A Potato Sus3 Sucrose Synthase Gene Contains a Context-Dependent 3' Element and a Leader Intron with Both Positive and Negative Tissue-Specific Effects

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To examine which sequences are involved in regulating the potato sucrose synthase gene Sus3-65, we examined a series of deletion and substitution constructs in transgenic potato and tobacco plants. In a construct containing 3.9 kb of 5' flanking region, substitution of the native 3' sequence with the nopaline synthase 3' sequence and deletion of the leader intron did not significantly affect expression in vegetative tissues. However, in a construct containing only 320 bp of 5' flanking region, these changes had marked effects. Replacing the native 3' sequences with nopaline synthase 3' sequences caused a six- to 20-fold increase in expression in vascular tissue, and removing the leader intron almost completely abolished expression in potato plants. Surprisingly, removal of the leader intron from either the full-length construct or a construct containing only 320 bp of 5' flanking sequence reduced expression in vascular tissue of tobacco anthers at later stages of development but increased expression in pollen by more than 100-fold.

INTRODUCTION

Sucrose is the major transport form of photosynthate in most plants and the initial substrate for carbohydrate metabolism in nonphotosynthetic tissues (Avigad, 1982). Because cleavage of the glycosidic bond is a prerequisite step for sucrose metabolism, regulation of the sucrose cleavage enzymes sucrose synthase (EC 2.4.1.13) and invertase (EC 3.2.1.26) is of considerable interest.

We have shown previously that sucrose synthase in potato is encoded by two differentially expressed gene classes (Fu and Park, 1995). These cannot be classified as Sus1 and Sus2 types based on sequence homology, but both have the same overall gene structure as sucrose synthase genes from other species (Werr et al., 1985; Chopra et al., 1992; Wang et al., 1992; Yu et al., 1992; Martin et al., 1993; Shaw et al., 1994), including the long leader intron characteristic of all the sucrose synthase genes examined thus far except Arabidopsis Asus1 (Martin et al., 1993).

Using RNA gel blots and transgenic plants, we have shown that potato Sus4 genes are expressed at the highest levels in potato tubers, root caps, and the basal tissues of axillary buds and shoots, suggesting that they play a role in sink function (Fu and Park, 1995). This is consistent with the critical role of sucrose synthase in sink strength suggested by experiments with transgenic potato plants (Zrenner et al., 1995). In contrast, we found that Sus3 genes are expressed at the higher levels in stem and roots. A Sus3-β-glucuronidase (GUS) construct containing 3.9 kb of 5' flanking sequence, the leader intron, and 0.7 kb of 3' sequence was expressed at highest levels in the vascular tissues of leaves, stems, roots, and tubers of transgenic potato plants, particularly in the internal phloem of stems. This pattern of expression suggests involvement of Sus3 sucrose synthase in the phloem-related functions indicated by previous biochemical and immunolocalization studies (Hawker and Hatch, 1965; Lowell et al., 1989; Tomlinson et al., 1991; Nolte and Koch, 1993) and by recent experiments in transgenic plants (Yang and Russell, 1990; Martin et al., 1993; Lerchl et al., 1995).

For most of the plant genes that have been examined, 1 to 1.5 kb of 5' flanking sequence is sufficient to confer the proper pattern of expression in transgenic plants. However, using deletion and replacement constructs, we found that sequences upstream of position -1500, 3' sequences, and the leader intron are all critical for high-level expression of Sus4 constructs in tubers of transgenic plants and for sucrose inducibility in leaves (Fu et al., 1995).

