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High-Level Tuber Expression and Sucrose Inducibility of a Potato *Sus4* Sucrose Synthase Gene Require 5′ and 3′ Flanking Sequences and the Leader Intron

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The 3.6 kb of 5′ flanking sequence, leader intron, and 0.7 kb of 3′ sequence from the potato sucrose synthase gene *Sus4*-16 are sufficient to direct high-level expression in developing tubers, in basal tissues of axillary buds and shoots, and in meristems and caps of roots, and to confer sucrose inducibility in leaves. By examining a series of deletion and substitution constructs in transgenic potato plants, we found that this pattern of expression requires 5′ flanking sequences both upstream and downstream of position −1500 and that sequences between positions −1500 and −267 are essential for sucrose induction. Replacement of the native 3′ sequence with the nopaline synthase 3′ sequence resulted in the loss of sucrose inducibility and of expression in basal tissues of axillary buds. A general decrease in expression in other tissues was also observed. Removal of the 1612-bp leader intron also had a dramatic effect on both the pattern and level of expression.

INTRODUCTION

Sucrose synthase (EC 2.4.1.13) catalyzes the reversible conversion of sucrose and UDP into UDP-glucose and fructose (Avigad, 1982). It is the predominant sucrose cleavage enzyme in potato tubers (Sung et al., 1989) and also plays a key role in providing substrates for respiration and metabolism in a variety of other tissues (e.g., Chourey and Nelson, 1976; Springer et al., 1986; Hendrix, 1990; Maas et al., 1990; Geigenberger et al., 1993; Martin et al., 1993; Nolte and Koch, 1993). Potato sucrose synthase cDNA was first isolated by Salanoubat and Belliard (1987). These investigators showed that potato sucrose synthase mRNA is most abundant in developing tubers and subsequently showed that the steady state level of sucrose synthase mRNA is dependent on wounding, anaerobiosis, and sucrose concentration (Salanoubat and Belliard, 1989).

Recently, two differentially regulated classes of potato sucrose synthase genes, *Sus3* and *Sus4*, have been isolated (Fu and Park, 1995). The coding regions of both classes of potato sucrose synthase genes are interrupted by 12 introns, and, like most other sucrose synthase genes that have been examined in other species (Werr et al., 1985; Chopra et al., 1992; Wang et al., 1992; Yu et al., 1992; Shaw et al., 1994), both classes of genes also contain a long leader intron. The coding regions of the two classes of potato sucrose synthase genes are 87% homologous. However, other than a few blocks of sequence in the 5′ and 3′ flanking regions, the two classes of genes are not significantly homologous outside the coding region. The *Sus4* class genes correspond to the cDNA isolated by Salanoubat and Belliard (1987). When examined in transgenic potato plants, these genes are expressed at the highest levels in developing tubers, in the basal tissues of axillary buds and shoots, and in the root cap and meristem. In contrast, the *Sus3* class genes are expressed at higher levels in roots and stems. Expression of a *Sus3* potato sucrose synthase gene in transgenic potato plants is associated with vascular tissues of all organs that have been examined, suggesting a role in supplying energy for phloem loading. *Sus4* genes are not normally expressed in leaves, but their expression can be induced by high levels of sucrose. In contrast, *Sus3* genes are normally expressed at low levels in leaves but are not sucrose inducible.

Expression in transgenic potato plants of a β-glucuronidase (*GUS*) construct, SS-IV-3.6/3′, which is based on the *Sus4* class gene *Sus4*-16, was in good agreement with that predicted from RNA gel blot analysis, suggesting that most critical sequences were included in this construct (Fu and Park, 1995). The *GUS* gene in this construct is driven by 3.6 kb of 5′ flanking region, the leader intron, and 0.7 kb of 3′ sequence from the *Sus4* gene. By examining a series of deletion and substitution constructs in transgenic potato plants, the leader intron and combined functions of the 5′ flanking and 3′ sequences were all shown to be essential for high-level expression in tubers, sucrose-inducible expression in leaves, and other aspects of proper *Sus4* gene expression.

