Received: 20 December 2022 | Revised: 2 May 2023 | Accepted: 8 May 2023

DOI: 10.1002/pld3.501

ORIGINAL RESEARCH

Genetic evidence that brassinosteroids suppress pistils in the maize tassel independent of the jasmonic acid pathway

Norman Best[1] | Brian Dilkes[2,3]

[1]Agriculture Research Service, Plant Genetics Research Unit, USDA, Columbia, Missouri, USA

[2]Department of Biochemistry, Purdue University, West Lafayette, Indiana, USA

[3]Center for Plant Biology, Purdue University, West Lafayette, Indiana, USA

Correspondence
Norman Best, Plant Genetics Research Unit, Agriculture Research Service, USDA, Columbia, MO 65211, USA.
Email: norman.best@usda.gov

Brian Dilkes, Department of Biochemistry, Purdue University, West Lafayette, IN 47907, USA.
Email: bdilkes@purdue.edu

Funding information
U.S. Department of Agriculture, National Institute of Food and Agriculture, Grant/Award Numbers: 2017-67011-26077, 2019-67012-29655; National Science Foundation, Grant/Award Number: 1755401

Abstract

The developmental genetics of reproductive structure control in maize must consider both the staminate florets of the tassel and the pistillate florets of the ear synflorescences. Pistil abortion takes place in the tassel florets, and stamen arrest is affected in ear florets to give rise to the monoecious nature of maize. Gibberellin (GA) deficiency results in increased tillering, a dwarfed plant syndrome, and the retention of anthers in the ear florets of maize. The *silkless1* mutant results in suppression of silks in the ear. We demonstrate in this study that jasmonic acid (JA) and GA act independently and show additive phenotypes resulting in androecious *dwarf1*;*silkless1* double mutant plants. The persistence of pistils in the tassel can be induced by multiple mechanisms, including JA deficiency, GA excess, genetic control of floral determinacy, and organ identity. The *silkless1* mutant can suppress both silks in the ear and the silks in the tassel of JA-deficient and AP2 transcription factor *tasselseed* mutants. We previously demonstrated that GA production was required for brassinosteroid (BR) deficiency to affect persistence of pistils in the tassel. We find that BR deficiency affects pistil persistence by an independent mechanism from the *silkless1* mutant and JA pathway. The *silkless1* mutant did not prevent the formation of pistils in the tassel by *nana plant2* in double mutants. In addition, we demonstrate that there is more to the *silkless1* mutant than just a suppression of pistil growth. We document novel phenotypes of *silkless1* mutants including weakly penetrant ear fasciation and anther persistence in the ear florets. Thus, the JA/AP2 mechanism of pistil retention in the tassel and silk growth in the ear are similarly sensitive to loss of the SILKLESS1 protein, while the BR/GA mechanism is not.

KEYWORDS
brassinosteroids, gibberellins, jasmonic acid, maize, reproductive development

1 | INTRODUCTION

In the maize reproductive structures, two complex inflorescences give rise to an iteratively branched structure that ultimate terminates in pairs of florets. In the tassel, both florets of each pair are retained, but all pistils abort mid-development, resulting in staminate flowers at maturity. In the ear, only the upper floret of each pair is retained, and stamen development arrests mid-development, resulting in pistillate flowers. In maize flowers, silks are a long structure that grow from pistils and contain transmitting tracts and stigmata. These are characteristic of the ear florets but can be induced in the tassel if the process of pistil abortion is suppressed (Dellaporta & Calderon-Urrea, 1993;

Irish, 1996; Irish & Nelson, 1989). When this persistence of pistils in the tassel results in seed production, the phenotype is referred to as "tasselseed." The persistence of pistils in the tassel can occur due to a suppression of abortion without subsequent development, as occurs in *grassy tillers1* (*gt1*) (Whipple et al., 2011) and enhanced by some *ramosa3* mutants (Klein et al., 2022), or can result in silk and even seed development in the tassel, as seen in *tasselseed* (*ts*) mutants (Acosta et al., 2009; Delong et al., 1993), BR-deficient dwarfs (Best et al., 2016; Hartwig et al., 2011; Makarevitch et al., 2012), JA deficient mutants (Yan et al., 2012), and the application of exogenous GA (Nickerson, 1959, 1960).

It has been nearly a century since gibberellin was first isolated (Kurosawa, 1926) and two centuries since it was first described in a dictated agronomy book by Koinishi; see discussion in (Stowe & Yamaki, 1959). Within a few years of its structural solution (Cross et al., 1961) and synthesis (Stowe & Yamaki, 1959), bioactive GA were applied to maize tassels and discovered to result in pistil retention in the tassel florets (Nickerson, 1959). Two dwarf mutants, *nana plant1* (*na1*) and *na2*, that retained pistils in their tassel florets were first identified in 1922 by Hutchison (Hutchison, 1922) and 1924 by Suttle (Suttle, 1924). These were later determined to be mutants in two steps in brassinosteroid biosynthesis (Best et al., 2016; Hartwig et al., 2011). We subsequently demonstrated that the retention of pistil phenotype of these mutants required GA by a combination of genetic and biochemical approaches (Best et al., 2016, 2017). Thus, these two hormones act in a concerted manner as a pathway affecting pistil development in the tassel. In addition to affecting floral organ retention in the tassel, reduced GA results in retention of anthers in ear florets (Evans & Poethig, 1995). As a result, some of the genes defined by mutants affecting steps in the GA pathway have been named *anther ear* in maize (*anther ear1* (*an1*), *an2*) (Bensen et al., 1995; Emerson & Emerson, 1922; Phinney, 1956), but this phenotype is more widely distributed and affected by all GA-deficient and insensitive *dwarf* mutants of maize (*dwarf1* (*d1*), *d3*, *d5*, *D8*, and *D9*) (Chen et al., 2014; Fu et al., 2016; Fujioka et al., 1988; Winkler & Freeling, 1994; Winkler & Helentjaris, 1995).