Here, we examine the role of the corresponding sequences in the expression of a Sus3–GUS construct in both transgenic potato and tobacco plants. Replacing the native 3' sequences with the nopaline synthase (NOS) 3' sequence or deleting the leader intron from a construct containing 39 kb of 5' flanking sequence did not significantly affect expression in vegetative tissues. However, in a construct containing only 320 bp of 5' flanking region, these changes had marked effects. Surprisingly, deleting the leader intron from either the full-length or proximal promoter had both positive and negative tissue-specific effects on Sus3 expression in flowers.
RESULTS

Deletion of the Sus3 3’ Sequence Does Not Significantly Affect Expression in the Context of the Full-Length Promoter

Replacing the native Sus3 3’ sequence of construct SS-III-3.9/3’ (Fu and Park, 1995) with the 3’ sequence of the NOS gene (designated here as NOS-ter for NOS terminator sequence; Jefferson et al., 1987) to yield construct SS-III-3.9/N (Figure 1A) had only a small effect on the level of GUS expression (Table 1) and did not change the pattern of GUS expression in any of the vegetative tissues of transgenic potato plants. With either construct (Figure 2A; only SS-III-3.9/N is shown), GUS activity in roots was observed in the cell division zone and cortex and showed higher intensity in vascular tissues. In leaves, GUS activity was detected in all cell types but showed higher intensity in primary veins. GUS activity in stems was localized in the phloem, especially the internal phloem tissues. In tubers, GUS activity was detected in all tissues inside the epidermis, and stronger activity was associated with vascular tissues.

Proximal Promoter and Leader Intron Are Sufficient for Expression in Vascular Tissues

To examine whether the proximal 5’ promoter and leader intron of Sus3-65 are sufficient to confer tissue-specific expression, construct SS-III-0.32/N was made by removing the upstream sequence -3900 to -323 from SS-III-3.9/N (Figure 1A). The GUS staining patterns in various organs of plants containing SS-III-0.32/N were similar to those in potato plants.

(C) The junction sequence between the leader exon and exon 2 of leader intron−deleted constructs. Lowercase letters indicate bases altered by introducing the PstI site (underlined).

Table 1. GUS Activity in Different Organs of Transgenic Potato Plants Containing Various Sus3−GUS Constructs

<table>
<thead>
<tr>
<th>Construct</th>
<th>n</th>
<th>Leaves</th>
<th>Stems</th>
<th>Roots</th>
<th>Tubers</th>
</tr>
</thead>
<tbody>
<tr>
<td>SS-III-3.9/3’</td>
<td>7</td>
<td>1,713 ± 612</td>
<td>5,263 ± 995</td>
<td>71,903 ± 6,257</td>
<td>6,847 ± 491</td>
</tr>
<tr>
<td>SS-III-3.9/N</td>
<td>8</td>
<td>2,190 ± 485</td>
<td>7,734 ± 1,501</td>
<td>53,339 ± 15,891</td>
<td>6,342 ± 1,359</td>
</tr>
<tr>
<td>SS-III-0.32/3’</td>
<td>4</td>
<td>46 ± 17</td>
<td>297 ± 137</td>
<td>2,720 ± 347</td>
<td>112 ± 20</td>
</tr>
<tr>
<td>SS-III-0.32/N</td>
<td>8</td>
<td>257 ± 84</td>
<td>3,014 ± 958</td>
<td>23,574 ± 5,543</td>
<td>2,215 ± 732</td>
</tr>
<tr>
<td>SS-III-3.9 ΔLI/3’</td>
<td>8</td>
<td>1,133 ± 434</td>
<td>6,514 ± 2,208</td>
<td>128,616 ± 26,910</td>
<td>12,162 ± 3,338</td>
</tr>
<tr>
<td>SS-III-3.9 ΔLI/N</td>
<td>10</td>
<td>849 ± 284</td>
<td>3,599 ± 884</td>
<td>75,833 ± 16,775</td>
<td>14,308 ± 3,364</td>
</tr>
<tr>
<td>SS-III-0.32 ΔLI/3’</td>
<td>6</td>
<td>58 ± 24</td>
<td>317 ± 158</td>
<td>566 ± 345</td>
<td>149 ± 138</td>
</tr>
<tr>
<td>SS-III-0.32 ΔLI/N</td>
<td>8</td>
<td>28 ± 6</td>
<td>572 ± 332</td>
<td>717 ± 263</td>
<td>400 ± 236</td>
</tr>
<tr>
<td>Wild type</td>
<td>6</td>
<td>6 ± 0</td>
<td>13 ± 1</td>
<td>20 ± 4</td>
<td>2 ± 0</td>
</tr>
</tbody>
</table>

GUS activity is given in picomoles of 4-methylumbelliferone per minute per milligram of protein.

