Maize is monecious, with separate male and female inflorescences. Maize flowers are initially bisexual but achieve separate sexual identities through organ arrest. Loss-of-function mutants in the jasmonic acid (JA) pathway have only female flowers due to failure to abort silks in the tassel. Tasselseed5 (Ts5) shares this phenotype but is dominant. Positional cloning and transcriptomics of tassels identified an ectopically expressed gene in the CYP94B subfamily, Ts5 (ZmCYP94B1). CYP94B enzymes are wound inducible and inactivate bioactive jasmonoyl-L-isoleucine (JA-Ile). Consistent with this result, tassels and wounded leaves of Ts5 mutants displayed lower JA and JA-Ile precursors and higher 12OH-JA-Ile product than the wild type. Furthermore, many wounding and jasmonate pathway genes were differentially expressed in Ts5 tassels. We propose that the Ts5 phenotype results from the interruption of JA signaling during sexual differentiation via the upregulation of ZmCYP94B1 and that its proper expression maintains maize monoecy.

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Most plants produce hermaphroditic flowers with the male organs, stamens, surrounding an inner whorl of female organs, the pistils. Fertilization occurs when pollen from the stamens successfully reaches the ovule within the pistil. While this arrangement assures successful seed production, it may also lead to inbreeding and reduced fitness. Plants have evolved several mechanisms to avoid inbreeding. One mechanism is genetic self-incompatibility, in which distinct alleles in the male pollen and female pistil are required for successful seed set. Another mechanism is physical separation, which involves the development of unisexual flowers. In monocot, male and female flowers are on the same plant. It is estimated that 30% of the plant species produce some unisexual flowers, including maize. In contrast, dioecious plants, such as Cannabis, Corylus, or Asparagus, have separate male and female plants.

Maize produces separate staminate (male) and pistillate (female) inflorescences called the tassel and the ear, respectively. All grasses contain flowers (referred to as florses) flanked by sterile bracts called glumes within spikelets, units of the grass inflorescence. During early floral development, the two florses in both the tassel and the ear spikelets are hermaphroditic, and monoecy is conferred by the selective abortion of pistillate organs in tassel florets and the arrest of staminate organs in the ear floret.

A large number of sex-determination mutants have been identified in maize, given the easy visibility of tassel/seed mutants in which silks (pistils) and kernels (seeds) are found in the normally male tassel. Recessive mutants, ts1 and ts2, and dominant mutants, Ts3 and Ts5, fail to abort carpels in tassels and also fail to abort the lower floret in ears. ts1 encodes a lipoygenase, also called ZmLox8, that acts in jasmonic acid (JA) biosynthesis, and ts2 encodes a monocot-specific short-chain alcohol dehydrogenase. Both ts1 and ts2 have a reduced plant height. Another tassel/seed mutant was created by knocking out the duplicated orthologs of OPR3, a major OPR (12-oxo-phytodienoic acid reductase) gene in Arabidopsis that acts in JA biosynthesis. The resulting maize opr7opr8 mutants are phenotypically similar to ts1 and ts2 mutants.

Other mutants with silks in the tassels include ts4, which encodes a miR172 microRNA, and Ts6, which has a mutation in the ts4-binding site. Brassinosteroid biosynthetic mutants and epigenetic mutants such as required to maintain repression (rrm6) also show feminized tassel. Clearly, many modes of regulation are necessary to keep hormone action balanced for proper sex determination.

Here, we use positional cloning, transcriptomics, and metabolomics to identify the Ts5 gene and its role in jasmonate metabolism. The mechanism of Ts5 function reveals a role for jasmonate catabolism through the ω-oxidation pathway in both attenuating response to wounding and specification of sexual identity.

Results

Ts5 tassels are feminized and ear florets fail to abort. Maize bears a terminal stamineate inflorescence (tassel) and lateral pistillate inflorescences (ears), which arise in the axils of leaves. Early development of ear and tassel is similar except that the tassel initiates branches prior to initiating spikelets. Both male and female spikelets initiate two floral meristems inside the sterile glumes. In the tassel spikelet, the pistil aborts producing two male florets. In the ear, the stamens arrest and the pistil of the lower floret aborts, leaving a single pistil to grow out and receive pollen in the female spikelet.