Persistence of pistils in tassel florets had been discovered as a mutant phenotype among the morphological mutants originally described by Emerson (1920), the first two of which were called *ts1* and *ts2*. These are not affected in GA production or BR production, and despite the name of these mutants, they alter more than the tassel florets. Both mutants affect the retention of pistils in the tassel florets, resulting in silk in the tassel and occasional seed formation on tassels. They also affect development to maturity of the floret from the lower of the two florets on the spikelet. This phenotype has been known since these mutants' discovery and is described in the original paper where the tassel-like retention of the lower floret of each spikelet on ears is referred to as the "tassel ear" phenotype (Emerson, 1920). This has been observed in multiple studies since then including a manuscript foundational to our understanding of the linkage map of maize (Emerson et al., 1935). Reversed germ orientation and disorganized kernel rows on the dried ear, resulting from a retention of the lower floret, is also a feature of *ts4*, *Ts5*, and *Ts6* (aka *Reversed germ orientation2/Indeterminant Spikelet1*) (Chuck et al., 2007; Irish, 1997; Irish et al., 1994; Lunde et al., 2019; Wang et al., 2020). Of these genes, *ts1*, *ts2*, and *ts5* have been cloned and determined to function in the JA pathway by having lower JA levels (Acosta et al., 2009; Delong et al., 1993; Lunde et al., 2019; Wang et al., 2020). The other two, *ts4* and *Ts6* (also known as *indeterminate spikelet*), are caused by a loss of microRNA 172e and a dominant mutant of one of its targets, which has an altered target site, respectively (Chuck et al., 2007). The mutants currently named as *ts* mutants in maize have a cohesive set of phenotypes. All of these mutants are relatively normal in height, display silk in the tassel floret, and retain florets in the ear spikelets, suggesting that the AP2 transcription factor and JA hormone are working in a similar pathway to manipulate floral organ determinacy. In addition to this set of cohesive phenotypes, *ts4* and *Ts6* mutants also have an indeterminate spikelet phenotype.

Attempts have been made to reconcile the *ts* group of mutants with the GA pathway. In a tour de force of double mutants (Irish et al., 1994), the interaction between GA deficiency and *ts* mutants *ts1*, *ts2*, *ts4*, and *Ts5* was tested. In all cases, the GA-deficient dwarf phenotypes were additive with the *ts* mutant phenotypes indicating no interaction between these two pathways. This demonstrated that the JA and GA pathways act independently to alter the floral organ complements retained in the mature tassel and ear florets. Likewise, JA acts independently of GA to affect continued development of the lower floret in the ear.

The *silkless1* (*sk1*) mutants, which lack pistils in ear florets, identified another gene in the JA biosynthetic pathway, encoding a glycosyltransferase. The biochemical function of this enzyme is yet unknown, but *sk1* mutants have high JA and overexpression of SK1 resulted in low JA and retention of silk in the tassel (Hayward et al., 2016; Zhao et al., 2018). Double mutants between *sk1* and the *ts* mutants demonstrated that *sk1* is epistatic to the *ts* mutants and suppressed the formation of silk in the tassel (Irish et al., 1994). It is unknown if *sk1* acts upstream of the JA pathway in which case it might also be epistatic to the pistil retention phenotype in the BR biosynthetic mutants or inhibit the response of the tassel florets to GA. However, *sk1* does not suppress the pistil development in the ear florets of the *ts2* mutant.

We explore whether the JA pathway and BR/GA pathways intersect via a series of double mutants of maize. We find no evidence for interaction between *sk1* and the GA and BR pathways confirming that GA and JA act independently on pistil retention in the maize tassel and ear florets. BR appears to be acting on these phenotypes exclusively via GA and does not interact with or potentiate JA effects on tassel development. In addition, we demonstrate that *sk1* mutants result in ear fasciation and mild anther ear, demonstrating heretofore unappreciated roles for this UDP-glucuronosyltransferase, and possibly JA, in meristem regulation and anther arrest in the ear florets.

2 | METHODS

2.1 | Plant material

The *sk1* mutant seeds were obtained from the Maize Genetics Cooperation Stock Center as stock 214J (Hayward et al., 2016). The *d1*

mutant was obtained from the Carolina Biological Supply Company. The *na2* mutant was obtained from the Maize Genetics Cooperation Stock Center as stock 506 G (*na2-1*) (Best et al., 2016). The *ts1* mutant was obtained from the Maize Genetics Cooperation Stock Center as stock 217A (Acosta et al., 2009). The *ts2* mutant was obtained from the Maize Genetics Cooperation Stock Center as stock 106E (Delong et al., 1993). All mutant stocks were backcrossed to B73 at least two times before making F1 crosses between mutant combinations.

2.2 | Plant growth conditions and phenotyping

F2 segregating double mutant plants were phenotyped under standard field conditions as managed by the Purdue Agronomy Center for Research and Education farm in the summer of 2015. Replicates of the F2 plants were grown in the summer of 2016 to confirm prior observations. Phenotypes were assessed and photographs were taken at maturity. Segregation ratios for phenotypes were assessed by chi-squared analysis when each category was $n > 5$ or by Fisher's exact tests when any category was $n < 5$. Comparison of phenotype penetrance was also tested by Fisher's exact tests.

3 | RESULTS

3.1 | The gibberellin and the jasmonic acid pathway defined by silkless1 act independently to control reproductive development

Reduced JA or excess GA can result in persistence of pistils in the tassel (Acosta et al., 2009; Nickerson, 1959). Based on prior experiments, reduced JA levels also results in pistil persistence in the ear (Emerson, 1920). Reduced GA results in persistence of anthers in both ear florets (Chen et al., 2014). Based on prior experiments, JA and GA effects appear unrelated and additive phenotypes for persistence of pistils in the tassel have been observed in *ts*;*d1* double mutants (Irish et al., 1994), indicating that GA is not required for JA deficiency to result in persistence of pistils in the tassel. The *sk1* gene encodes a UDP-glycosyltransferase that regulates endogenous jasmonic acid levels (Hayward et al., 2016; Zhao et al., 2018). The *sk1* gene is predicted to encode an enzyme responsible for inactivating JA via conjugation. Knockout mutants of *sk1* results in an absence of pistils in the normally pistillate ears and high levels of JA. Overexpression of SK1 results in persistence of pistils in the tassel, development of pistils in the ear, and low levels of JA (Hayward et al., 2016). Reduction of GA levels, on the other hand, results in severely dwarf plants, outgrowth of tillers, and anther development in the ear (Emerson & Emerson, 1922). This results in the upper floret producing a perfect flower and the lower floret, resulting in an androecious flower. Thus, GA-deficient mutants of maize are andromonoecious, unlike the normal maize plant's monoecious habit.