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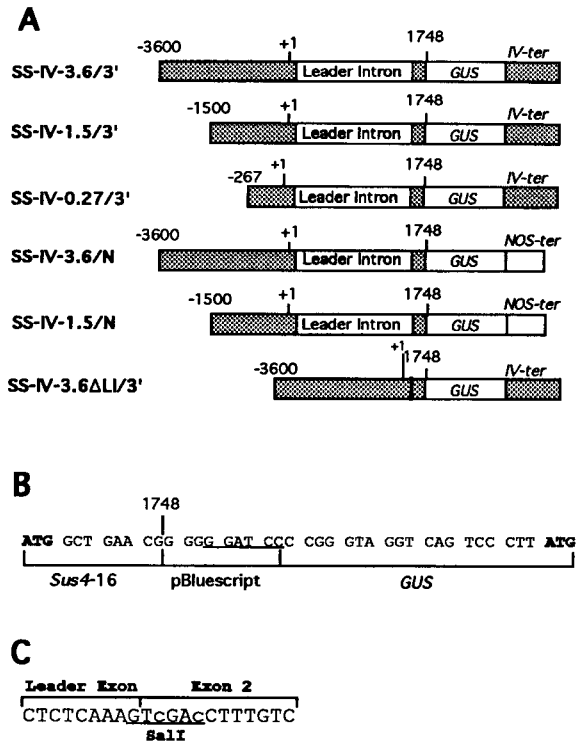

Figure 1. Schematic Diagram of Chimeric *Sus4–GUS* Constructs.

(A) Constructs. The numbers indicate nucleotide positions relative to the transcription start site of *Sus4*-16. *IV-ter* indicates the 3′ sequence of *Sus4*-16; *NOS-ter*, the 3′ sequence of *NOS*.

(B) The junction sequence between the *Sus4* gene and *GUS*. The sequence derived from pBluescript SK+ is also shown. The underlined sequence is the BamHI site used to join the potato *Sus4* sucrose synthase gene and *GUS*. The start codons of the potato sucrose synthase gene and *GUS* are indicated by boldface letters.

(C) The junction sequence between the leader exon and the exon 2 of *Sus4*-16 in SS-IV-3.6ΔLI/3′ is shown. Lowercase letters indicate bases altered as a result of introducing the SalI site (underlined).

RESULTS

The 1.5 kb of 5′ Flanking Sequence Is Not Sufficient for High-Level Expression in Tubers or for Sucrose Inducibility

To dissect the regulatory sequences required for proper expression of the potato *Sus4* class sucrose synthase gene, *Sus4*-16, we first compared SS-IV-3.6/3′, which contains 3.6 kb of 5′ flanking sequence, including the leader intron and 0.7 kb of 3′ untranslated and flanking sequence, with construct SS-IV-1.5/N (Figure 1A). This construct contains only 1.5 kb of the 5′ flanking sequence, including the leader intron, and the 3′ sequence of the nopaline synthase (*NOS*) gene (designated here as *NOS-ter* for *NOS* terminator sequence; Jefferson et al., 1987) rather than the native 3′ sequence of the *Sus4* class gene *Sus4*-16. As shown in Table 1, these changes led to a 60-fold reduction in expression in tubers, a 15-fold reduction in stems, and three- and sixfold reductions in leaves and roots, respectively. The GUS staining pattern in root vascular tissues observed with SS-IV-1.5/N was generally similar to that seen with SS-IV-3.6/3′, but staining in the root cap and root apical meristem could not be observed (Figures 2A and 2B). Also, in tubers, *GUS* expression was restricted to vascular tissues. In addition, GUS activity in leaves remained at background levels after incubation in Murashige and Skoog (MS; Murashige and Skoog, 1962) basal medium supplemented with 0.25 M of sucrose, in contrast with the 16-fold sucrose induction seen with SS-IV-3.6/3′ (Figure 3).

Sequences between Positions −3600 and −1500 Are Required for Expression in Root Tips and in Storage Parenchyma of Tubers

To examine the basis for the differences in expression seen with SS-IV-1.5/N as compared with SS-IV-3.6/3′, we next examined construct SS-IV-3.6/N (Figure 1A). This construct contains

Table 1. GUS Activity in Different Organs from Transgenic Potato Plants Containing Various *Sus4–GUS* Constructs