Ts5 tassels are feminized; the pistil fails to abort and, consequently, stamen development is arrested in affected florets. Although covered in silks, the Ts5 tassel remains branched (Fig. 1a–e) as previously described. Scanning electron micrographs reveal that in developing tassels of Ts5/+ and glumes are short and glabrous (Fig. 1b), resembling those of ear glumes, a phenotype shared with ts2, and indicating that the entire spikelet is feminized in the Mo17 background. In contrast, tassel glumes of normal siblings are long and produce trichomes (Fig. 1b). Ts5/+ ears are also abnormal; they fail to abort the lower floret, leading to disordered vertical kernel files (Fig. 1c–f). In cases of reduced seed set, unfilled pericarps are visible, appressed to the kernel of the upper floret (Fig. 1d); these are present but hidden by filled kernels in fully pollinated ears as in Fig. 1c, f.

In crossing Ts5 to different inbred backgrounds, we observed strong differences in expressivity. In the Mo17 background, the tassel is completely feminized and produces no pollen (Fig. 1a). In B73 tassels, Ts5 displays a mild phenotype and is male fertile (Fig. 1e). Spikelets in B73 Ts5 tassels are a mix of either male or female identity, but female florets can be found inside male glumes. The weak expressivity seen in B73 tassels, however, is not true for the ear because lower floret abortion still fails and the rows are uneven (Fig. 1f). The phenotype of Ts5 in A188 is intermediate between that of Mo17 and B73. The tassel is highly feminized, but still produces pollen. We quantified tassel/feminization traits by measuring two ratios, the number of feminized branches/total branch number (FBN/TBN) and the length of the main spike that was feminized/spike length (FSL/SL) and found that feminization increases with increase in copies of Ts5. In A188, homozygotes have more feminization along the main rachis and heterozygotes have an intermediate phenotype (Supplementary Fig. 1). Raw data used to create each graph are available in Supplementary Data 4.

Ts5 plants in A188 are noticeably shorter. Plants heterozygous for the dominant Ts5 allele have intermediate heights and Ts5 homozygotes are the shortest (Fig. 1g). We quantified height differences between Ts5/+ and wild type (A188, BC4) and found that mutants were nearly 16 cm shorter (Fig. 1h). Using these same individuals, we measured the internode length to determine the cause of the height difference and found a reduction in elongation of the four internodes immediately subtending the tassel (Fig. 1i). Genotype did not affect the lengths of the lower internodes.

Ts5 maps to ZmCYP94B1 and has JA-deficient phenotypes. Ts5 appears on a genetic linkage map published by R.A. Emerson. Current genetic mapping data placed the Ts5 mutation within bin 4.03 (www.maizegdb.org). Using molecular markers, we found a lack of recombination between umc2039 and adjacent markers. In our mapping population, TIDP218 was distal to MS13.14, compared to its reported location in B73 AGPv3. We fine-mapped Ts5 to a 15 Mb interval containing 65 genes between umc2039 and our custom indel marker JW35.36 (Fig. 2a) for primer sequences, see Supplementary Data 1).

