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Note

sparse inflorescence1, *barren inflorescence1* and *barren stalk1* Promote Cell Elongation in Maize Inflorescence Development

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ABSTRACT

The *sparse inflorescence1* (*spi1*), *Barren inflorescence1* (*Bif1*), *barren inflorescence2* (*bif2*), and *barren stalk1* (*ba1*) mutants produce fewer branches and spikelets in the inflorescence due to defects in auxin biosynthesis, transport, or response. We report that *spi1*, *bif1*, and *ba1*, but not *bif2*, also function in promoting cell elongation in the inflorescence.

AUXIN is essential for lateral organ and axillary meristem initiation in plants (Barazesh and McSteen 2008b; Delker *et al.* 2008). The maize (*Zea mays*) mutants, *sparse inflorescence1* (*spi1*), *Barren inflorescence1* (*Bif1*), *barren inflorescence2* (*bif2*), and *barren stalk1* (*ba1*) produce fewer branches and spikelets in the inflorescence due to defects in axillary meristem initiation (McSteen and Hake 2001; Ritter *et al.* 2002; Barazesh and McSteen 2008a; Gallavotti *et al.* 2008). *spi1* functions in localized auxin biosynthesis, while *bif1* and *bif2* regulate auxin transport (McSteen *et al.* 2007; Barazesh and McSteen 2008a; Gallavotti *et al.* 2008). *spi1; bif2* and *Bif1; bif2* double mutants have a synergistic interaction producing dwarf plants with fewer leaves, indicating that *spi1*, *bif1*, and *bif2* also function in leaf initiation during vegetative development (Barazesh and McSteen 2008a; Gallavotti *et al.* 2008). Synergistic interactions between mutants affecting auxin biosynthesis and auxin transport have also been reported in Arabidopsis (*Arabidopsis thaliana*) (Cheng *et al.* 2007a,b).

Investigation of tassel-length reduction in *spi1* mutants: An interesting aspect of the *spi1* phenotype is that the length of the tassel (male inflorescence) is reduced compared to a normal tassel (Figure 1, A and F). Previous analysis revealed that spikelets grow over the tip of the tassel (arrowhead in Figure 1C) (Gallavotti *et al.* 2008). Development of spikelets over the tip of the tassel could consume the apical inflorescence meristem, which would inhibit growth of the tassel. To test whether the production of spikelets over the tip causes the short inflorescence phenotype, we utilized *spi1; bif2* double mutants, which produce tassels with no spikelets (Figure 1A) (Gallavotti *et al.* 2008). SEM analysis verified that *spi1; bif2* mutants fail to initiate spikelet pair meristems (SPMs) (Figure 1, B–E). However, there was no significant difference in the tassel length of *spi1; bif2* double mutants compared to *spi1* single mutants (Figure 1F, $P = 0.366$), showing that the growth of spikelets over the tip of the inflorescence does not the cause the reduction in tassel length in *spi1* mutants.

***spi1*, *bif1*, and *ba1* function in cell elongation in the tassel:** To determine if the reduced tassel length in *spi1* mutants was due to defective cell elongation, impressions were taken of epidermal cells of mature *spi1* tassels, and cell length was quantified. Cell length was significantly decreased in the epidermal cells of *spi1* tassels compared to normal (Figure 2, Table 1). However, cell length in the epidermis of the leaf was unaffected (data not shown).

The reduced tassel length of *spi1* prompted us to investigate if other *barren inflorescence* mutants had this defect. We discovered that *bif2* did not affect tassel length (Figure 1F) or cell elongation (Table 1). However, both *Bif1* and *ba1* mutants had shorter tassels than normal (Figure 3, Table 2, and Table 3), and epidermal cell length was significantly reduced (Figure 2, Table 1). As *Bif1* and *ba1* affected tassel length, we investigated the interaction between *spi1* and each of these mutants.