* n, number of independent transgenic plants assayed.

\(^{b}\) Mean ± SE.
Figure 2. Histochemical Staining of GUS Activity in Various Organs of Transgenic Potato Plants Containing Various Sus3-GUS Constructs.

The GUS staining patterns in leaves, stems, roots, and tubers from transgenic potato plants containing Sus3 sequences are shown.

(A) SS-III-39/N.
(B) SS-III-032/N.
(C) SS-III-032/3’.
(D) SS-III-39/ALII/3’.
(E) SS-III-032/ALII/N.
containing either SS-III-3.9/3' or SS-III-3.9/N, except that GUS activity was undetectable in the cell division zone of roots and was limited to vascular tissues in tubers (Figure 2B). As expected, removal of the upstream sequence affected quantitative GUS expression. GUS activity decreased twofold in roots, ninefold in leaves, threefold in stems, and fourfold in tubers (Table 1). Although the proximal promoter contains most of the regulatory sequences necessary for vascular expression, the upstream sequence appears to contain the cis element(s) necessary for expression in the cell division zone of roots and in nonvascular tuber tissues.

Sus3 3' Sequences Negatively Affect Expression of the Proximal 5' Promoter and Leader Intron

As shown earlier, deletion of Sus3 3' sequences did not substantially alter expression of a construct containing the leader intron and 3.9 kb of 5' flanking sequence. However, the Sus3 3' sequences had a marked effect on expression when sequences upstream of −320 were deleted. Compared with the construct SS-III-0.32/N containing the NOS-ter, expression from the construct SS-III-0.32/3' (Figure 1A) containing the native 3' sequences was reduced sixfold in leaves, 10-fold in stems, ninefold in roots, and 20-fold in tubers (Table 1). In agreement with the fluorometric assays, GUS staining was observed in fewer tissues, with significantly reduced intensity (Figure 2C). These results indicate that, in addition to the cis elements involved in expression in the cell division zone of roots and storage tissue of tubers, the 5' flanking sequence from −3900 to −320 also mitigates the potential negative influence of the Sus3 3' sequences on the expression in vascular tissues.

Effect of Leader Intron on Expression in Vegetative Tissues of Potato Is Context Dependent

Deleting the leader intron from constructs containing 3.9 kb of 5' flanking sequence and either the native 3' sequence, construct SS-III-3.9/LI/3'; or NOS-ter, construct SS-III-3.9/LI/N (Figure 1A), did not significantly change their pattern of expression. The average GUS activity observed in all organs was within twofold of that seen in plants harboring the corresponding leader intron-containing construct (Table 1). The GUS staining patterns were also very similar (Figure 2D; only SS-III-3.9/LI/3' is shown).

Deleting the leader intron from the construct containing the Sus3 3' sequences and only 320 bp of 5' flanking region (SS-III-0.32/LI/3'; Figure 1A) did not significantly change the level of expression in leaves, stems, or tubers (Table 2). In roots, however, average GUS activity decreased approximately fivefold. The tissue specificity of GUS staining in organs of plants containing SS-III-0.32/LI/3' was generally similar to the specificity seen in plants containing SS-III-0.32/3'; in agreement with the decrease in the average GUS activity, weaker GUS staining was detected in roots of the majority of plants containing SS-III-0.32/LI/3' (data not shown).

Deleting the leader intron from constructs containing NOS-ter and only 320 bp of 5' flanking sequence (SS-III-0.32/LI/N; Figure 1A), however, significantly decreased expression in all tissues. Average GUS activity decreased ninefold in leaves, fivefold in stems, 33-fold in roots, and sixfold in tubers (Table 1). GUS activity was restricted to main veins of leaves of plants containing SS-III-0.32/LI/N (Figure 2E). The pattern of GUS activity in other organs was similar to that in the corresponding construct with the leader intron (SS-III-0.32/N), but the intensity was significantly reduced.

