer. Fragments were transferred in 0.4 M NaOH to Zeta-Probe GT (Bio-Rad) membranes, and hybridized to TS2 cDNA probe as described by Dellaporta and Moreno (1993). Each reaction was also subject to PCR amplification under identical conditions using actin gene primers P213 (5’CATAGGCCACGTACAACTCCATC3’) and P214 (5’TCTACCTCTCCCTTGGAGATCCAC3’).

RESULTS

Stage-specific cell death during pistil abortion

We examined cellular changes associated with pistil elimination by analyzing sections of paraffin-embedded immature florets with the fluorescent DNA stain 4’,6-diamidine-2-phenylindole dihydrochloride (DAPI). We refer to the relative positions of the florets as the T1 (primary) and T2 (secondary) florets of the tassel spikelets; and the E1 (primary) and E2 (secondary) florets of the ear spikelets. Features of maize inflorescence development allowed us to analyze several stages of unisexual spikelet formation within a single inflorescence. Spikelet maturation proceeds in an acropetal fashion with more advanced spikelets at the base than at the tip of the growing inflorescence. A second developmental asynchrony exists within each maturing spikelet with primary florets more developmentally advanced than secondary ones.

Regardless of their ultimate destiny, immature bisexual florets were observed to have brightly staining nuclei, indicative of nuclear DNA integrity (Fig. 2A,E). As tassel spikelets matured, however, nuclear loss, as judged by greatly diminished DAPI fluorescence, was observed in subepidermal cells of T1 pistils (Fig. 2B,C). The pattern of loss occurred in a specific spatial and temporal pattern within the degenerating pistil. The earliest indications of nuclear DNA loss began in just a few subepidermal cells within the central region of the pistil at a developmental stage that coincided with the formation of the gynoecial ridge (as shown for T1 pistils in Fig. 2B). As spikelets matured, a greater region of the subepidermis lost nuclei until all, or nearly all, subepidermal nuclear fluorescence was missing (Fig. 2C). Even though a large number of subepidermal cells were affected, overlying epidermal cells had brightly staining nuclei until late in the pistil abortion process when the entire pistil collapsed due to a loss of structural integrity (not shown). Within individual...
tassel spikelets, there was a delay in the degeneration of T2 pistils with respect to T1 pistils. Nuclear DNA loss in T2 pistils began when T1 pistil degeneration was well underway, and in a similar subepidermal pattern (not shown). Nuclear loss observed in T2 pistils began in subepidermal cells at a stage of pistil maturation equivalent to that seen when T1 pistil nuclei degenerated (i.e. during formation of the gynoecial ridge). In ear spikelets, no signs of cell death or nuclear loss were observed in wild-type E1 pistils (Fig. 2E-G). E2 pistils, however, showed nuclear loss in a spatial and temporal pattern similar to T1 and T2 pistils (Fig. 2F,G). Analogous results to the data reported here were obtained with the DNA stains propidium iodide (an example is shown in Fig. 3F) and toluidine blue (data not shown).

tasselseed2 is required for pistil cell death

The ts2-R mutation blocks the process of pistil elimination in both tassel and secondary ear florets. In contrast to pistil abortion in wild-type plants, the T1, T2 and E2 pistils of ts2-R plants reach sexual maturity and are functional. Hence, loss of ts2 activity prevents all pistil elimination in the maize plant. We examined mutant tassel and ear spikelets by DAPI staining to determine how the ts2-R mutation interferes with the pistil cell death process. In ts2-R mutant tassels, the integrity of nuclei in cells in both T1 and T2 pistils was maintained throughout spikelet maturation, as judged by uniform distribution of DAPI staining throughout the epidermal and subepidermal cells (Fig. 2D). Likewise, in ts2-R ear spikelets, there was no evidence for nuclear loss in E2 pistils (Fig. 2H). These results indicate that the pistil cell death process associated with nuclear loss requires a functional ts2 gene.

The tasselseed2 gene of maize is expressed in T1 pistils prior to cellular degeneration (DeLong et al., 1993). To determine whether ts2 expression coincided with nuclear loss and cell death in other pistils, we followed its expression, by in situ hybridization, in both tassel and ear spikelets (Fig. 3). In tassel spikelets, ts2 expression was first detected in T1 pistil cells shortly after reaching the bisexual stage (Fig. 3A) and at a similar stage of floret maturation in T2 pistils (Fig. 3B). Cellular expression of ts2 coincided with the loss of nuclei, as judged by TS2 mRNA in situ hybridization and counterstaining tissue sections with propidium iodide (Fig. 3E,F). In ear spikelets ts2 expression was detected in the subepidermal cells of both E1 and E2 pistil primordia (Fig. 3C,D). E1 expression was unexpected because these pistils are functional and are not eliminated during spikelet maturation.

