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Transcriptional responses to gibberellin in the maize tassel and control by DELLA domain proteins

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SUMMARY

The plant hormone gibberellin (GA) impacts plant growth and development differently depending on the developmental context. In the maize (*Zea mays*) tassel, application of GA alters floral development, resulting in the persistence of pistils. GA signaling is achieved by the GA-dependent turnover of DELLA domain transcription factors, encoded by *dwarf8* (*d8*) and *dwarf9* (*d9*) in maize. The *D8-Mpl* and *D9-1* alleles disrupt GA signaling, resulting in short plants and normal tassel floret development in the presence of excess GA. However, D9-1 mutants are unable to block GA-induced pistil development. Gene expression in developing tassels of *D8-Mpl* and *D9-1* mutants and their wild-type siblings was determined upon excess GA_3 and mock treatments. Using GA-sensitive transcripts as reporters of GA signaling, we identified a weak loss of repression under mock conditions in both mutants, with the effect in *D9-1* being greater. *D9-1* was also less able to repress GA signaling in the presence of excess GA_3. We treated a diverse set of maize inbred lines with excess GA_3 and measured the phenotypic consequences on multiple aspects of development (e.g., height and pistil persistence in tassel florets). Genotype affected all GA-regulated phenotypes but there was no correlation between any of the GA-affected phenotypes, indicating that the complexity of the relationship between GA and development extends beyond the two-gene epistasis previously demonstrated for GA and brassinosteroid biosynthetic mutants.

Keywords: Maize, Hormones, Genetics, Gibberellins, Meristem, Branching, Floral development, Plant height, DELLA transcription factor, Transcriptomics.

INTRODUCTION

Gibberellic acid (GA) is a plant hormone, best known for the induction of directional cell elongation by GA excess (Brian, 1958; Phinney, 1956). The anisotropic expansion of cells due to GA excess results in a dramatic increase in plant organ length and height. Similarly, loss of GA biosynthesis and blocks in GA signal transduction result in dwarfed plants (Evans & Poethig, 1995). GA signaling is accomplished by relief of transcriptional repression in every plant system investigated to date. The GA receptors, the GIBBERELLIN INSENSITIVE DWARF1 proteins, physically associate with GRAS family transcription factors encoding a DELLA domain in a GA-dependent manner (Arizumi et al., 2011; Griffiths et al., 2006; Nakajima et al., 2006). The DELLA domain is critical for their protein–protein association with this GA receptor. Binding of DELLA domain transcription factors by GA–GID complexes recruits SCF proteins and the Cullin1 ubiquitin ligase, resulting in DELLA domain protein ubiquitination and degradation by the 26S proteosome (Dill et al., 2001). Degradation of DELLA domain transcription factors relieves promoters from transcriptional repression and stimulates GA-dependent responses (Dill & Sun, 2001). Dominant alleles of the DELLA domain transcriptional repressors that block GA signal transduction have been identified in every plant genetic model and are an important source of crop architecture improvement (Peng et al., 1997; Silverstone et al., 1997; Winkler & Freeling, 1994). In Arabidopsis, there are five DELLA genes and a quintuple recessive mutant maintained some GA-responsiveness for some traits, suggesting a GA-independent signaling pathway (Fuentes et al., 2012). This interpretation requires that none of the alleles be leaky. The DELLA quintuple mutant was unresponsive to GA in the presence of the 26S proteasome inhibitor MG132 (Fuentes et al., 2012), which would be consistent with a leaky allele of one of the DELLA genes.

Dominant alleles of DELLA domain transcription factors played a key role in the 20th century improvement of crop architecture, including wheat (*Triticum aestivum*), rice (*Oryza sativa*), and barley (*Hordeum vulgare*) (Ashikari et al., 2002; Hedden, 2003; Sasaki et al., 2002). These dominant negative alleles decrease plant height, reduce lodging, and reduce plant responsiveness to other cues that increase plant size such as high nitrogen and increased plant densities (Hedden, 2003; Wu et al., 2020). Changing plant stature and architecture permitted plant breeders to rapidly increase the yield per acre of these crops (Pinstrup-Andersen & Hazell, 2009). But there is ample evidence of pleiotropy for these alleles, as should be expected for perturbing a major phytohormone signal transduction pathway. Changes in root depth and angle, seed micronutrients, and branching are also affected by these alleles (Liao et al., 2019; Schaefer et al., 2018; Ubeda-Tomas et al., 2008). While some of the traits affected by these DELLA domain transcription factor alleles contribute to increases in plant yield, others may limit their commercial potential.

In no other species is the negative pleiotropy of GA mutants effects on yield clearer than in maize (*Zea mays*). The monoecious reproductive habit of maize is sensitive to GA. During floral development flowers are initially perfect but undergo selective abortion of stamen or pistil primordia in the ear and tassel florets, respectively (Cheng et al., 1983; Kim et al., 2007). In pistillate ear florets, GA is required to suppress anther production, whereas in tassel florets, GA excess results in pistil retention (Bensen et al., 1995; Nickerson, 1959). It is likely that as a result of this negative pleiotropy, reduced height alleles of the DELLA domain proteins were not utilized in the 20th century improvement of maize (Larsson et al., 2013; Thornsberry et al., 2001; Yu et al., 2008).

Much of what we know about the influence of GA on transcription is the result of work done on Arabidopsis hypocotyls, where the overwhelming influence of GA is anisotropic cell expansion (Chiang et al., 1995; Koornneef & van der Veen, 1980; Sun et al., 1992). Previous work in maize demonstrated that GA and brassinosteroid (BR) pathways have differing genetic interactions for plant height, tiller branch development, retention of stamens in the ear, and retention of pistils in the tassel. Even if the lessons of the Arabidopsis hypocotyl are completely generalizable to maize cell elongation, the same regulator hierarchy could only be conserved for one of the three developmental contexts investigated so far (Best et al., 2016; Best et al., 2017). In Arabidopsis, cell cycle genes are regulated by DELLAs and mutants in these genes block meristem control but not cell elongation (Serrano-Mislata et al., 2017). This demonstrates that mutants in DELLA targets can uncouple GA regulation of cell division and cell elongation. KNOX genes in both Arabidopsis and maize upregulate GA catabolic genes to keep GA levels low in developing meristems (Bolduc & Hake, 2009), suggesting an important role of GA in maintaining meristem function.

The maize genome harbors *dwarf8* (*d8*) and *dwarf9* (*d9*), which encode two paralogous GRAS family transcription factors that contain DELLA domains. Both paralogs were identified from dominant mutant alleles (Lawit et al., 2010; Winkler & Freeling, 1994). Thus far, the height of all tested dominant alleles at these two loci is GA-insensitive (Winkler & Freeling, 1994), indicating that the GA response requires properly functioning DELLA domain proteins in maize. The D8 transcripts are roughly 4 to 5 times more abundant than D9 across the publicly available maize transcriptome analyses (Lawrence et al., 2004; Portwood 2nd et al., 2019). Consistent with this, many alleles have been described for the *d8* locus but only one dominant allele of *d9* is known, *Dwarf9-1* (*D9-1*). Among the dominant *d8* alleles compared by Winkler & Freeling, 1994, the weakest, *D8-Miniplant* (*D8-Mpl*), was similar in height to *D9-1* and completely GA-insensitive (Winkler & Freeling, 1994).

Because of the strong influence of GA excess on tassel development and the direct effect of DELLA domain protein repression of transcription on GA signaling, we explored gene expression in the maize tassel following GA application. We utilized the *D8-Mpl* and *D9-1* dominant mutants and their wild-type siblings and found that both mutants decreased the transcriptional responses to excess GA. Surprisingly, both mutants were also ineffective transcriptional repressors in the absence of GA excess. Because of our previous reports of non-concordance of genetic effects of loss of GA and BR on different developmental outputs (Best et al., 2016; Best et al., 2017), we explored the effects of GA excess on multiple plant organs across the diversity of maize. Genotype influenced all GA-dependent phenotypes but, consistent with previous findings, there was no concordance in the genotypic effects across different organs of maize. This demonstrated that the impacts of GA on height are genetically separable from effects on other phenotypes, opening up the opportunity to mitigate the negative effects of pleiotropy affected by dominant alleles at the DELLA domain proteins.

RESULTS

Floral organ retention and tassel development are differentially sensitive to GA in *D8-Mpl*/+ and *D9-1*/+

To determine the GA-regulated gene expression associated with floral organ retention induced by GA excess, tassel florets were compared between GA_3-treated wild-type plants and *D8-Mpl*/+ or *D9-1*/+ mutant siblings. *d8* and *d9* encode GRAS domain transcription factors that contain the DELLA domains critical for GA-regulated transcriptional responses. Both genes are expressed in a variety of tissues, but the D8 mRNA expression level was approximately 4 times higher than that of D9 mRNA across

developing tissues (Figure S1). Dominant alleles of both genes have been recovered from mutagenesis experiments in maize, and all tested to date were insensitive to GA for stem elongation (Winkler & Freeling, 1994). Only one dominant mutant allele has been described at *d9*, *D9-1*, and it results in a moderately dwarfed plant. Multiple severe dominant alleles at *d8* have been described (Lawit et al., 2010; Winkler & Freeling, 1994) but we selected the moderately dwarfed *D8-Mpl* allele to provide the closest comparison to the *D9-1* allele.

D8-Mpl/+ and *D9-1*/+ displayed several morphological changes in the tassel. In the genetic backgrounds obtained directly from the maize stock center, mock-treated *D8-Mpl*/+ and *D9-1*/+ had fewer primary tassel branches than mock-treated wild-type siblings (Figure 1; Table S2). Both wild-type siblings treated with GA_3 had more primary tassel branches as compared to mock-treated wild-type siblings. *D8-Mpl*/+ and *D9-1*/+ mutants were also both insensitive to GA_3 with respect to the number of primary tassel branches. Both *D8-Mpl*/+ and *D9-1*/+ in their own original backgrounds and in a third backcross to B73 (B73BC3) generation produced anthers in the ear florets, though the *D9-1*/+ phenotype was less severe than that of *D8-Mpl*/+ (Table S2). GA_3 treatment was unable to suppress this phenotype in all genetic backgrounds. This was most likely the result of the method of treatment to the apex of the plant and the inability of the applied GA_3 to reach the developing axillary ear inflorescences that are initiated well below the shoot apical meristem.

GA_3 was able to prevent pistil abortion in the wild-type siblings of *D8-Mpl*/+ in their original background. The wild-type *D9-1*/+ siblings were sterile as a result of both smooth barren patches and abortion of florets; as a result these did not produce pistils (Figure 1). In B73BC3 families, wild-type siblings' tassel florets retained pistils following GA_3 treatment (Table 1). The *D8-Mpl*/+ mutant was completely insensitive to GA_3 treatment in both backgrounds and all plants produced viable pollen. Interestingly, *D9-1*/+ tassel florets retained pistils in both backgrounds. This indicates that *D9-1*/+ was GA-responsive and could not prevent pistil abortion. This difference in phenotypes may be due to the specificity in function of the two DELLA proteins or differences in the two alleles.

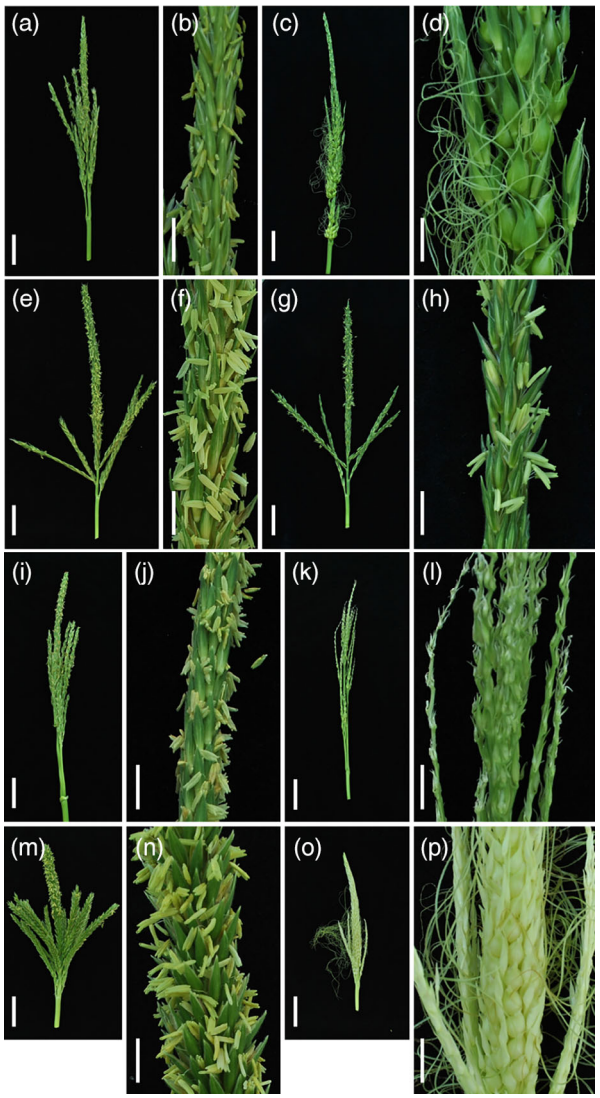

Figure 1. GA_3 whorl application of *D8-Mpl*/+ and *D9-1*/+ results in divergent floral phenotypes.
(a–d) Isogenic wild type of *D8-Mpl*/+ at anthesis with (a, b) mock treatment and (c, d) GA_3 treatment. (e–h) *D8-Mpl*/+ at anthesis with (e, f) mock treatment and (g, h) GA_3 treatment. (i–l) Isogenic wild type of *D9-1*/+ at anthesis with (i, j) mock treatment and (k, l) GA_3 treatment. (m–p) *D9-1*/+ at anthesis with (m, n) mock treatment and (o, p) GA_3 treatment. (a, c, e, g, i, k, m, o) Scale bar is 5 cm. (b, d, f, h, j, l, n, p) Scale bar is 1 cm.