To test the interaction between reduction of GA and excess of JA, we constructed double mutants between *d1* and *sk1*. The single *d1* mutants were dwarf, tillered, and had developed anthers in the ear florets (Figure 1a). The single *sk1* mutants failed to develop silks in the ear (Figure 2b). In addition to the previous role in silk growth, *sk1* mutants also displayed some anther persistence in the ear florets, similar to a reduction in GA signaling (Figure S1a–e and Table 1). Anther ear was visible in five of the 116 *sk1* single mutant plants, which was significantly different from the complete lack in normal ears (Fisher's exact test p value < .001; anther ear; normal; *sk1* 5:111; WT 0:326). Remarkably, three of the five *sk1* ears that displayed anther ear were also fasciated (Figure S1c–e). This fasciation was not solely a feature of *sk1* ears that displayed anther ear and was also found on numerous *sk1* ears (Figure S2a,b). Thus, in addition to suppressing pistil growth, *sk1* also displayed weakly penetrant anther ear and ear fasciation phenotypes demonstrating previously unknown roles for JA in ear and anther development. Double mutants between *d1* and *sk1* were completely additive, resulting in dwarf plants with normal tassel

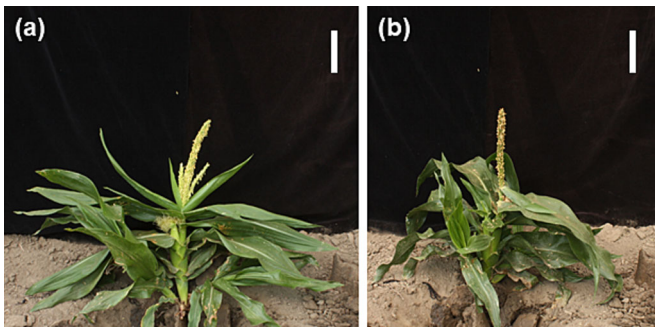

FIGURE 1 Morphological features of *d1* and *d1*;*sk1* double mutants. (a) Mature *d1* mutant and (b) *d1*;*sk1* double mutant. (a,b) Scale bar indicates 20 cm.

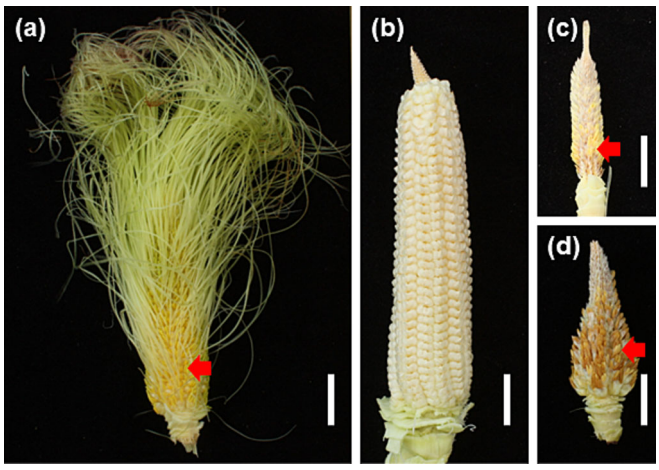

FIGURE 2 Mature ears of *d5*, *sk1*, and *d5*/*sk1* mutants. (a) Mature *d1* ear, (b) *sk1* ear, and (c,d) *d1*;*sk1* ears. (a–d) Red arrows point to anthers. Scale bar indicates 2 cm.

TABLE 1 Effect of *dwarf1* and *silkless1* on mature phenotypes of ear florets in a *d1*/+;*sk1*/+ F2.

	WT	d1	sk1	*d1;sk1*
n	326	101	116	48
Plants with anther ear (%)	0 (0%)	101 (100%)	5 (4%)	48 (100%)
Plants with sk (%)	0 (0%)	0 (0%)	116 (100%)	48 (100%)

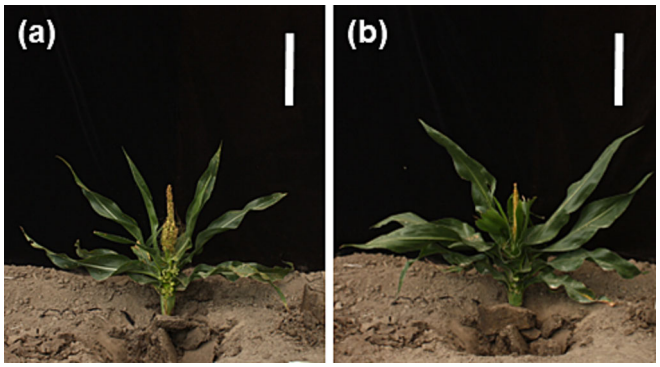

FIGURE 3 Morphological features of normal, *na2*, and *na2;sk1* mutants. (a) Mature normal plant, (b) *na2* single mutant, and (c) *na2;sk1* double mutant. (a–c) Scale bar indicates 20 cm.

florets, tiller outgrowth, and ears with staminate florets with no pistil development (Figures 1b and 2c,d and Table 1). This converted maize into an androecious plant as both inflorescences only produced staminate flowers. This demonstrates a simple path to transform both maize inflorescences to produce an androecious maize plant with two recessive alleles.

3.2 | Brassinosteroids act independent of the silkless1 pathway to affect silk formation in maize flowers

Loss of *sk1* is sufficient to suppress persistence of pistils in the tassel of JA-deficient recessive *ts* mutants (*ts1* and *ts2*), which in turn restore pistil production in *sk1* ears (Irish et al., 1994; Jones, 1934). The *na2* single mutants exhibited a dwarf stature and a subset of florets retained pistils (Figure 3b). Double mutants of *na2;sk1* were dwarf and had pistils in the tassel, like na2 single mutants, but lacked pistils in the ear, like sk1 single mutants (Figure 4c,e and Table 2). Thus, *sk1* was unable to suppress the persistence of pistils in the tassel of *na2;sk1* double mutants as pistils developed in a subset of tassel florets just as was observed in *na2* single mutants (Fisher's exact test p value = .456; persistence of pistils in the tassel; normal; *na2* 34:66; *na2;sk1* 8:11). Thus, *sk1* mutants cannot suppress the pistil development in the tassel induced by a reduction in BR levels. This also demonstrates that unlike in the tassel, a reduction in BR levels does not induce pistil development in the ears of *sk1* mutants. These results set BR deficiency apart from the effects of *Ts6*, where *sk1* completely suppressed pistil production in the tassel of *sk1;Ts6* double mutants (Irish et al., 1994). This also sets BR deficiency apart from the JA deficiency phenotypes of *ts2*, where *sk1;ts2* double mutants partially suppress the *ts2* phenotype in the tassel (Irish et al., 1994). Similarly, *ts2*, but not BR deficiency (Figure 4c and Table 2), suppressed the *sk1* phenotype in the ear (Irish et al., 1994). Thus, although the JA and BR pathways both can affect pistil retention, they are not part of the same floral organ retention pathway and have distinct genetic interactions in both inflorescences.

