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Both developmental and metabolic signals activate the promoter of a class I patatin gene

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Patatin is one of the major soluble proteins in potato tubers and is encoded by a multigene family. Based on structural considerations two classes of patatin genes are distinguished. The 5′-upstream regulatory region of a class I gene contained within a 1.5 kb sequence is essential and sufficient to direct a high level of tuber-specific gene activity which was on average 100- to 1000-fold higher in tubers as compared to leaf, stem and roots in greenhouse grown transgenic potato plants when fused to the β-glucuronidase reporter gene. Histochemical analysis revealed this activity to be present in parenchymatic tissue but not in the peripheral phellem cells of transgenic tubers. Furthermore the promoter fragment can be activated in leaves under conditions that simulate the need for the accumulation of starch in storage organs, i.e. high levels of sucrose. The expression is restricted to both mesophyll and epidermal cells in contrast to vascular tissue or hair cells.

Key words: patatin/potato/tuber-specific/β-glucuronidase/sucrose induction

Introduction

Potato tubers represent underground stems which have undergone a morphological differentiation due to a drastic radial expansion (Artschwager, 1924). Under field conditions, induction of this differentiation process is controlled by a number of environmental conditions such as photo-period, temperature and nitrogen supply (for summary see Ewing, 1985).

The morphological process of tuberization is accompanied by a variety of biochemical changes, the most dramatic ones being the accumulation of starch (which is typical for the storage function of the potato tuber) and the appearance of new proteins.

Up to 40% of the total soluble protein of potato tubers is represented by a family of immunologically identical glycoproteins with a mol. wt of ~40 kd which have been given the trivial name 'patatin' (Racusen and Foote, 1980; Park, 1983). The high amount of patatin in potato tubers argues for its function as a storage protein, however, unlike most other storage proteins, patatin also has an enzymatic activity (lipid acyl hydrolase) (Racusen, 1984, 1986; Rosahl *et al.*, 1987; Andrews *et al.*, 1988).

In potato plants grown under either greenhouse or field conditions expression of patatin is as a rule restricted to tubers and to stolons associated with growing tubers (Paiva *et al.*, 1983; Rosahl *et al.*, 1986a). In addition, patatin is also expressed in roots, albeit at a 100-fold lower level (Pikaard *et al.*, 1987). Although tuberization is always accompanied by patatin expression, there are several instances where patatin expression is observed in non-tuberizing tissues. Patatin is expressed in petioles and stems of potato plants induced for tuberization upon removal of tubers and stolons (Paiva *et al.*, 1983). In addition patatin accumulates to considerable levels in leaves of potato plantlets growing under axenic conditions on media supplied with high levels of sucrose (Rocha-Sosa *et al.*, submitted). In these cases therefore induction of patatin expression is independent from the morphological differentiation process of tuberization.

Patatin is encoded by a gene family of more than 10 members per haploid genome. Several cDNA and genomic clones have been isolated and the complete nucleotide sequence of both the promoter and the coding region has been determined for some of them (Bevan *et al.*, 1986; Rosahl *et al.*, 1986b; Pikaard *et al.*, 1986; Mignery *et al.*, 1988; Twell and Ooms, 1988). The protein coding regions and the promoter region up to position −87 of all genes analysed so far are very homologous, whereas upstream from this point the promoters diverge, allowing the genes to be divided into at least two classes. Apart from homologies reaching far into the 5′ direction class II genes are also characterized by the presence of a 22 bp sequence in the untranslated leader which is absent in class I genes. Most interestingly, however, class I and class II genes have a different expression pattern: class I genes are mainly expressed in tubers, whereas class II gene expression is found in both tubers and roots albeit at a much lower level as compared to class I genes (Pikaard *et al.*, 1987).

In order to understand the molecular mechanism underlying the complex control of patatin expression as well as the differential expression of the two classes of patatin genes, we have isolated several members of the patatin gene family representing class I and class II genes. In particular we want to determine whether specific *cis*-acting upstream regulatory elements are responsible for the differential expression of these genes.

Here we report results obtained with a chimeric gene composed of the upstream sequence of a class I patatin gene and the β-glucuronidase gene of *Escherichia coli* after *Agrobacterium* mediated transfer into potato plants.

