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 (bal) 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; Cheng et al. 2007b).

Investigation of the 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 normal (Figure 1 A, 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 1B-E). However, there was no significant difference in the tassel length of \textit{spi1; bif2} double mutants compared to \textit{spi1} single mutants (Figure 1F, P = 0.366), showing that the growth of spikelets over the tip of the inflorescence does not cause the reduction in tassel length in \textit{spi1} mutants.

\textbf{\textit{spi1}, \textit{Bif1} and \textit{ba1} function in cell elongation in the tassel:} To determine if the reduced tassel length in \textit{spi1} mutants was due to defective cell elongation, impressions were taken of epidermal cells of mature \textit{spi1} tassels and cell length was quantified. Cell length was significantly decreased in the epidermal cells of \textit{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 \textit{spi1} prompted us to investigate if other \textit{barren inflorescence} mutants had this defect. We discovered \textit{bif2} did not affect tassel length (Figure 1F) or cell elongation (Table 1). However, both \textit{Bif1} and \textit{ba1} mutants had shorter tassels than normal (Figure 3, Table 2, 3), and epidermal cell length was significantly reduced (Figure 2, Table 1). As \textit{Bif1} and \textit{ba1} affected tassel length, we investigated the interaction between \textit{spi1} and each of these mutants.

\textbf{\textit{spi1} interaction with \textit{Bif1}:} \textit{spi1; Bif1} double mutants had a severe tassel phenotype, with no tassel branches and very few spikelets, similar to the \textit{spi1; bif2} inflorescence phenotype (Figure 3A, Table 2) (GALLAVOTTI et al. 2008). However, the tassel length defect in \textit{spi1; Bif1} was not statistically different from \textit{spi1} single mutants (P=0.464), suggesting that \textit{spi1} and \textit{Bif1} may function in the same pathway to promote tassel length. Unlike the \textit{spi1; bif2} double mutants (GALLAVOTTI et al. 2008), the \textit{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; Bifl double mutants compared to spi1 single mutants (P = 0.429 and 0.066, respectively).

**spi1 interaction with ba1:** The spi1; 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 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.

**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). Over expression 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 paper, we show 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 where 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 bal 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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TABLE 1 - *spi1, Bif1* and *ba1* affect cell elongation.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Mean cell length (μm) ± se</th>
<th>N</th>
<th>P-value&lt;sup&gt;d&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>156.51 ± 6.01</td>
<td>5</td>
<td>__</td>
</tr>
<tr>
<td><em>spi1</em></td>
<td>127.63 ± 6.81</td>
<td>5</td>
<td>0.015</td>
</tr>
<tr>
<td><em>bif2</em></td>
<td>157.83 ± 8.36</td>
<td>5</td>
<td>0.902</td>
</tr>
<tr>
<td><em>Bif1</em></td>
<td>109.66 ± 7.83</td>
<td>7</td>
<td>0.001</td>
</tr>
<tr>
<td><em>ba1</em></td>
<td>101.86 ± 4.80</td>
<td>7</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

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 20X magnification using a Nikon 80i microscope and photographed with a Nikon DM1200F camera. Approx 25 cells per 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 is shown.

<sup>a</sup> mean calculated for each biological replicate and then each genotype.

<sup>b</sup> se = standard error of mean.

<sup>c</sup> N = number of biological replicates.