Construct	*n*[a]	Leaves	Stems	Roots	Microtubers
SS-IV-3.6/3′	8	27 ± 6[b]	616 ± 219	9,081 ± 1,633	58,186 ± 4,013
SS-IV-1.5/3′	11	54 ± 12	4,677 ± 2,034	5,178 ± 1,366	7,812 ± 2,246
SS-IV-0.27/3′	9	16 ± 7	241 ± 187	437 ± 167	759 ± 251
SS-IV-3.6/N	13	6 ± 1	14 ± 1	1,102 ± 412	8,119 ± 2,197
SS-IV-1.5/N	5	8 ± 1	40 ± 13	1,640 ± 1,179	993 ± 475
SS-IV-3.6ΔLI/3′	3	64 ± 55	3,280 ± 3,241	2,159 ± 315	7,215 ± 950
PS20A-G[c]	3	7 ± 2	16 ± 2	39 ± 14	23,376 ± 929
Wild type	9	6 ± 0	12 ± 1	25 ± 4	2 ± 0

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

[a] Number of independent transgenic plants analyzed.

[b] Mean ± SE.

[c] Plants containing a class I patatin gene construct (Wenzler et al., 1989) were included in the assay as a control.

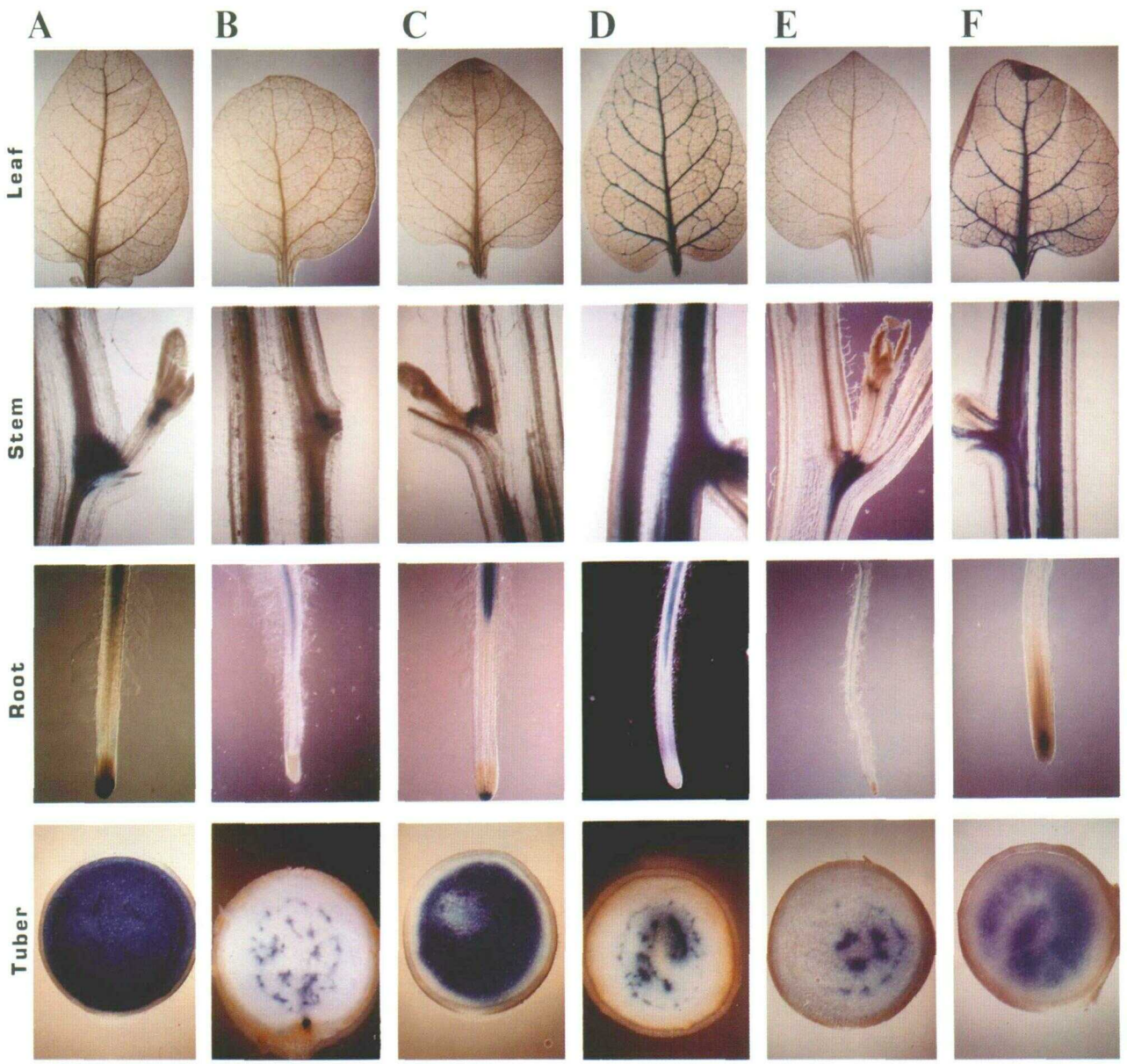

Figure 2. Histochemical Staining of GUS Activity in Different Organs of Transgenic Potato Plants Containing Various *Sus4–GUS* Constructs.

GUS staining patterns of different organs of transgenic potato plants containing various *Sus4* constructs are shown.
(A) SS-IV-3.6/3′.
(B) SS-IV-1.5/N.
(C) SS-IV-3.6/N.
(D) SS-IV-1.5/3′.
(E) SS-IV-0.27/3′.
(F) SS-IV-3.6ΔLI/3′.