To identify the potential mutations within these genes, we performed RNA sequencing (RNA-Seq) using 9–11 mm developing tassels from Ts5/+ and normal siblings in the phenotypically express Mo17 background. This approach enriched for potential transcripts causal for the phenotype and excluded secondary transcripts simply related to ectopic silk production. Plants were genotyped by polymerase chain reaction (PCR) using flanking markers. A CYP94B1 gene GRMZM2G177668 was not expressed in the normal sibling pool but was highly expressed in two Ts5/+ pools when comparing mapped reads (logFC 11.69) (Fig. 2b; Supplementary Data 2). In addition to an increase in read number, ectopic 5’ reads were detected, mapping to an area
Fig. 1 Inflorescence and vegetative phenotype of Ts5. a Normal and TsS/+ mature tassels in Mo17. b SEM micrograph of central spike of 4 cm tassel of normal and TsS/+ in Mo17. c Normal and TsS/+ ears in Mo17. d A poorly pollinated TsS/+ ear in Mo17 showing empty pericarps. Arrow: appressed empty pericarp of the lower floret. e Normal and TsS/+ mature tassels in B73. f Normal and TsS/+ ears in B73. g Mature plants of normal, TsS/+ , and TsS/TsS in A188. b Plant height (cm) of normal siblings (n = 30) and TsS/+ (n = 31) in A188. Height was significantly different by two-tailed unpaired t test, P < 0.0001 (t = 6.778, df = 59; 95% CI −20.21 to −11.00). Bar, mean; whiskers, SD. i Graph of upper internode lengths of the same plants graphed in panel (h), normal siblings (n = 30) and TsS/+ (n = 31) in A188. The entry 1st internode is measured from the last tassel branch to the first subtending node. Means of the first four internodes measured were significantly different, one-way ANOVA with Sidak’s multiple comparison test adjusted for multiple comparisons: 1st, t = 13.96, P < 0.0001, 95% CI = 4.778–7.0137; 2nd, t = 3.3874, 95% CI = 0.31771–2.548113, P = 0.0047; 3rd, t = 4.162688, 95% CI = 0.6405964–2.875533, t = 4.287441, 95% CI = 0.6932846–2.928221, P = 0.0002; 4th, P = 0.0001, global DF = 353, ns = non-significant. Bar, mean; whiskers, SD. SEM, scanning electron microscope.
annotated as being 5′ of the canonical transcriptional start site of GRMZM2G177668. These 5′ reads are unique to Ts5 and were not found in other published transcriptomes, suggesting that the Ts5 phenotype is the result of a novel transcript at this locus. Because Ts5 behaves as a dominant mutation, the expression of a novel transcript or the over-expression of a normal transcript could be causal. The sequence reads of adjacent genes were similar in the Ts5/+ pools compared to the normal pool (Fig. 2b), implying that although the order of two markers (MS13.14 and TIDP9218) is reversed in the mapping population, a gross rearrangement affecting linked genes had not occurred. Two other genes within the mapping interval were slightly upregulated in Ts5 mutant pools. GRMZM2G079452 (logFC 1.35) is annotated as a hypothetical protein whereas GRMZM2G112795 (logFC 1.07) is annotated as a putative uncharacterized protein. Otherwise, the expression
of nearby genes was unchanged between Ts5 and normal plants, suggesting that the chromosomal rearrangement did not broadly impact regional transcription, and that the increase in GRMZM2G177668 expression is most likely the specific cause of Ts5 phenotypes.

We sought to confirm that Ts5 is in the JA pathway given that GRMZM2G177668 encodes a paralog of an enzyme known to catabolize bioactive jasmonate, and Ts5 shares phenotypes with jasmonic acid biosynthetic mutants. We tested if an exogenous JA application could block the growth of silks in homozygous Ts5 mutant tassels and suppress the mutant phenotype (Fig. 2c). We used tasselseed1 (ts1), a mutation in a JA biosynthetic enzyme, as a control. The feminization of that mutant was previously shown to be reversed by the exogenous application of JA9. JA or a mock solution was applied directly into the whorls of 4-week-old plants every 2 days for 2 weeks at a concentration of 1 mM as they transitioned from vegetative to inflorescence development. FBN/TBN and FSL/SL were quantified and compared to the reduction, after treatment, of ts1 mutants (Fig. 2c). Ts5 tassels responded similarly to ts1 tassels, revealing that high concentrations of exogenous JA can rescue the feminization of the Ts5 phenotype. We applied JA at a concentration of 1 mM, but endogenous levels are much lower. This finding is consistent with Ts5 encoding an enzyme that catabolizes JA, though it is unable to metabolize an excess of JA applied directly to the developing tassel. Similar cases have been reported with transgenic lines overexpressing enzymes in the JA catabolic pathways26–28.