***spi1* interaction with *Bif1*:** *spi1; Bif1* double mutants had a severe tassel phenotype, with no tassel branches and very few spikelets, similar to the *spi1*; *bif2* inflorescence phenotype (Figure 3A, Table 2) (Gallavotti

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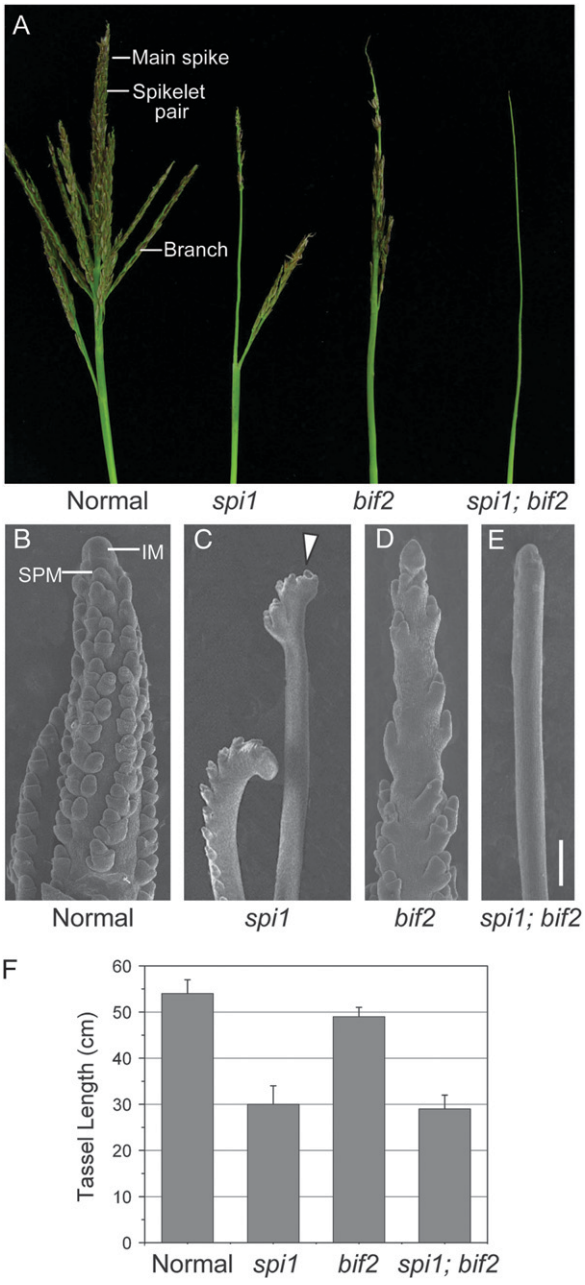

FIGURE 1.—Genetic interaction of *spi1* with *bif2*. Double mutants were constructed in the B73 background with *bif2-77* and *spi1-ref* alleles, which were genotyped as previously described (GALLAVOTTI *et al.* 2008). For analysis of immature *spi1; bif2* double mutants, tassels were dissected from 5-week-old plants, and fixation and SEM was carried out as previously described (BARAZESH and MCSTEEN 2008a). For mature plant analysis, all plants were grown in the field to maturity. Two families of 120 kernels were planted at two different field locations. (A) Mature tassel phenotype of all genetic classes in a family segregating for both *spi1* and *bif2*. (B–E) SEM analysis of developing inflorescences of (B) normal, (C) *spi1*, (D) *bif2*, and (E) *spi1; bif2* double mutants. Arrowhead indicates spikelets growing over the tip of the *spi1* mutant tassel. IM, inflorescence meristem; SPM, spikelet pair meristem. Bar, 100 μm. (F) Mature tassel length of all genetic classes in a family segregating for both *spi1* and *bif2*. Tassel length was measured from the node at the base of the flag leaf to the tip of the tassel. Sample size was 10 for each genetic class.