the entire 3.6 kb of the 5′ flanking region and the leader intron; however, as in SS-IV-1.5/N, the native 3′ sequences are replaced by the *NOS-ter.* Adding more 5′ flanking sequence did not appear to significantly change the level of GUS activity in leaves, stems, and roots, but it increased expression in tubers approximately eightfold when compared with SS-IV-1.5/N (Table 1). However, relatively large variation in *GUS* expression was observed in stems and roots (as shown by the large standard

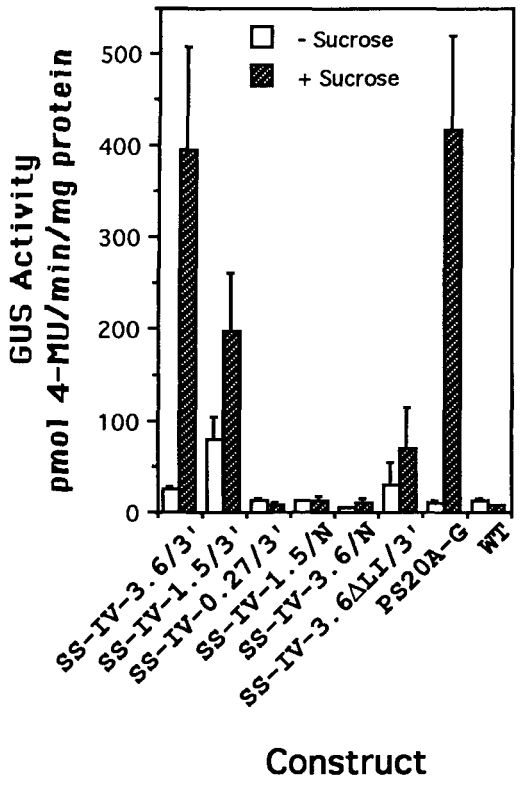

Figure 3. Effect of Sucrose on GUS Activity in Leaves of Transgenic Potato Plants Containing Various Constructs.

The histograms show average GUS activity (in picomoles of 4-methylumbelliferone [MU] per minute per milligram of protein) after incubation in either 0 (−) or 250 (+) mM of sucrose. Bars are standard errors. Eight, eight, nine, six, 13, three, and four independent plants were analyzed for constructs SS-IV-3.6/3′, SS-IV-1.5/3′, SS-IV-0.27/3′, SS-IV-1.5/N, SS-IV-3.6/N, SS-IV-3.6ΔLI/3′, and the wild type (WT), respectively. As a positive control, GUS activities in leaves from potato plants containing class I patatin–*GUS* constructs (PS20A-G; Wenzler et al., 1989) were also measured.

errors). Thus, the possibility of small but significant quantitative differences in *GUS* expression in stems and roots between SS-IV-1.5/N–containing and SS-IV-3.6/N–containing plants cannot be ruled out.