All GA-induced genes in developing tassels are DELLA-regulated

Tassel morphology and floral organ development are affected by excess GA_3 (Best et al., 2016; Nickerson, 1959; Nickerson, 1960) and variation in the DELLA transcriptional repressors (Figure 1). To identify the consequences of *D8-Mpl* and *D9-1* on GA-regulated gene expression we performed an RNA-Seq analysis of developing tassels from heterozygous mutants and their wild-type siblings with and without GA_3 treatment. Tassel primordia of approximately 0.5–1 cm were taken at 40 DAP from plants treated with 866 μM GA_3 or mock by injection into the whorl every third day starting at 20 DAP. *Bona fide* GA-responsive genes were identified as follows. First, all significantly differentially expressed genes (DEGs) were identified between GA_3-treated and untreated samples from the wild-type siblings of *D8-Mpl*/+ and *D9-1*/+. Genes were retained as GA-responsive if they (i) were identified as significantly differentially expressed at a *P*-value of <0.05 after Benjamini–Hochberg correction, (ii) had a nominal *P*-value

Table 1 Effects of GA_3 on floral development of *D8-Mpl*/+, *D9-1*/+, and respective wild-type siblings from BC3F1 with B73

Source[†]	Genotype	Treatment	*n*	Plants with barren tassels[‡]	Plants with pistils in the tassel[§]
B73 × *D8-Mpl*/+	+/+	Mock	10	0 (0%)[a]	0 (0%)[a]
B73 × *D8-Mpl*/+	+/+	GA_3	9	9 (100%)[b]	9 (100%)[b]
B73 × *D8-Mpl*/+	*D8-Mpl*/+	Mock	8	0 (0%)[a]	0 (0%)[a]
B73 × *D8-Mpl*/+	*D8-Mpl*/+	GA_3	9	0 (0%)[a]	0 (0%)[a]
B73 × *D9*-1/+	+/+	Mock	10	0 (0%)[a]	0 (0%)[a]
B73 × *D9*-1/+	+/+	GA_3	9	9 (100%)[b]	9 (100%)[b]
B73 × *D9*-1/+	*D9-1*/+	Mock	8	0 (0%)[a]	0 (0%)[a]
B73 × *D9*-1/+	*D9-1*/+	GA_3	9	0 (0%)[a]	9 (100%)[b]

[†]Male pollen was selected for the presence of mutation in the BC2 generation.
[‡]Number of plants with barren tassels, with percentage of plants in parentheses. Lowercase letters indicate significant differences as determined by Fisher's exact test with *P*-value < 0.001.
[§]Number of plants with pistils in the tassel, with percentage of plants in parentheses. Lowercase letters indicate significant differences as determined by Fisher's exact test with *P*-value < 0.001.

of <0.05 in both experiments, and (iii) were affected by GA_3 application in the same direction in both wild-type backgrounds. In this way, 366 genes were identified, hereafter referred to as the *bona fide* GA-responsive genes, which were reproducibly affected by GA in the two wild-type backgrounds, of which 277 were upregulated and 89 were downregulated after GA_3 treatment (Data Files S1–S3).

The most obvious result from the analysis of this group of *bona fide* GA-regulated genes in tassels was that none of them were DELLA-independent. The two genetic backgrounds of *D8-Mpl*/+ and *D9-1*/+ responded similarly to GA treatment. No genes identified as *bona fide* GA-responsive genes were identified as DEGs in the comparison of *D8-Mpl*/+ GA_3-treated compared to mock treatment nor *D9-1*/+ GA_3-treated compared to mock treatment. Furthermore, the GA effect on every one of the 366 GA-regulated genes was suppressed by the *D8-Mpl* and *D9-1* mutations as compared to their respective GA-treated wild-type siblings (Figure 2).

GA-regulated genes demonstrate auto-regulation and hormonal interactions

Among the *bona fide* GA-regulated genes were several regulators of GA biosynthesis, catabolism, and response. Transcripts encoding D8 (GRMZM2G144744) and D9 (GRMZM2G024973) were accumulated in wild-type plants following GA_3 treatment, indicating a release from negative feedback regulation by degradation of these repressors. We also expect that the restoration of GA homeostasis should be affected by the accumulation of GA catabolic enzymes. We observed accumulation of transcripts encoding the GA-catabolizing enzymes GA2-OXIDASE2 (GA2-OX2; GRMZM2G006964) and GA2-OX9 (GRMZM2G152354) following GA_3 treatment. There was no observable decrease in the accumulation of transcripts encoding GA biosynthetic enzymes, suggesting that GA biosynthesis may already be very low in tassels at this developmental stage. A maize homolog of a gene identified as GA-responsive in Arabidopsis, *gibberellic acid stimulated-like1* (*GSL1*; GRMZM2G062527) (Zhang & Wang, 2008), was also identified among the genes with increased transcript accumulation following GA_3 treatment.

The GA and BR pathways interact to control tassel development in maize (Best et al., 2016; Best et al., 2017). The BRASSINAZOLE RESISTANT (BZR) transcription factors involved in BR-responsive gene regulation have been shown to interact with DELLA proteins (Gallego-Bartolome et al., 2012; Li et al., 2012). Two orthologs of the Arabidopsis DELLA protein interactors, a BZR homolog (encoded by *BZR10*; GRMZM2G102514) (Yu et al., 2018) and a GRAS domain transcription factor (encoded by *GRAS54*; GRMZM2G106548), were increased in their expression. This suggests that these partners of the DELLA proteins carry out conserved roles in the GA response and hormone integration. mRNA encoding a known interactor of the BZRs in rice and Arabidopsis, dwarf and low tillering1 (DLT1), accumulates following GA application (Tong et al., 2009; Tong et al., 2012) and the maize ortholog (AC234164.1_FG004) also accumulates following GA treatment of wild-type tassels, suggesting that it is a downstream target of the DELLA domain repressors. These changes indicate that some of the integration of GA and BR signaling occurs directly due to DELLA-dependent transcriptional regulation of BR signaling components and transcriptional networks.

In addition to factors involved in BR signaling, genes with demonstrated roles in auxin signaling and transport were differentially expressed following GA_3 treatment. The ABC transporter gene responsible for the *brachytic2* (*br2*; GRMZM2G315375) mutant of maize (Multani et al., 2003) was increased in expression in GA_3-treated tassels. Similarly, expression of the auxin transporter PINFORMED1D (PIN1D; GRMZM2G171702) was increased, suggesting that both BR2 and PIN1D are upregulated with excess GA_3 and therefore may be direct DELLA targets. In contrast, expression of the AUXIN RESPONSE FACTOR29 (ARF29;

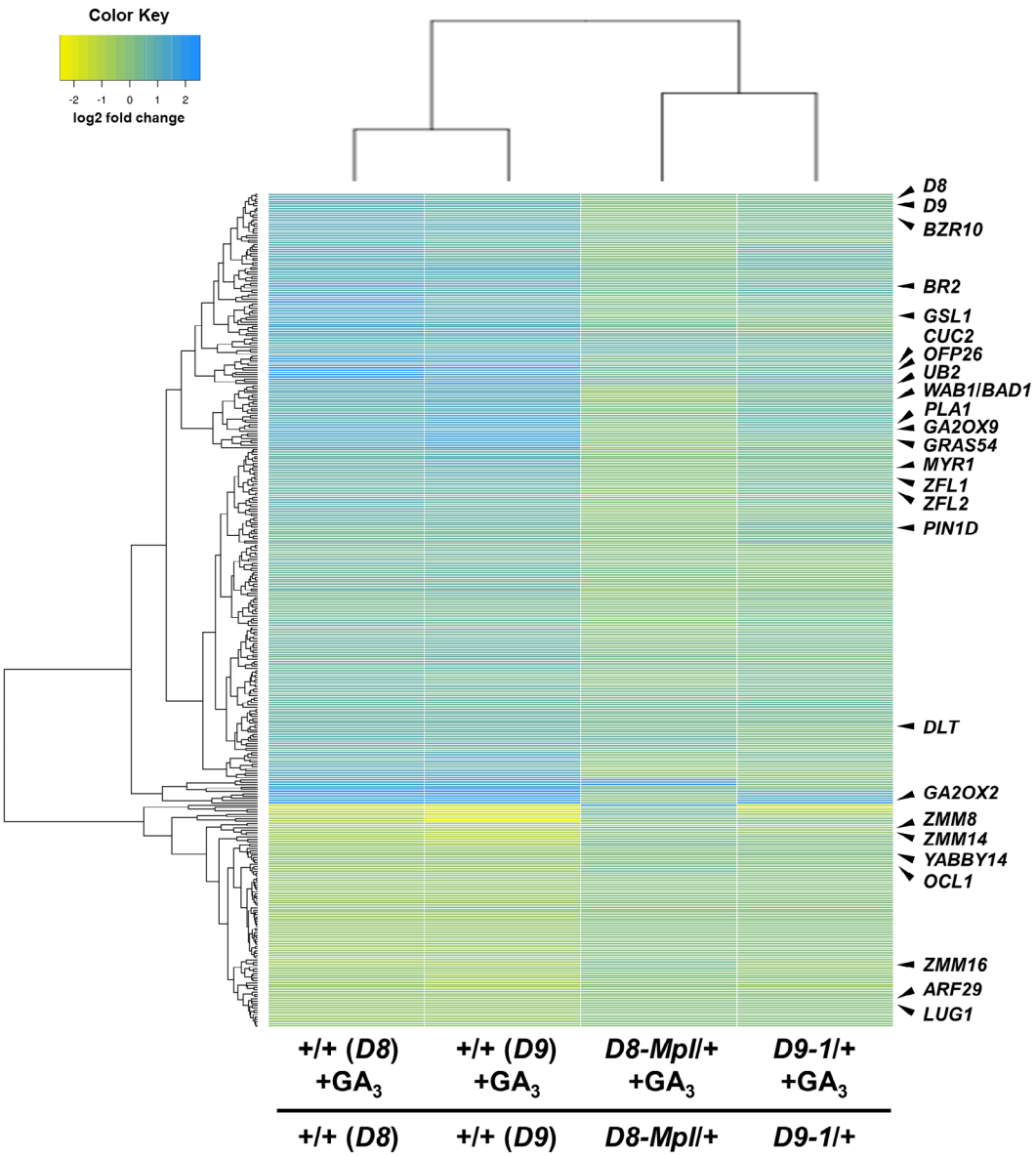

Figure 2. Heatmap of 366 *bona fide* filtered GA-responsive genes.
Genes were first filtered for being differentially expressed in +/+ (*D8*) treated with GA_3 compared to mock at a Benjamini–Hochberg genome-wide significant *P*-value of <0.05. The same genes then had to be significant at a nominal *P*-value of <0.05 in +/+ (*D9*) and in the same direction as +/+ (*D8*). Log_2-transformed fold change expression value heatmap of 366 filtered differentially expressed genes in GA-treated samples compared to mock samples within the respective genotype. Dodgerblue1 indicates high expression and magenta represents low expression in GA-treated samples. Genes are clustered by the expression across all four genetic samples. Selected genes are indicated on the right of the heatmap.

GRZM2G086949) transcriptional activator (Galli et al., 2015; Matthes et al., 2019) was decreased in tassels following GA_3 treatment.

Effect of GA treatment on floral organ identity and inflorescence development

Consistent with the pistil retention in tassel florets following GA treatment, several transcripts with roles in floral organ identity and the control of floral transition and patterning were differentially expressed in tassels following GA_3 application. Transcripts of the two *Zea mays leafy* (*ZFL*) homologs, *ZFL1* and *ZFL2* (Bomblies et al., 2003), were more abundant following GA_3 treatment. This was consistent with GA accelerating flowering time (Evans & Poethig, 1995). In addition, the expression levels of MADS box genes that are downstream of ZFL1 and ZFL2 (Danilevskaya et al., 2008) were altered. Expression of the MADS box transcription factors ZEA MAYS MADS8 (ZMM8;

GRMZM2G102161), ZMM14 (GRMZM2G099522), and ZMM16 (GRMZM2G110153) was decreased following GA_3 application. Both ZMM8 and ZMM14 are abundantly expressed in tassel and ear florets (Cacharron et al., 1999). In tassel florets, ZMM8 is restricted to the upper floret primordia, where it accumulates in the carpels prior to abortion (Cacharron et al., 1999). ZMM16 is encoded by *sterile tassel silky ear1* (*STS1*), and loss of this *pistillata* homolog results in a loss of stamens but no gain of carpels in the tassel (Bartlett et al., 2015). Similarly, the maize homolog of the LEUNIG (LUG) repressor (GRMZM2G361398), which represses both the MADS box transcription factor AGAMOUS and microRNA172 (miR172) in Arabidopsis (Aukerman & Sakai, 2003; Grigorova et al., 2011), was decreased in abundance following GA_3 treatment. The homolog of the known LUG interactor and YABBY transcription factor ABNORMAL FLORAL ORGANS1/FILAMENTOUS FLOWER1 (Chen et al., 1999) (GRMZM2G005353) was also decreased in abundance following GA_3 treatment.

Several other genes with predicted effects on flowering time were also differentially expressed in tassels following GA_3 treatment. Among these, CYTOCHROME P450 78A (CYP78A) transcripts, encoded by the maize *plastochron1* gene (*PLA1*; GRMZM2G167986) (Sun et al., 2017), were increased in abundance following GA_3 treatment. This is consistent with GA-induced accelerated flowering time (Lang, 1957; Wittwer et al., 1957). Expression of *golden-like transcription factor6* (*GLK6*; GRMZM2G117193), which encodes a homolog of the Arabidopsis MYB-RELATED PROTEIN1 (MYR1), a regulator of flowering time under low light (Zhao et al., 2011), was increased following GA_3 treatment. In addition, expression of the *outer cell layers1* (*OCL1*; GRMZM2G026643) gene was decreased following GA_3 treatment. This gene was identified based on its accumulation in the outer cell layer of all maize meristems and tissues investigated (Ingram et al., 1999; Ingram et al., 2000) and was shown to delay flowering time when overexpressed (Depege-Fargeix et al., 2011).

Although tissue was collected too late to investigate the suppression of tassel branching, effects on axillary meristems such as spikelet and floret meristems and meristem determinacy may be visible as changes in gene expression in these experiments. Multiple genes with known or predicted effects on axillary meristem determinacy and growth were affected by GA_3 treatment, and this was dependent on D8 and D9. Among these genes, two have demonstrated roles in axillary meristems in the tassel. Both the squamosa promoter binding protein-like (SPL) transcription factor gene *unbranched2* (*UB2*; GRZM2G160917) and the teosinte branched1-cincinnata-proliferating cell factor (TCP) domain transcription factor gene *Wavy auricle in blade1*/*branch angle defective1* (*WAB1*/*BAD1*; GRMZM2G110242) accumulated following GA_3 treatment. Both *UB2* and *WAB1*/*BAD1* were previously shown to be responsible for spikelet pair meristem and spikelet meristem determinacy (Bai, Reinheimer, et al., 2012a; Chuck et al., 2014; Hay & Hake, 2004). Though multiple other SPLs were altered in their expression, no genes with previous roles in pistil retention in the tassel (e.g., *tasselseed1* [*ts1*] through *ts6*) were differentially expressed in response to GA_3. This was consistent with genetic studies of double mutants between GA mutants and *ts1*, *ts2*, *ts4*, *ts5*, and *ts6* mutants demonstrating independent mechanisms regulate pistil retention in the maize tassel (Acosta et al., 2009; Chuck et al., 2007; Delong et al., 1993; Irish et al., 1994; Lunde et al., 2019).

GA_3 treatment of *D8-Mpl*/+ and *D9-1*/+ fails to identify any DEG

GA_3 treatment had no discernable effect on gene expression in *D8-Mpl*/+ mutants as assessed by differential gene expression analysis at a Benjamini–Hochberg adjusted *P*-value threshold of <0.05. This corresponded well with the phenotypic observation of no stem elongation upon GA_3 treatment and pistil abortion in *D8-Mpl*/+ tassels treated with GA_3 (Figure 1). Similarly, and somewhat surprisingly given the effect of GA_3 treatment on *D9-1*/+ tassels, no genes were significantly affected by GA_3 application in *D9-1*/+ compared to mock controls at a Benjamini–Hochberg *P*-value of <0.05. Yet, these results were consistent with the lack of DELLA-independent transcriptional effects of GA in the maize tassel (Figure 2; Data Files S4 and S5). Given the phenotypic impact of GA on *D9-1*/+ tassels and the absence of DEG at this threshold, we carried out a series of careful, and more sensitive, assessments of GA-regulated gene expression by considering pathways and gene sets.