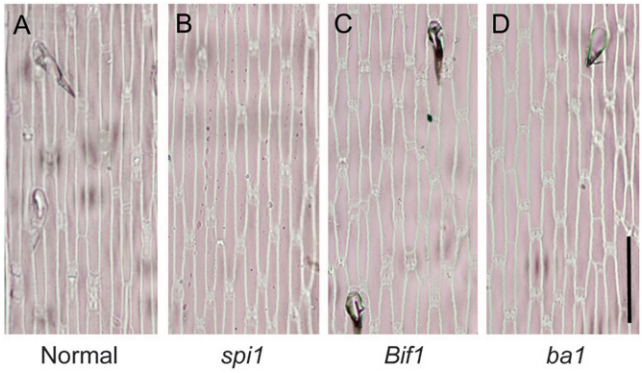

FIGURE 2.—*spi1*, *Bif1*, and *ba1* have reduced cell elongation. Nail polish impressions of epidermal cells from the base of the mature tassel in (A) normal, (B) *spi1*, (C) *Bif1*, and (D) *ba1*. Bar, 100 μm.

et al. 2008). However, the tassel length defect in *spi1; Bif1* was not statistically different from *spi1* single mutants ($P = 0.464$), suggesting that *spi1* and *Bif1* may function in the same pathway to promote tassel length. Unlike the *spi1; bif2* double mutants (GALLAVOTTI *et al.* 2008), the *spi1; Bif1* double mutants did not have a synergistic effect on vegetative development (Table 2). Plant height and leaf number were not significantly different in *spi1; Bif1* double mutants compared to *spi1* single mutants ($P = 0.429$ and 0.066, respectively).

***spi1* interaction with *ba1*:** The s*pi1; ba1* double mutant was similar to *ba1* single mutants, with no ears and no tassel branches (Figure 3B, Table 3). The reduction in spikelet number in the tassel was more

TABLE 1

***spi1*, *Bif1*, and *ba1* affect cell elongation**

Genotype	Mean cell length (μm)[a]	±SE	*N*	*P*-value[b]
Normal	156.51	6.01	5	—
spi1	127.63	6.81	5	0.015
bif2	157.83	8.36	5	0.902
Bif1	109.66	7.83	7	0.001
ba1	101.86	4.80	7	<0.001

To measure the length of epidermal cells of mature tassels, clear nail polish was used to make impressions from the surface of the tassel rachis. Double-sided tape was used to lift the nail polish from the surface of the tassel and adhere it to a slide. Impressions were viewed at ×20 magnification using a Nikon 80i microscope and photographed with a Nikon DM1200F camera. Approximately 25 cells/biological replicate were measured in three regions of the tassel. Similar results were obtained for each region, and data for one region near the base are shown. SE, standard error of mean; *N*, number of biological replicates.

[a] Mean calculated for each biological replicate and then for each genotype.

[b] *P*-value indicates the significance of the difference between the mutant and the normal calculated using a Student's *t* test.