Expression in Vegetative Organs of Transgenic Tobacco Plants

We also examined GUS expression in tobacco. GUS activity was detected in leaves, stems, and roots of transgenic tobacco plants harboring SS-III-3.9/3' at levels comparable with those detected in transgenic potato plants (compare Table 2 with Table 1). Also, the spatial expression pattern observed in stems and roots of tobacco plants containing this construct was very similar to that observed in potato plants (Figures 3A and 3B). GUS activity was localized in stem phloem tissues, especially the internal phloem, and was also detected in root cell division zones, the cortex, and root vascular tissues.

Table 2. GUS Activity in Different Organs of Transgenic Tobacco Plants Containing Various Sus3-GUS Constructs

<table>
<thead>
<tr>
<th>Construct</th>
<th>n</th>
<th>Leaves</th>
<th>Stems</th>
<th>Roots</th>
</tr>
</thead>
<tbody>
<tr>
<td>SS-III-3.9/3'</td>
<td>6</td>
<td>1,747 ± 905</td>
<td>9,054 ± 4,062</td>
<td>44,461 ± 9,451</td>
</tr>
<tr>
<td>SS-III-3.9/LI/3'</td>
<td>3</td>
<td>980 ± 40</td>
<td>13,885 ± 405</td>
<td>113,998 ± 6,808</td>
</tr>
<tr>
<td>SS-III-0.32/N</td>
<td>6</td>
<td>90 ± 15</td>
<td>759 ± 158</td>
<td>2,631 ± 469</td>
</tr>
<tr>
<td>SS-III-0.32/LI/N</td>
<td>5</td>
<td>136 ± 90</td>
<td>981 ± 704</td>
<td>1,503 ± 854</td>
</tr>
<tr>
<td>pBI121*</td>
<td>2</td>
<td>3,945 ± 493</td>
<td>9,615 ± 3,470</td>
<td>11,323 ± 2,949</td>
</tr>
<tr>
<td>Wild type</td>
<td>8</td>
<td>6 ± 0</td>
<td>10 ± 1</td>
<td>12 ± 1</td>
</tr>
</tbody>
</table>

GUS activity is given in picomoles of 4-methylumbelliferone per minute per milligram of protein.

* n, number of independent transgenic plants assayed.
* Mean ± SE.
* As a control, plants containing pBI121, which has a GUS gene driven by the cauliflower mosaic virus 35S promoter (Jefferson et al., 1987), were included in the assays.
Figure 3. Histochemical Staining of GUS Activity in Various Organs of Transgenic Tobacco Plants.

(A) to (E) GUS staining patterns in the stem, root, pistil, anther, and pollen from transgenic tobacco plants containing SS-III-3.9/3'.
(F) to (J) GUS staining patterns in the same organs from transgenic tobacco plants containing SS-III-0.32/N.
(K) and (L) GUS staining in anthers one day before anthesis and pollen the day of anthesis, respectively, from plants containing SS-III-3.9ΔLI/3'.
(M) and (N) GUS staining in anthers as given for (K) and (L), respectively, from plants containing SS-III-0.32ΔLI/N.
(O) to (R) Temporal GUS expression pattern in anthers and pollen. (O) shows an anther at stage 2 from a plant containing SS-III-3.9ΔLI/3'. (P) shows a stage 2 anther from a plant containing SS-III-3.9ΔLI/3'. (Q) shows pollen at stage 6 as well as the anther from a plant containing SS-III-3.9ΔLI/3'.
(R) shows pollen at stage 7 from a plant containing SS-III-3.9ΔLI/3'.
As in potato, removing the leader intron in construct SS-III-3.9AL1/3' changed GUS activity <2.5-fold in the various vegetative tissues of transgenic tobacco plants, as compared with the GUS activity in SS-III-3.9/3' (Table 2). The spatial expression pattern in stems and roots was also very similar between tobacco plants containing either SS-III-3.9/3' or SS-III-3.9AL1/3' (data not shown). As in potato, the proximal promoter and leader intron in construct SS-III-0.32/N were also sufficient to confer the basic pattern of expression in vascular tissue of both stems and roots but not in the cell division zone of roots (Figures 3F and 3G). The primary difference was that, in tobacco, expression from SS-III-0.32/N was less dependent on the presence of the leader intron than it was in potato (compare SS-III-0.32/N and SS-III-0.32AL1/N in Tables 1 and 2).