D8-Mpl strongly represses GA-regulated gene expression

To determine what effects these mutant alleles have on GA-regulated gene expression, transcript levels were compared between GA_3-treated wild-type siblings and *D8-Mpl*/+ or *D9-1*/+ mutants. A comparison between GA_3-treated *D8-Mpl*/+ and GA_3-treated wild-type siblings identified 561 DEGs at a Benjamini–Hochberg adjusted *P*-value of <0.05 (Data Files S6 and S7). Of these genes, 560 were present in the comparison of GA_3- and mock-treated wild-type siblings and 131 were among the 366 *bona fide* GA-regulated genes (Data Files S3 and S6). Of these 131 genes, 81 genes were both induced by GA_3 in wild-type samples and suppressed by *D8-Mpl*/+ treated with GA_3. Expression of the 50 genes repressed by GA_3 application in wild-type samples was increased in the GA_3-treated *D8-Mpl*/+ samples (Figure 2). Thus, all genes were affected in the expected direction for a dominant negative mutant blocking GA signaling.

An additional 268 GA-regulated genes were identified in the wild-type siblings of *D8-Mpl*/+ following GA_3 treatment. These represent putative GA-regulated genes even

though they were not identified in the congenic wild-type siblings of *D9-1*/+ treated with GA_3, and were therefore not included the *bona fide* gene set. Of these 268 genes, the 158 genes induced by GA_3 in wild type were suppressed by *D8-Mpl*/+ and the 110 genes repressed by GA_3 application in wild type were increased in the *D8-Mpl*/+ samples. Thus, all genes affected by GA_3 treatment reacted to *D8-Mpl*/+ as expected for genes controlled by a constitutive repressor of GA-induced gene expression.

DEG overlaps can be problematic in the presence of high false negative rates. A more complete analysis of the effects of GA on the 560 *D8-Mpl*/+ sensitive genes during GA_3 treatment was performed by looking at the direction of their expression in the comparison of congenic wild-type samples with and without GA_3 treatment (Table 2). Of the 560 genes, 545 genes were affected in the expected direction if *D8-Mpl*/+ encodes a repressor of GA-induced gene expression (Table 2). Of the 15 genes not affected by GA_3, but altered in the mutant, six genes were on chromosome 1 linked to the position of *d8*, which was consistent with introgression of expression quantitative trait loci (eQTLs) from the original progenitor of *D8-Mpl*/+ affecting these changes in expression. These consonant effects on gene expression by *D8-Mpl*/+ and GA were far more than expected (expectation of 1:1 for consonant:dissonant expression patterns; chi-squared *P*-values of $<4.2 \times 10^{-111}$) and the direction of the effects indicated that the protein encoded by the *D8-Mpl* allele represses GA-induced gene expression.

The 268 DEGs that were repressed by *D8-Mpl*/+ treated with GA_3 and significantly affected by GA_3 application of the wild type included 131 genes in the *bona fide* GA-regulated gene list (Figure 2; Data Files S1–S3, see discussion above) as well as 137 additional genes (Figure S2; Data Files S5 and S6). Among these 137 additional genes altered by the *D8-Mpl*/+ mutant were multiple genes with possible functions in GA signaling and tassel development. Among these was an additional BZR homolog (*BZR7*; AC194970.5_FG002) (Yu et al., 2018) that was suppressed by *D8-Mpl*/+ in the GA_3-treated samples relative to GA_3-treated wild types. This was similar to the GA-induced BZR homolog described above (*BZR10*; GRMZM2G102514; Figure 2; Data File S3), further suggesting that the interaction between DELLA domain transcription factors and the BZR transcription factors observed in Arabidopsis (Bai, Shang, et al., 2012b; Gallego-Bartolome et al., 2012; Li et al., 2012) is conserved in maize. In addition, a link with ethylene signaling was suggested by the decreased accumulation of transcripts encoding an ETHYLENE INSENSITIVE3 (EIN3) homolog (EIN-LIKE6; GRMZM2G151811) in GA_3-treated *D8-Mpl*/+ mutants as compared to GA_3-treated wild type (Figure S2; Data Files S5 and S6).

A set of floral-associated transcription factors was also present in this list. Several MADS box transcription factors were differentially expressed, consistent with complete suppression of pistils in GA_3-treated *D8-Mpl*/+ mutants. Among these were three MADS box genes, all of which were decreased in abundance in wild-type plants treated with GA_3 as compared to GA_3-treated *D8-Mpl*/+ mutants. As these genes were repressed by GA, they were likely altered in their expression because of pistil retention in the tassel, rather than being direct targets of D8-Mpl transcriptional repression. These genes included *bearded ear1* (*BDE1*; GRMZM2G160565) (Thompson et al., 2009), mutants of which display floral meristem indeterminacy and other defects in floral organ production. Expression of another member of the same *AG*-like subfamily, *zea agamous5* (*ZAG5*; GRMZM2G003514), was also decreased. The third MADS box gene was *ZMM24* (GRMZM2G087095), which is known to respond to floral induction (Danilevskaya et al., 2008). Other genes with known roles in floral development that may encode indirect targets included a *cincinnata-like* gene, *tcp transcription factor7* (*TCPTF7*; GRMZM2G035944), and a gene encoding the maize homolog of the flower-specific phytochrome-associated protein (GRMZM2G11 9720). Expression of another meristem-associated transcript encoding an aintegumenta (Klucher et al., 1996) paralog of maize (*AP2/EREBP-transcription factor184* [*EREB184*]; GRMZM2G028151) was decreased in *D8-Mpl*, suggesting it may be a direct target of DELLA-domain protein-mediated repression.

Other indications of changes in specialized metabolic pathways were visible when GA_3-treated wild-type and *D8-Mpl*/+ plants were compared. A second CYP78A gene that is closely related to *PLA1* and *KLUH* (Anastasiou et al., 2007; Stransfeld et al., 2010; Sun et al., 2017), the CYP78A9-like gene GRMZM2G092823, was accumulated in wild-type treated plants as compared to GA_3-treated *D8-Mpl*/+ mutants. This mirrors the GA-induced genes in the two wild-type datasets for the *PLA1* transcripts and suggests a close link between the KLUH pathway, tassel development, and GA signaling in maize. Expression of the

Table 2 Effect of *D8-Mpl* on GA-regulated gene expression compared to congenic wild types

Effect of GA_3[a]	Effect of *D8-Mpl*[b]	Number of DEGs[c]
Increase	Decrease	244
Decrease	Decrease	6
Increase	Increase	9
Decrease	Increase	301

[a]Indicates if gene expression increases or decreases following GA_3 treatment of wild-type tassels.
[b]Indicates if gene expression increases or decreases in *D8-Mpl* relative to wild type following GA_3 treatment.
[c]Genes selected based on differential expression at a Benjamini–Hochberg *P*-value of <0.05 in *D8-Mpl* relative to wild type following GA_3 treatment.

indole monooxygenase-encoding gene *BENZOXAZINONE SYNTHESIS2* (*BX2*; GRMZM2G085661) (Frey et al., 1995, 1997) was decreased in GA_3-treated wild type compared to GA_3-treated *D8-Mpl*/+ mutants. This gene may be a direct target of D8-mediated gene repression.

Even in the presence of GA_3, *D9-1* has few consequences for transcript accumulation in tassels

Remarkably, only 28 DEGs were identified between wild-type siblings and *D9-1*/+ mutants treated with GA_3 at a Benjamini–Hochberg adjusted *P*-value of <0.05 (Figure S3; Data Files S8 and S9). Of these, 15 genes are located on chromosome 5, 14 of which were on the short arm, where *d9* is located. These are potentially *cis*-eQTLs introgressed from the original mutant background into our *D9-1*/+ stock introgressed into B73. Only two of the genes in Table S9, *dehydrin3* (*DHN3*; GRMZM2G373522) and *c-terminal encoded peptide1* (*CEP1*; GRMZM2G007969), are among the *bona fide* GA-regulated genes and neither of these two are located on chromosome 5. Both genes were more accumulated in GA_3-treated wild-type siblings than in GA_3-treated *D9-1*/+ mutants. These two genes were both upregulated in the wild-type GA_3-treated compared to the mock dataset. The very small number of DEGs demonstrates the *D9-1* mutant allele prevents GA-regulated gene expression changes but is less able to repress GA-regulated gene expression than *D8-Mpl*. The lack of differences in transcript abundance between *D9-1*/+ and wild-type controls mirrors the retention of pistils in *D9-1*/+ and wild-type tassels following GA_3 application (Figure 1 and Table 1).

D8-Mpl and *D9-1* have consistent effects on expression in the absence of GA_3 treatment

Minor transcriptional effects were observed between mock-treated *D8-Mpl*/+ and wild-type siblings with only 17 transcripts (five upregulated and 12 downregulated in *D8-Mpl*/+) being differentially accumulated between the two samples (Data Files S10 and S11). Of these genes, 10 are located on chromosome 1, the chromosome harboring *d8*, suggesting that linked expression polymorphisms contributed to the expression differences. These data suggest that *D8-Mpl*/+ had little effect on gene expression in the absence of GA_3 application, perhaps because the endogenous level of GA signaling in the developing maize tassel was below the level of detection by genome-wide mRNA profiling.

The mock-treated *D9-1*/+ mutant exhibited a stronger transcriptional effect with 93 differentially accumulated transcripts (30 upregulated and 63 downregulated in *D9-1*/+) between mock-treated *D9-1*/+ mutant and wild-type siblings (Data Files S12 and S13). Of these 93 genes, 72 were affected in the same direction in the mock-treated *D8-Mpl*/+ compared to the wild-type dataset. Of the remaining genes, 19 were in opposing directions and two were not expressed in the *D8-Mpl*/+ experiments. This indicates that the *D8-Mpl* and *D9-1* alleles have similar effects on transcription in the absence of GA (chi-squared test *P*-value = 2.7×10^{-08}) but that *D9-1* alters transcription to a greater degree. This was the opposite of what was observed above for the two alleles in the presence of GA_3. Because linkage drag may have introduced *cis*-effects linked to *d9* and *d8* in these comparisons, we removed chromosomes 1 and 5 from the comparison and re-calculated the overlaps between DEG lists. Removing these genes resulted in 45 overlapping genes, of which 42 moved in the same direction in both mutants and three moved in opposing directions.

A closer look at GA-regulated genes among the *D9-1*/+-affected transcripts returned a surprise. Of the 93 DEGs affected by *D9-1*/+ in comparison to wild-type under mock conditions, 83 were altered in the same direction by *D9-1*/+ and by GA_3 application compared to the wild type (Table 3; chi-squared test *P*-value = 3.7×10^{-14}; Data Files S5, S8, and S13). This demonstrates that under mock conditions, *D9-1*/+ did not completely repress GA-responsive genes and did not activate GA-repressed genes. Removal of the 28 genes on chromosome 5, which may contribute to DEGs by linkage drag of strong *cis*-eQTLs, resulted in 61 of the remaining 65 genes being similarly affected by GA_3 treatment and the *D9-1*/+ genotype (chi-squared test *P*-value = 1.5×10^{-12}; Table S7). A similar result was obtained when comparing the expression of 93 genes in the wild-type siblings from the *D8-Mpl*/+ mutant with and without GA_3 treatment (64 the same, 27 opposing, two genes not expressed in the comparison; chi-squared test *P*-value = 1.1×10^{-4}; Table 4; Data Files S4, S6, and S11). Despite the absence of DEGs in *D8-Mpl*/+, we used the mock comparison of wild type and *D8-Mpl*/+ to determine if the genes differentially expressed in *D9-1*/+ were also impacted by the *D8-Mpl*/+ mutation. Of the 93 genes affected by *D9-1*/+ in mock conditions, 72 were similarly impacted by the *D8-Mpl*/+ mutant (chi-squared

Table 3 Effect of *D9-1* on GA-regulated gene expression compared to congenic wild types

Effect of GA_3[a]	Effect of *D9-1*[b]	Number of DEGs[c]
Increase	Decrease	8
Decrease	Decrease	55
Increase	Increase	28
Decrease	Increase	2

[a]Indicates if gene expression increases or decreases following GA_3 treatment of wild-type tassels.
[b]Indicates if gene expression increases or decreases in *D9-1* relative to wild type following mock treatment.
[c]Genes selected based on differential expression at a Benjamini–Hochberg *P*-value of <0.05 in *D9-1* relative to wild type following GA_3 treatment.

Table 4 Effect of GA on gene expression in the wild-type siblings of *D8-Mpl* for the 93 DEGs affected by *D9-1*

Effect of GA_3[a]	Effect of *D9-1*[b]	Number of DEGs[c]
Increase	Decrease	19
Decrease	Decrease	42
Increase	Increase	22
Decrease	Increase	8

[a]Indicates if gene expression increases or decreases following GA_3 treatment of wild-type tassels.
[b]Indicates if gene expression increases or decreases in *D9-1* relative to wild type following mock treatment.
[c]Genes selected based on differential expression at a Benjamini–Hochberg *P*-value of <0.05 in *D8-Mpl* relative to wild type following GA_3 treatment.

Table 5 Impact of *D8-Mpl* on transcript accumulation for the 93 genes affected by *D9-1*

Effect of *D9-1*[a]	Effect of *D8-Mpl*[b]	Number of transcripts
Increase	Increase	26
Increase	Decrease	4
Decrease	Increase	15
Decrease	Decrease	46

[a]Indicates if gene expression increases or decreases in *D9-1* relative to wild type following mock treatment.
[b]Indicates if gene expression increases or decreases in *D8-Mpl* relative to wild type following mock treatment.

test *P*-value = 3.8×10^{-8}; Table 5; Data Files S10, S12, and S13). These findings of differential expression under mock conditions of *D9-1*/+, the similarity of the direction of the effects of GA_3 treatment, and the significant overlap observed in the *D8-Mpl*/+ mutant were not predicted by the 'dominant-negative' interpretation of these alleles' phenotypes (Winkler & Freeling, 1994). Our data were consistent with *D9-1*, and to a lesser extent *D8-Mpl*, encoding hypofunctional repressors of GA-regulated genes. This resulted in a higher expression status in *D9-1*/+ of GA-responsive genes under mock conditions compared to wild-type siblings (see additional analysis below). Together our results demonstrate that the protein encoded by *D9-1* is an inhibitor of GA signaling at high GA concentrations and a poor repressor of GA-regulated genes under mock conditions. We carried out additional analysis to explore this further.

Assessment of GA-responsive gene expression using an aggregate index of expression differences

The 366 *bona fide* GA-responsive genes represented an annotated set of genes reproducibly affected by GA excess. The chi-squared tests, as employed on DEGs, use the direction of expression to determine whether the overall patterns of effects were consonant, dissonant, or indistinguishable from other gene expression patterns. For example, do effects of the *D9-1*/+ mutant resemble treating wild-type plants with excess GA_3? As such, these tests are non-parametric and do not respond to the degree of change in expression. We hypothesized that a parametric estimate of expression similarity might be able to estimate the degree of GA response in a tissue. We performed a parametric quantification of GA-responsiveness by calculating an index of the aggregate effect of a treatment on the expression levels of the 366 *bona fide* GA-responsive genes (Figure 3). If our previous analyses were correct, then *D9-1* should encode a defective repressor and result in a weak loss of repression of the 366 *bona fide* GA-responsive genes in mock conditions. Thus, among the 366 *bona fide* GA-responsive genes, we should observe an increase in the index value calculated for the genes that increased in their accumulation upon GA_3 treatment and a decrease in the index value calculated for the genes that decreased in their accumulation upon GA_3 treatment in the *D9-1*/+ mutant.