Since Ts5 could be corrected by the addition of JA, we measured root traits, known to be affected in other JA biosynthetic mutants such as opr7/opr8 in maize14 or allene oxide cyclase mutants in rice29. In addition, the overexpression of either CYP94B1 or CYP94B3 in Arabidopsis has been shown to display a similar long root phenotype26,27. Dark-grown Ts5 homozygous (produced after seven backcrosses to B73 (BC7)) seedlings have prematurely elongated coleoptiles and longer roots than B73 (Fig. 2d), similar to those found in opr7/opr8 double mutants. This suggests that Ts5 plants have reduced bioactive jasmonate and that bioactive jasmonate negatively regulates root growth, as previously reported14,15,29. To assess the similarity in the function of ZmCYP94B1 and CYP94B3, we overexpressed the maize gene in Arabidopsis. ZmCYP94B1-OE lines had a similar ratio of under-developed to developed siliques as CYP94B3-OE lines (Fig. 2e). Arabidopsis ZmCYP94B1-OE lines have been reported to display similar flower-development defects as CYP94B3-OE26.

From this experiment, we conclude that ZmCYP94B1, like CYP94B1 or CYP94B3, functions to hydrolyze JA-Ile to control flower and fruit development in planta.

**Ts5 mutants accumulate metabolites of jasmonate ω-oxidation.** We profiled jasmonate metabolites in the developing tassels of Ts5 homozygous plants (B73 BC7) and B73 wild-type controls using liquid chromatography–mass spectrometry to test whether they are predictably altered. Arabidopsis CYP94B126 and CYP94B327 are known to convert JA-Ile, the most bioactive form of jasmonate30–33 to the inactive form 12OHEJA-Ile during oxidative catabolism of JA34,35 as summarized in Fig. 3a. Comparison of CYP94B protein sequences from maize and Arabidopsis did not distinguish whether GRMZM2G177668 is more similar to Arabidopsis CYP94B3 or to CYP94B1 (Fig. 3b). Both the Arabidopsis proteins, however, oxidize JA-Ile to 12OHEJA-Ile semi-redundantly leading to less-bioactive jasmonate26,27,34,36. Based on the phylogeny and evidence presented in this paper, we named GRMZM2G177668 ZmCYP94B1. Our phylogenetic analysis also revealed that GRMZM2G164074 is a close paralog, although not differentially expressed in Ts5 heterozygous tassels (Supplementary Data 2).

Compared to B73 wild type, steady-state levels of JA and JA-Ile are lower in Ts5 tassels, whereas steady-state levels of 12OHEJA-Ile, the product of JA-Ile oxidation by Arabidopsis CYP94B1 and CYP94B3, are higher in Ts5 mutants (Fig. 3c). This result is consistent with the increased expression of GRMZM2G177668 promoting catalysis of active jasmonates in Ts5 tassels. In Arabidopsis, the prolonged catabolism of JA-Ile provides more substrate for CYP94C1, which preferentially catalyzes carboxy-derivative formation, explaining the increased levels of its downstream product 12COOH-JA-Ile34,35, as seen in Fig. 3c. 12OHEJA-Ile is a wound-induced jasmonate, also known as tuberonic acid37, that results either from the hydrolytic cleavage of 12OHEJA-Ile by amidohydrolases38,39 or from the direct oxidation of JA40,41. We also profiled wild-type B73 ear jasmonates and found that the levels of all tested metabolites (JA, JA-Ile, 12OHEJA-Ile, 12COOH-JA-Ile, and 12COOH-JA-Ile) were decreased significantly as wild-type ears grew from 6 mm (comprised of spikelet and spikelet pair meristems) to 20 mm (comprised of floral meristems) (Fig. 3d).