Results

Isolation of class I patatin genes

A genomic library established in the λ-phage derived EMBL 4 vector was screened with a patatin cDNA pcT58 probe (Rosahl *et al.*, 1986b) and yielded 13 different

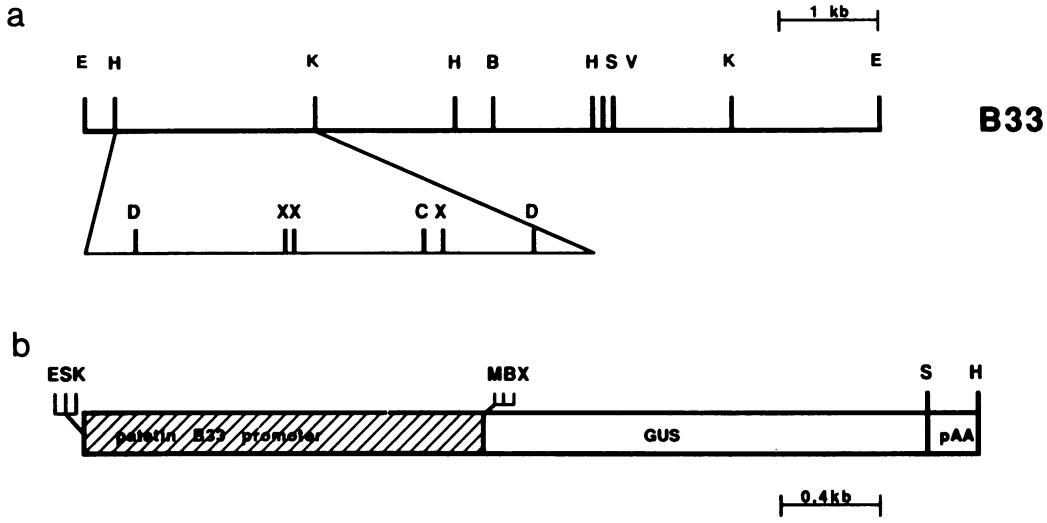

Fig. 1. Restriction map of the genomic clone encoding the class I patatin gene B33 (**a**) and structure of the chimeric gene composed of the promoter region of the B33 gene, the coding sequence of the β-glucuronidase gene (*gus*) and the 3'-terminal region of the nopaline synthase gene (pAA) (**b**). Abbreviations used: E = *Eco*RI, H = *Hin*dIII, K = *Kpn*I, B = *Bam*HI, S = *Sst*I, V = *Eco*RV, M = *Sma*I, X = *Xba*I, C = *Cla*I, D = *Dra*I.

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        AAGCTTATGTTGCCATATAGAGTAGTTTGTGATGGTATACTTCATAAACTTTAACTTATGTTAAATTTGTAATGATAAAATTTTTATTGTAAATTAAAA
        ATTACTTATAAAATTGGGCATTATAACATATGAAAGACAAATTGTGTTACATATTTTACTTTTGACTTTAATATGAATATTTCAATTTAAATCATTGTTT
-1500  TATTTTCTCTTTCTTTTTACAGGTATAAAAGGTGAAAATTGAAGCAAGATTGATTGCAAGCTATGTGTCACCACGTTATTGATACTTTGGAAGAAATTTT
        TACTTATATGTCTTTGTTTAGGAGTAATATTTGATATGTTTTAGTTAGATTTTCTTGTCATTTATGCTTTAGTATAATTTTAGTTATTTTTATTATATGA
        TCATGGGTGAATTTTGATACAAATATTTTTGTCATTAAATAAATTAATTTATCACAACTTGATTACTTTCAGTGACAAAAAATGTATTGTCGTAGTACCC
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-1000  TTTTTTATATATAAAAAATAGTCTTTAGTGACGATCGTAGTGTTGAGTCTAGAAATCATAATGTTGAATCTAGAAAAATCTCATGCAGTGTAAAATAAAC
        CTCAAAAAGGACGTTCAGTCCATAGAGGGGGTGTATGTGACACCCCAACCTCAGCAAAAGAAAACCTCCCTTCAACAAGGACATTTGCGGTGCTAAACAA
                                                                    lr1
        TTTCAAGTCTCATCACACATATATTTATTATATAATACTAATAAAGAATAGAAAAGGAAAGGTAAACATCATTAAATCGTCTTTGTATATTTTTAGTGAC
                                                  cm1
        AACTGATTGACGAAATCTTTTTCGTCACACAAAATTTTTAGTGACGAAACATGATTTATAGATGATGAAATTATTTGTCCCTCATAATCTAATTTGTTGT
        AGTGATCATTACTCCTTTGTTTGTTTTATTTGTCATGTTAGTCCATTAAAAAAAAATATCTCTCTTCTTATGTACGTGAATGGTTGGAACGGATCTATTA
- 500  TATAATACTAATAAAGAATAGAAAAAGGAAAGTGAGTGAGGTTCGAGGGAGAGAATCTGTTTAATATCAGAGTCGATCATGTGTCAATTTTATCGATATG
            cm2
        ACCCTAACTTCAACTGAGTTTAACCAATTCCGATAAGGCGAGAAATATCATAGTATTGAGTCTAGAAAAATCTCATGTAGTGTGGGGTAAACCTCAGCAA
        GGACGTTGAGTCCATAGAGGGGGGTGTATGTGACACCCCAACCTCAGCAAAAGAAAACCTCCCCTCAAGAAGGACATTTGCGGTGCTAAACAATTTCAAG
                                                                    lr2
        TCTCATCACACATATATATATTTATATAATACTAATAAATAATAGAAAAAGGAAAGGTAAACATCACTAACGACAGTTGCGGTGCAAACTGAGTGAGGT
                                                  cm3
- 100  AATAAACATCACTAACTTTTATTGGTTATGTCAAACTCAAAGTAAAATTTCTCAACTTGTTTACGTGCCTATATATACCATGCTTGTTATATGCTCAAAG
                                                                    tata-box
        CACCAACAAAATTTAAAAACACTTTGAACATTTGCAAAATGGCAACTACTAAAACTTTTTTAATTTTATTTTTTATGATATTAGCAACTACTAGTTCAAC
        *tc                                                  tl
        ATGTGCTAAGTTGGAAGAAATGGTTACTGTTCTAAGTATTGATGGAGGTGGAATTAAGGGAATCATTCCAGCTATCATTCTCGAATTTCTTGAAGGACAA
        CTTCAGGTATTGTAAAAATATTTTTTAATGTATGTGCGTAAGTGTGACACTACTACTATAGTCATTCTGGGTACCT
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Fig. 2. Nucleotide sequence of the first exon and 1.7 kb of the upstream region of the B33 gene. The supposed translation start site (tl) and transcription start site (tc) as deduced from a comparison with other patatin genes (pgT5, Rosahl *et al.*, 1986b) are indicated. Homology to the TATA-box is underlined and the 205 bp repeat bc (lr 1,2) and the common motif (cm 1,2,3) are marked by arrows.