How we calculate an index that summarizes gene expression will strongly impact the sensitivity to gene expression distributions. Summing the gene length-normalized counts of all up- or downregulated genes, for example, will result in an index that is disproportionately affected by the most abundant transcripts. The accumulation distributions of transcripts were highly skewed among genes (Data File S3; Figure S4). Within the 366 *bona fide* GA-regulated genes, the top accumulated transcripts were GRMZM2G326111 and GRMZM5G815894, each of which contributes more than 6% of the total transcript counts across all samples (Figure S4). The GRMZM2G326111 gene encodes a PEPTIDYL PROLYL ISOMERASE-LIKE protein and GRMZM5G815894 was predicted to encode a RIBOSOMAL L5 protein involved in ribosomal RNA export from the nucleus. Overall, only 20 genes of the 366 genes account for 50% of the total transcripts from the *bona fide* GA-regulated gene set (Figure S4). To avoid the effect of the unequal distribution of read counts per gene but maintain the utility of the index as a measure of GA-responsiveness, we chose to calculate *Z*-scores for each gene in each treatment as the input value for the index. Thus, the index value was the sum of the *Z*-scores for each gene in each treatment. This places the estimate of each gene on the same scale, relative to its expression level, with weights determined by the degree of expression difference in each sample relative to the others. We calculated indices measuring the overall GA-responsiveness as a sum of the *Z*-scores for all genes that increased with GA_3 treatment (hereafter called the 'up-index'), that decreased with GA_3 treatment (hereafter called the 'down-index'), and a joint value of responsiveness by adding the up-index values and subtracting the down-index values (Figure 3; Data File S3).

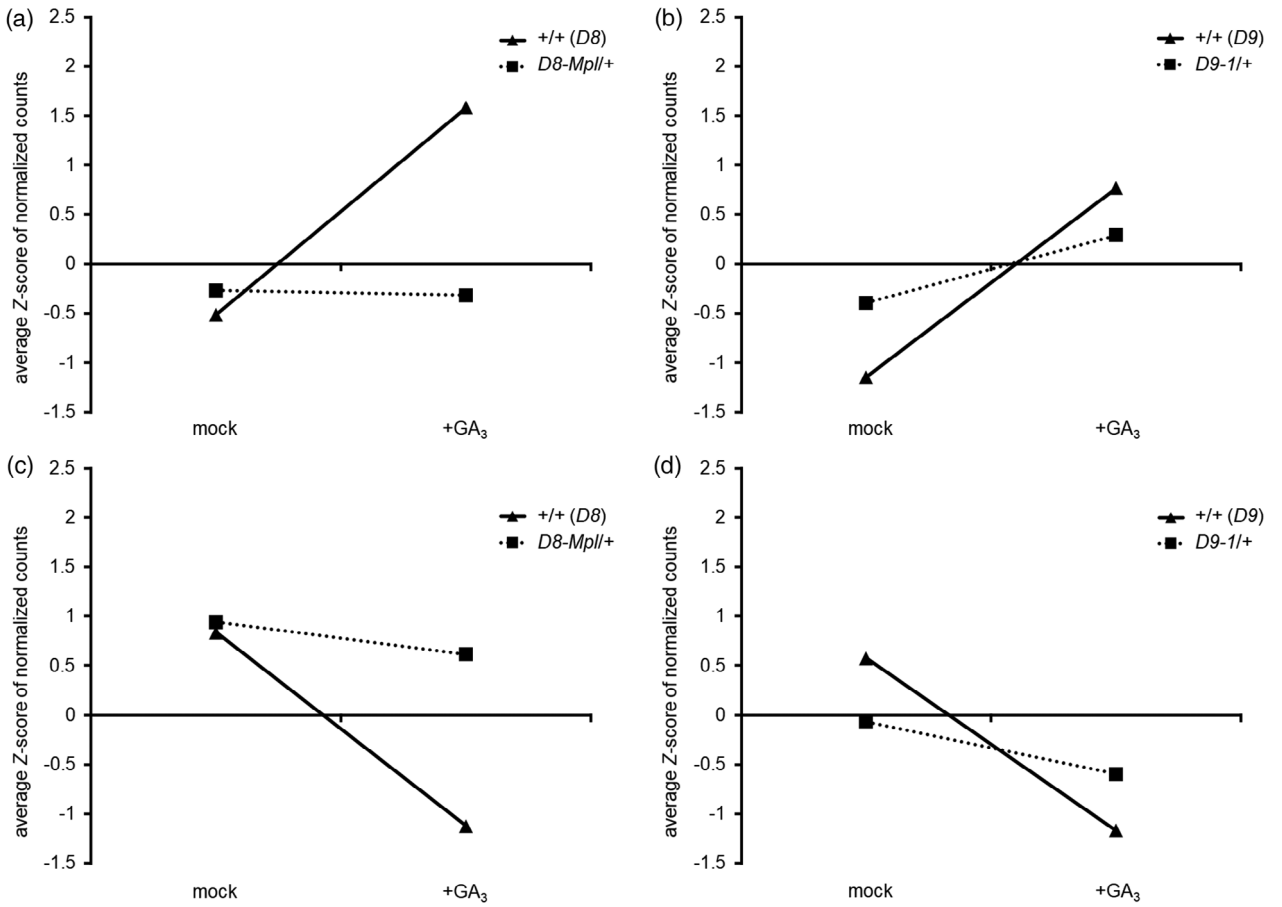

Figure 3. Average *Z*-score of normalized counts of 366 *bona fide* misregulated genes by GA_3 treatment.
(a) Average *Z*-score of normalized count values of 268 upregulated genes by GA treatment of +/+ in the *D8* background (solid black line with triangle) and *D8-Mpl*/+ (dashed black line with square). (b) Average *Z*-score of normalized count values of 268 upregulated genes by GA treatment of +/+ in the *D9* background (solid black line with triangle) and *D9-1*/+ (dashed black line with square). (c) Average *Z*-score of normalized count values of 98 downregulated genes by GA treatment of +/+ in the *D8* background (solid black line with triangle) and *D8-Mpl*/+ (dashed black line with square). (d) Average *Z*-score of normalized count values of 98 downregulated genes by GA treatment of +/+ in the *D9* background (solid black line with triangle) and *D9-1*/+ (dashed black line with square).

The index values calculated from GA_3- and mock-treated wild-type plants fit expectations. The up-index value increased and the down-index value decreased following GA_3 treatment in each set of wild-type samples. Moreover, as stated above, all 366 genes in the *bona fide* GA-responsive gene set were selected on the criteria that they were consistent in their effect on the directional change in both wild-type backgrounds.

The index values were substantially impacted in the *D8-Mpl*/+ and *D9-1*/+ mutants. In contrast to the wild types, and as expected from the *D8-Mpl*/+ mutant phenotype (Figure 1), the up-index did not move following GA_3 treatment of *D8-Mpl*/+ mutants and the down-index was only nominally decreased (Figure 3). When we analyzed the 366 *bona fide* GA-responsive genes as a set, we found evidence that the D8-MPL protein is not completely insensitive to GA and the mutant is mildly GA-responsive. Of the genes that were consistently increased in their abundance following GA_3 treatment, the expression levels of 200 genes were increased by GA_3 treatment in *D8-Mpl*/+ and the expression levels of 68 genes were decreased. Of the genes whose abundance was consistently decreased following GA_3 treatment, the abundance of 22 genes was decreased in *D8-Mpl*/+ while the abundance of 76 genes was increased. The total number of genes consistently affected by GA_3 treatment in *D8-Mpl*/+ and wild-type samples was 222, while 144 were dissonantly affected (chi-squared *P*-value = 4.6×10^{-5}, Table 6). This indicates that despite the very small effect on transcript accumulation, whether assessed by DEG analysis or by our *Z*-score index, a very small but consistent GA response was visible among the genes induced by GA_3 treatment.

The effects of GA_3 treatment were clearer in the *D9-1*/+ mutant RNA-Seq experiments and corroborated our hypothesis that it encodes a weak repressor. Index values calculated from the RNA-Seq data from the *D9-1*/+ mutants

were GA-responsive, resulting in the up-index increasing following treatment and the down-index values decreasing (Figure 3). These index values were the result of consistent effects on most genes in the index. Of the 366 genes, 321 responded to GA_3 in the same direction in wild-type and *D9-1*/+ samples (chi-squared *P*-value = 3.5×10^{-47}; Table 7). Thus, along with the tassel floret phenotype and DEG analysis presented above, the index effects indicate that *D9-1*/+ is mildly GA-responsive and the chi-squared analysis indicates that these effects extend across most of the GA-responsive genes.

If *D9-1* encodes a weak repressor, one might expect a loss of repression of GA-induced genes under mock conditions. Consistent with this, the index value calculated for the up-index gene set in *D9-1*/+ under mock conditions was greater than that of the wild type and the index calculated for the down-index genes was lower in *D9-1*/+ than in wild type. When the set of GA-responsive genes was looked at by chi-squared analysis of direction of expression in the *D9-1*/+ mutant compared to wild type under mock conditions, 318 of the 366 GA-responsive genes' expression levels were affected by *D9-1*/+ in the same direction as by GA_3 treatment in the wild-type samples, while only 48 moved in opposite directions (chi-squared *P*-value = 3.2×10^{-45}; Table 8). Thus, *D9-1*/+ plants exhibited weak constitutive GA-regulated gene expression, consistent with the hypothesis that it encodes a weak repressor.

A much weaker pattern was visible by chi-square analysis of the 366 genes in *D8-Mpl*/+ compared to wild type. In mock-treated *D8-Mpl*/+ compared to the wild type, 216 genes were affected in the same direction as upon GA_3 treatment of the wild type and 150 went in the opposite direction (chi-squared *P*-value = 5.6×10^{-4}; Table 9). While no significant effect of *D8-Mpl*/+ was observed on the index values under mock conditions (Figure 3), the chi-squared results are consistent with a very weak loss of transcriptional repression in *D8-Mpl*/+ in the absence of GA_3.

Taken together, our transcript analyses mirror the developmental responsiveness of tassel florets to GA_3 treatment in *D9-1*/+ and the insensitivity of *D8-Mpl*/+ floret development to GA_3 application (Figure 1). These data are consistent with our proposed explanation for the *D9-1*/+ DEGs that were similarly affected by GA levels in their expression direction and the lack of DEGs between GA_3-treated wild type and *D9-1*/+. Our results indicate that *D9-1* encodes a constitutive ineffective repressor, resulting in weak activation of GA-responsive genes in the absence of GA while also exhibiting a lesser increase in transcriptional response following GA_3 application than observed in wild-type plants.

Table 6 Impact of GA on transcript accumulation in *D8-Mpl* for the 366 *bona fide* GA-regulated genes

Effect of GA_3[a]	Effect of *D8-Mpl*[b]	Number of transcripts
Increase	Increase	200
Increase	Decrease	68
Decrease	Increase	76
Decrease	Decrease	22

[a]Indicates if gene expression increases or decreases following GA_3 treatment of wild-type tassels.
[b]Indicates if gene expression increases or decreases in *D8-Mpl* relative to wild type following GA_3 treatment.

Table 7 Impact of GA in *D9-1* on transcript accumulation for the 366 *bona fide* GA-regulated genes

Effect of GA_3[a]	Effect of *D9-1*[b]	Number of transcripts
Increase	Increase	250
Increase	Decrease	18
Decrease	Increase	27
Decrease	Decrease	71

[a]Indicates if gene expression increases or decreases following GA_3 treatment of wild-type tassels.
[b]Indicates if gene expression increases or decreases in *D9-1* relative to wild type following GA_3 treatment.

Table 8 Impact of mock-treated *D9-1* and wild type on transcript accumulation for the 366 *bona fide* GA-regulated genes

Effect of GA_3 on *D9-1*[a]	Effect of *D9-1* under mock[b]	Number of transcripts
Increase	Increase	200
Increase	Decrease	68
Decrease	Increase	76
Decrease	Decrease	22

[a]Indicates if gene expression increases or decreases following GA_3 treatment of *D9-1* tassels.
[b]Indicates if gene expression increases or decreases in *D9-1* relative to wild type following mock treatment.

Table 9 Impact of mock-treated *D8-Mpl* and wild type on transcript accumulation for the 366 *bona fide* GA-regulated genes

Effect of GA_3 on *D8-Mpl*[a]	Effect of *D8-Mpl* under mock[b]	Number of transcripts
Increase	Increase	175
Increase	Decrease	93
Decrease	Increase	57
Decrease	Decrease	41

[a]Indicates if gene expression increases or decreases following GA_3 treatment of *D8-Mpl* tassels.
[b]Indicates if gene expression increases or decreases in *D8-Mpl* relative to wild type following mock treatment.

Plant height of *D8-Mpl*/+ and *D9-1*/+ is unresponsive to GA application

Though *D8-Mpl*/+ and *D9-1*/+ are taller than other semidominant alleles of *d8*, they were previously reported to be insensitive to GA application (Winkler & Freeling, 1994). As there were background effects on tassel floret development, we sought to determine if genetic background effects were visible for plant height. To test this, mutant and wild-type siblings were treated with 866 μM GA_3 every 3 days by direct application into the whorl from 25 DAP until reproductive maturity. GA_3 application to wild-type siblings of *D8-Mpl*/+ and *D9-1*/+ increased plant height by 91% and 86%, respectively (Figures 4 and 5). Neither *D8-Mpl*/+ nor *D9-1*/+ mutants showed an increased plant height after GA_3 treatment in their original backgrounds. Additionally, when backcrossed to B73 three times, wild-type siblings of *D8-Mpl*/+ and *D9-1*/+ increased in plant height by 33% and 25% after GA_3 treatment, respectively. Backcrossing to B73 did not alter the mutants' sensitivities to GA_3 as both *D8-Mpl*/+ and *D9-1*/+ mutants were insensitive to GA_3. This indicates that the mutations leading to *D8-Mpl* or *D9-1* constitutively repress GA signaling in maize stem tissues that contribute to plant height. Thus, *D8-Mpl*/+ and *D9-1*/+ responses to high GA levels are similar for plant height (Figure 5) but diverge for tassel floret retention (Figure 1). The *D9-1* allele results in different outcomes depending on the developmental output being measured. In addition, the background dependence in elongation response in wild-type siblings indicates standing variation in maize for GA-responsiveness affecting both plant height (Figures 4 and 5) and production of sterile tassels following GA_3 application in some backgrounds but not others (Figure 1).