Although the overall level of *GUS* expression in roots did not appear to increase significantly in SS-IV-3.6/N relative to SS-IV-1.5/N, the characteristic *Sus4* gene staining pattern in the root apical meristem and root cap was restored by adding more 5′ flanking sequences (Figure 2C). The GUS staining in tubers was also no longer restricted to the vascular tissue with SS-IV-3.6/N as it was with SS-IV-1.5/N. However, the level of GUS activity in tubers with SS-IV-3.6/N was still eightfold lower than the level in tubers with SS-IV-3.6/3′, and the pattern of GUS staining was often not evenly distributed across the tuber. Even with 3.6 kb of 5′ flanking sequence, characteristic *Sus4* gene expression in the basal tissue of axillary buds was not seen in the absence of the *Sus4* 3′ sequences, and GUS activity in leaves was still not sucrose inducible (Figure 3).

The 3′ Sequence Is Required for Sucrose Inducibility and Causes Ectopic Expression in the Absence of Distal 5′ Sequences

Replacing the *NOS-ter* in SS-IV-1.5/N with native 3′ sequences of the *Sus4* gene in construct SS-IV-1.5/3′ (Figure 1A) led to an eightfold increase in GUS activity in tubers and a threefold increase in roots (Table 1). However, GUS activity was still two- and eightfold lower, respectively, in these organs when compared with plants containing SS-IV-3.6/3′. Also, similar to SS-IV-1.5/N, *GUS* expression was mostly restricted to the vascular tissues in roots and tubers of plants containing SS-IV-1.5/3′, and expression was not seen in root caps and root apical meristems (Figure 2D).

Replacing the *NOS-ter* in SS-IV-1.5/N with native *Sus4* 3′ sequences in construct SS-IV-1.5/3′ also led to a sevenfold increase in expression in leaves and a 120-fold increase in activity in stems (Table 1). GUS activity in these organs was two- and eightfold higher, respectively, than in plants containing SS-IV-3.6/3′. Unlike the "full-length" construct SS-IV-3.6/3′ in which GUS staining is restricted to the basal tissue of axillary buds and generally cannot be detected in leaves, with SS-IV-1.5/3′, GUS staining was observed throughout the vascular tissue of stems and also in leaves (Figure 2D). In leaves, GUS staining was detected in primary and secondary veins and, although weak, in guard cells as well (data not shown).

Unlike *GUS* expression in plants containing either construct lacking *Sus4* 3′ sequences, *GUS* expression in leaves of plants containing SS-IV-1.5/3′ was sucrose inducible (Figure 3). However, the relative induction was 2.4-fold rather than the 16-fold, as observed with plants containing SS-IV-3.6/3′. The reduced ratio was the result of both increased *GUS* expression in uninduced leaves and a twofold decrease in the level of *GUS* expression in the presence of 0.25 M of sucrose.

Sequences Necessary for Sucrose Induction Are Present between Positions −1500 and −267

To determine whether the *Sus4* 3′ sequences are sufficient for sucrose induction and other aspects of the expression pattern seen with SS-IV-1.5/3′ or whether the 5′ sequences were also required, a deletion was made between positions −1500 and −267 of SS-IV-1.5/3′ to give SS-IV-0.27/3′ (Figure 1A). Because expression in leaves either in the absence or presence of 0.25 M of sucrose did not rise above background levels with SS-IV-0.27/3′, the *Sus4* 3′ flanking sequences were clearly not sufficient for sucrose induction (Figure 3). Removal of the sequences between positions −1500 and −267 also caused a 20-fold decrease in GUS activity in stems, a 12-fold decrease in roots, a 10-fold decrease in tubers, and a threefold decrease in leaves (Table 1) and loss of the ectopic expression observed

in leaves and stems with the construct SS-IV-1.5/3′ (Figure 2E). Whether the expression seen with SS-IV-0.27/3′ is dependent on the presence of *Sus4* 3′ sequences is not yet known. However, 3′ sequences of the *Sus4* gene are likely to be required for expression in basal vascular tissues of axillary buds, based on the lack of staining of this tissue in plants containing either SS-IV-3.6/N or SS-IV-1.5/N.