hybridizing clones. These were subsequently screened by Southern blot analysis for class I genes using a class specific oligonucleotide which was derived from sequences around the 22 bp insertion within the 5'-untranslated leader (see Materials and methods). Two different class I patatin genes were identified named B24 and B33. Here we report on the analysis of clone B33, the restriction map of which is shown in Figure 1. The patatin cDNA pcT58 and the class I-specific oligonucleotide only hybridized to the 8 kb *Eco*RI fragment, which was subcloned and used for further analysis.

Figure 2 shows part of the sequence of the first exon as well as 1.7 kb of the upstream region of the B33 gene. Comparison with published sequences of other genomic clones reveals a high degree of homology of both class I and class II genes in the region of the first exon and in part of the upstream sequence (see Figure 2). Based on this high degree of homology the transcription start site was allocated as indicated in Figure 2. The homology to class II patatin genes however ends after position −86, whereas the homology to other class I patatin genes (Bevan *et al.*, 1986) extends for at least 1.7 kb only differing by a duplication of a 208 bp sequence in B33.

The upstream region of the gene B33 described here contains motifs homologous to the TATA- and CAAT-box at position −31 and −63, respectively. The most outstanding feature however is the occurrence of a long direct repeat of 208 nucleotides, which is itself composed of several subrepeats. One of the subrepeats is present at three different positions in the B33 upstream sequence (i.e. at position −775, −505 and −175) and has also been found in all other

class I patatin genes analysed so far (see Mignery *et al.*, 1988) the consensus sequence being TATTATATAATAC-TAA TAAAGAATAGAAAG as indicated in Figure 2.

Construction of a chimeric gene containing the 5′-upstream region of the patatin gene B33 fused to the coding region of the β-glucuronidase gene and transfer into potato

In order to analyse whether the upstream region of gene B33 is sufficient to obtain a controlled expression in transgenic potato plants, a *Dra*I fragment extending from position +14, ~1.5 kb into the upstream region, was fused to the β-glucuronidase gene from *E.coli* (Figure 1) and the poly(A) site of the nopaline synthase gene added to the 3′-end of this gene.

This construct was cloned into the binary vector pBin 19 (Bevan, 1984) and transferred into the *Agrobacterium tumefaciens* strain LBA 4404. By reisolating the plasmid DNA and digestion with appropriate enzymes (data not shown) the correct structure of the chimeric gene in *Agrobacterium* was proven.