Maize standing variation results in altered sensitivity to GA_3 for plant height

To test if maize inbred lines differ in sensitivity to GA_3, plant height and tassel architecture were measured in the

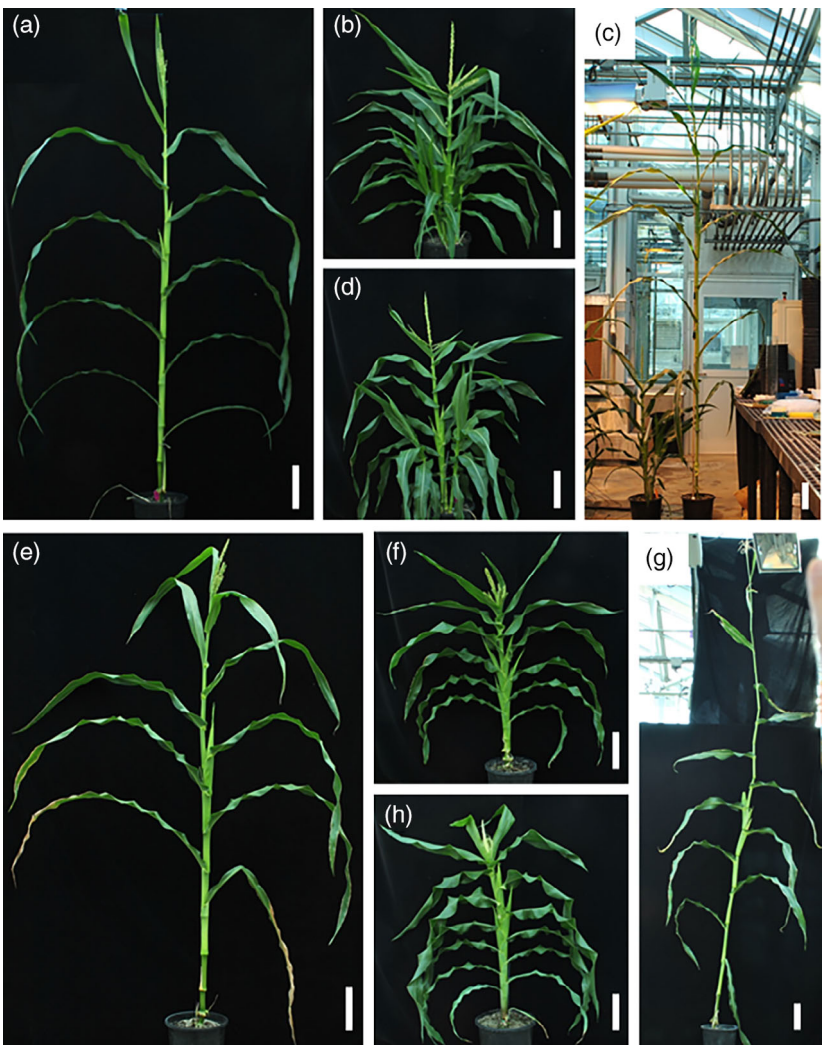

Figure 4. Mature plant phenotypes of *D8-Mpl*/+, *D9-1*/+, and isogenic wild-type sibling plants with or without GA_3 application.
(a) Mature +/+ (*D8* background) and (b) *D8-Mpl*/+ mock-treated. (c) Mature +/+ (*D8* background; shown next to a *D8-Mpl*/+ plant) and (d) *D8-Mpl*/+ treated with GA_3. (e) Mature +/+ (*D9* background) and (f) *D9-1*/+ mock-treated. (g) Mature +/+ (*D9* background) and (h) *D9*/−1+ treated with GA_3. (a–h) Scale bar is 20 cm.

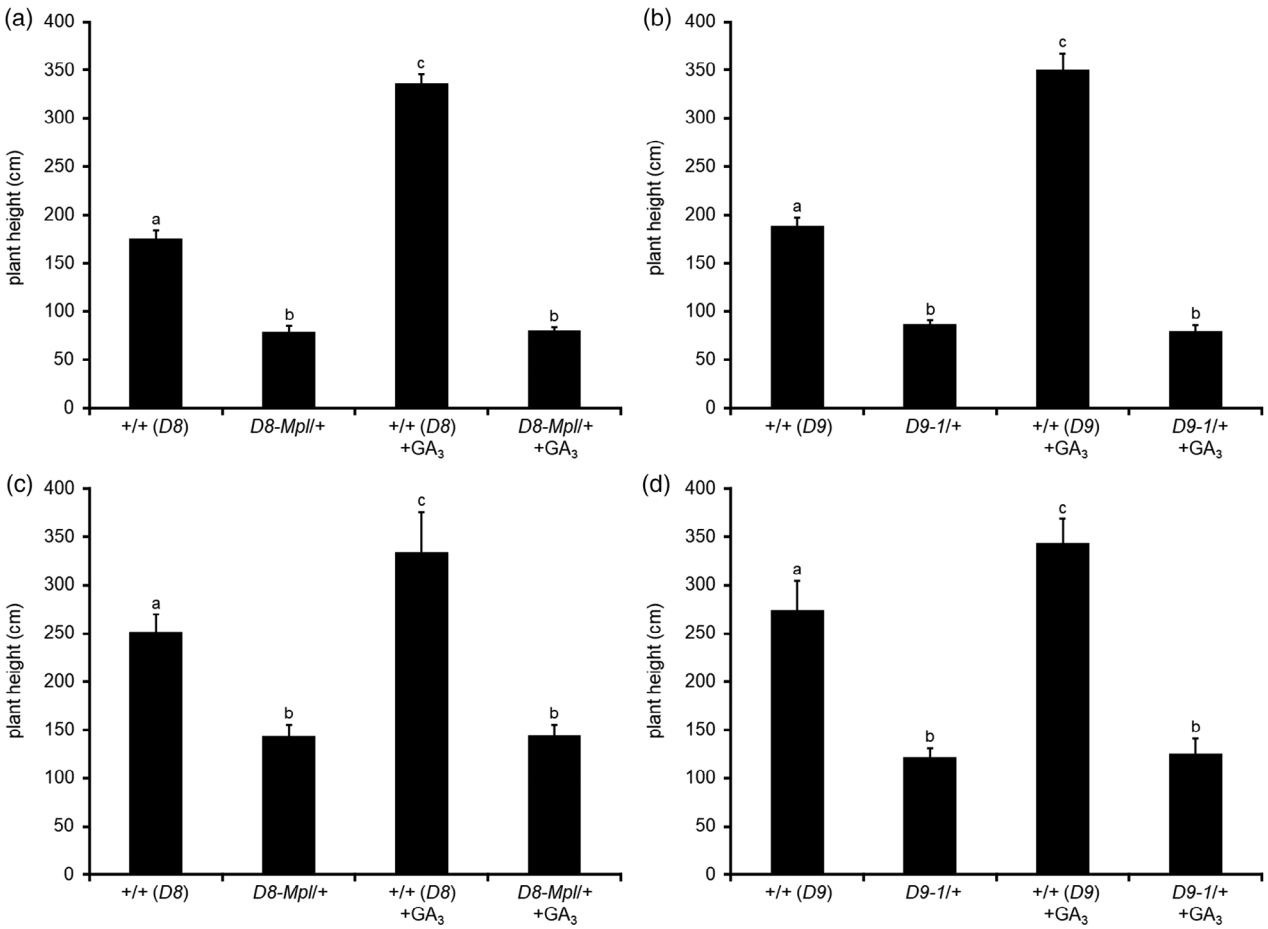

Figure 5. Plant height response of *D8-Mpl*/+, *D9-1*/+, and wild-type siblings treated with and without GA_3.
(a) Plant height of isogenic +/+ and *D8-Mpl*/+ treated with or without GA_3. (b) Plant height of isogenic wild type and *D9-1*/+ treated with or without GA_3. (c) Plant height of BC3F1 with B73 +/+ and *D8-Mpl*/+ treated with or without GA_3. (d) Plant height of BC3F1 with B73 +/+ and *D9-1*/+ treated with or without GA_3. (a–d) Error bars indicate standard deviation. Different lowercase letters indicate *P*-value < 0.05, as determined by analysis of variance with a post hoc test using the Holm–Sidak algorithm.

25 maize NAM founders and B73 repeatedly treated with excess GA_3. Plants were treated every 3 days by applying 1 ml of 866 μM GA_3 as described above. Treatments were continued until tassels emerged. The B73 inbred line was sensitive to GA_3, which showed an increase of 33.8% or 68.8 cm in plant height compared to mock treatment (Figure 6a,b). The B97 inbred line was the least responsive and exhibited an increase of only 11.5% or 25.1 cm when treated with GA_3 compared to mock treatment (Figure 6c,d). The CML322 inbred line was the most sensitive for plant height with an increase of 63.7% or 103.8 cm when treated with GA_3 (Figure 6e,f). Not only was there a wide range of sensitivity to GA_3 (Figure 6g), but there was also large variation in mock height as previously reported by Peiffer et al. (2014). There was no correlation between mock plant height and GA_3 sensitivity (*P*-value of slope of line = 0.9945; Figure 6h). There was a significant negative correlation between mock plant height and percent change in plant height upon GA_3 treatment (Figure S5). This was not surprising as the mock plant height was the denominator in the calculation of percent change in plant height. These results demonstrate that GA-responsiveness of plant height varied across the NAM founder lines.

Maize standing variation affects tassel architecture and floret sensitivity to GA_3

Similar to our results in Figure 1, previous reports demonstrated that GA affects maize tassel branching and results in retention of pistils in the tassel and that these effects are sensitive to the genetic background (Nickerson, 1959; Nickerson, 1960). To determine whether this results from a similar difference in sensitivity as observed for height, we measured tassel branch number and floral organ persistence in GA_3-treated and untreated tassels in the same panel of NAM founders. The effect of GA_3 on tassel branch number was entirely dependent on genetic background.

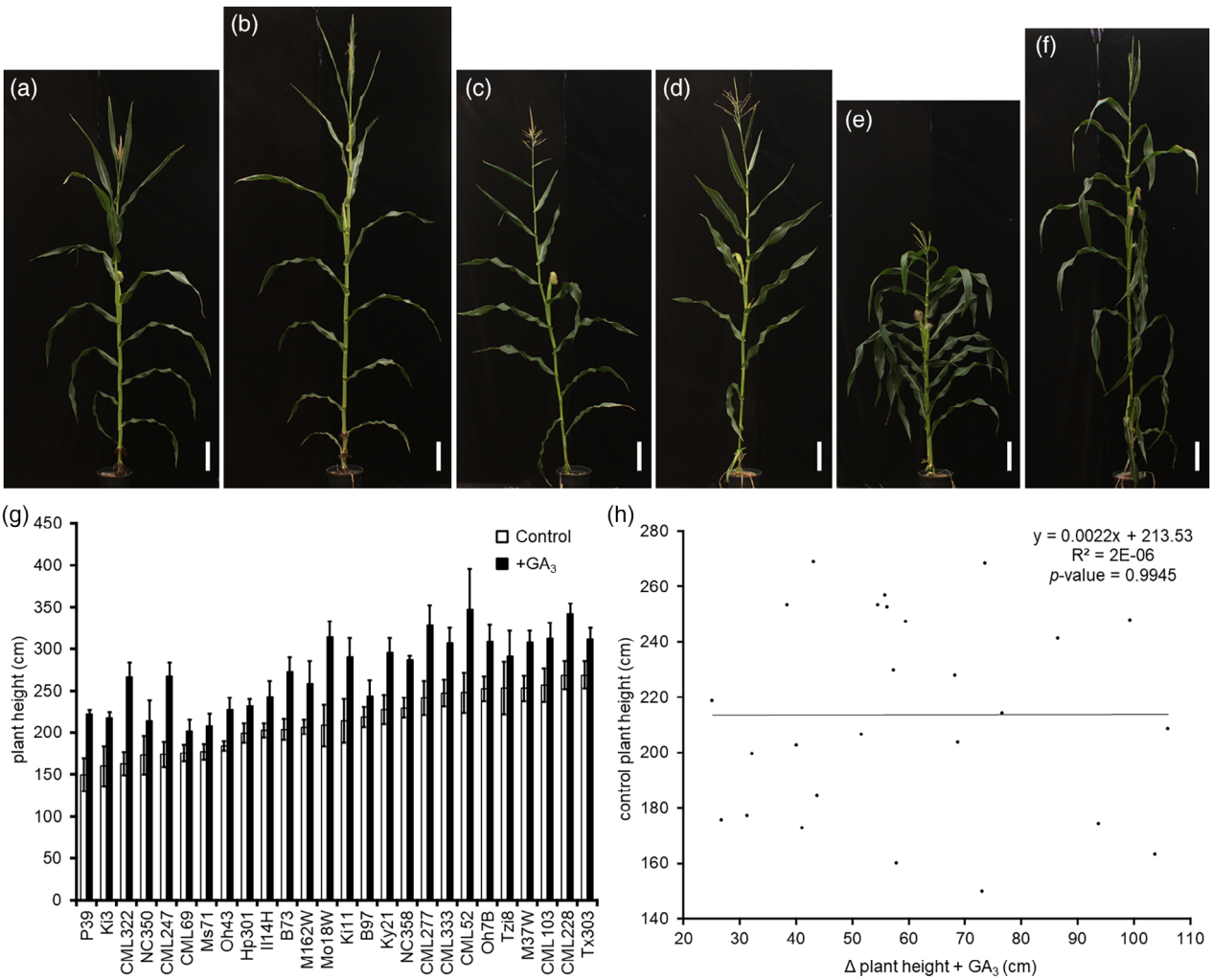

Figure 6. Effects of GA_3 on plant height of NAM founders and B73.
(a–f) Photographs of selected inbred lines at maturity. (a) B73 mock-treated plant and (b) B73 plant treated with GA_3. (c) B97 mock-treated plant and (d) B97 plant treated with GA_3. (e) CML322 mock-treated plant and (f) CML322 plant treated with GA_3. (g) Plant height measured at maturity of the 25 NAM founders and B73 with mock or GA_3 treatment. Error bars indicate standard deviation, and in all inbred lines tested a significant difference was observed between GA_3 treatment and mock treatment, as determined by Student's *t*-test (*P*-value < 0.05). (h) Linear regression plot of mock-treated plant height compared to the change in plant height upon GA_3 application relative to mock-treated plant height at maturity. A best-fit straight line is shown with equation, R^2, and *P*-value for the slope of the line. (a–f) Scale bar represents 20 cm.

Much like it was for plant height, the B73 inbred line was similarly sensitive to GA_3 treatment for these traits and treatment increased the primary tassel branch number from 6.9 to 12.0 (Figure 7a,b,g). The majority of inbred lines, such as Ki3 (Figure 7c,d,g), showed no change in tassel branch number following GA_3 treatment. Three lines (CML322, CML33, and M162W) exhibited a decrease in tassel branch number upon GA_3 treatment (Figure 7e–g), indicating that GA can promote or inhibit tassel branch development depending on the genetic make-up of the inbred line.

In addition to tassel branch number, the effect of GA_3 on floral organ persistence was analyzed. Nickerson showed that a high concentration of GA_3 resulted in retention of pistils in the tassel of four inbred lines (Parker's Flint, Zapalote Chico, CC5, and L317) and their hybrids (Nickerson, 1959). Retention of pistils in the tassel has been previously described in some inbred lines grown in various conditions, whereas day length, light intensity, water availability, and temperature have been shown to influence this phenotype (Richey & Sprague, 1932). In our growth conditions, one mock-treated P39 inbred plant retained pistils in a small number of tassel florets. In addition, mock-treated plants from the inbred lines NC350, B97, and CML103 exhibited barren patches primarily on tassel branches (Figure 7h). As expected, many inbred lines exhibited some degree of pistil retention in the tassels following GA_3 treatment (17 out of 25 inbred lines; Figure 7i).