tasselseed1 regulates expression of tasselseed2

The tasselseed1 locus is defined by a recessive mutation (ts1-R) which transforms the staminate florets of the tassel into pistillate ones (Emerson, 1920). Mutant ts1-R plants have a phenotype indistinguishable from ts2-R plants – tassel and ear spikelets contain double pistillate florets. The similarity of ts1-R and ts2-R mutant phenotypes suggests that these genes may lie within the same sex determination pathway. Formal genetic epistasis analysis is not possible due to the similarity of ts1-R and ts2-R mutant phenotypes. Therefore, to address this possiblility, we examined ts2 expression in ts1-R mutant plants by in situ hybridization (Fig. 4). ts2 expression was undetected in ts1-R mutant tassel pistils (Fig. 4A,C) at a developmental stage when expression was expected in wild-type pistils (Fig. 2D).
4B,D). A similar result was seen in E2 pistils (not shown). These results suggest that TS1 action was required for TS2 RNA accumulation.

To confirm these results, RT-PCR analysis for TS2 mRNA was performed on RNA extracted from wild type, ts2-R and ts1-R tassels (Fig. 5). The expected 455 bp amplification product was seen in wild-type tassel cDNA (Fig. 5A, lane 5), but nearly absent in ts1-R (lane 7) and ts2-R (lane 9) tassel cDNA. A minute amount of a TS2 amplification product was detected in the ts1-R tassels after long exposures of the autoradiograph. Based on the relative intensities of these products, we estimate that ts1-R tassels must contain at least 100-fold less TS2 mRNA. As a control for the quality and quantity of cDNA used in each PCR reaction, actin cDNA was co-amplified using gene-specific primers. Fig. 5B shows that amplification of an actin cDNA product was uniform with each template, indicating that the cDNA quality and quantity used in each reaction was roughly equivalent. These results eliminate the possibility that the previous in situ data was due to precocious or delayed expression of ts2 in the ts1-R mutant since RT-PCR analysis was done on the entire inflorescence cDNA representing a continuum of spikelet development. Taken together, these in situ hybridization and RT-PCR data indicate that the tasselseed1 gene regulates the synthesis or accumulation of TS2 mRNA in pistil cells.

**silkless1 protects pistils from tasselseed-induced cell death**

The expression of ts2 in the functional E1 pistil appears to conflict with its role as a mediator of cell death. This apparent contradiction may indicate that ts2 expression is an insufficient indicator of cell death, or that, unlike all other pistils, E1 pistils are protected from tasselseed-induced cell death. To address this issue, we examined the fate of E1 pistils in plants homozygous for the recessive silkless1 mutation (sk1-R) in which E1 pistils are non-functional (Jones, 1925). Previous studies indicated that the ear phenotype of the ts2 sk1 double mutant was similar to that of the ts2 single mutant, while the tassel had predominantly staminate florets with pistillate and perfect florets at the base of the tassel (Irish et al., 1994; Jones, 1934). These studies concluded partial epistasis between the ts2 and sk1. We noted that after self pollinating double mutant plants (sk1-R; ts2-R) for two generations, the ts2-R mutation showed complete epistasis to sk1-R. The partial epistasis may be due to incomplete expressivity of one or both mutations in heterogeneous genetics backgrounds.

In sk1-R ears, we found that the failure of E1 pistils to develop involves stage-specific cell death with features indistinguishable from tasselseed-induced abortion of other pistils. For instance, loss of nuclei in sk1-R E1 pistil cells occurred in the subepidermal cells of the pistil, leaving epidermal cells relatively intact (Fig. 6A). Subepidermal ts2 expression coincided with the timing of nuclear degeneration and cell death in sk1-R E1 pistils (Fig. 6B) suggesting that loss of sk1 activity does not interfere with the synthesis or accumulation of TS2 RNA. Asynchrony in the initiation of subepidermal cell death in E1 and E2 pistils was similar to that seen in tassel spikelets (not shown). These studies demonstrate that E1 pistil abortion in sk1-R mutant plants occurs in a similar spatial and temporal pattern as during tasselseed-induced cell death of other pistils. Taken together, the pattern of E1 pistil cell death in sk1-R mutants, ts2 expression in E1 pistils, and the double mutant ts2-R;sk1-R phenotype all indicate that sk1 activity is required to protect E1 pistils from tasselseed-mediated cell death.