The Leader Intron Is Required for Proper Expression

The presence of a long leader intron is a conserved structural feature of sucrose synthase genes (Werr et al., 1985; Chopra et al., 1992; Yu et al., 1992; Shaw et al., 1994; Fu and Park, 1995), indicating a possible regulatory role. When the leader intron was removed from construct SS-IV-3.6/3′ to give construct SS-IV-3.6ΔLI/3′ (Figure 1A), several differences in expression were observed. The level of expression in tubers was reduced eightfold (Table 1). Also, the overall level of expression in roots decreased fourfold (Table 1), and the location of expression in root tips changed from the root cap and apical meristem to the procambium (Figure 2F). Although two of the three transformants containing SS-IV-3.6ΔLI/3′ had low GUS activity in leaves and stems, the remaining plant showed high GUS activity in these organs. Histochemical staining of this plant showed ectopic expression in the vascular tissue of leaves and stems similar to that seen in plants containing SS-IV-1.5/3′ (Figures 2D and 2F). When leaves from any of the SS-IV-3.6ΔLI/3′ plants were incubated with 0.25 M of sucrose, there was only a modest level of induction (Figure 3).

DISCUSSION

For many plant genes, 1 kb of 5′ sequence is sufficient to confer the appropriate pattern of expression in transgenic plants. However, proper expression of the potato *Sus4* sucrose synthase genes appears to require the 5′ flanking sequences both proximal and distal of position −1500, the leader intron, and 3′ sequences.

That 3′ sequences are essential for proper expression has also been shown for the potato proteinase inhibitor II (*PI-II*) gene (Thornburg et al., 1987), petunia ribulose bisphosphate carboxylase small subunit gene (Dean et al., 1989), oilseed rape *AX92* (Dietrich et al., 1992), and Arabidopsis *GLABROUS1* (Larkin et al., 1993). However, the potato *Sus4* sucrose synthase gene provides a particularly clear example of how combined functions of the 5′ and 3′ sequences are required for the proper gene expression. For example, replacing the *Sus4* 3′ sequences of SS-IV-3.6/3′ with the *NOS-ter* in SS-IV-3.6/N led to an eightfold decrease in expression in tubers but not to a dramatic change in the pattern of expression in tubers. Removal of the sequences upstream of position −1500 in construct SS-IV-1.5/3′ also led to an eightfold reduction in expression in tubers and caused most of the expression to become localized to vascular tissue. Removal of the sequences upstream of −1500 and replacing the *Sus4* 3′ sequences with the *NOS-ter* led to a 60-fold decrease in expression in tubers and to strict vascular localization of the remaining activity.

The dramatic decrease in expression seen in stems when *Sus4* 3′ sequences were replaced with the *NOS-ter* in SS-IV-3.6/N and SS-IV-1.5/N clearly shows that 3′ sequences play a key role in conferring the proper pattern of expression in stems. However, 5′ sequences also appear to play a key role. Removal of the sequences upstream of position −1500 from a construct containing *Sus4* sucrose synthase 3′ sequences (Figure 1A, construct SS-IV-1.5/3′) resulted in high levels of ectopic expression throughout the vascular tissue of the stems as well as in leaves. Deletion of additional sequences upstream of −267 in construct SS-IV-0.27/3′ resulted in a pattern of expression in stems and leaves that was again similar to that seen in SS-IV-3.6/3′. This suggests that the 5′ sequences upstream of −1500 contain a negative element that suppresses the *Sus4* 3′ sequence-enhanced expression in stems and leaves to give the proper tissue specificity and level of expression. The degree to which expression seen in stems and other tissues in SS-IV-0.27/3′ reflects a basal activity of proximal 5′ sequences rather than that of sequences farther downstream is not known. However, 3′ flanking sequences are likely to be required for the observed expression in the basal vascular tissue of axillary buds, based on the lack of expression in this tissue in both SS-IV-3.6/N–containing and SS-IV-1.5/N–containing plants.

Finally, both the 3′ and 5′ sequences between positions −1500 and −267 are required for sucrose-inducible expression in detached leaves. Interestingly, the sequence between −1500 and −267 contains an 18-bp sequence element (Figure 4) that, other than a single base pair insertion, is identical with the 3′ end of the 37-bp repeat of the class I patatin promoter (Mignery et al., 1988). This conserved sequence contains the sucrose-responsive element (SURE1) identified with the potato class I patatin promoter (Grierson et al., 1994). This sequence has been recognized as a conserved motif among a number of sucrose-responsive genes (Ishiguro and Nakamura, 1992; Kim et al., 1994). Interestingly, sequences highly homologous with SURE1 are also present in other sucrose-inducible sucrose synthase genes, such as Arabidopsis *Asus1* (position −901 to −891; Martin et al., 1993), maize *Sus1* (−159 to −151; Shaw et al., 1994), and rice *Sus1* (−826 to −817, −475 to −466, and −163 to −154; Yu et al., 1992). Also, the 3′ sequence contains a sequence element, located in a previously identified repeat region (3′B; Fu and Park, 1995), that, when in reverse orientation, is highly homologous with a sequence in the same repeat region of the class I patatin promoter (Figure 4). This conserved sequence contains another sucrose-responsive element, SURE2, identified with the potato class I patatin promoter (Grierson et al., 1994). Whether these conserved sequences are involved in sucrose induction of the *Sus4* gene as they exist in other sucrose-inducible genes remains to be determined.