The CYP94B3 enzyme is known to be wound inducible in Arabidopsis27,35. To evaluate our hypothesis that Ts5 is a maize functional homolog of CYP94B, we assayed its gene expression and profiles JA metabolites via a wounding time-course experiment.
We found that GRMZM2G177668 is expressed at higher levels in Ts5 homozygous (B73 BC7) leaves even before wounding (Fig. 3e), consistent with the increase seen in our RNA-Seq experiments in Ts5 tassels (Fig. 2b). Post-wounding, GRMZM2G177668 levels increased within 2 h in both wild-type and Ts5 plants. In wild type, GRMZM2G177668 expression levels dropped to pre-wounding levels within 24 h, yet GRMZM2G177668 expression levels remained high in Ts5 (Fig. 3e). These results suggest that the regulation of the mutant allele is altered in Ts5, causing enhanced ZmCYP94B1 transcript accumulation.
The JA metabolite profile in wounded Ts5 homozygous leaves, in the B73 genetic background, is consistent with the prolonged and increased expression of ZmCYP94B1. JA-like and JA levels were lower in Ts5, but 12OH-JA-like and 12COOH-JA-like levels are increased (Fig. 3f). Similar to reports in Arabidopsis (27), the jasmonate caballides showed a slight time lag behind the increase in JA and JA-Ile. One hour post-wounding, JA and JA-Ile had significantly less JA-Ile at 1 h (t = 4.802854, P = 0.0030, df = 6, 95% CI = [−75.41736 to −24.50798]) post-wounding than B73 leaves. Ts5 leaves had significantly more 12OH-JA-Ile at 6 h (t = 6.354809, P = 0.0028, df = 4, 95% CI = [−19.91499] after wounding than B73 leaves. Ts5 leaves had significantly less JA-Ile at 1 h (t = 4.490589, P = 0.0003, df = 6, 95% CI = [−13.2358 to −10.497]) after wounding than B73 leaves. Ts5 leaves had significantly more 12COOH-JA-Ile at 6 h (t = 5.007114, P = 0.00021, df = 4, 95% CI = [−148.6606–64.41580]) at 24 h (t = 8.622493, P = 0.0003, df = 5, 95% CI = [626.7214–1159.130]) post-wounding than B73 leaves. * Error bars, SD of four biological replicates. LC-MS, liquid chromatography-mass spectrometry.

Fig. 3 A wound-inducible CYP94B is upregulated in Ts5. a A diagram of steps of JA-like catabolism via the γ-oxidation pathway. b A phylogenetic tree of CYP450 genes from maize (blue) and Arabidopsis (brown). CYP94 genes (blue-gray) and CYP84B genes (yellow) are shaded. A blue dot at a node indicates branches with >95% support. c Graph of jasmonate levels in developing tassels (2 cm) in B73 (black) and Ts5 homozygotes (blue). The amount of 12OH-JA-Ile is significantly higher in Ts5 than in B73 using a one-tailed Student’s t test with Welch’s correction, P = 0.0109, t = 3.438524 df = 3.037953, 95% CI = [112.0289 to 707.7894]. d JA and JA-Ile levels in three sizes of B73 ears during early development. The amount of JA in T5-15 mm ears is significantly less than that in 4–10 mm ears by one-way ANOVA; F = 7.078, P = 0.008, DF = 18. Bar, mean; whiskers, SD. e Relative expression of GRMZM2G177668 measured by qRT-PCR at 0, 2, and 24 h after wounding in pooled (n = 4) second leaves of Ts5 homozygotes (BC7 B73) and B73. Graph depicts means of three technical replicates. Error bars, SD. f Graphs depicting a time-course of LC-MS outputs of JA, JA-Ile, 12OH-JA-like, and 12COOH-JA-Ile accumulation (pmol/gFW) in wounded leaves of Ts5 homozygotes (B73) and B73 at 0, 1, 4, 6, and 24 h. Ts5 leaves had significantly less JA at 1 h (t = 4.567575, P = 0.0038, df = 6, 95% CI = [−188.1638 to −56.88620]) and 4 h (t = 3.548059, P = 0.0238, df = 4, 95% CI = [−163.2358 to −19.91499]) after wounding than B73 leaves. Ts5 leaves had significantly lower JA-Ile at 1 h (t = 4.802854, P = 0.0030, df = 6, 95% CI = [−75.41736 to −24.50798]) post-wounding than B73 leaves. Ts5 leaves had significantly more 12OH-JA-Ile at 6 h (t = 6.354809, P = 0.0028, df = 4, 95% CI = [−19.91499] after wounding than B73 leaves. Ts5 leaves had significantly less JA-Ile at 1 h (t = 4.490589, P = 0.0003, df = 6, 95% CI = [−13.2358 to −10.497]) after wounding than B73 leaves. Ts5 leaves had significantly more 12COOH-JA-Ile at 6 h (t = 5.007114, P = 0.00021, df = 4, 95% CI = [−148.6606–64.41580]) at 24 h (t = 8.622493, P = 0.0003, df = 5, 95% CI = [626.7214–1159.130]) post-wounding than B73 leaves. f Error bars, SD of four biological replicates. LC-MS, liquid chromatography-mass spectrometry.
These classes were not significantly different (ns) by one-way ANOVA. Given the novel 5’ transcripts found by RNA-Seq and the reduction in recombination around the locus, it is likely to be a genomic rearrangement. Reduced recombination can be found in heterozygous genomic regions of differing retrotransposon haplotypes. Our mapping population, made by backcrossing a Ts5+/parent in an unknown progenitor background to Mo17, may indeed have a structural variation that affects genetic distances by contracting or expanding physical and genetic distances between markers. Moreover, we were unable to amplify the 5’-end of the gene using PCR, possibly due to the presence of a large insertion such as a transposon. Unique dominant alleles often result from large rearrangements that alter cis regulatory sequences, such as Abphyt2, which is a transposition, Kn1-O, which is a tandem duplication, or Wab1-R, which is ectopically expressed in the leaf due to unknown cis regulatory changes.