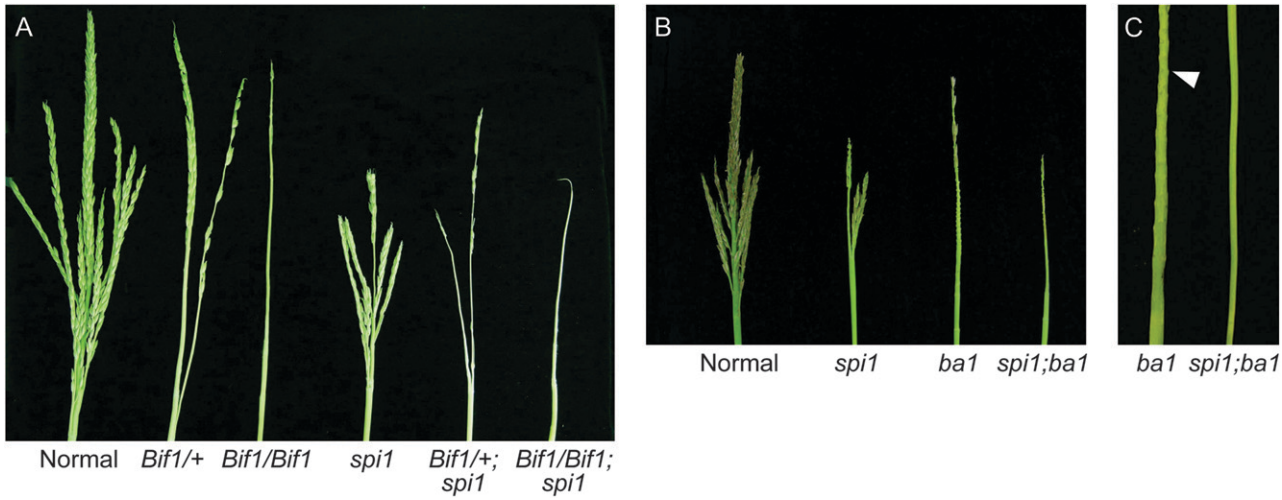

FIGURE 3.—Genetic interaction of *spi1* with *Bif1* and of *spi1* with *ba1*. (A) Mature tassel phenotype of a *spi1*, *Bif1* segregating family. Double mutants were constructed in the B73 genetic background with the *spi1-ref* and *Bif1-N1440* alleles (BARAZESH and MCSTEEN 2008a; GALLAVOTTI *et al.* 2008). Plants were genotyped for the *spi1-ref* allele as reported (GALLAVOTTI *et al.* 2008). A total of 120 plants were analyzed in two different field locations. (B) Mature tassel phenotype of a *spi1*, *ba1* segregating family. Double mutants were constructed in the B73 genetic background with the *spi1-ref* and *ba1-ref* alleles and genotyped as described (BARAZESH and MCSTEEN 2008a; GALLAVOTTI *et al.* 2008). A total of 120 plants were analyzed in two different field locations. (C) Close-up of the surface of the tassel rachis showing prominent bract leaf primordia in *ba1* (arrowhead), which are not present in *spi1; ba1*.

severe than either *spi1* ($P < 0.0001$) or *ba1* single mutants ($P < 0.001$). Furthermore, the double-mutant tassels were more severely reduced in length than either *spi1* ($P < 0.005$) or *ba1* single mutants ($P < 0.001$). We infer that *spi1* and *ba1* play independent roles in spikelet formation and tassel elongation although, as neither of these mutants are known to be null alleles, it is also possible that they function in the same pathway.

TABLE 2

***spi1; Bif1* double-mutant analysis**

Genotype	Tassel length (cm)	Branch no.	Spikelet no.	Plant height (cm)	Leaf no.
Normal	52.73 ± 1.74	8.46 ± 0.57	685.1 ± 23	215.91 ± 6.48	22.90 ± 0.21
Bif1/+	48.5 ± 0.9[a]	2.67 ± 0.31[a]	112.17 ± 8.32[a]	179.24 ± 6.02[a]	21.53 ± 0.34[a]
Bif1/Bif1	44.33 ± 1.55[a]	0.556 ± 0.18[a]	5.667 ± 0.83[a]	161.56 ± 6.59[a]	21 ± 0.37[a]
spi1	31.79 ± 0.86[a]	4.43 ± 0.42[a]	68.71 ± 7.86[a]	152.13 ± 9.43[a]	21.62 ± 0.50[a]
spi1; Bif1/+	31.5 ± 0.97[b]	2.286 ± 0.27[c]	29.5 ± 4.15[b,c]	148.11 ± 5.3	20.67 ± 0.40
spi1; Bif1/Bif1	30.89 ± 0.84[b]	0.11 ± 0.11[c]	0.22 ± 0.22[b,c]	143.64 ± 3.99[b]	20.18 ± 0.53

For quantification of inflorescence characters, the sample size was normal 13, *Bif1/+* 12, *Bif1/Bif1* 9, *spi1* 14, *Bif1/+; spi1/spi1* 14, and *Bif1/Bif1; spi1/spi1* 9. For quantification of vegetative characters, the sample size was Normal 11, *Bif1/+* 17, *Bif1/Bif1* 9, *spi1* 4, *Bif1/+; spi1/spi1* 6, and *Bif1/Bif1; spi1/spi1* 6.