It is not clear whether involvement of 3′ sequences in sucrose induction is peculiar to the potato *Sus4* sucrose synthase

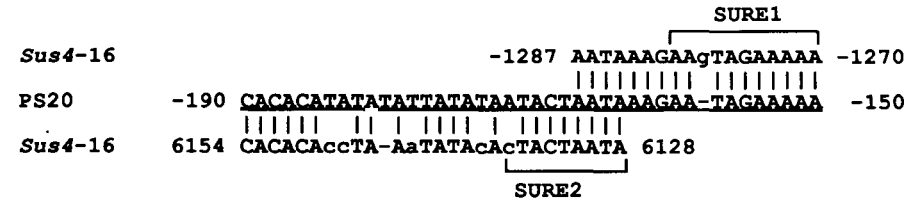

Figure 4. Sequence Comparison between the *Sus4* Sucrose Synthase Gene *Sus4*-16 and the Class I Patatin Gene PS20.

An 18-bp sequence in the 5′ flanking region of *Sus4*-16 (top) is highly homologous with the 3′ end of the 37-bp repeat of the class I patatin promoter (PS20, middle; Mignery et al., 1988). Also, a sequence (bottom) in the antisense strand of the 3′ repeat region in *Sus4*-16 (3′B; Fu and Park, 1995) is highly homologous with the same patatin promoter repeat region. Uppercase letters of *Sus4*-16 shown with vertical bars are bases matching PS20, and lowercase letters are mismatched bases. Gaps are indicated by dashes. SURE1 and SURE2 are indicated. The sequences of part of the 33-bp (left) and 37-bp (right) PS20 repeat are underlined.

gene. In the patatin and sporamin genes, 5′ flanking sequence alone appears to be sufficient for sucrose induction (Wenzler et al., 1989; Ohta et al., 1991). The sucrose-responsive sequence identified in the potato *PI-II* gene was also found in the 5′ flanking sequence (Kim et al., 1991). However, of the *PI-II* constructs tested, all contained *PI-II* 3′ sequences. Whether these sequences were necessary for the observed sucrose induction was not directly tested.

In addition to the 5′ and 3′ sequences, the leader intron of the *Sus4* gene is also required for proper expression in transgenic potato plants. Removal of the leader intron resulted in an eightfold reduction of expression in tubers, a reduction in sucrose inducibility, and in some cases, in ectopic expression in the vascular tissue of stems and leaves. Two single base differences were introduced into SS-IV-3.6ΔLI/3′ in the process of deleting the leader intron. Because this construct bypasses splicing of the leader intron, these differences should not have affected RNA processing. However, the possibility of some other effect on expression of the construct cannot be ruled out.

That the leader intron should be functionally important is not surprising. A leader intron >1 kb in length has been found in all of the sucrose synthase genes isolated thus far except for *Asus1* from Arabidopsis (Martin et al., 1993). There have also been several examples of regulation of gene expression by an intron (Callis et al., 1987; McElroy et al., 1990), including reports that the maize *Shrunken1* sucrose synthase leader intron can increase the reporter gene expression in transient assays up to 100-fold (Vasil et al., 1990; Maas et al., 1991; Clancy et al., 1994). Also, as shown in a companion paper (Fu et al., 1995), the potato *Sus3* sucrose synthase leader intron has both positive and negative tissue-specific effects in flowers.

The degree to which the leader intron is involved in interaction with regulatory elements in the 5′ and 3′ sequence is not yet known. However, the possible interaction between the 5′ sequence of −3600 to −1500 and the leader intron is suggested by the effect of deleting the leader intron or deleting the sequence of −3600 to −1500 on expression in root tips. The full-length construct SS-IV-3.6/3′ has a characteristic pattern of expression in the root apical meristem and root cap that is lost when the 5′ flanking sequences upstream of position −1500 are removed. In contrast, removing the leader intron from SS-IV-3.6/3′ resulted in a novel pattern of expression in the procambium of roots that differed from those patterns seen with any of the other constructs containing 3.6 kb of 5′ flanking sequence.