Subsequently leaf discs of potato plants cv. Désirée were infected and transformed calli cells were selected on kanamycin containing medium. Regenerated plantlets were transferred to the greenhouse for further analysis.

The upstream region of the patatin gene B33 directs tuber-specific expression of the fused β-glucuronidase gene

Because of the high variability in the level of expression of genes in plants independently transformed with the same construct (see, e.g. Willmitzer, 1988), we decided to analyse a statistically significant number of transformed potato plants for expression of the chimeric patatin gene. Thus in a first screening leaves and tubers of 40 independently derived transgenic potato plants were assayed for glucuronidase activity using the fluorescent assay. As expected the glucuronidase activity varied considerably from plant to plant (Table I and data not shown). Southern analysis of transformed plants showed in all cases analysed the presence of intact non-rearranged chimeric genes. The copy number varied between 2 and 10, in a few cases more than one integration site was seen. No correlation between the level of expression and the copy number integrated was observed (data not shown). The mean value for the glucuronidase activity in tubers of these 40 plants was 8500 pmol MU/mg protein/min as compared to a value of 94 pmol MU/mg protein/min in leaves (MU = methylumbelliferrol).

In order to further analyse the organ specificity of the expression of the transferred gene, 10 plants exhibiting a high level of expression in tubers were also analysed for glucuronidase activity in stem and root tissue (Table I). In all cases, the expression was highest in tubers followed by stems and roots and lowest in leaves. The mean value in tubers from all 10 plants was ~90-fold higher when compared to stem, 150-fold higher than in roots and 1500-fold higher than in leaves. The discrepancy between the average value for the activity in the leaves of the 40 plants described above and the data for the 10 plants given here (which represent a subset of the 40 plants) will be discussed later.

The organ specificity of expression on the protein level is sustained by Northern type experiments (Figure 3 and data not shown) thus excluding the possibility that the differential expression is due to an altered stability of the glucuronidase protein in different organs.

Table I. Glucuronidase activities in different organs of 10 transgenic potato plants

Transformant	Root	Stem	Leaf	Tuber
33G-12	137	55	0	16882
33G-19	138	7	14	2047
33G-21	155	1034	25	19471
33G-23	0	50	0	12149
33G-24	0	14	0	4530
33G-27	86	8	4	7284
33G-38	30	14	6	3847
33G-52	69	10	0	2864
33G-61	31	10	2	14916
33G-62	133	151	24	18620
x	76	135	7.5	11948
cv. Désirée	0	2	1	0

Activities are given in pmol MU/mg protein/min.
cv. Désirée indicates data obtained for a non-transformed control plant.

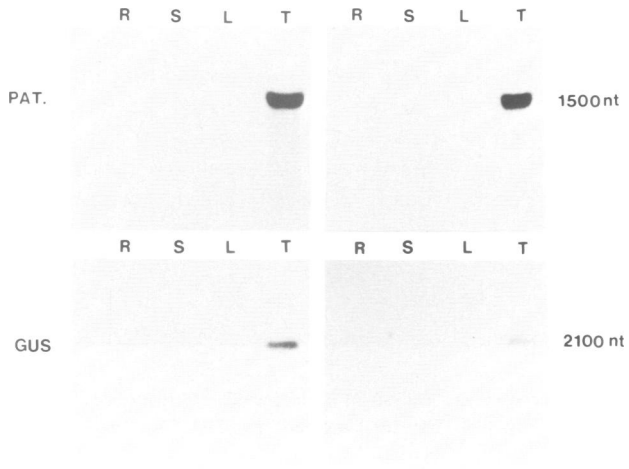

Fig. 3. Northern-type analysis of two transgenic potato plants. Total RNA (50 μg each) extracted from leaf (1), stem (s), root (r) and tuber (t) of greenhouse grown transgenic potato plants was separated by gel electrophoresis using formaldehyde gels and transferred to nylon membranes. They were subsequently hybridized with nick-translated patatin-cDNA [pcT58 (Rosahl *et al.*, 1986a)] and the *Eco*RI−*Hin*dIII fragment of the β-glucuronidase gene from pGUS. The size of the hybridizing RNAs is given in nucleotides.

Glucuronidase activity of the chimeric patatin gene is restricted to parenchymatic cells of the potato tuber

We have analysed the expression of the patatin protein in various tissues of potato tubers by immunocytochemical methods showing that patatin is present in the parenchymatic cells of the potato tuber but not in the peripheral phellem. (Sonnewald *et al.*, in press). In order to analyse whether this tissue specificity is retained for the expression of the chimeric patatin gene, sections from tubers of greenhouse grown plants were assayed for glucuronidase activity. An intensive blue colour is visible over the whole section containing parenchymatic cells but not in the peripheral phellem (see Figure 4).