If higher jasmonate levels lead to pistil abortion in the tassel, what keeps it from eliminating the pistils in the ear? This quandary was first investigated in 1925 in the analysis of silkless1 (sk1)42,52. Tassels of sk1 mutants are normal, but ears lack silks, and they retain other female features such as stamen suppression and short glumes. The double mutant with ts2 restores the silks to the ear, while the tassel remains mostly staminate8,43. Although the exact substrate of SK1 is unknown, its overexpression leads to a transposon phenotype and reduces 12-oxophytodienoic acid (OPDA, a precursor of JA) and JA-Ile44. The authors hypothesize that SK1 inactivates JA or a precursor in a tissue-specific manner. The rescue of silks in the sk1;ts2 double mutant occurs because the loss of SK1 has no effect in the ear. STS5 or JA-Ile, the active jasmonate metabolite that binds to the nuclear localization sequence of the jasmonate co-receptor complex25. Transcript levels of ZmCYP94B1 are dramatically upregulated in Ts5 mutant tassels, and jasmonate metabolites are altered consistently with the increased catabolism of JA-Ile through the ω-oxidation pathway25. Ts5 leaves have ~4-fold more ZmCYP94B1 transcripts prior to wounding, and transcript levels continue to rise over time, failing to return to non-wounded levels even a day after wounding. Indeed, the catabolic products of JA-Ile remain high in wounded Ts5 leaves, unlike those of wild types. The increase in ZmCYP94B1 transcripts in Ts5 tassel and leaf, along with changes in JA metabolism, support the hypothesis that the feminization of Ts5 tassels is due to reduced JA-Ile levels, similar to the observations of ts1 or opr7opr89,14.

The genetic lesion of Ts5 is unknown. The first mention of Ts5 is on a genetic map in 1932 without further description21. Given the novel 5’ transcripts found by RNA-Seq and the reduction in recombination around the locus, it is likely to be a genomic rearrangement. Reduced recombination can be found in heterozygous genomic regions of differing retrotransposon haplotypes48. Our mapping population, made by backcrossing a Ts5+/parent in an unknown progenitor background to Mo17, may indeed have a structural variation that affects genetic distances by contracting or expanding physical and genetic distances between markers48. Moreover, we were unable to amplify the 5’-end of the gene using PCR, possibly due to the presence of a large insertion such as a transposon. Unique dominant alleles often result from large rearrangements that alter cis regulatory sequences, such as Abphyt2, which is a transposition, Kn1-O, which is a tandem duplication, or Wab1-R, which is ectopically expressed in the leaf due to unknown cis regulatory changes51.