METHODS

Plant Materials

Potato plants (*Solanum tuberosum* cv FL1607), including transgenic plants, were grown axenically in Murashige and Skoog (MS) basal medium (Murashige and Skoog, 1962) supplemented with 2% sucrose and 0.8% agar at 25°C under a 16-hr photoperiod. Microtubers were induced by placing nodal cuttings in 0.5 × MS basal medium supplemented with 8% sucrose, 5 mg kinetin, and 5 mg ancymidol (Elanco, Indianapolis, IN) per liter of medium.

Sucrose Induction

Leaves were cut from tissue culture plantlets and were placed abaxial side up in 2 mL of either MS basal medium (control) or the same medium supplemented with 0.25 M sucrose in a 24-well microtiter plate. The leaves were incubated in the dark at 20°C for 5 days.

Construction of Chimeric Potato Sucrose Synthase *Sus4-GUS* Genes

To prepare SS-IV-3.6/N and SS-IV-1.5/N, a genomic DNA fragment of *Sus4*-16 either from position −3600 or −1500 to 8 bp downstream of the start codon (+1748) was cloned into pBluescript SK+ and fused in frame to the 5′ end of the β-glucuronidase (*GUS*) coding region of pBI101.2 (Jefferson et al., 1987) by using HindIII and BamHI sites. Construct SS-IV-1.5/3′ was prepared in the same way as SS-IV-1.5/N, except that pBI101.2-IV-ter was used. As described by Fu and Park (1995), the pBI101.2-IV-ter is derived from pBI101.2 in which the 3′ sequence of the nopaline synthase gene was replaced by the native 3′ sequence of the *S. tuberosum* sucrose synthase gene *Sus4*-16. To prepare construct SS-IV-0.27/3′, an ExoIII-deleted genomic DNA fragment from

position −267 to 8 bp downstream of start codon (+1748) was cloned into pBluescript SK+ and fused in frame to the *GUS* coding region of pBI101.2-IV-ter.

The leader intron-deleted construct SS-IV-3.6ΔLI/3′ was prepared in several steps. To delete the leader intron, two polymerase chain reaction–amplified fragments were joined. One contained the leader exon (from position −172 to the 3′ end of the leader exon), and the other contained part of the exon 2 (from the 5′ end of the exon 2 to 8 bp downstream of the start codon, the XmnI site). In the resulting fragment, the leader intron was cleanly removed, and two bases at the 5′ end of the exon 2 were changed as a result of introducing a SalI site to join the two fragments (Figure 1C). Farther upstream sequences from positions −3600 to −173 were introduced stepwise to the 5′ end of this fragment. The final intronless fragment was fused in frame to the 5′ of the *GUS* coding region in pBI101.2-IV-ter.

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

Transformation of *Agrobacterium tumefaciens* LBA4404 and potato was performed as described by Fu and Park (1995). Fluorometric assays were performed as described by Jefferson (1987) using 4-methylumbelliferyl β-D-glucuronide as substrate. Plant materials were ground in a 1.5-mL microcentrifuge tube using a Kontes pestle (Kontes Scientific Glassware/Instruments, Vineland, NJ) or with a mortar and pestle. Protein concentrations in the extracts were determined by the method of Bradford (1976); ~20 μg protein was used per assay. Histochemical assays of GUS activity were performed according to Jefferson (1987) using 5-bromo-4-chloro-3-indolyl β-D-glucuronide (X-gluc; Biosynth, Staad, Switzerland). Plant materials were placed in 2 mM X-gluc, vacuum infiltrated for 1 min, and incubated overnight at 37°C in the dark. After staining was completed, leaves and stem segments were cleared with ethanol at room temperature. Photographs were taken with an Olympus (Tokyo, Japan) dissection microscope. Figure 2 demonstrates the typical GUS staining pattern for plants containing the different constructs. While the pattern of expression for a given construct was generally consistent, the amount of expression varied. Thus, to better demonstrate qualitative differences in expression between constructs, in some cases data from plants with higher than usual levels of expression are shown.

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