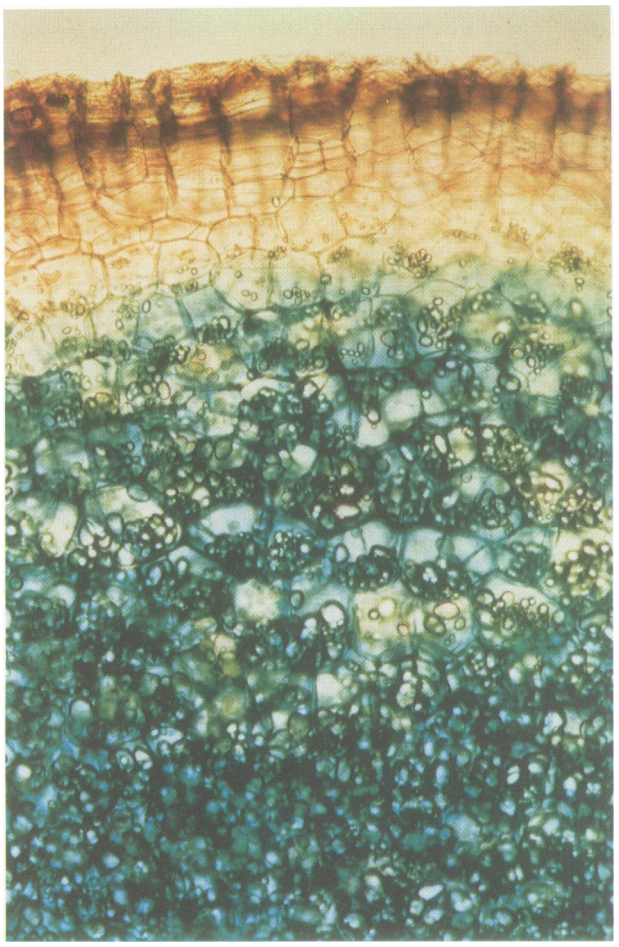

Fig. 4. Histochemical analysis of β-glucuronidase activity in cross-section of a tuber derived from a greenhouse transgenic potato plant (transformant No. 33-G-23).

The promoter region of the B33 gene leads to a tissue-specific induction of expression of the β-glucuronidase gene B33-GUS in potato plantlets axenically cultivated on high sucrose containing media

The expression of patatin is subject to a variety of developmental and environmental influences. Recently we observed that the expression of patatin can be induced in leaves of axenically cultured potato plantlets when these are transferred from medium containing the (normally used) 2% sucrose to a medium containing a much higher level of sucrose, i.e. 7% (Rocha-Sosa *et al.*, submitted). In order to test whether or not the promoter fragment of the B33 gene is capable of mediating this response, four independently derived transgenic potato plantlets containing B33-GUS were first propagated and amplified on medium containing low sucrose levels (2%) and subsequently several explants of each clone were transferred in parallel to medium either containing high (7%) or low (2%) levels of sucrose and assayed after 2 weeks for the activity of the β-glucuronidase indicator gene (Table II). For three plants tested the shift from a low to a high sucrose containing medium is accompanied by a significant (between 20- and 100-fold) increase in β-glucuronidase activity.

Figure 5 shows a histochemical analysis of leaves from potato plants kept either on high or on low sucrose levels. Whereas low sucrose did not lead to any (histochemically) detectable glucuronidase activity (Figure 5a), a strong staining was observed with sections from leaves of plants kept on 7% sucrose. The staining is seen in both spongy and palisade mesophyll cells (Figure 5b) as well as in epidermal cells (Figure 5c) but is absent or rarely detectable in different types of hair cells as well as in guard cells surrounding the stomata openings (Figure 5b and c). Furthermore no or very little expression is detectable in the vascular tissue (Figure 5d).

Table II. Expression of the B33-β-glucuronidase gene in leaves of transgenic potato plantlets kept axenically on Murashige and Skoog medium supplemented with either 2 or 7% sucrose

	gus-activities	
Plant No.	2% sucrose	7% sucrose
33G-TC1	12	1259
33G-TC2	13	464
33G-TC3	41	1110
33G-TC4	8.9	18.8

Glucuronidase activities are given as pmol MU/mg protein/min.