3.3 | Reduction of brassinosteroids and the short-chain dehydrogenase tasselseed2 independently control maize development

Brassinosteroid-deficient mutants result in a dwarf phenotype with mildly penetrant persistence of pistils in the tassel (Best et al., 2016). The *ts2* mutant does not affect plant height (Figure 5b) but results in highly penetrant persistence of pistils in the tassel (Figure 6b). The *ts2* gene encodes a putative alcohol dehydrogenase of unknown function but application of JA suppresses the phenotype, suggesting that it may be required for JA biosynthesis (Acosta et al., 2009). We constructed double mutants to test if *ts2* and *na2* displayed any genetic interactions. As we have shown previously, *na2* single mutants had incomplete conversion of their tassel such that a subset of florets retained their pistils and developed silks (Figure 6a and Table 3). All of the *ts2* single mutants had tassel florets that retained their pistils (Figure 6b and Table 3). Double mutants of *na2* and *ts2* were additive, resulting in plants with a dwarf stature similar to *na2* (Figure 5c,d), with highly penetrant persistence of pistils in the tassel, similar to *ts2* (Figure 5b,d). Segregation ratios failed a chi-square analysis of 9:3:3:1 predominantly due to a low number of *na2* single mutant plants. We have repeatedly observed altered segregation ratios due to poor seedling establishment of BR-deficient mutants under field conditions. Taken together, no interaction was detected between *na2* and *ts2*.

3.4 | Jasmonic acid and brassinosteroid deficiencies independently control maize development

To further test for any interactions between JA and BR deficiency for plant height and persistence of pistils in the tassel, double mutants between the BR-deficient mutant *na2* and the JA-deficient mutant *ts1* were created. The *ts1* mutants had no effect on plant height, and the *na2;ts1* double mutants stature was similar to the *na2* single mutant dwarfs (Figure 7a–d). The *na2* single mutants had mildly penetrant

FIGURE 4 Mature ears and tassels of *na2*, *sk1*, and *na2*;*sk1* mutants. (a) Mature *sk1* ear, (b) *na2* ear, and (c) *na2*;*sk1* double mutant. (d) Mature tassel of *na2* and (e) *na2*;*sk1* double mutant. (a–e) Scale bar indicates 2 cm.

TABLE 2 Effect of *nana plant2* and *silkless1* on mature phenotypes of florets in a *na2*/+;*sk1*/+ F2.

	WT	na2	sk1	*na2;sk1*
n	275	100	92	19
Plants with tasselseed (%)	0 (0%)	34 (34%)	0 (0%)	8 (42%)
Plants with silkless (%)	0 (0%)	0 (0%)	92(100%)	19(100%)

FIGURE 5 Morphological features of normal, *ts2*, *na2*, and *na2*;*ts2* mutants. (a) Mature normal plant, (b) *ts2* mutant, (c) *na2* mutant, and (d) *na2*/*ts2* double mutant. (a–d) Scale bar indicate 20 cm.

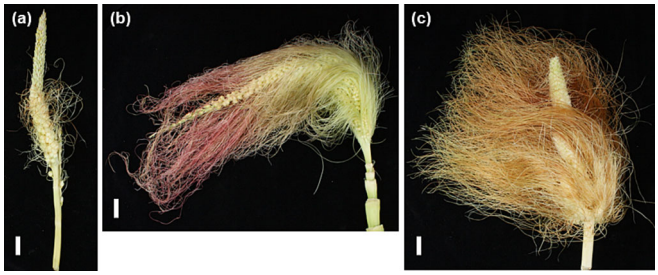

FIGURE 6 Mature tassels of *na2*, *ts2*, and *na2*;*ts2* mutants. (a) Mature *na2* tassel, (b) *ts2* tassel, and (c) *na2*;*ts2* double mutant tassel. (a–c) Scale bar indicates 2 cm.

persistence of pistils in the tassel affecting 58% of single mutant tassels primarily at the base of the main rachis and base of tassel branches (Figure 8a and Table 4). The variable penetrance of *na2-1* for persistence of pistils in the tassel is visible in comparisons between Tables 2–4 as a lower percentage of *na2* single mutants exhibited persistence of pistils in the tassel in the cross to *sk1* (Table 2) as compared with the *na2* single mutants in the *ts1* (Table 4) and *ts2* populations (Table 3). One hundred percent of *ts1* single mutants exhibited high penetrance of persistence of pistils in the tassel with suppression of anther development (Figure 8b and Table 4). Double mutants between *na2* and *ts1* also exhibited a high penetrance of persistence of pistils in the tassel with suppression of anther development, like the more severe *ts1* single mutants. There were an over representation of wild-type (WT) and double mutant plants within the population that resulted in a failed chi-test for segregation ratios. Despite this, there is no evidence of an interaction between JA and BR.

4 | DISCUSSION

Our results are consistent with the finding that GA excess and BR deficiency form a single pathway affecting floral organ persistence (Best et al., 2016, 2017), distinct from the JA pathway. We have summarized the published genetic interactions affecting floral organ persistence (specifically *ts*, *sk*, BR *na*, and GA *d* classes of mutants) in maize in Table 5. The double mutants with *sk1* and *na2* also demonstrate a separation between the BR and JA pathways with respect to floral organ persistence and floral meristem persistence in maize

TABLE 3 Effect of *nana plant2* and *tasselseed2* on mature phenotypes of tassel florets in a *na2*/+;*ts2*/+ F2.