**The Sus3 Leader Intron Confers Both Positive and Negative Regulation in Tobacco Anthers**

We were unable to examine expression of the potato Sus3 sucrose synthase sequences in potato flowers, because the potato variety FL1607, which was used for generating transgenic plants, does not flower under our normal growth conditions. However, in transgenic tobacco plants containing SS-III-3.9/3' that were examined one day before anthesis, strong GUS expression in the pistil was detected in the epidermis of the ovary wall and basal petal of the pistil, and in the vascular tissues of the receptacle (Figure 3C). Strong GUS staining was detected in the vascular tissues of anthers (Figure 3D), and very weak staining was observed in pollen (Figure 3E). In agreement with the histochemical staining, GUS activity was low in pollen (Figure 4). As in vegetative tissues, the pattern of expression in pistils, anthers, and pollen observed with SS-III-0.32/N was very similar to that seen with SS-III-3.9/3'; although the level of expression was reduced (Figures 3H to 3J and Figure 4).

Removing the leader intron from SS-III-3.9/3' or SS-III-0.32/N did not significantly affect GUS expression in the pistil (data not shown) but, surprisingly, had a major effect in anthers and pollen. Strong GUS staining observed in vascular tissues of anthers was no longer observed in plants harboring either construct lacking the leader intron (Figures 3K and 3M). Furthermore, very strong GUS staining was observed in pollen from plants containing either SS-III-3.9AL1/3' or SS-III-0.32AL1/N (Figures 3L and 3N). In agreement with the histochemical assays, GUS activity detected in pollen from plants containing either SS-III-3.9AL1/3' or SS-III-0.32AL1/N was ~160- or 100-fold higher, respectively, than the activity detected in plants containing SS-III-3.9/3' or SS-III-0.32/N (Figure 4).

**Effects of the Sus3 Gene Leader Intron in Tobacco Flowers Are Developmentally Regulated**

To determine whether the Sus3 leader intron in tobacco might be involved in developmental control, flowers from plants containing either SS-III-3.9/3' or SS-III-3.9AL1/3' were examined at stages 1 to 12, as defined by Koltunow et al. (1990). GUS staining was observed in the vascular tissues of anthers at stages 1 and 2 with both constructs (Figures 3O and 3P). With construct SS-III-3.9AL1/3'; GUS staining started to decrease at approximately stage 8 (data not shown) and disappeared during later stages (Figure 3K). With construct SS-III-3.9/3', strong GUS staining was still detectable even in later stages (Figure 3D). GUS activity was also detected in the central cluster cells at early stages, approximately stage 1 or 2, with plants containing either construct (Figures 3O and 3P). In pollen from plants containing SS-III-3.9AL1/3'; GUS activity was not visible before stage 6 (Figure 3Q) but became visible at approximately stage 7 (Figure 3R), reaching the highest level at the day of anthesis (Figure 3L). A very similar temporal expression pattern was observed in anther vascular tissues and pollen from plants harboring SS-III-0.32AL1/N (data not shown).

**DISCUSSION**

A long 5’ leader intron is a characteristic of sucrose synthase genes (Werr et al., 1985; Chopra et al., 1992; Wang et al., 1992;
Yu et al., 1992; Shaw et al., 1994; Fu and Park, 1995). It is present in all of the sucrose synthase genes isolated thus far, except for asus1 from Arabidopsis (Martin et al., 1993). The leader intron from the Shrunken gene of maize has been shown to increase expression of a heterologous promoter in transient assays up to 100-fold (Vasil et al., 1990; Maas et al., 1991; Clancy et al., 1994). Our experiments with potato sucrose synthase genes have shown that removal of the leader intron also changes both the level and pattern of expression of sucrose synthase constructs in transgenic plants. Removing the leader intron from GUS constructs derived from the sus4 gene causes a marked reduction in expression in tubers and sucrose inducibility and also changes the pattern of expression in vegetative tissues of transgenic potato plants (Fu et al., 1995). Removing the leader intron from GUS constructs derived from the sus3 gene does not have a significant effect on expression in vegetative organs but results in both a decrease in expression in the vascular tissues of tobacco anthers and a dramatic increase in expression in pollen at later stages of development. Our data demonstrate the involvement of a plant intron in both positive and negative regulation and support a functional role for the conserved, long leader introns in sucrose synthase genes.