<sup>d</sup> P-value indicates the significance of the difference between mutant and normal calculated using a students *t* test.
TABLE 2 – *spi1; Bif1* double mutant analysis.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Tassel length (cm)</th>
<th>Branch number</th>
<th>Spikelet number</th>
<th>Plant height (cm)</th>
<th>Leaf number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>52.73 ± 1.74</td>
<td>8.46 ± 0.57</td>
<td>685.1 ± 23</td>
<td>215.91 ± 6.48</td>
<td>22.90 ± 0.21</td>
</tr>
<tr>
<td><em>Bif1/+</em></td>
<td>48.5 ± 0.9‡</td>
<td>2.67 ± 0.31‡</td>
<td>112.17 ± 8.32‡</td>
<td>179.24 ± 6.02‡</td>
<td>21.53 ± 0.34‡</td>
</tr>
<tr>
<td><em>Bif1/Bif1</em></td>
<td>44.33 ± 1.55‡</td>
<td>0.556 ± 0.18‡</td>
<td>5.667 ± 0.83‡</td>
<td>161.56 ± 6.59‡</td>
<td>21 ± 0.37‡</td>
</tr>
<tr>
<td><em>spi1</em></td>
<td>31.79 ± 0.86‡</td>
<td>4.43 ± 0.42‡</td>
<td>68.71 ± 7.86‡</td>
<td>152.13 ± 9.43‡</td>
<td>21.62 ± 0.50‡</td>
</tr>
<tr>
<td><em>spi1; Bif1/+</em></td>
<td>31.5 ± 0.97§</td>
<td>2.286 ± 0.27†</td>
<td>29.5 ± 4.15§†</td>
<td>148.11 ± 5.3</td>
<td>20.67 ± 0.40</td>
</tr>
<tr>
<td><em>spi1; Bif1/Bif1</em></td>
<td>30.89 ± 0.84§</td>
<td>0.11 ± 0.11†</td>
<td>0.22 ± 0.22§†</td>
<td>143.64 ± 3.99§</td>
<td>20.18 ± 0.53</td>
</tr>
</tbody>
</table>

‡ Value is significantly different from normal, $P < 0.05$

§ Value is significantly different from *Bif1/+* and *Bif1/Bif1*, $P < 0.05$

† Value is significantly different from *spi1*, $P < 0.05$

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.
TABLE 3 – *spi1; ba1* double mutant analysis.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Tassel length (cm)</th>
<th>Branch number</th>
<th>Spikelet number</th>
<th>Ear number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>52.32 ± 1.01</td>
<td>8 ± 0.30</td>
<td>646.7 ± 12.6</td>
<td>1.71 ± 0.07</td>
</tr>
<tr>
<td><em>spi1</em></td>
<td>32.32 ± 1.2‡</td>
<td>3.18 ± 0.32‡</td>
<td>36.36 ± 3.59‡</td>
<td>1.38 ± 0.15‡</td>
</tr>
<tr>
<td><em>ba1</em></td>
<td>36.8 ± 1.18‡</td>
<td>0 ± 0‡</td>
<td>21.7 ± 3.24‡</td>
<td>0 ± 0‡</td>
</tr>
<tr>
<td><em>spi1; ba1</em></td>
<td>25.1 ± 1.8§</td>
<td>0 ± 0</td>
<td>1.5 ± 1.19§</td>
<td>0 ± 0</td>
</tr>
</tbody>
</table>

‡ Value is significantly different from normal, P < 0.05

§ Value is significantly different from either single mutant, P < 0.05

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.
FIGURE LEGENDS

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 five-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. Scale bar = 100µm. (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 *bal* have reduced cell elongation. Nail polish impressions of epidermal cells from the base of the mature tassel in (A) Normal, (B) *spi1*, (C) *Bif1*, (D) *bal*. Scale bar = 100µm.

FIGURE 3 - Genetic interaction of *spi1* with *Bif1*, and *spi1* with *bal*. (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
Plants were genotyped for the *spi1-ref* allele as reported (GALLAVOTTI *et al.* 2008). 120 plants were analyzed in two different field locations. (B) Mature tassel phenotype of a *spi1, bal* segregating family. Double mutants were constructed in the B73 genetic background with the *spi1-ref* and *bal-ref* alleles, and genotyped as described (BARAZESH and McSTEEN 2008a; GALLAVOTTI *et al.* 2008). 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 *bal* (arrowhead) which are not present in *spi1; bal*. 
FIGURE 1
FIGURE 3