	WT	na2	ts2	*na2;ts2*
n	127	19	50	19
Plants with tasselseed (%)	0 (0%)	12 (63%)	50 (100%)	19 (100%)

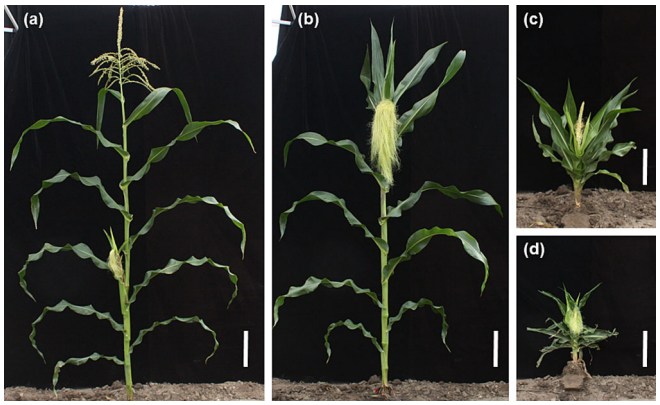

FIGURE 7 Morphological features of normal, *ts1*, *na2*, and *na2*; *ts1* mutants. (a) Mature normal plant, (b) *ts1* mutant, (c) *na2* mutant, and (d) *na2*;*ts1* double mutant. (a-d) Scale bar indicate 20 cm.

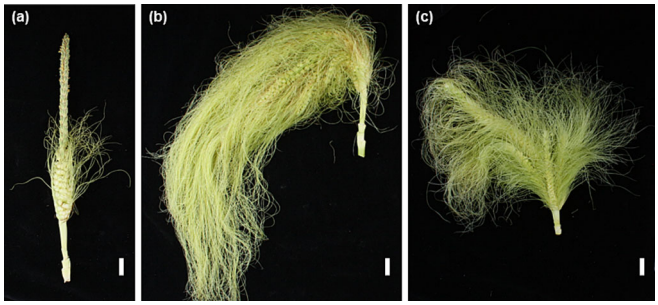

FIGURE 8 Mature tassels of *na2*, *ts1*, and *na2*;*ts1* mutants. (a) Mature *na2* tassel, (b) *ts1* tassel, and (c) *na2*;*ts1* double mutant tassel. (a–c) Scale bar indicates 2 cm.

inflorescences. Prior suggestions that GAs are a pistil specific factor that is involved in the retention of pistils in the JA and AP2 transcription factor *ts* mutants (Dellaporta & Calderon-Urrea, 1994) are not supported by the phenotypes of the double mutants. Further experiments manipulating BR, GA, and JA levels through exogenous application of these hormones to *na*, *ts*, and *sk1* mutants remain as follow-up experiments. Even though blocks in GA production, but not the JA accumulating *sk1*, can suppress pistil retention in the BR mutants, it is unknown if JA application to BR mutants can result in pistil retention in BR mutant tassel florets. Likewise, it is unknown if excess GA application can induce silk formation in the ears or tassels of *sk1* mutants.

The *ts2*;*sk1* double mutants display substantially less retention of pistils in their tassel florets than *ts2* single mutants (Table 5) (Irish et al., 1994). The reversed-germ orientation phenotype in the ear, however, is not *sk1* dependent (Irish et al., 1994). The suppression of pistils in tassel florets of *ts2*;*sk1* double mutants contrasts with an earlier anecdote from D.F. Jones (Jones, 1934) claiming "when homozygous *tasselseed2* and *silkless* are together in the same individual the *silkless* gene has no apparent effect," given the clear images in Irish et al., 1994 we think this claim by D.F. Jones is in error.

The *sk1* mutant is unique among the JA affecting mutants in that it raises JA levels (Hayward et al., 2016; Zhao et al., 2018). Reduction of JA production results in more than just pistil retention in the tassel florets but also affects continued development of the lower floret on the lower branch in the ear spikelet. Likewise, *sk1* mutants were affected by more than just the loss of silk in the ear. We noticed ear fasciation in a subset of *sk1* ears (Figures S1c–e and S2a,b). In other mutants, this phenotype results from meristem over proliferation (Du et al., 2021; Pautler et al., 2015; Taguchi-Shiobara et al., 2001). This phenotype has not been previously mentioned in work on *sk1* mutants. We think this owes to the phenotype arising infrequently and being weakly penetrant as it was observed in both the *d1* and *na2* F2 populations. It is formally possible that there was a second mutation segregating in the *sk1* background and that this second mutation caused fasciation in a *sk1*-dependent manner. In this context, it is worth noting that some *sk1* ears, including a subset of fasciated and unfasciated ears, also exhibited florets with retained anthers. The arrest of anther development in ear florets is associated with the expression of the cell cycle inhibitor *wee1* (Kim et al., 2007; Sun et al., 1999). If cell cycle inhibition is abrogated in the *sk1* mutants, this might explain both the anther ear and fasciation phenotypes. Like the retention of lower florets in *ts* mutants, it may be that the name of the *sk1* mutant has caused people to unnecessarily focus only on pistils. A careful assessment of ear fasciation in the *ts* mutants remains to be done to determine if this phenotype is limited to *sk1* or also is affected by the genetically interacting *ts* mutants. Meristem growth control is also affected by the *ts4* and *ts6* mutants which exhibit ear branching (Chuck et al., 2007; Irish, 1997), as does *ramosa3*, which can also affect pistil retention in the tassel via an interaction with *gt1* (Klein et al., 2022). Future experiments may clarify the relationships of meristem growth control in the ear and pistil retention in the tassel.

What could JA and GA be influencing? Several mutants in MADS box genes with roles in floral organ identity control pistil formation. Tassels of mutants in the floral MADS box transcription factors *bearded ear1* produce pistils (Thompson et al., 2009), and *silky1* results in transformation of stamens into pistils and the retention of these pistils in the tassel florets (Ambrose et al., 2000). As might be expected for a mutant in miRNA processing, the *fuzzy tassel* mutant, defective in a dicer-like homolog, also results in pistil-like floral organs with stigmatic papillae (Thompson et al., 2014). Similarly, mutants in the epigenetic regulators *required to maintain repression6*, encoding a component of the small RNA pathway, and *mediator of paramutation1*,

TABLE 4 Effect of *nana plant2* and *tasselseed1* on mature phenotypes of tassel florets in a *na2*/+;*ts1*/+ F2.

	WT	na2	ts1	*na2;ts1*
n	323	64	70	37
Plants with tasselseed (%)	1 (0.3%)	37 (58%)	70 (100%)	37 (100%)

TABLE 5 Summary of genetic interactions between mutants affecting reproductive development.