Involving the leader intron in regulation may be achieved by cis elements present in the leader intron. There are numerous examples of genes regulated at the transcriptional level by intronic enhancer sequences, including the mouse a2 (type I) collagen gene (Rossi and De Crombrugghe, 1987), the quail tropolin I gene (Konieczny and Emerson, 1987), the human keratin 18 gene (Oshima et al., 1990), the immunoglobulin heavy-chain gene (Gillies et al., 1983), and the chicken a1 crystallin gene (Goto et al., 1990). Alternatively, RNA processing may be a critical aspect of this regulation. More specifically, the splicing of the leader intron may be both positively and negatively regulated in a tissue-specific and temporal manner. Several examples of such on/off regulation mediated by splicing are known in Drosophila, including the P element (Rico, 1991), the suppressor-of-white-apricot locus (Bingham et al., 1988), and the transformer locus (Boggs et al., 1987). In the case of the P element, expression of the transposase, encoded by the element itself, is turned on or off in a tissue-specific fashion by regulating third intron splicing. Expression of the functional transformer gene product required for sex determination is regulated by selecting alternative 3' splicing sites. In plants, the possible involvement of RNA processing in the regulation of gene expression has been implied in many cases, including the maize genes Shrunken1 and Alcohol dehydrogenase1 and the rice actin gene (Callis et al., 1987; McElroy et al., 1990; Vasil et al., 1990; Maas et al., 1991; Clancy et al., 1994); however, the specific mechanisms involved are not yet known. Two single-base differences were introduced into the leader intron–deleted constructs in the process of deleting the leader intron. Because the constructs bypass splicing of the leader intron, these differences should not have affected RNA processing. However, the possibility of some other effect on expression of the constructs cannot be ruled out.

Removing Sus3 3' sequences from Sus3 constructs containing the full-length promoter and leader intron did not have the dramatic effect on expression seen with the Sus4 constructs. However, in the context of only 320 bp of 5' flanking sequence, replacement of the native 3' sequences with the NOS-ter caused a dramatic increase in expression in leaves, stems, roots, and tubers. This indicates that, as in the Sus4 gene, the combined functions of Sus3 3' and 5' elements are critical in regulating expression. Requirement of such combined functions in both classes of potato sucrose synthase genes is perhaps not surprising, because some of the 3' and 5' flanking sequences are conserved between Sus3 and Sus4 genes (Fu and Park, 1995). However, although replacement of the Sus3 3' sequences with the NOS-ter caused a marked increase in expression in the context of only proximal 5' sequence elements, replacement of the Sus4 3' sequences with NOS-ter caused a decrease in expression. Thus, although the presence of conserved sequences in flanking regions of the Sus3 and Sus4 potato sucrose synthase genes suggests conservation of regulatory mechanisms, at least some of them are distinct. The effect of 3' sequences could possibly be exerted by effects on transcription, RNA processing, or RNA stability. However, because this effect was not observed in the context of the full-length promoter, effects on RNA stability appear less likely.

Despite the important role of both 3' sequences and the leader intron in various contexts, 3.9 kb of 5' flanking sequence from the Sus3 gene was sufficient to confer essentially the same pattern of expression seen in the full-length construct in the absence of both the leader intron and 3' sequences. Also, in the absence of both the leader intron and 3' sequences, expression in the vascular tissues of vegetative organs and in tobacco pistils in the construct containing only 320 bp of 5' flanking sequence was also similar to that seen with the full-length construct. However, expression in the cell division zone of roots and in nonvascular tissues of leaves and tubers was lost. This indicates that the proximal 5' flanking sequence contains cis elements involved in expression in vascular tissues but that cis elements directing expression in the cell division zone of roots and in nonvascular tuber tissues are located farther upstream. It also indicates that all the sequences necessary to mediate both the positive and negative effects of the Sus3 leader intron in tobacco flowers are located between positions -320 and +1648 (8 bp downstream of the ATG in the exon 2).