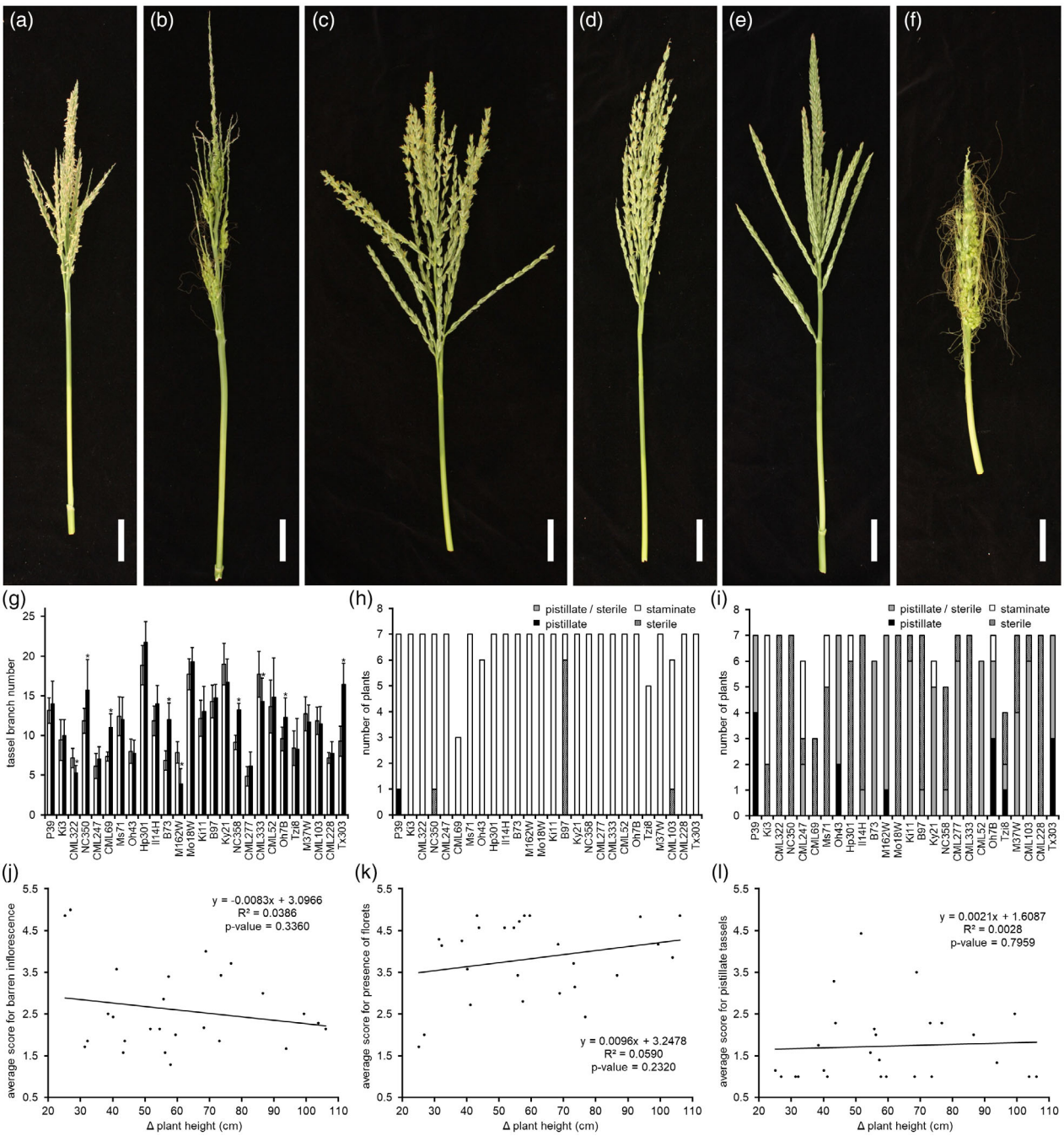

Figure 7. Tassel phenotypes and quantitative tassel phenotype data of selected NAM founders and B73 plants treated with GA_3.
(a-f) Photographs of selected inbred tassels at maturity. (a) B73 mock-treated tassel and (b) GA_3-treated tassel. (c) Ki3 mock-treated tassel and (d) GA_3-treated tassel. (e) M162W mock-treated tassel and (f) GA_3-treated tassel. (g) Average tassel branch number with (black bars) or without (white bars) GA_3 treatment. Error bars indicate standard deviation, and significant differences between inbred lines with or without GA_3 treatment are indicated by asterisks, as determined by Student's *t*-test (*P*-value < 0.05). (h, i) Number of plants with pistils in the tassel, retained pistils and partially sterile tassels, partially sterile tassels, or completely staminate tassels with (h) mock treatment or (i) GA_3 treatment. (j–l) Linear regression plots of average score (1–5) with GA_3 treatment for (j) barren inflorescence, (k) presence of floret development, or (l) retained pistils in the tassel compared to change in plant height with GA_3 application relative to mock plant height at maturity. (a–f) Scale bar represents 5 cm. (j–l) A best-fit straight line is shown with equation, R^2, and *P*-value for the slope of the line.

The most dramatic of these was M162W, as the tassel was primarily pistillate and had reduced tassel branches (7.6 to 3.9), rendering the tassel architecture reminiscent of a maize ear (Figure 9e–g,i). Just like it was for tassel branch number, Ki3 florets were almost insensitive to GA_3 treatment and this was the only inbred line to produce ample

pollen after GA_3 treatment (Figure 7c,d,i). In addition to branching and retention of pistils in the tassel florets, GA_3 treatment also resulted in a varying degree of sterile tassel phenotypes in all inbred lines (Figure 7i). Treated tassels were variously sterile and any tassel with a sterile patch was scored as such. Sterile tassels had a variety of defects including missing spikelets, male sterility, and failure to develop functional florets. As a result, every inbred line had at least one individual with at least a partially sterile tassel (Figure 7a–f,i).

Tassel and plant height traits are under independent genetic control

If the variability in these traits' GA response was due to genetic variation in GA sensitivity, these traits should co-vary across genotypes. On the other hand, if the variation was due to differential responsiveness of developmental programs to GA signals, we do not expect a correlation between traits. We performed regression analyses to compare the change in plant height with tassel phenotypes and test if common allelic variation causes these differences (Figure 7j–l; Figure S6). We found no association of any tassel phenotype (retention of pistils in tassel florets, presence of florets, or barren phenotype) with an increase in plant height following GA_3 treatment (Figure 7j–l; Figure S6). No correlation was observed between tassel branch number and any other phenotype as well (Figure S7). These results indicate that the alleles encoding the genetic variation in response to GA_3 are developmentally distinct. The fact that the response of plant height to GA_3 did not predict the effect on tassel architecture or floral organ persistence indicates that the natural variation we have described encodes complex and downstream signaling circuits that independently control these distinct developmental processes in response to GA.

DISCUSSION

We utilized two mutants which were similar with respect to dwarfing in the untreated plants as well as in the insensitivity of plant height to GA_3 application (Figures 4 and 5). The *D8-Mpl* allele is the weakest among the *d8* alleles (Winkler & Freeling, 1994) and the *D9-1* allele is the only described dominant allele of *d9*. These mutants are also similar in the production of anthers in the ear florets, a stereotypical phenotype observed in GA loss-of-function mutants (Bensen et al., 1995; Chen et al., 2014; Emerson & Emerson, 1922; Fujioka et al., 1988; Phinney, 1956; Winkler & Freeling, 1994). Part of the motivation to use the alleles with the weakest phenotype was to begin our experiments in a system where the GA response could potentially go up (resulting in taller plants) or go down (resulting in shorter plants). The finding that the heights of both mutants were completely insensitive to GA_3 demonstrates that GA signaling in plant cell elongation was more complicated than this simple vision. It may be that the weak activation of GA-regulated gene expression, visible when the genes were assessed as a set, was relevant to their intermediate phenotype (Figure 3).

Complete insensitivity of *D9-1* to GA for elongation does not block the effects of GA on floral organ persistence

The extremely few genes that were differentially affected by *D9-1* in the GA_3-treated tassel RNA-Seq experiments match the observation of GA_3 treatment effects on this mutant's tassels. Retention of pistils in tassel florets following GA supplementation is well documented (Best et al., 2016; Nickerson, 1959). Unlike the complete suppression of GA-induced plant height by *D9-1*/+ (Figure 4), the application of GA_3 to *D9-1*/+ developing tassels resulted in a stereotypical GA response, including the conversion of tassel florets to pistillate flowers (Figure 1). As a result, both wild-type and *D9-1*/+ GA_3-treated tassels had similar terminal phenotypes (Figure 1). They also accumulated GA-regulated genes to similar levels (Figures 1 and 2). The lack of correspondence between *D9-1*/+ effects on GA-induced changes in plant architecture highlights the tissue-specific effect of GA, and presumably GA signaling networks, on plant development. Whether this was the result of weak expression of the *d9* gene in the cells that respond to GA controlling tassel floret development relative to expression in elongating cells in stems is currently not clear. Previous observations of opposing epistatic interactions and physiological interactions between GA and BR signaling (Best et al., 2016; Best et al., 2017) provide precedence for this and strongly argue that developmental context-specific experiments are required to unravel the signaling mechanisms affecting GA responses and that solely observing cell elongation will not uncover the targets, transduction mechanisms, and consequences of this hormone. This context dependence may emerge from a concentration dependence of the effect of GA on gene expression and growth. For example, different GA concentration thresholds may be needed to disrupt protein–protein interactions, direct proteolysis, and affect gene expression and cell elongation. Gating of any of these processes by the signaling status of other pathways, for example via a DELLA–BZR physical interaction (Bai, Shang, et al., 2012b; Gallego-Bartolome et al., 2012; Li et al., 2012), could conceivably affect this more complex and developmentally specific effect of the *D9-1* mutant in the absence of any tissue-specific accumulation of its gene product.

Parametric analysis of GA-induced gene expression as a readout of GA signal status

We processed our genotype and hormone treatment experiments to identify DELLA-responsive and GA-induced genes. All GA-regulated genes were suppressed by the *D8-Mpl* mutant, even as a heterozygote, demonstrating that there were no DELLA-independent GA-regulated genes in

maize tassels. The absence of any DELLA-independent GA-regulated genes lends credence to the interpretation that the Arabidopsis DELLA quintuple mutant contained a leaky allele (Fuentes et al., 2012). The analysis of these genes as a set of reproducibly GA-responsive genes provides an assessment of the GA signaling status in each sample. This approach, rather than looking at each gene individually, provided a hypothesis test about the status of GA-responsive genes in the four genotypes even in the absence of exogenously applied GA_3. The *Z*-score sums calculated in Figure 3 provide a quantitative assessment of how similar the GA response was in each transcriptome. This composite value has averaged the measurement error across the gene set and has the potential to uncover patterns in our experiments not immediately obvious from a traditional differential gene expression analysis. Among the genes changed in their expression are many consistent with expectations and predictions from other systems such as auto-regulation of the DELLA domain transcription factors and an increase in genes encoding enzymes in GA catabolism. By using the gene sets in aggregate, we observe that both wild-type backgrounds tested responded similarly to GA_3 and displayed a similar quantitative output when we calculated the composite value. Unexpectedly, these composite values identified slightly higher expression of DELLA-repressed and GA-induced genes in both the *D9-1*/+ and *D8-Mpl*/+ mutants under mock conditions. This effect was most evident in *D9-1*/+, and at first glance seems incompatible with the dwarf phenotype and GA insensitivity (see below). This quantitative assessment of pathway activation status used a combination of DEG analysis in the treatment of interest, GA_3 application, and the direction of the effect on gene expression. This was similar to, and extends, our previous work using the set of genes coding for the members of a protein complex to assess the effects of loss of one subunit on the expression of the complex as a set (Best et al., 2021). The extension of this set to analyze DEGs opens up the possibility of constructing indices of gene expression to test physiological hypotheses about pathway activation states, signal levels, or responses using gene expression as an integrative and quantitative output. For the open questions about GA signaling identified here, future work combining loss-of-function mutants in GA biosynthesis and a lower concentration of GA could be used to draw a response curve for GA-regulated gene expression in a specific tissue.

How could the *D9-1* and *D8-Mpl* mutants result in both GA insensitivity and low-level constitutive expression?

The surprising finding that the expression of the 366 *bona fide* GA-responsive genes was higher in the dominant mutants invites a hypothesis to help direct further research. The DELLA repressors have two purposes: repress gene expression and undergo GA-dependent turnover. In other systems, dominant DELLA mutants are the result of poor protein turnover resulting in constitutive repression of GA-regulated gene expression and dwarfism. A mutation that abrogated the ability of a DELLA protein to both repress gene expression and interact with the GID1 proteins responsible for targeting DELLA proteins for degradation would result in different phenotypes in the presence and absence of excess GA. Given the moderate dwarfism in *D9-1*/+ and *D8-Mpl*/+, as compared to the other known *d8* alleles, this may be the case for these alleles. In the absence of GA, this would result in a slight de-repression of GA-regulated gene expression, as we observe most clearly in *D9-1*/+, where GA-induced genes were identified as DEGs that were upregulated in the mutant mock-treated samples. An inability to proteolyze and completely remove the DELLA proteins from promoters and protein interactors in the presence of GA (Figure 8) would result in the continued repression of GA-regulated gene expression resulting in no change in the levels of GA-regulated genes between these two GA levels. DELLA proteins have no DNA binding activity. If structural changes that interfere with proteolysis also decrease in the affinity for the transcription factors that DELLA proteins use to bind DNA, this would result in weak activation of these sites in the presence of the mutant alleles in the absence of GA. Most dominant DELLA mutants are defective in the DELLA domain or the adjacent TVHYNP domain, which are required for the binding to GID1 and the targeting of the DELLA domain protein for degradation (Ueguchi-Tanaka et al., 2007). These two alleles are caused by a point mutation at a domain of unknown importance far from the DELLA domain in *D9-1* and an N-terminal truncation of 106 amino acids that comprise the DELLA motif and 101

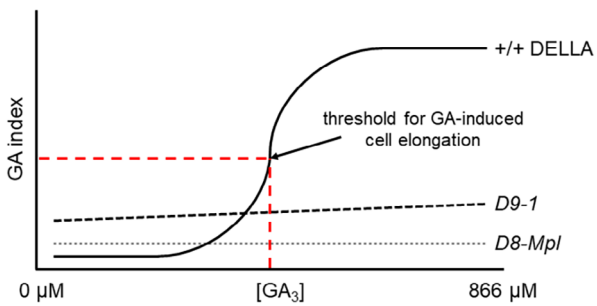

Figure 8. Model for GA-induced cell elongation by +/+ DELLA, *D8-Mpl*, and *D9-1*.
Model for the threshold of GA-induced cell elongation in maize. The *x*-axis depicts the concentration of exogenously applied GA_3 up to 866 µM and the *y*-axis depicts the transcript levels of the 366 GA-responsive genes (GA index). The solid black line indicates wild-type (+/+) DELLA proteins, the black dashed line indicates the mutant D9-1 protein, and the gray dashed line indicates the mutant D8-Mpl protein. The red dashed line shows a relative threshold necessary for GA_3 application to induce cell elongation by transcriptional change of the GA index genes. The model depicts that the mutant D9-1 and D8-Mpl proteins inhibit the necessary change in the GA index to induce cell elongation with GA_3 application.

additional residues in *D8-Mpl* (Lawit et al., 2010). Loss of functions critical to DELLA regulation of transcription other than GID1 binding is possible, given the locations and sizes of the two mutants, and seems very likely based on the gene expression analysis in Figures 2 and 3.