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Although sex determination in maize is dependent upon jasmonate suppression of pistils, regulating pistil outgrowth does not explain why tasselseed florets have pistils and no stamens. The function of jasmonate in promoting stamen development is well established in Arabidopsis. Mutants that are deficient in jasmonate biosynthesis, perception, or signaling are also defective in stamen differentiation12,32,53. In fact, the overexpression of CYP94B1 or CYP94B3 in Arabidopsis leads to a partial loss of fertility. The stigma is extended, the anther filament is shortened, and pollen...
viability is reduced. We also saw a similar phenotype when overexpressing Ts5 in Arabidopsis. The high levels of JA and JA-Ile in wild-type tassels compared to ears and the repression of stamens in JA maize mutants suggest that jasmonate is needed in both maize and Arabidopsis for proper stamen development.

Additional jasmonate-deficient phenotypes are described in rice that consist in sex-determination phenotypes in maize. The allene oxide cyclase mutants of rice have elongated sterile lemmas and, occasionally, longer pales or additional bract-like organs similar to tassel glumes. The eg1 mutant, which encodes a plastid-targeted lipase, a homolog of DEFECTIVE IN ANOTHER DEHISCENCE1 (DAD1) of Arabidopsis, and the eg2 mutant, defective in JAZMONATE ZIM-DOMAIN (JAZ) gene, have extra glume-like organs and altered floral organ numbers. In Sorghum, pedicellate spikelets are normally sterile but not in multiseeded (msd) mutants that produce extra spikelets. The increase in fertile spikelets in msd1 mutants is lost with the addition of JA. Clearly, jasmonate plays a role in blocking the growth of pistils, leaf-like organs, and spikelets in addition to its well-established role in stamen development.

Our analysis of Ts5 adds to the growing understanding of how jasmonates are involved in maize sex determination and that not only its biosynthesis but also its turnover plays a role by contributing to its homeostasis (Fig. 4c). The lipoygenase TS1 putatively converts α-linolenic acid (18:3) to an intermediary and then to cis-(-) + 12-oxophytodienoic acid (OPDA), a precursor to JA-Ile that is further metabolized to JA via the function of OPRL and OPRR, which are partially and functionally redundant. The activation of the short-chain dehydrogenase/reductase TS2 requires TS1. Although, its exact substrates are unknown, TS2 promotes JA production. Once the bioactive jasmonate, JA-Ile, is made, it relieves transcription factors from JAZ repression, leading to the activation of several pathways, including those necessary for floral development.

ZmCYP94B1 then acts to inactivate JAs-Ile by oxidation. In conclusion, study of the gain-of-function mutant, Ts5, has revealed that the proper expression of ZmCYP94B1 is necessary to maintain maize monosity. The conversion of JA-Ile to 12OH-JA-Ile via the ZmCYP94B1 enzyme is an important regulatory mechanism for sex determination in normal ear and tassel development. Given that this enzyme also has a role in ameliorating wound-induced JA response in leaves, it provides an elegant example of a gene product having multiple tissue-specific functions.

**Methods**

**Plant materials and growth conditions.** The Ts5-ref allele was obtained from the Maize Cooperative Center and backcrossed at least seven times to B73 and Mo17 and four times to A188. Mapping was performed after backcrossing seven times into Mo17 population. The ts2-ref and ts1-ref alleles were obtained from the Maize Cooperative Center. Genetic analysis of TS5;ts2 double mutants was performed in the A188 background after three backcrosses and genotyping of all possible alleles. Introgressed stocks of sk1-R in Mo17 were a gift from Erik Vollbrecht (Iowa State). Genetic analysis of TS5;sk1 double mutants was performed after crossing sk1-R in Mo17 to Ts5 in Mo17 and self-pollinating the F1 to create an F2 population. Ecotype Col-0 was used for all Arabidopsis thaliana experiments, and plants were grown under standard greenhouse conditions. Primers CL 682 and CL 758 (Supplementary Data 1) were used to amplify Ts5 (GRMZM2G177668), which has no introns, from B73 genomic DNA. The resulting amplicon was cloned into the pEarleyGate10356 was linearized with EcoRV and recombined with the Ts5 entry clone via LR Clonase II Reaction. The resulting clone places the Ts5 ORF under control of the cauliflower mosaic virus 35S promoter. Plants were transformed using the floral dip method. Transformants were also genotyped and transgene expression was confirmed with primers CL 682 and CL 758 (Supplementary Data 1). CYP94B3-OE lines have been described.