Discussion

A variety of environmental, metabolic and developmental factors are involved in the control of patatin gene expression. Although the formation of storage organs (tuberization) by potato plants is always associated with a high level of patatin expression in tubers this does not mean that this is strictly dependent on tuber specific factors since patatin synthesis has also been observed in other organs under a variety of conditions. The study of the patatin gene regulation has been complicated by the fact that the tetraploid genome of potato carries ~40–60 patatin genes. The 5′-upstream regions of a number of cloned patatin genes did thus far not allow the identification of *cis*-acting elements essential for a high level of tuber-specific expression. Twell and Ooms (1987) described a class II patatin gene the promoter of which can drive the expression of a reporter gene in transgenic potato plants. The observed level of expression was however rather low being ~5-times above background in tubers induced on media containing high levels of cytokinins and sucrose whereas no activity was observed in roots. This observation made for a single class II gene differs from the data described for the sum of all endogenous class II genes being predominantly expressed in roots and, though to a lower extent compared to class I genes, in tubers (Pikaard *et al.*, 1987).

The data presented here show that a class I patatin gene (B33) carries a 5′-upstream promoter region of 1.5 kb which on average is 100- to 1000-fold more active in tubers as compared to stems, roots or leaves.

In this respect we would like to comment upon the 10-fold difference found for the level of expression of the B33-glucuronidase gene in leaves of transgenic potato plants observed during our first screening of the 40 potato plants and the mean average as given in Table I. As described in the Results section screening of the 40 plants was done before testing of the 10 plants taken up in Table I. Both screenings were done within a time interval of ~4 weeks, which seemingly led to the reduction in the B33 driven glucuronidase activity in leaves. When a more close analysis of some of the plants was performed by assaying the leaves,