[a] Value is significantly different from normal, $P < 0.05$.

[b] Value is significantly different from *Bif1/+* and *Bif1/Bif1*, $P < 0.05$.

[c] Value is significantly different from *spi1*, $P < 0.05$.

TABLE 3

***spi1; ba1* double-mutant analysis**

Genotype	Tassel length (cm)	Branch no.	Spikelet no.	Ear no.
Normal	52.32 ± 1.01	8 ± 0.30	646.7 ± 12.6	1.71 ± 0.07
spi1	32.32 ± 1.2[a]	3.18 ± 0.32[a]	36.36 ± 3.59[a]	1.38 ± 0.15[a]
ba1	36.8 ± 1.18[a]	0 ± 0[a]	21.7 ± 3.24[a]	0 ± 0[a]
spi1; ba1	25.1 ± 1.8[b]	0 ± 0	1.5 ± 1.19[b]	0 ± 0

For quantification of inflorescence characters, the sample size was normal 11, *spi1* 12, *ba1* 11, and *spi1;ba1* 10. For quantification of ear number, the sample size was normal 56, *spi1* 16, *ba1* 24, and *spi1;ba1* 7.

[a] Value is significantly different from normal, $P < 0.05$.

[b] Value is significantly different from either single mutant, $P < 0.05$.

ba1 mutants produce a regular pattern of bumps on the surface of the tassel rachis, which are the bract leaf primordia that subtend axillary meristems in the tassel (Figure 3C) (Ritter *et al.* 2002). The surface of the *spi1; ba1* tassel rachis was smooth, similar to that of the *spi1* single mutant, indicating that the bract leaf bumps were missing (Figure 3C). Similarly, the *Bif1; ba1* and *bif2; ba1* double mutants had a smooth tassel rachis (Barazesh and McSteen 2008a; Skirpan *et al.* 2008). Therefore, both auxin biosynthesis and transport are required for bract leaf initiation.

Conclusions: Auxin is known to function in cell expansion (Jones *et al.* 1998; Christian *et al.* 2006). A link between auxin biosynthesis and cell expansion was illustrated by experiments involving the *erecta* (*er*) mutants of Arabidopsis, which are defective in internode and pedicel elongation (Woodward *et al.* 2005). Overexpression of the auxin biosynthesis gene, *YUC5*, suppressed the *er* phenotype by increasing the elongation of epidermal pavement cells, showing that an increase in localized auxin biosynthesis led to an increase in cell elongation. In this article, we have shown that a decrease in localized auxin biosynthesis led to a decrease in cell elongation, with *spi1* epidermal cells significantly reduced in length compared to normal. Mutations in other auxin biosynthesis genes in Arabidopsis and petunia (*Petunia inflata*) also cause short inflorescences (Tobena-Santamaria *et al.* 2002; Cheng *et al.* 2006; Stepanova *et al.* 2008), implying that these mutations may also affect cell elongation.

spi1 is expressed in a very restricted pattern in the inflorescence (Gallavotti *et al.* 2008). As *spi1* appears to function in tissues in which the gene is not expressed, we infer that auxin synthesized by *spi1* is transported rapidly to other cells and therefore that *spi1* functions in a non-cell-autonomous manner. This is consistent with the finding that a homologous gene in Petunia acts non-cell autonomously (Tobena-Santamaria *et al.* 2002).

Previously, it was shown that auxin transport functions in cell elongation during vegetative development (Multani *et al.* 2003). Here, we show that *spi1*, *Bif1*, and *ba1* mutants also have defects in cell elongation in the inflorescence. This emphasizes the importance of both auxin biosynthesis and transport in cell elongation during inflorescence development.

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