Despite this weak activation of GA-induced genes in *D9-1*/+, and to a lesser extent *D8-Mpl*/+, the mutants are very clearly GA-insensitive dwarfs. The effect of these mutants on plant growth indicates that this weak loss of repression was below the threshold necessary to induce elongation (Figure 8). We suspect, although a critical experiment to test this is lacking, that the effects of GA on cell elongation are mediated by localized high levels of GA. If this was the case, our ability to measure changes in gene expression was sensitive enough to detect changes in gene expression affected by signaling below the threshold required to induce elongation of cells. The effect of this across our experiments was expected to be a weak activation of the GA signaling pathway in the *D9-1*/+ mutant that was insufficient to result in elongation, and an inability of high concentrations to turn over the encoded mutant protein resulting in the classic GA dwarf phenotypes. The transcript level of *d9* was approximately 4-fold lower than that of *d8* (Figure S1). The insensitivity of elongation in *D9-1*/+ to GA_3 application suggests that the mutant protein is sufficient to repress GA-induced gene expression at high GA_3 application. If wild-type alleles are effectively turned over by the signaling cascade but the mutant proteins are not, endogenous GA levels would result in the accumulation of mutant protein. At the time of GA application, repressor complexes present would be disproportionately comprised of mutant subunits. Alternatively, the weak GA signaling could be the result of residual wild-type protein encoded by the wild-type gene *d9* and the two wild-type copies of *d8*. Steady-state measurements of D8 and D9 protein abundances in the mutants should confirm or rule out this latter possibility.

Identification of GA-responsive genes in the maize tassel and GA control of floral organ identity and meristem determinacy

The maize MADS box genes *ZMM8*, *ZMM14*, and *ZMM16* were all downregulated in the presence of GA_3. These three genes have previously been shown to be expressed in developing floret meristems in both the tassel and the ear and ZMM16 was shown to be expressed in carpels right before their abortion in the tassel. Thus, the expression of these MADS box genes was consistent with pistil retention and aberrant determinacy in the tassel floret meristems. The reduction in the transcripts of these genes following GA_3 application indicates that they are not pistil-expressed genes and are more likely to control meristem determinacy than organ identity. This was also consistent with the timing of tassel collection for the RNA-Seq experiments. Previous work has demonstrated that loss of determinacy and proliferation of meristem types results in misregulation of GA metabolic enzymes. The *KN1* gene controls meristem maintenance via GA regulation in specific cells in the meristems (Kessler et al., 2006; Thomas et al., 2005). Bolduc and Hake (2009) showed that *KN1* represses GA via direct upregulation of catabolic genes and GA represses meristem determinacy (Bolduc & Hake, 2009). These MADS box genes were downregulated upon GA_3 treatment and therefore are not direct DELLA targets due to the fact that DELLAs are repressors. This suggests that there is an upstream regulator of the MADS box genes that is a direct DELLA target. *ZFL1* and *ZFL2* transcripts accumulated upon GA_3 treatment and have been shown to regulate expression of MADS box genes, and both could be direct DELLA targets responsible for their regulation.

Mutation of the *UB2* and *WAB1*/*BAD1* genes in maize results in fewer tassel branches (Chuck et al., 2014; Du et al., 2020). The *UB2* and *WAB1*/*BAD1* transcripts accumulated upon exogenous application of GA_3 to wild-type plants. The *CUC2* gene has been shown to control axillary meristem initiation in Arabidopsis (Raman et al., 2008). Similar to *UB2* and *WAB1*/*BAD1*, transcripts of the *CUC2* homolog also accumulated in wild-type samples treated with GA_3. All three of these genes' transcripts are downstream of GA function and may be targeted for repression by the DELLA repressor complex. There was an increase in tassel branch number in wild-type B73 plants (Figure 7g). Altered expression of these genes may be the mechanism by which GA promotes axillary branch development. Further experiments are necessary to test this hypothesis. Alternatively, these genes may also be indirect targets of GA downstream of other GA-misexpressed genes such as *ZFL1*, *ZFL2*, or the MADS box genes, which may also be important for the regulation of meristem determinacy.

A recent study identified downregulation of the GA-inactivating GA2-OXs in *tasselsheath1* (*tsh1*), *tsh4*, and their double mutants (Xiao et al., 2022). The *TSH1* and *TSH4* genes encode transcription factors that control meristem determinacy, branching, and bract formation (Bommert & Whipple, 2018; Chuck et al., 2010; Whipple et al., 2010). The lower levels of *GA2-OXs* might result in higher GA levels or might be a transcriptional response to a decrease in GA signaling affected by these mutants. In addition to *GA2-OXs*, the *d8* transcript level is also decreased in these mutants. In both cases, these changes in gene expression are consistent with a decrease in GA signaling, rather than an increase in GA levels mediated by a loss of GA catabolism. The full set of GA-regulated transcripts in tassels identified here (Figure 2) should provide a clearer picture of any changes in GA levels affected by these mutants. Of the 366 GA-regulated genes (Table S3), 332 were reported by Xiao et al. (2022), and the gene expression directions of 218, 181, and 217 genes were

consistent with low GA signaling in *tsh1*, *tsh4*, and their double mutant, respectively. Thus, the *tsh* mutants appear to have low GA signaling, resulting in decreased accumulation of GA catabolic enzyme transcripts as well as other GA-induced genes. This insight is another demonstration of the value of looking at the consequences on gene expression of mutants and physiological treatments, as well as analyzing gene sets in aggregate.

Paralogous genes within the DEG lists indicate multiple pathways impacted by DELLA-regulated GA signaling

The detection of multiple paralogs with likely redundant functions highlights the importance of considering pathway-level and physiological hypotheses when analyzing differential gene expression data. The reproducibly GA-responsive genes (Figure 3; Data File S3) and genes that were repressed in the *D8-Mpl*/+ mutant following GA_3 treatment (Data File S7) were broadly similar. Many of the same genes were differentially expressed in both, and the direction of the effect fit the expectation for *D8-Mpl* encoding a constitutive repressor of GA-regulated gene expression. Among those genes that were only found in one of the two experiments were several gene pairs encoding paralogs or multiple members of an established pathway. For example, a broad complement of BZR-like bHLH transcription factors were among the significant DEGs in the two experiments. In Arabidopsis, DELLAs and BZR transcription factors have been shown to physically interact to control GA and BR gene expression (Bai, Shang, et al., 2012b; Gallego-Bartolome et al., 2012; Li et al., 2012). The rice gene encoding the GRAS domain transcription factor DLT has been demonstrated to control GA and BR responses in rice (Li et al., 2010; Tong et al., 2009; Tong et al., 2012; Xiao et al., 2017). The maize ortholog of *DLT* was upregulated upon GA_3 treatment to wild-type plants (Figure 3). The *DLT* ortholog in Arabidopsis, *SCL28*, regulates mitotic cell cycles and this gene may be the connection point between BR and GA and meristem determinacy. Unlike predictions in Arabidopsis (Serrano-Mislata et al., 2017), there no *CYCLIN-DEPENDENT INTERACTING* transcripts were differentially accumulated upon GA_3 treatment. In addition to the maize *DLT1* ortholog, *ovate family protein26* was also upregulated upon GA_3 treatment. A homolog of this gene, *OSOFP1*, interacts with *DLT* in rice (Xiao et al., 2017). These data demonstrate that DELLA domain proteins in maize and GA signaling impact gene expression and affect the BR pathway directly in maize.

In addition to regulatory pathways that are biochemically understood, GA_3 application had unexpected impacts on the pathway affected by the CYP78A family. This pathway was genetically defined originally by the Arabidopsis mutant *kluh* (Anastasiou et al., 2007) and later by the *plastochron1* mutant of rice (Miyoshi et al., 2004) and maize (Sun et al., 2017). Loss of CYP78A family members variously affects organ size and the timing of leaf initiation and flowering (Anastasiou et al., 2007; Ito & Meyerowitz, 2000; Miyoshi et al., 2004; Nagasawa et al., 2013; Stransfeld et al., 2010). This family of CYP P450s is conserved across embryophytes (Katsumata et al., 2011). Biochemical assays demonstrated that they can hydroxylate fatty acids (Kai et al., 2009), but how the activity of these enzymes influences plant physiology and development is unknown. We identified multiple, and different, CYP78A paralogs among the DEGs in the *bona fide* GA-regulated genes (Figure 2), as well as in the GA-regulated and D8-dependent (Figure 2; Figure S2) datasets. The *bona fide* GA-regulated genes include the maize homolog of *PLA1*. The findings that GA increased the expression of *PLA1* and that GA application decreased the days to tassel emergence (Best et al., 2016) suggest that the KLUH/PLA1 pathway may act downstream of GA in floral induction. The *CYP78A* paralog present in the GA-regulated D8-dependent genes is related to the sister clade to *KLUH*. The non-overlap of these two paralogs likely stems from the relatively strict controls for false positives employed in RNA-Seq experiments, as the direction of the effect on gene expression was the same for both genes in both experiments. Future work to determine the molecular mechanism by which CYP78A proteins affect organ growth and flowering may help clarify how and why GA regulates the abundance of these transcripts.

No correlation between phenotypic responses to GA_3 across multiple tissues

Similar to the non-correspondence of the *D9-1*/+ mutant's effects on tassel floret organ persistence and stem elongation, natural variation affecting the GA response for each phenotype we tested displayed no correlation. GA_3 treatment of inbred lines representing a diversity of maize (Flint-Garcia et al., 2005; Peiffer et al., 2014) resulted in a variety of responses. Across the variation affected by these lines, none of the measured phenotypes were correlated, demonstrating that the genetic basis of this variation must be independent. This ruled out global changes in the pre-existing levels of GA, changes in GA catabolism, or changes in GA signaling (e.g., perception and initial transduction) as variation in these would exhibit stereotypical responses across tissues. It is possible that alleles responsible for tissue-specific expression of core GA biosynthetic or signaling genes are responsible for the non-correspondence between phenotypes. It was also possible that the lack of correlation between these different phenotypes' responses to GA_3 was due to variation in developmentally specific regulators affecting these different GA outputs. For example, alleles encoded by genes critical to the effects of GA on floral organ identity and retention, such as the *leafy*/*floricula* orthologs *ZFL1* and *ZFL2* and various floral-expressed MADS box genes, may not have any impact on plant cell elongation. Future work to

genetically map the variation in tassel response or plant height responses to GA will be required to discover the molecular basis of this complexity. Our results demonstrate that the generalization of one signaling network for a single GA response in a single tissue (e.g., only cell elongation or only floral development) will be naive and generalizations will be misleading.

CONCLUSION

We conducted RNA-Seq analysis to identify GA-regulated genes in the developing maize tassel. This identified DELLA targets during tassel development in maize and allows for future hypotheses to be tested on GA regulation of plant development across development pathways. This gene set was leveraged to calculate GA response index values in dominant mutants of DELLA domain proteins and their wild-type siblings confirmed their role as GA excess-resistant mutants. Analysis in untreated tissue uncovered that these alleles are weak repressors of GA-regulated gene expression in the absence of signal. The finding that natural variation altering the consequences of GA signaling was independent across three aspects of maize development demonstrates that alleles affecting GA can be used to modify plant architecture in maize without strong effects on floral organ persistence and negative impacts on reproduction.

MATERIALS AND METHODS

Plant material and growth conditions

Seeds of *D8-Mpl* (accession 120F) and *D9-1* (*D9-N2319*; accession 502C) were obtained from the Maize Genetics COOP (Champaign-Urbana, Illinois). All studies were conducted with the semidominant mutants as heterozygotes. GA_3 treatments were given and morphometric analyses were conducted in the original genetic backgrounds from the stock center maintained by sib-mating. RNA sequencing (RNA-Seq) analyses were conducted on material backcrossed three times to B73 and maintained by backcrossing. Seeds of inbred lines comprising the parents of the Nested Association Mapping population (e.g., NAM founders) (Flint-Garcia et al., 2005; Peiffer et al., 2014) were obtained from the U.S. National Plant Germplasm System. Plants were grown in two gal pots with a 1:1 mixture of peat germinating mix (Sun Gro Horticulture, Bellevue, WA) and Turface® (Profile Products LLC, Buffalo Grove, IL). Greenhouse conditions were a 16/8-h light/dark photoperiod with supplemental lighting by high-pressure sodium growth lamps with a day-time temperature of 27°C and a night-time temperature of 21°C. Plants were fertilized with pH 6 adjusted 200 ppm Miracle-Gro Excel (Scotts, Maryville, OH).

GA_3 treatments

For morphological measurements and RNA-Seq, *D8-Mpl*/+, *D9-1*/+, and wild-type siblings were treated with 1 ml of 866 μM GA_3 (Gold Biotechnology, St. Louis, MO), 0.02% ethanol, and 0.005% Silwet L-77 (Sigma-Aldrich, St. Louis, MO). Solutions were applied directly into the whorl using a pipette every 3 days starting at 20 days after planting (DAP) and continuing until tassel emergence. Mock plants were treated with a solution with the same total volume and concentrations of ethanol and Silwet L-77 but lacking GA_3. Plants were treated a total of seven times prior to the collection of RNA-Seq samples. For morphological measurements of NAM inbred lines, a total of 14 plants (seven mock and seven treated) per inbred line were planted in a complete randomized block design at the Purdue Horticultural Greenhouse Facility. Bamboo stakes and a clothesline were used to ensure stability of GA_3-treated wild-type siblings due to their extreme elongation.

RNA sequencing and analysis

Developing tassels (0.5–1 cm) of *D8-Mpl*/+, *D9-1*/+, and wild-type siblings treated with or without GA_3 were collected at 40 DAP in the morning hours of 8:00 to 11:00 a.m. For each genotype, three biological replicates of eight immature tassels were collected, frozen in liquid nitrogen, and ground, and total RNA was extracted. Stranded cDNA libraries were created with a TruSeq mRNA HT Sample Prep Kit and sequenced on an Illumina HiSeq 2500 platform (Illumina, San Diego, CA) using TruSeq SBS v3-HS reagents (Illumina, San Diego, CA). Sequenced reads were aligned to the B73 reference genome (v3.31) using tophat2 (version 2.1.1) along with bowtie2 (version 2.3.2) (Kim et al., 2013; Langmead & Salzberg, 2012). Sequencing results and alignment rates are described in Table S1. Aligned reads were used to create count tables using HTSeq (version 0.7.0) (Anders et al., 2015), which were then inputted into DESeq2 (version 1.27.31) (Love et al., 2014) to determine differential expression using a negative binomial likelihood ratio test with parametric gene-wise dispersion. Genes were annotated by the Basic Local Alignment Search Tool for protein sequences (BLASTP) (Mahram & Herbordt, 2015) of maize transcripts against the Arabidopsis genome. Top hits were used for annotation with a 1e−05 cutoff; if the top hit BLASTP e-value was greater it was not included as an annotation and was indicated as an asterisk in supplementary data files. Annotation descriptions of genes were downloaded from Arabidopsis.org (TAIR10). For expression comparison of D8 and D9 across developmental tissues, normalized counts were obtained from Bolduc et al. (2012).