Double mutant	Pistil retention tassel	Floret retention ear	Pistil retention ear	Stamen retention ear	Fasciated ear	Reference
d1;sk1	n/a	n/a	Additive	Additive	Additive	This study
na2;sk1	*na2* epistatic	n/a	Additive	n/a	Additive	This study
na2;ts1	Additive	Additive	n/a	n/a	n/a	This study
na2;ts2	Additive	Additive	n/a	n/a	n/a	This study
sk1;ts1	*sk1* epistatic	Additive	Additive	n/a	?	Irish et al. (1994)
sk1;ts2	*sk1* epistatic	Additive	Additive	n/a	?	Irish et al. (1994)
sk1;ts4	*sk1* epistatic	Additive	Additive	n/a	?	Irish et al. (1994)
sk1;Ts5	*sk1* epistatic	Additive	Additive	n/a	?	Irish et al. (1994)
na2;d5	*d5* epistatic	n/a	n/a	Additive	n/a	Best et al. (2016)
d1;ts1	Additive	Additive	n/a	Additive	?	Irish et al. (1994)
d1;ts2	Additive	Additive	n/a	Additive	?	Irish et al. (1994)
d1;ts4	Additive	Additive	n/a	Additive	?	Irish et al. (1994)
d1;Ts6	Additive	Additive	n/a	Additive	?	Irish et al. (1994)

Note: n/a indicate no interaction can be assessed because the phenotype is not present and question marks indicates that an interaction has not been assessed.

encoding RNA-dependent RNA polymerase, similarly result in pistil retention in the tassels indicating a requirement for these genes in miRNA-regulated gene repression (Parkinson et al., 2007). One area that needs clarification is the order of controls over floral organ persistence. If the MADS box genes act upstream of either of these two hormone transduction cascades, then biochemical experiments should readily display sensitivity of mutant phenotypes. One MADS box mutant that has been assessed for its interactions with JA is *sterile tassel silky ear1*(*sts1*)*/zmm16* (Bartlett et al., 2015). The *sts1* mutant transforms the identity of stamens into lodicules in the tassel. Double mutants between *sts1* and either *ts1* or *gt1* demonstrate that *gt1* and *ts1* are required to abort pistils in the tassel florets but also that they were negatively regulating pistil identity. This results in sterile tassel florets in *sts1* single mutants and a persistence of pistils in *sts1*;*gt1* and *sts1*;*ts1* double mutants. Interestingly *sts1*;*ts1* double mutants also exhibit a greater number of silks in ear and tassel florets than either single mutant, consistent with our finding that JA affects both florets similarly (Bartlett et al., 2015). A role for GA has yet to be established in this pathway. If GA is an important player in the MADS box *sts1*;*gt1* and *gt1*;*ra1* mutants, then uniconazole and paclobutrazol should block the changes in pistil retention. If they are primarily affected by the JA pathway, then JA application should inhibit silk production in these mutants. If, however, the mutants affected by mutations in MADS box transcription factors and their regulators are downstream of these hormone pathways, exogenous applications of pistil suppressing compounds (e.g., paclobutrazol or JA) should have no effect on the floral organ persistence in these mutants. The fact that the JA accumulating *sk1* mutant can suppress the AP2 transcription factor mutant *Ts6* and its miRNA complement *ts4* suggests that these floral regulatory transcription factors are upstream of JA.

This work demonstrates that the persistent pistil phenotype of GA excess induced by BR deficiency is separate and additive with the roles of JA and the miR172/AP2 pathway. This integrates our understanding of these pathways. Unlike the JA and miR172/AP2 pathways, sk1 could not prevent silk production due to BR deficiency in the tassel.

AUTHOR CONTRIBUTIONS

Norman Best and Brian Dilkes designed the experiments, analyzed data, and wrote the manuscript. Norman Best performed the experiments.

ACKNOWLEDGMENTS

We would like to thank Jim Beaty and the crew at the Purdue University ACRE for help with production of field-grown maize used in these studies. Mention of trade names or commercial products in this publication was solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture. The U.S. Department of Agriculture is an equal opportunity provider and employer. This work was supported by funds to NBB (NIFA Nos. 2017-67011-26077 and 2019-67012-29655) from the U.S. Department of Agriculture, National Institute of Food and Agriculture and the U.S. Department of Agriculture, Agriculture Research Service and to BPD (National Science Foundation award 1755401).

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Norman Best https://orcid.org/0000-0002-6572-5999
Brian Dilkes https://orcid.org/0000-0003-2799-954X

REFERENCES

Acosta, I. F., Laparra, H., Romero, S. P., Schmelz, E., Hamberg, M., Mottinger, J. P., Moreno, M. A., & Dellaporta, S. L. (2009). tasselseed1 is a lipoxygenase affecting jasmonic acid signaling in sex determination of maize. *Science*, *323*, 262–265. https://doi.org/10.1126/science.1164645

Ambrose, B. A., Lerner, D. R., Ciceri, P., Padilla, C. M., Yanofsky, M. F., & Schmidt, R. J. (2000). Molecular and genetic analyses of the Silky1 gene reveal conservation in floral organ specification between eudicots and monocots. *Molecular Cell*, *5*, 569–579. https://doi.org/10.1016/S1097-2765(00)80450-5

Bartlett, M. E., Williams, S. K., Taylor, Z., DeBlasio, S., Goldshmidt, A., Hall, D. H., Schmidt, R. J., Jackson, D. P., & Whipple, C. J. (2015). The maize PI/GLO ortholog Zmm16/sterile tassel silky ear1 interacts with the zygomorphy and sex determination pathways in flower development. *Plant Cell*, *27*, 3081–3098. https://doi.org/10.1105/tpc.15.00679

Bensen, R. J., Johal, G. S., Crane, V. C., Tossberg, J. T., Schnable, P. S., Meeley, R. B., & Briggs, S. P. (1995). Cloning and characterization of the maize An1 gene. *Plant Cell*, *7*, 75–84.