**DISCUSSION**

Pistil elimination is a developmentally programmed and tasselseed-mediated cell death process

In both ear and tassel florets, the elimination of pistils involves a mechanism associated with loss of nuclear DNA as one of its prominent and early characteristics. Our results indicate that...
pistil cell death, as judged by nuclear DNA loss, begins at a particular stage of floret maturation, just as the gynoecial ridge is beginning to form, and in a specific spatial pattern initially restricted to only subepidermal cells. In the tassel, as spikelets mature, the T1 pistil primordium degenerates, later followed by degeneration of the developmentally delayed neighboring T2 pistil. The gradient of spikelet maturation on the inflorescence axis and the asynchrony of the maturation of primary and secondary florets within each spikelet result in two overlapping acropetal waves of pistil cell death. The first wave, acting on the primary pistils, is followed by a second delayed wave, acting on the secondary pistils. This pattern of cell death strongly argues that cell death is developmentally programmed in maize pistils.

The observation that most subepidermal nuclei were lost while overlying epidermal cells continued to show good nuclear integrity suggests that the mechanism of pistil cell death may function cell-autonomously. These complex patterns of pistil abortion suggest that the cell death signaling process may be self-initiating, rather than mediated by an exogenous signal perceived by all pistils. Alternatively, an exogenous signal may exist but its perception may be delayed until the pistil cells reach a certain state of competency.

**tasselseed genes are required for cell death**

Genetic and molecular evidence shows that ts2 is required for pistil elimination in both tassel and ear spikelets. ts2 was expressed in pistil cells coincident with loss of nuclear integrity. ts2 expression and loss of nuclear integrity, as judged by loss of DAPI (and propidium iodide) staining, were the first indications of cell death in pistils. Finally, all pistil cell death was absent in both ears and tassels of ts2-R mutant plants. The coincident expression of ts2 in degenerating cells and its mutant phenotype provide strong evidence that ts2 is a mediator (direct or indirect) of cell death.

Yet, it is still not clear how the ts2 gene product may be functioning in a cell death pathway. On the basis of its similarity to short-chain alcohol dehydrogenases, especially to hydroxysteroid dehydrogenases (DeLong et al., 1993), two possibilities come to mind. The ts2 product may metabolize a substrate, perhaps a steroid, required for cell viability.

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**Fig. 3.** TS2 in situ hybridization in wild-type tassel and ear spikelets. Serial sections were hybridized with a TS2 antisense (A-D) and sense probes (not shown); no expression was detected with a TS2 sense probe. Tassel spikelets (A,B) and ear spikelets (C,D) are shown. TS2 expression is detected in T1 (A) and T2 (B) pistils of tassel spikelets, and in E1 (C) and E2 (D) pistils of ear spikelets. See legend to Fig. 1 for abbreviations. E and F are the same section first hybridized with TS2 antisense probe (E) then counterstained with propidium iodide (F) to visualize the nuclear loss pattern. Note the loss of nuclei in cells at the site of TS2 expression. See legend of Fig. 1 for abbreviations. Scale bars, 50 μm.

**Fig. 4.** In situ hybridization analysis of TS2 mRNA expression in ts1-R and wild-type tassels. Sections of ts1-R mutant (A,C) and wild-type (B,D) tassel spikelets were hybridized to TS2 antisense probe. As a positive control for the quality of tissue sections, serial sections were hybridized to an antisense probe of the maize MADS gene zag1, a homologue of the Arabidopsis AGAMOUS gene, that is expressed in both stamen and pistils primordia (Schmidt et al., 1993) (not shown). See legend to Fig. 1 for abbreviations. Scale bars, 50 μm.
Amplification either with (+) or without (−) the reverse transcriptase other substrates indicated (lanes 3-9) were subjected to PCR. DNA, maize genomic DNA; WT, wild-type tassel cDNA; (Gibco, BRL); RT, reverse transcriptase treatment; Ps, primers alone; control for cDNA quality. Abbreviations: M, 123 base pair ladder.

Amplification of substrates as in A using actin-specific primers to cDNA probe. (B) Ethidium bromide stained gel after PCR.

**Fig. 6.** Analysis of cell death and TS2 expression in *skl-R* ear spikelets. (A) DAPI staining of longitudinal sections of *skl-R* ear spikelets showing subepidermal nuclear loss in E1 pistils (P). (B) In situ hybridization with TS2 antisense probe to sections of *skl-R* ear spikelets. In the *skl-R ts2-R* double mutants, no sign of nuclear loss is seen in ear or tassel spikelets (not shown). See legend to Fig. 1 for abbreviations. Scale bars, 50 μm.

Alternatively, TS2 action may result in the formation of a signaling molecule that activates a cell death response. For instance, several signaling molecules are known to induce the process of apoptotic cell death in animal cells (Roy et al., 1992; Wyllie et al., 1980). There is no evidence, however, that the process of pistil cell death is related, in any way, to apoptosis.

Our data suggests a functional role for *tsl* in the cell death process as a regulator of *ts2* expression. The most parsimonious explanation for these results is that the *tsl* gene product may be a transcriptional regulator of the *ts2* gene. Another possible mode of action is that the regulation of *ts2* by *tsl* is an indirect one. For instance, other factors that directly regulate *ts2* expression may be under *tsl* control, or that the *tsl* gene product may be required for the stability of the TS2 mRNA. The cloning of *tsl* should help elucidate its role in the sex determination process.