METHODS

Plant Materials

Wild-type and transgenic potatoes (Solanum tuberosum cv FL1607) and tobacco (Nicotiana tabacum cv Xanthi) were maintained in tissue culture under a 16-hr-light/8-hr-dark regime at 25°C on Murashige and Skoog medium (Murashige and Skoog, 1962) containing 2% (w/v) sucrose and 0.8% agar. To examine expression in flowers, tobacco
plants were grown in soil in a growth chamber under a 16-hr-light/8-hr-dark cycle.

Chimeric β-Glucuronidase Gene Constructs

To prepare SS-III-3.9/3' and SS-III-0.32/3', a genomic fragment of the S. tuberosum sucrose synthase Sus3 class Sus3-65 gene from either position -3900 or -322 to 8 bp downstream of the start codon (+1648) was cloned into pBluescript KS+ and fused in frame to the 5' end of the β-glucuronidase (GUS) coding region of pB101.2 (Jefferson et al., 1987), using either SalI-BamHI or HindIII-BamHI sites. The junction sequence between the potato Sus3-65 and GUS is shown in Figure 1B. Construct SS-III-0.32/3' was prepared in the same way as SS-III-0.32/N, except that pB101.2-III-ter was used instead of pB101.2. As described by Fu and Park (1995), the pB101.2-III-ter is derived from pB101.2 in which the 3' sequence of the nopaline synthase (NOS) gene was replaced by the native 3' sequence of the S. tuberosum Sus3 sucrose synthase gene Sus3-65.

The leader intron-deleted constructs SS-III-3.9ALI/3' and SS-III-0.32ALI/3' were prepared in several steps. First, to delete the leader intron, two polymerase chain reaction–amplified products were joined. One contained the leader exon (from position -322 to the 3' end of the leader exon), and the other contained part of the exon 2 (from 5' end of the exon 2 to 8 bp downstream of the start codon, XmnI site). This removed the leader exon cleanly. Two bases at the 3' end of the leader exon were changed as a result of introducing a PstI site to join the two fragments (Figure 1C). Second, to give SS-III-0.32ALI/3', the leader intron-deleted proximal promoter fragment was attached in frame to the 5' end of the GUS coding region in pB101.2-III-ter. Third, to construct SS-III-3.9ALI/3', a sequence farther upstream (-3900 to -322) of Sus3-65 was attached to the leader intron-deleted proximal promoter fragment, which was cloned into pBluescript and then attached in frame to the 5' end of the GUS coding region in pB101.2-III-ter. To prepare constructs SS-III-0.32ALI/N and SS-III-3.9ALI/N, these leader intron-deleted, proximal, and full-length promoter fragments were attached individually in frame to the 5' end of the GUS coding region in pB101.2.

Potato and Tobacco Transformation and Analysis of GUS Expression in Transgenic Plants

Transformation of Agrobacterium tumefaciens LBA4404 and potato were performed as described by Fu and Park (1995). Transformation of tobacco was performed according to Horsch et al. (1985). Fluorometric assays were performed as described by Jefferson (1987), using 4-methylumbelliferyl β-D-glucuronide as substrate. Preparation of crude extract and sampling of axenically grown plant materials of both potato and tobacco were performed as described by Fu and Park (1995). Tobacco pollen was collected by washing three to six anthers from each plant in GUS extraction buffer. The anthers were discarded after washing. Plant materials were placed in 2 mM of X-gluc, vacuum infiltrated for 1 min, and incubated overnight at 37°C in the dark. Photographs were taken with either an Olympus (Tokyo, Japan) dissection microscope or an Olympus IMT-2 inverted research microscope.

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