Best, N. B., Hartwig, T., Budka, J., Fujioka, S., Johal, G., Schulz, B., & Dilkes, B. P. (2016). Nana plant2 encodes a maize ortholog of the *Arabidopsis* brassinosteroid biosynthesis gene DWARF1, identifying developmental interactions between brassinosteroids and gibberellins. *Plant Physiology*, *171*, 2633–2647. https://doi.org/10.1104/pp.16.00399

Best, N. B., Johal, G., & Dilkes, B. P. (2017). Phytohormone inhibitor treatments phenocopy brassinosteroid-gibberellin dwarf mutant interactions in maize. *Plant Direct*, *1*, pld3.9. https://doi.org/10.1002/pld3.9

Chen, Y., Hou, M., Liu, L., Wu, S., Shen, Y., Ishiyama, K., Kobayashi, M., McCarty, D. R., & Tan, B. C. (2014). The maize DWARF1 encodes a gibberellin 3-oxidase and is dual localized to the nucleus and cytosol. *Plant Physiology*, *166*, 2028–2039. https://doi.org/10.1104/pp.114.247486

Chuck, G., Meeley, R., Irish, E., Sakai, H., & Hake, S. (2007). The maize tasselseed4 microRNA controls sex determination and meristem cell fate by targeting Tasselseed6/indeterminate spikelet1. *Nature Genetics*, *39*, 1517–1521. https://doi.org/10.1038/ng.2007.20

Cross, B. E., Grove, J. F., McCloskey, P., MacMillan, J., Moffatt, J. S., & Mulholland, T. P. C. (1961). The structures of the fungal gibberellins. In *Gibberellins* (Vol. 28) (pp. 3–17). American Chemical Society. https://doi.org/10.1021/ba-1961-0028.ch001

Dellaporta, S. L., & Calderon-Urrea, A. (1993). Sex determination in flowering plants. *The Plant Cell*, *5*, 1241–1251.

Dellaporta, S. L., & Calderon-Urrea, A. (1994). The sex determination process in maize. *Science*, *266*, 1501–1505. https://doi.org/10.1126/science.7985019

Delong, A., Calderonurrea, A., & Dellaporta, S. L. (1993). Sex determination gene Tasselseed2 of maize encodes a short-chain alcohol-dehydrogenase required for stage-specific floral organ abortion. *Cell*, *74*, 757–768. https://doi.org/10.1016/0092-8674(93)90522-R

Du, Y., Lunde, C., Li, Y., Jackson, D., Hake, S., & Zhang, Z. (2021). Gene duplication at the fascicled ear1 locus controls the fate of inflorescence meristem cells in maize. *Proceedings of the National Academy of Sciences*, *118*, e2019218118. https://doi.org/10.1073/pnas.2019218118

Emerson, R. A. (1920). Heritable characters of maize II.-Pistillate flowered maize plants. *Journal of Heredity*, *11*, 65–76. https://doi.org/10.1093/oxfordjournals.jhered.a101971

Emerson, R. A., Beadle, G. W., & Fraser, A. C. (1935). Summary of linkage studies in maize. *Cornell University Agricultural Experiment Station Memoir*, *180*, 1–83.

Emerson, R. A., & Emerson, S. H. (1922). Genetic interrelations of two andromonoecious types of maize, dwarf and anther ear. *Genetics*, *7*, 203–236. https://doi.org/10.1093/genetics/7.3.203

Evans, M. M. S., & Poethig, R. S. (1995). Gibberellins promote vegetative phase-change and reproductive maturity in maize. *Plant Physiology*, *108*, 475–487. https://doi.org/10.1104/pp.108.2.475

Fu, J., Ren, F., Lu, X., Mao, H., Xu, M., Degenhardt, J., Peters, R. J., & Wang, Q. (2016). A tandem array of ent-kaurene synthases in maize with roles in gibberellin and more specialized metabolism. *Plant Physiology*, *170*, 742–751. https://doi.org/10.1104/pp.15.01727

Fujioka, S., Yamane, H., Spray, C. R., Katsumi, M., Phinney, B. O., Gaskin, P., Macmillan, J., & Takahashi, N. (1988). The dominant non-gibberellin-responding dwarf mutant (D8) of maize accumulates native gibberellins. *Proceedings of the National Academy of Sciences of the United States of America*, *85*, 9031–9035. https://doi.org/10.1073/pnas.85.23.9031

Hartwig, T., Chuck, G. S., Fujioka, S., Klempien, A., Weizbauer, R., Potluri, D. P., Choe, S., Johal, G. S., & Schulz, B. (2011). Brassinosteroid control of sex determination in maize. *Proceedings of the National Academy of Sciences of the United States of America*, *108*, 19814–19819. https://doi.org/10.1073/pnas.1108359108

Hayward, A. P., Moreno, M. A., Howard, T. P. 3rd, Hague, J., Nelson, K., Heffelfinger, C., Romero, S., Kausch, A. P., Glauser, G., Acosta, I. F., Mottinger, J. P., & Dellaporta, S. L. (2016). Control of sexuality by the sk1-encoded UDP-glycosyltransferase of maize. *Science Advances*, *2*, e1600991. https://doi.org/10.1126/sciadv.1600991

Hutchison, C. B. (1922). The linkage of certain aleurone and endosperm factors in maize, and their relation to other linkage groups. *Cornell University Agricultural Experiment Station Memoir*, *60*, 1419–1473.

Irish, E. E. (1996). Regulation of sex determination in maize. *BioEssays*, *18*, 363–369. https://doi.org/10.1002/bies.950180506

Irish, E. E. (1997). Experimental analysis of tassel development in the maize mutant tassel seed 6. *Plant Physiology*, *114*, 817–825. https://doi.org/10.1104/pp.114.3.817

Irish, E. E., Langdale, J. A., & Nelson, T. M. (1994). Interactions between tassel seed genes and other sex-determining genes in maize. *Developmental Genetics*, *15*, 155–171. https://doi.org/10.1002/dvg.1020150206

Irish, E. E., & Nelson, T. (1989). Sex determination in monoecious and dioecious plants. *The Plant Cell*, *1*, 737–744. https://doi.org/10.2307/3868981

Jones, D. F. (1934). Unisexual maize plants and their bearing on sex differentiation in other plants and in animals. *Genetics*, *19*, 552–567. https://doi.org/10.1093/genetics/19.6.552

Kim, J. C., Laparra, H., Calderon-Urrea, A., Mottinger, J. P., Moreno, M. A., & Dellaporta, S. L. (2007). Cell cycle arrest of stamen initials in maize sex determination. *Genetics*, *177*, 2547–2551. https://doi.org/10.1534/genetics.107.082446

Klein, H., Gallagher, J., Demesa-Arevalo, E., Abraham-Juarez, M. J., Heeney, M., Feil, R., Lunn, J. E., Xiao, Y., Chuck, G., Whipple, C., Jackson, D., & Bartlett, M. (2022). Recruitment of an ancient branching program to suppress carpel development in maize flowers. *Proceedings of the National Academy of Sciences of the United States of*

America, *119*, e2115871119. https://doi.org/10.1073/pnas.2115871119

Kurosawa, E. (1926). Experimental studies on the nature of the substance secreted by the "bakanae" fungus. *Natural History Society of Formosa*, *16*, 213–227.