**Fig. 7.** Model pathway for pistil fate in maize sex determination. In primary and secondary florets in the tassel (T1 and T2), and in secondary ear florets (E2) pistil fate is controlled by the action of *tasselseed* genes. TS2 acts on an unknown substrate, possibly a steroid, to mediate a cell-autonomous cell death response. The function of TS1 is to promote *ts2* expression. It is not known whether TS1 activates genes needed for cell death signaling in addition to *ts2*. In primary ear florets (E1) pistils are protected from *tasselseed*-mediated cell death by *skl* action. SK1 may protect pistils by directly interfering with TS2 activity or by blocking a downstream step in the cell death response. This model predicts that *skl* will be differentially expressed or activated in E1 pistils.

**Role of silkless1 in sex determination**

On the basis of epistasis results (Jones, 1932, 1934), it has previously been proposed that *skl* suppresses *ts2*’s potential to suppress silk (pistil) development (Veit et al., 1993). Our data show that *skl* specifically protects E1 pistils from *tasselseed*-mediated cell death although the mechanism of *skl* protection is unknown. Several examples of cell protection genes are known in animal systems. Protection from cell death by direct inactivation of cell death factors is thought to occur in blocking apoptosis. For instance, the ICE/CED3 family of programmed cell death factors in animals are required for apoptotic cell death (reviewed by Martin and Green, 1995; Steller, 1995). The prototypes of this family of cysteine proteases, called caspaspe, are ICE and CED3 (Alnemri et al., 1996). A protein of baculoviruses (i.e. p35) has been shown to block ICE/CED3-induced apoptotic cell death by forming stable inactive complexes (Bump et al., 1995). In *C. elegans*, the *ced3* and *ced4* gene functions are required in cells undergoing programmed cell death (reviewed by Ellis et al., 1991). The *ced9* gene, a member of the same gene family as the human proto-oncogene *BCL-2* (Hengartner, 1995; Hengartner et al., 1992), protects against *ced3*- and *ced4*-induced cell death. The CED4 protein binds in vitro to CED9 (Spector et al., 1997; Wu et al., 1997) and, after interaction, the CED4/CED9 complex localizes primarily to the membrane fractions in the cytoplasm of mammalian 293T cells (Wu et al., 1997). CED4 can also bind CED3, both independently of and simultaneously with CED9 (Chinnaiyan et al., 1997). These results suggest a possible scenario for the regulation of cell death in animals in which CED9/BCL2, localized at the mitochondrial membrane, binds and modulates CED4 which in turn binds CED3/ICE.
caspases. If CED4 is not bound by CED9/BCL2, binding of CED3/ICE caspases by CED4 may activate them, leading to cell death (Goldstein, 1997).

In the case of sk1, several possibilities are consistent with our results. For instance, the product of sk1 may function in a fashion similar to anti-apoptotic proteins by directly complexing with the ts2 gene product to prevent pistil cell death or it may interfere with a downstream step in a cell death signaling pathway. Also, similarly to CED3/CED4/CED9 cell death regulation, SK1 may function by binding and sequestering TS2, or other cell death factors, to an organelle compartment, thus preventing pistil cell death. To distinguish among these various mechanisms, the isolation of the sk1 gene, its product localization pattern and interaction, if any, with TS2 will be important to determine.

A model for the control of pistil fate

A model for the control of pistil fate by two alternative pathways is presented in Fig. 7 that is consistent with all available genetic and molecular observations. In this model, pistil fate is determined by the action of three genes: ts1, ts2, and sk1; other genes may yet be discovered. Consistent with the inability to detect the TS2 mRNA in ts1-R pistils, we propose that ts1 is a positive regulator of ts2 gene expression. TS2 action, either directly or indirectly, elicits a cell death response, presumably through the modification of an unknown substrate. This substrate may be related to steroids based on TS2 protein similarity to bacterial and mammalian hydroxysteroid dehydrogenases. To protect the functional E1 pistils, an alternative pathway is evoked involving the action of the cell protection gene sk1 required to block tasselseed-induced cell death signaling. sk1 may function directly to inactivate TS2 action or block a downstream effector of the TS2 signaling. A role for sk1 in modulating the availability of a TS2 substrate is inconsistent with the wild-type tassel phenotype of sk1-R mutant plants.

This model predicts that sk1 functions only in E1 and not in E2 or tassel pistils. The distribution of the sk1 gene product may be extremely informative in this regard. Several other key issues, such as the biochemical function of the TS2 protein, its substrate and product, and the isolation of sk1 and ts1 will be of central importance in achieving a greater understanding of the sex determination process in maize.

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