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AUTHOR CONTRIBUTIONS

NBB and BPD designed the experiments and analyzed the data; NBB performed the experiments; NBB and BPD wrote the manuscript; NBB and BPD agree to serve as the authors responsible for contact and ensure communication.

DATA AVAILABILITY STATEMENT

All raw reads are available at the Short Read Archive under BioProject ID PRJNA784941 (D8/D9 Maize Transcriptome Data). Raw read files are described in Table S1.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article.

Figure S1 RNA expression levels of *dwarf8* (*d8*) and *dwarf9* (*d9*) from the Bolduc et al. (2012) dataset. Normalized RNA expression values from previously published RNA-Seq data from Bolduc et al. (2012) for *d8* (GRMZM2G144744) and *d9* (GRMZM2G024973). Data were obtained from GEO accession GSE38487.

Figure S2. Heatmap of 561 responsive genes in +/+ (*D8*) compared to *D8* treated with GA_3. Genes were filtered for being differentially expressed in +/+ (*D8*) treated with GA_3 compared to *D8* treated with GA_3 at a Benjamini–Hochberg genome-wide significant *P*-value of <0.05. Heatmap of log_2-transformed fold change values of 561 differentially expressed genes. Dodgerblue1 indicates high expression and magenta represents low expression in GA-treated samples. Genes are clustered by the expression across all six comparisons. Selected genes are indicated on the right of the heatmap.

Figure S3. Heatmap of 28 responsive genes in +/+ (*D9*) compared to *D9* treated with GA_3. Genes were filtered for being differentially expressed in +/+ (*D9*) treated with GA_3 compared to *D9* treated with GA_3 at a Benjamini–Hochberg genome-wide significant *P*-value of <0.05. Heatmap of log_2-transformed fold change values of 28 differentially expressed genes. Dodgerblue1 indicates high expression and magenta represents low expression in GA-treated samples. Genes are clustered by the expression across all six comparisons. Selected genes are indicated on the right of the heatmap.

Figure S4. Transcript accumulation distribution of the filtered 366 *bona fide* GA-responsive gene loci averaged across all samples. Average normalized read count across all samples of RNA-Seq data of the filtered 366 *bona fide* GA-responsive gene loci ordered by transcript abundance on the *x*-axis from 1 (most abundant) to 366 (least abundant). The two most abundant transcripts (GRMZM2G32611 and GRMZM5G815894) are labeled on the graph. The large red point denotes the 20th gene locus in order of transcript abundance indicating that the first 20 loci represent more than 50% of the total average normalized read counts for the entire set of 366 *bona fide* GA-regulated genes.

Figure S5. Linear regression plot of mock plant height compared to percent increase in plant height with GA_3 application related to mock plant height at maturity. Plot of NAM founders and B73. A best-fit straight line is shown with equation, R^2, and *P*-value for the slope of the line.

Figure S6. Linear regression plots of tassel phenotypes compared to percent increase in plant height with GA_3 treatment. (a–c) Linear regression plots of average score (1–5) with GA_3 treatment for (a) barren inflorescence, (b) presence of floret development, or (c) pistillate tassels compared to percent increase in plant height with GA_3 application related to mock plant height at maturity. (a–c) A best-fit straight line is shown with equation, R^2, and *P*-value for the slope of the line.

Figure S7. Linear regression plots of tassel branch number compared to other tassel phenotypes and response in plant height with GA_3 treatment. (a–j) Linear regression plots of change in tassel branch number or percent change in tassel branch number with GA_3 treatment compared to changes in plant height or inflorescence phenotypes with GA_3 treatment. A best-fit straight line is shown with equation, R^2, and *P*-value for the slope of the line.

Table S1. RNA-Seq information and statistics.

Table S2. Morphological measurements of *D8-Mpl*/+, *D9-1*/+, and wild-type siblings with and without GA_3.

Data File S1. DESeq2 results of differential mRNA expression of tassels from the congenic wild types of the *D8-Mpl* mutant sized 0.5–1 cm treated with GA_3 compared to tassels of the congenic wild types of the *D8-Mpl* mutant sized 0.5–1 cm treated with mock treatment. Column 1 shows the maize B73 gene identification number (v3.31), column 2 shows the baseMean read count for all samples, column 3 shows the log_2-transformed fold change values (where positive numbers indicate higher expression in GA_3-treated tassels and negative numbers indicate lower expression in GA_3-treated tassels), column 4 shows the log_2-transformed fold change standard error, column 5 shows the Wald statistic, column 6 shows the Wald test *P*-value, column 7 shows the Benjamini–Hochberg multiple testing adjusted *P*-value, column 8 shows the Arabidopsis gene identification number for the closest homolog in Arabidopsis, column 9 shows the e-value for the BLASTp result of comparison between maize and Arabidopsis, and column 10 shows the Arabidopsis annotation. An asterisk in columns 8–10 indicates that there was not a homologous protein sequence of the maize gene in Arabidopsis with an e-value score of 1.00e−05.

Data File S2. DESeq2 results of differential mRNA expression of tassels from the congenic wild types of the *D9-1* mutant sized 0.5–1 cm treated with GA_3 compared to tassels of the congenic wild types of the *D9-1* mutant sized 0.5–1 cm treated with mock treatment. Column 1 shows the maize B73 gene identification number (v3.31), column 2 shows the baseMean read count for all samples, column 3 shows the log_2-transformed fold change values (where positive numbers indicate higher expression in GA_3-treated tassels and negative numbers indicate lower expression in GA_3-treated tassels), column 4 shows the log_2-transformed fold change standard error, column 5 shows the Wald statistic, column 6 shows the Wald test *P*-value, column 7 shows the Benjamini–Hochberg multiple testing adjusted *P*-value, column 8 shows the Arabidopsis gene identification number for the closest homolog in Arabidopsis, column 9 shows the e-value for the BLASTp result of comparison between maize and Arabidopsis, and column 10 shows the Arabidopsis annotation. An asterisk in columns 8–10 indicates that there was not a homologous protein sequence of the maize gene in Arabidopsis with an e-value score of 1.00e−05.

Data File S3. The filtered 366 *bona fide* GA-responsive genes. Column 1 shows the maize B73 gene identification number (v3.31), column 2 shows the log_2-transformed fold change values from the comparison of the congenic wild types of the *D8-Mpl* mutant treated with GA_3 compared to tassels of the congenic wild types of the *D8-Mpl* mutant treated with mock treatment (where positive numbers indicate higher expression in GA_3-treated tassels and negative numbers indicate lower expression in GA_3-treated tassels), column 3 shows the Benjamini–Hochberg multiple testing adjusted *P*-value for the comparison in column 2, column 4 shows the log_2-transformed fold change values from the comparison of the congenic wild types of the *D9-1* mutant treated with GA_3 compared to tassels of the congenic wild types of the *D9-1* mutant treated with mock treatment (where positive numbers indicate higher expression in GA_3-treated tassels and negative numbers indicate lower expression in GA_3-treated tassels), and column 5 shows the Benjamini–Hochberg multiple testing adjusted *P*-value for the comparison in column 4.

Data File S4. DESeq2 results of differential mRNA expression of tassels from the *D8-Mpl* mutants sized 0.5–1 cm treated with GA_3 compared to tassels of the *D8-Mpl* mutants sized 0.5–1 cm treated with mock treatment. Column 1 shows the maize B73 gene identification number (v3.31), column 2 shows the baseMean read count for all samples, column 3 shows the log_2-transformed fold change values (where positive numbers indicate higher expression in GA_3-treated tassels and negative numbers indicate lower expression in GA_3-treated tassels), column 4 shows the log_2-transformed fold change standard error, column 5 shows the Wald statistic, column 6 shows the Wald test *P*-value, column 7 shows the Benjamini–Hochberg multiple testing adjusted *P*-value, column 8 shows the Arabidopsis gene identification number for the closest homolog in Arabidopsis, column 9 shows the e-value for the BLASTp result of comparison between maize and Arabidopsis, and column 10 shows the Arabidopsis annotation. An asterisk in columns 8–10 indicates that there was not a homologous protein sequence of the maize gene in Arabidopsis with an e-value score of 1.00e−05.

Data File S5. DESeq2 results of differential mRNA expression of tassels from the *D9-1* mutants sized 0.5–1 cm treated with GA_3 compared to tassels of the *D9-1* mutants sized 0.5–1 cm treated with mock treatment. Column 1 shows the maize B73 gene identification number (v3.31), column 2 shows the baseMean read count for all samples, column 3 shows the log_2-transformed fold change values (where positive numbers indicate higher expression in GA_3-treated tassels and negative numbers indicate lower expression in GA_3-treated tassels), column 4 shows the log_2-transformed fold change standard error, column 5 shows the Wald statistic, column 6 shows the Wald test *P*-value, column 7 shows the Benjamini–Hochberg multiple testing adjusted *P*-value, column 8 shows the Arabidopsis gene identification number for the closest homolog in Arabidopsis, column 9 shows the e-value for the BLASTp result of comparison between maize and Arabidopsis, and column 10 shows the Arabidopsis annotation. An asterisk in columns 8–10 indicates that there was not a homologous protein sequence of the maize gene in Arabidopsis with an e-value score of 1.00e−05.

Data File S6. DESeq2 results of differential mRNA expression of tassels from the congenic wild types of the *D8-Mpl* mutant sized 0.5–1 cm treated with GA_3 compared to tassels of the *D8-Mpl* mutants sized 0.5–1 cm treated with GA_3. Column 1 shows the maize B73 gene identification number (v3.31), column 2 shows the baseMean read count for all samples, column 3 shows the log_2-transformed fold change values (where positive numbers indicate higher expression in wild-type GA_3-treated tassels and negative numbers indicate lower expression in wild-type GA_3-treated tassels), column 4 shows the log_2-transformed fold change standard error, column 5 shows the Wald statistic, column 6 shows the Wald test *P*-value, column 7 shows the Benjamini–Hochberg multiple testing adjusted *P*-value, column 8 shows the Arabidopsis gene identification number for the closest homolog in Arabidopsis, column 9 shows the e-value for the BLASTp result of comparison between maize and Arabidopsis, and column 10 shows the Arabidopsis annotation. An asterisk in columns 8–10 indicates that there was not a homologous protein sequence of the maize gene in Arabidopsis with an e-value score of 1.00e−05.

Data File S7. Differentially expressed genes in congenic wild-type tassels treated with GA_3 compared to *D8-Mpl* tassels treated with GA_3. Column 1 shows the maize B73 gene identification number (v3.31), column 2 shows the log_2-transformed fold change values (where positive numbers indicate higher expression in wild-type GA_3-treated tassels and negative numbers indicate lower expression in wild-type GA_3-treated tassels), and column 3 shows the Benjamini–Hochberg multiple testing adjusted *P*-value.

Data File S8. DESeq2 results of differential mRNA expression of tassels from the congenic wild types of the *D9-1* mutant sized 0.5–1 cm treated with GA_3 compared to tassels of the *D9-1* mutants sized 0.5–1 cm treated with GA_3. Column 1 shows the maize B73 gene identification number (v3.31), column 2 shows the baseMean read count for all samples, column 3 shows the log_2-transformed fold change values (where positive numbers indicate higher expression in wild-type GA_3-treated tassels and negative numbers indicate lower expression in wild-type GA_3-treated tassels), column 4 shows the log_2-transformed fold change standard error, column 5 shows the Wald statistic, column 6 shows the Wald test *P*-value, column 7 shows the Benjamini–Hochberg multiple testing adjusted *P*-value, column 8 shows the Arabidopsis gene identification number for the closest homolog in Arabidopsis, column 9 shows the e-value for the BLASTp result of comparison between maize and Arabidopsis, and column 10 shows the Arabidopsis annotation. An asterisk in columns 8–10 indicates that there was not a homologous protein sequence of the maize gene in Arabidopsis with an e-value score of 1.00e−05.

Data File S9. Differentially expressed genes in congenic wild-type tassels treated with GA_3 compared to *D9-1* tassels treated with GA_3. Column 1 shows the maize B73 gene identification number (v3.31), column 2 shows the log_2-transformed fold change values (where positive numbers indicate higher expression in wild-type GA_3-treated tassels and negative numbers indicate lower expression in wild-type GA_3-treated tassels), and column 3 shows the Benjamini–Hochberg multiple testing adjusted *P*-value.

Data File S10. DESeq2 results of differential mRNA expression of tassels from the congenic wild types of the *D8-Mpl* mutant sized 0.5–1 cm treated with mock treatment compared to tassels of the *D8-Mpl* mutants sized 0.5–1 cm treated with mock treatment. Column 1 shows the maize B73 gene identification number (v3.31), column 2 shows the baseMean read count for all samples, column 3 shows the log_2-transformed fold change values (where positive numbers indicate higher expression in wild-type mock-treated tassels and negative numbers indicate lower expression in wild-type mock-treated tassels), column 4 shows the log_2-transformed fold change standard error, column 5 shows the Wald statistic, column 6 shows the Wald test *P*-value, column 7 shows the Benjamini–Hochberg multiple testing adjusted *P*-value, column 8 shows the Arabidopsis gene identification number for the closest homolog in Arabidopsis, column 9 shows the e-value for the BLASTp result of comparison between maize and Arabidopsis, and column 10 shows the Arabidopsis annotation. An asterisk in columns 8–10 indicates that there was not a homologous protein sequence of the maize gene in Arabidopsis with an e-value score of 1.00e−05.

Data File S11. Differentially expressed genes in congenic wild-type tassels treated with mock treatment compared to *D8-Mpl* tassels treated with mock treatment. Column 1 shows the maize B73 gene identification number (v3.31), column 2 shows the log_2-transformed fold change values (where positive numbers indicate higher expression in wild-type mock-treated tassels and negative numbers indicate lower expression in wild-type mock-treated tassels), and column 3 shows the Benjamini–Hochberg multiple testing adjusted *P*-value.

Data File S12. DESeq2 results of differential mRNA expression of tassels from the congenic wild types of the *D9-1* mutant sized 0.5–1 cm treated with mock treatment compared to tassels of the *D9-1* mutants sized 0.5–1 cm treated with mock treatment. Column 1 shows the maize B73 gene identification number (v3.31), column 2 shows the baseMean read count for all samples, column 3 shows the log_2-transformed fold change values (where positive numbers indicate higher expression in wild-type mock-treated tassels and negative numbers indicate lower expression in wild-type mock-

treated tassels), column 4 shows the $\log_2$-transformed fold change standard error, column 5 shows the Wald statistic, column 6 shows the Wald test *P*-value, column 7 shows the Benjamini–Hochberg multiple testing adjusted *P*-value, column 8 shows the Arabidopsis gene identification number for the closest homolog in Arabidopsis, column 9 shows the e-value for the BLASTp result of comparison between maize and Arabidopsis, and column 10 shows the Arabidopsis annotation. An asterisk in columns 8–10 indicates that there was not a homologous protein sequence of the maize gene in Arabidopsis with an e-value score of 1.00e−05.

Data File S13. Differentially expressed genes in congenic wild-type tassels treated with mock treatment compared to *D9-1* tassels treated with mock treatment. Column 1 shows the maize B73 gene identification number (v3.31), column 2 shows the $\log_2$-transformed fold change values (where positive numbers indicate higher expression in wild-type mock-treated tassels and negative numbers indicate lower expression in wild-type mock-treated tassels), and column 3 shows the Benjamini–Hochberg multiple testing adjusted *P*-value.

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