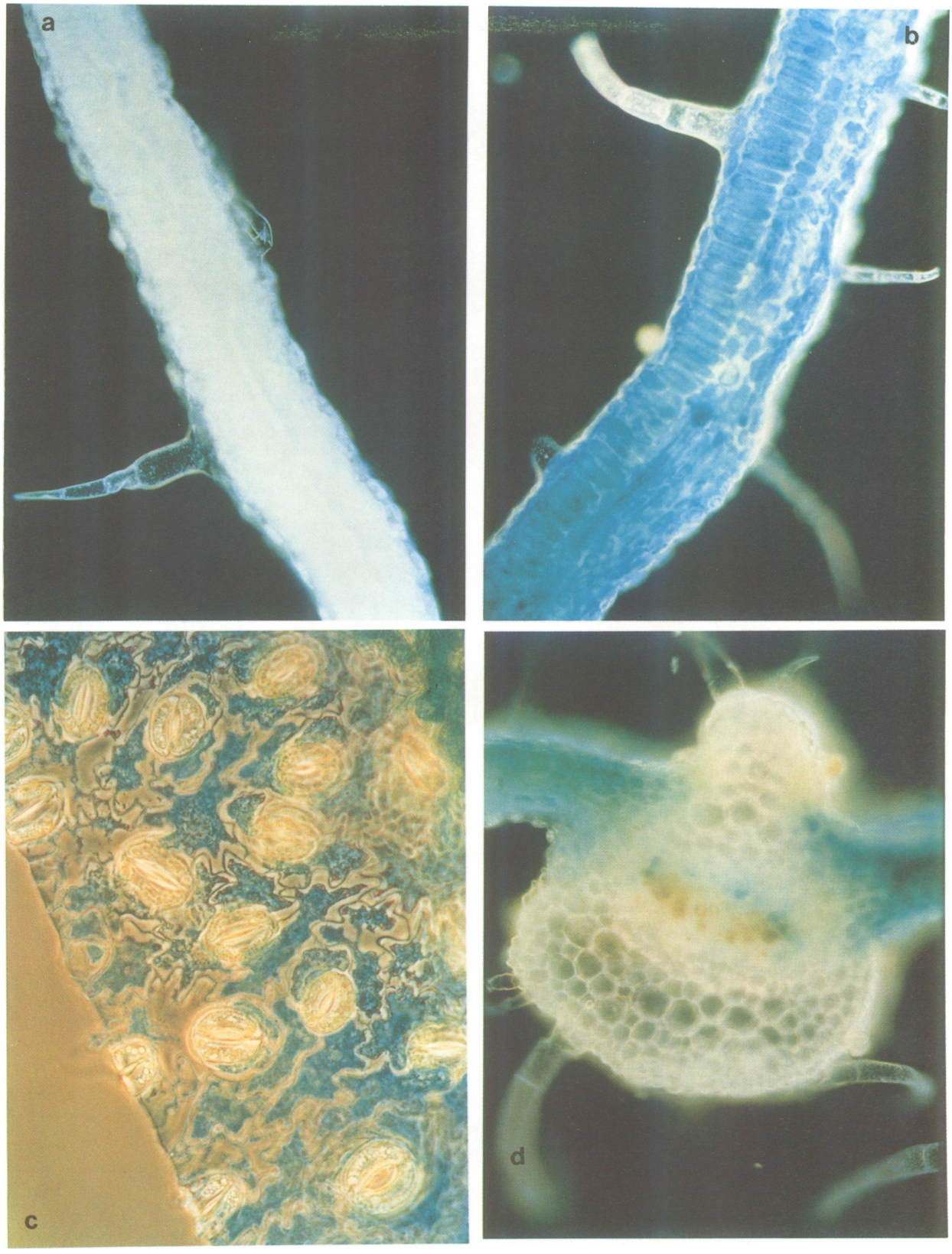

Fig. 5. Histochemical analysis of the β-glucuronidase activity in cross-sections of leaves from potato plantlets (transformant No. 33-G-TC-3) kept on media with low (2%; **a**) or high sucrose levels (7%; **b**–**d**). (a) Analysis of a section through a leaf obtained from the transgenic potato plant kept on MS-medium (see Materials and methods) containing only 2% sucrose. No glucuronidase activity is detectable above background. (b) Analysis of a section through a leaf obtained from clonal derivative of the same transformant, however, after 2 weeks' incubation on MS-medium containing 7% sucrose. A clear staining is visible in both palisade and spongy parenchymatic cells, but seemingly absent from hair cells. (c) Analysis of the epidermis of leaves from the plant described in (b). A clear glucuronidase activity is readily detectable in the epidermal cells but seemingly absent from the stomata. (d) Analysis of a section through the middle-rib of a leaf derived from the plant described in (b). Only very little activity of the glucuronidase is associated with the vascular tissue compared to the mesophyll.

every week, a continuous decrease of the glucuronidase activity was observed. In addition we observed that leaves displaying a B33 driven glucuronidase activity also contained endogenous patatin protein (data not shown). Thus the residual activity of the B33 driven glucuronidase in leaves observed during the first screening is most likely not due to an erroneous expression of this chimeric gene but is rather due to the creation of conditions which led to a (though low) activation of the endogenous patatin genes too.

The level of activity observed in tubers with the B33 patatin promoter (2000−15 000 pmol MU/mg protein/min) is within the same range as the one observed with other strong promoters such as the 35S promoter of the CaMV (average value in leaves; between 2000 and 15 000 pmol MU/mg protein/min) (data not shown) the photosynthetic ST-LS1 gene (J.Stockhaus, personal communication) or the potato proteinase inhibitor II in potato tubers (M.Keil, personal communication).

The B33 gene thus most likely represents an active tuber-specific gene since it carries a strong tuber-specific promoter region and since its coding-sequence upon fusion to the 35S promoter of the CaMV and transfer into tobacco plants yields a protein which immunologically crossreacts with patatin antibodies and gives rise to a new esterase activity (R.Höfgen, personal communication).

A comparative analysis of the 5′-upstream sequences of the B33 patatin gene reveals a high degree of homology to both class II and class I genes up to position −87, whereas upstream from this up to at least −1.7 kb, sequences are homologous only to class I genes (e.g. Bevan *et al.*, 1986; Mignery *et al.*, 1988). The major difference is the presence of the 208 bp direct repeat in B33. A possible role of this long repeat remains to be shown by means of deletions in expression studies.

One possible explanation for the presence of several different patatin genes in the potato genome is the assumption that different members of this multigene family carry different regulatory sequences and therefore react to different environmental, metabolic and developmental signals. The described differences in expression of class I and class II patatin genes support this assumption. Since the B33 class I gene carries a strong tuber-specific promoter it was of interest to test whether this particular patatin gene would also carry regulatory sequences that react to signals which are not organ-specific. We (Rocha-Sosa *et al.*, submitted) and others (Paiva *et al.*, 1983) have observed that patatin expression correlates closely with the formation of starch, a major storage product of potato tubers. Sucrose produced in leaves by photosynthesis is a precursor of starch synthesis and could thus be involved in the signal transduction.

High levels of externally supplied sucrose or glucose, but not sorbitol induce patatin gene activity in leaves (Rocha-Sosa *et al.*, submitted). Whereas activity of the B33 gene promoter is very low in leaves from plantlets supplied with 2% sucrose, an increase of up to 100-fold is found in leaves from plants supplied with 7% sucrose. This given activation is cell specific since it is observed in leaves of mesophyll and epidermal cells but not in vascular tissue and not in guard cells. These observations reflect true cell-specificity and are not due to differential accessibility of the substrate for β-glucuronidase in different leaf cell types because if the glucuronidase reporter gene is driven by other promoters such as one derived from the photosynthetic ST-LS1 gene (Stockhaus *et al.*, 1987) expression in leaf hairs and guard cells is readily detectable whereas glucuronidase activity in vascular tissue is observed when the promoter region of a wound-inducible gene is used (unpublished data).