**Plant treatments.** Plants for the rescue of tassel feminization were treated with JA as in Yan et al. 

For the root-growth assay, plants were grown on filter paper in the dark at 30 °C for 5 days or grown in the medium verniculite for 10 days under normal greenhouse conditions.

**Genetic mapping of the Ts5 locus.** We fine mapped Ts5 to a 15 Mb interval flanked by custom indel markers CLS89.590 and MS13.14 in a backcross population of Ts5 to Mo17 consisting of 273 plants. This was further narrowed to the interval flanked by umc2039 and TIPD9218. DNA extraction was performed using standard protocols (Lunde, 2018) using the primers listed in Supplementary Data 1.

**RNA-expression analysis.** RNA-Seq libraries were constructed as in Tsuda et al. except that 6 μg of total RNA was used rather than 3 μg. Three libraries were made of four pooled 9–11 mm tassels: two mutants and one wild type. Libraries were sequenced on a NextSeq Illumina platform with 75 bp paired-end reads. Raw reads were aligned to the maize B73 genome AGPv3.30 and A. thaliana Araport11 genomes. Canonical protein isoforms with blastp bit scores > 100 were run in an ETE3 pipeline that included alignment by Clustal Omega phylogeny model evaluation using PhyML and tree branchpoint evaluation using 100 bootstraps. Trees were visualized and annotated in R with the ggtree package.

**Phylogenetic analysis of maize CYP94 genes.** The amino-acid sequence of GRMZM2G177668, P01 was blasted against Zea mays AGPv3.30 and A. thaliana Araport11 genomes. Canonical protein isoforms with blastp bit scores > 100 were run in an ETE3 pipeline that included alignment by Clustal Omega phylogeny model evaluation using PhyML and tree branchpoint evaluation using 100 bootstraps. Trees were visualized and annotated in R with the ggtree package.

**Analytical methods and chemicals.** Hormone extraction from tissues was according to a previously described method with minor modifications for the quantification of jasmonate by mass spectrometry. Frozen leaf tissues and inflorescences were pulverized using metal beads in tissueLyser II (Qiagen) and extracted multiple times with 3 ml of ethyl acetate containing 0.5% acetic acid as an internal standard. The combined extracts were evaporated under a stream of nitrogen gas, and the dried residue was reconstituted in 0.2 ml of 70% methanol/water/acetic acid (v/v/v). The resulting tissue extract was cleared by centrifugation, reconstituted with 18,000 g for 30 min in 4 °C. Analysis and quantification of jasmonate were carried out based on methods described previously using an electrospray ionization triple quadrupole mass spectrometer (Xevo T-QS, Waters) interfaced with an ultra-performance liquid chromatography (ACQUITY H-class, Waters). Characteristic mass spectrometry transitions were detected using multiple reaction monitoring in electrospray ionization-negative mode for JA (m/z 209 > 59), dhJA (211 > 59), 12OH-JA (225 > 59), JA-Ile (322 > 130), [13C6]-JA-Ile (328 > 136), 12OH-JA-Ile (338 > 130), and 12COOH-JA-Ile (352 > 130). MassLynx 4.1 and TargetLynx (Waters) were used to analyze the data.

**Statistical analysis and plotting.** Student’s t tests, one-way analysis of variance, and graphs were made using GraphPad Prism software, with the exception of graphs in 3F, which were designed in core R packages. Raw data used to create graphs are available in Supplementary Data 4.

**Reporting Summary.** Further information on experimental design is available in the Nature Research Reporting Summary linked to this article.

**Data availability**

Transcriptomic data for 10 mm tassel RNA-Seq of Ts5/+ and normal siblings are available at the NCBI sequence read archive (SRA) under the accession code PRJNA495059.

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Author contributions

C.L., S.H. and A.J.K. designed the experiments. C.L. and A.K. performed the experiments. C.L., A.K. and S.L. analyzed the data. C.L. and S.L. prepared the figures. C.L. and S.H. wrote the manuscript. A.J.K. and S.L. edited the manuscript.

Additional information

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