Lunde, C., Kimberlin, A., Leiboff, S., Koo, A. J., & Hake, S. (2019). Tasselseed5 overexpresses a wound-inducible enzyme, ZmCYP94B1, that affects jasmonate catabolism, sex determination, and plant architecture in maize. *Communications Biology*, *2*, 114. https://doi.org/10.1038/s42003-019-0354-1

Makarevitch, I., Thompson, A., Muehlbauer, G. J., & Springer, N. M. (2012). Brd1 gene in maize encodes a brassinosteroid C-6 oxidase. *PLoS ONE*, *7*, e30798. https://doi.org/10.1371/journal.pone.0030798

Nickerson, N. H. (1959). Sustained treatment with gibberellic acid of five different kinds of maize. *Annals of the Missouri Botanical Garden*, *46*, 19–37. https://doi.org/10.2307/2394566

Nickerson, N. H. (1960). Sustained treatment with gibberellic acid of maize plants carrying one of the dominant genes teopod and corn-grass. *American Journal of Botany*, *47*, 809–815. https://doi.org/10.1002/j.1537-2197.1960.tb07168.x

Parkinson, S. E., Gross, S. M., & Hollick, J. B. (2007). Maize sex determination and abaxial leaf fates are canalized by a factor that maintains repressed epigenetic states. *Developmental Biology*, *308*, 462–473. https://doi.org/10.1016/j.ydbio.2007.06.004

Pautler, M., Eveland, A. L., LaRue, T., Yang, F., Weeks, R., Lunde, C., Je, B. I., Meeley, R., Komatsu, M., Vollbrecht, E., Sakai, H., & Jackson, D. (2015). Fasciated EAR4 encodes a bZIP transcription factor that regulates shoot meristem size in maize. *The Plant Cell*, *27*, 104–120. https://doi.org/10.1105/tpc.114.132506

Phinney, B. O. (1956). Growth response of single-gene dwarf mutants in maize to gibberellic acid. *Proceedings of the National Academy of Sciences of the United States of America*, *42*, 185–189. https://doi.org/10.1073/pnas.42.4.185

Stowe, B. B., & Yamaki, T. (1959). Gibberellins: Stimulants of plant growth. *Science*, *129*, 807–816. https://doi.org/10.1126/science.129.3352.807

Sun, Y., Dilkes, B. P., Zhang, C., Dante, R. A., Carneiro, N. P., Lowe, K. S., Jung, R., Gordon-Kamm, W. J., & Larkins, B. A. (1999). Characterization of maize (*Zea mays* L.) Wee1 and its activity in developing endosperm. *Proceedings of the National Academy of Sciences*, *96*, 4180–4185. https://doi.org/10.1073/pnas.96.7.4180

Suttle, A. D. (1924). *The genetic interrelations of different types of dwarf corn*. Cornell University.

Taguchi-Shiobara, F., Yuan, Z., Hake, S., & Jackson, D. (2001). The fasciated ear2 gene encodes a leucine-rich repeat receptor-like protein that regulates shoot meristem proliferation in maize. *Genes & Development*, *15*, 2755–2766. https://doi.org/10.1101/gad.208501

Thompson, B. E., Bartling, L., Whipple, C., Hall, D. H., Sakai, H., Schmidt, R., & Hake, S. (2009). Bearded-ear encodes a MADS box transcription factor critical for maize floral development. *Plant Cell*, *21*, 2578–2590. https://doi.org/10.1105/tpc.109.067751

Thompson, B. E., Basham, C., Hammond, R., Ding, Q., Kakrana, A., Lee, T.-F., Simon, S. A., Meeley, R., Meyers, B. C., & Hake, S. (2014). The dicer-like1 homolog fuzzy tassel is required for the regulation of meristem determinacy in the inflorescence and vegetative growth in maize. *The Plant Cell*, *26*, 4702–4717. https://doi.org/10.1105/tpc.114.132670

Wang, F., Yuan, Z., Zhao, Z., Li, C., Zhang, X., Liang, H., Liu, Y., Xu, Q., & Liu, H. (2020). Tasselseed5 encodes a cytochrome C oxidase that functions in sex determination by affecting jasmonate catabolism in maize. *Journal of Integrative Plant Biology*, *62*, 247–255. https://doi.org/10.1111/jipb.12826

Whipple, C. J., Kebrom, T. H., Weber, A. L., Yang, F., Hall, D., Meeley, R., Schmidt, R., Doebley, J., Brutnell, T. P., & Jackson, D. P. (2011). Grassy tillers1 promotes apical dominance in maize and responds to shade signals in the grasses. *Proceedings of the National Academy of Sciences of the United States of America*, *108*, E506–E512. https://doi.org/10.1073/pnas.1102819108

Winkler, R. G., & Freeling, M. (1994). Physiological genetics of the dominant gibberellin-nonresponsive maize dwarfs, Dwarf-8 and Dwarf-9. *Planta*, *193*, 341–348.

Winkler, R. G., & Helentjaris, T. (1995). The maize Dwarf3 gene encodes a cytochrome P450-mediated early step in gibberellin biosynthesis. *The Plant Cell*, *7*, 1307–1317.

Yan, Y., Christensen, S., Isakeit, T., Engelberth, J., Meeley, R., Hayward, A., Emery, R. J. N., & Kolomiets, M. V. (2012). Disruption of OPR7 and OPR8 reveals the versatile functions of Jasmonic acid in maize development and defense. *The Plant Cell*, *24*, 1420–1436. https://doi.org/10.1105/tpc.111.094151

Zhao, Y., Zhang, Y., Wang, L., Wang, X., Xu, W., Gao, X., & Liu, B. (2018). Mapping and functional analysis of a maize silkless mutant sk-A7110. *Frontiers in Plant Science*, *9*, 1227. https://doi.org/10.3389/fpls.2018.01227

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Best, N., & Dilkes, B. (2023). Genetic evidence that brassinosteroids suppress pistils in the maize tassel independent of the jasmonic acid pathway. *Plant Direct*, *7*(7), e501. https://doi.org/10.1002/pld3.501