The 1.5 kb B33 gene promoter must therefore also carry (a) *cis*-acting element(s) that react to metabolic signals. One and the same member of the gene family can therefore mediate patatin expression in tubers but also in other organs under defined metabolic conditions. It is conceivable that the availability of starch or one of its precursors is a signal not only to initiate the morphological changes typical for tuberization but also for the activation of 'tuber-specific' genes. If this hypothesis would turn out to be correct the B33 glucuronidase gene would provide a convenient marker to study the factors involved in the switching of a somatic tissue into a storage tissue.

Materials and methods

Material

Potato lines cv. Berolina and cv. Désirée were obtained through Vereinigte Saatzuchten eG, 3112 Ebsdorf, FRG. Leaves, stems, tubers and roots were harvested from greenhouse plants, whereas for sucrose induction the lowest leaves were taken from plantlets grown in sterile tissue culture on MS-medium (Murashige and Skoog, 1962) containing 2 and 7% sucrose (2MS, 7MS) respectively.

Cloning

Genomic DNA of *Solanum tuberosum* cv. Berolina was isolated (Rogers and Bendich, 1985), subjected to partial *Sau*3A digestion, ligated into *Bam*HI digested lambda EMBL4 arms and plated on bacterial strain P1K803. A total of 3×10^5 recombinant plaques were screened yielding 13 clones that hybridized to the patatin cDNA clone pcT58 (Rosahl *et al.*, 1986). Using a class I specific oligonucleotide (5′-TTTTGCAAATGTTCAAAGTGT-3′ synthesized on an Applied Biosystem DNA Synthesizer 380A) two class I genes were identified by blot hybridizations.

Constructions

A 1.5 kb *Dra*I fragment, as indicated in Figure 1, was ligated into the *Sma*I site of pGus, a plasmid derived from pBI101 (Jefferson 1987; Jefferson *et al.*, 1987). By digestion with *Eco*RI and *Sma*I the coding region of the β-glucuronidase gene together with the 3′-end of the nopaline synthase gene were excised and ligated into the *Hin*cII site of pUC 18 after treatment with Klenow fragment of DNA polymerase I (M.Köster, personal communication). The whole insert was excised with *Eco*RI and *Hin*dIII and cloned into the appropriate sites of pBin19 (Bevan, 1984). The recombinant vector was transformed into the *Agrobacterium* strain pGV2260 or LBA4404.

Histochemical localization

Hand sections of potato tubers or leaves were fixed by vacuum infiltration with 2% formaldehyde in 100 mM Na-phosphate, pH 7.0, 1 mM EDTA. Fixation was carried out for 30 min on ice and slices were extensively washed with phosphate buffer.

Histochemical assay

The histochemical assay for GUS was performed for times from 2 h to overnight according to Jefferson (Jefferson, 1987; Jefferson *et al.*, 1987).

Potato transformation

Leaf discs of *S.tuberosum* cv. Désirée were incubated in MS-medium (Murashige and Skoog, 1962) containing 2% sucrose, 50 μl of the recombinant *Agrobacterium* strain 33 G were added and mixed for 5 min and then incubated in the dark for 2 days. For callus induction leaf discs were transferred to MS-medium supplemented with 1.6% glucose, 2 mg/l zeantinribose, 20 μg/ml NAA, 20 g/l GA_3, 0.5 g/l Claforan, 50 mg/l Kanamycin and 0.8% Agar. After 1 week leaf discs were kept on the same medium with reduced Claforan concentration (0.25 g/l). Every 10 days leaf discs or developing calli were put into fresh medium. After shoot formation (after 3−4 transfers), shoots were cut off the leaf disc and transferred to MS-medium containing 2% sucrose and 250 μg/ml Claforan (C.Recknagel and A.O'Connors, personal communication).

RNA was isolated according to Logemann *et al.* (1987), protein concentration was determined according to Bradford (1976) and all other methods follow the protocols of Maniatis *et al.* (1982).

Assay for glucuronidase activity

Extracts of leaf, stem, root and tuber were assayed for glucuronidase activity using substrate as described by Jefferson *et al.* (1987). Activities are given in pmol (MU)/mg protein/min.

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