Plant Molecular Biology **14**: 595–604, 1990.

High-level expression of tuberous root storage protein genes of sweet potato in stems of plantlets grown *in vitro* on sucrose medium

Tsukaho Hattori, Shoko Nakagawa and Kenzo Nakamura*
*Laboratory of Biochemistry, School of Agriculture, Nagoya University, Fruo-cho, Chikusa-ku, Nagoya 464-01, Japan (*author for correspondence)*

Received 2 October 1989; accepted in revised form 12 December 1989

Key words: tuber storage protein, sporamin, sucrose, sweet potato, *Ipomoea batatas*

Abstract

Sporamin, the tuberous root storage protein of the sweet potato, accounts for about 60 to 80% of the total soluble protein of this organ. The amount of sporamin present in other organs is very low, or even not detectable, in the normal field-grown plants. However, the stem of sweet potato plantlets grown axenically on agar medium containing sucrose was found to accumulate large amounts of sporamin. Two-dimensional gel electrophoretic profiles of sporamin precursors synthesized *in vitro* by poly(A)$^+$ RNA are indistinguishable between tuberous roots of the field-grown plants and stems of the axenically cultured plants, suggesting that an essentially identical set of the members of sporamin multigene family are expressed in these two organs under different growth conditions. Transgenic tobacco plants having a CAT (chloramphenicol acetyltransferase) fusion gene with the 5′ upstream region of a sporamin A gene, gSPO-A1, show preferential expression of CAT activity in stems when the plants are maintained in axenic culture on sucrose medium as is the case for sporamin in sweet potato. Deletion analysis revealed that the DNA sequence of gSPO-A1 between −94 and −305, relative to the transcription start site, is important for its expression in tobacco. This region contains two of the previously postulated putative regulatory elements conserved between sporamin A and B genes.

Introduction

Underground tuberous organs, such as the tuber of the potato and the tuberous root of the sweet potato, develop as sink organs by accumulating large amounts of starch during the growth of the plant. Large portions of proteins in both of these storage organs can be accounted for by several major proteins which specifically accumulate upon tuberization. These major proteins may function at least in part as storage proteins, which are used as a nutritional source, together with starch, for the vegetative reproduction of new generations [11, 17, 19]. Thus, despite the fact that the potato tuber and the sweet potato tuberous root develop from different organs, underground stem in the former and root in the latter case, these two organs share some functional and biochemical properties.

Potato tubers contain several different major proteins. Among these is patatin which constitutes about 20 to 40% of the total soluble protein of the tissue and is the most abundant and most extensively studied protein [16–23]. In contrast, about 60 to 80% of the total soluble protein of the sweet potato tuberous root is accounted for

by sporamin [11]. Sporamin is actually a mixture of closely related proteins with M_r 20000, and it probably represents the major component of '*ipomoein*', described for the sweet potato globulin fraction as early as 1931 [9]. In normal field-growth plants, sporamin is not detectable or is present in only small amounts in tissues other than tuberous roots [11], and this organ-specific accumulation of sporamin is seen at the mRNA level [3].

The multigene family encoding sporamin can be divided into two major subfamilies, which are designated A and B based on nucleotide sequence homologies among the members [5, 13]; the hexaploid genome of the sweet potato contains about 10 copies of sporamin genes per haploid genome (Y. Kowyama, T. Hattori, T. Kumashiro and K. Nakamura, manuscript in preparation). Nuclear genes for sporamin do not contain introns, and comparison of nucleotide sequences of one each of sporamin A and sporamin B genes revealed the presence of two conserved sequence blocks in the highly diverged 5′ upstream region preceding the TATA box, which may be involved in the regulation of sporamin gene expression [4].

Maeshima *et al.* [11] previously reported the presence of a small amount of sporamin in stems of field-grown sweet potato, suggesting that sporamin gene expression may not be under strict organ-specific regulation. In the prsent paper, we describe evidence that the amount of sporamin in stems can become very high, comparable to that in tuberous roots, when sweet potato plantlets are cultured axenically on sucrose-containing agar medium. Axenically cultured transgenic tobacco plants with a sporamin promoter-CAT (chloramphenicol acetyltransferase) fusion gene preferentially expressed CAT activity in stems. These results strongly suggest that the expression of sporamin genes is regulated by one or more factors that are not tuberous root-specific *per se*.

Materials and methods

Plant materials

Sweet potatoes (*Ipomoea batatas* Lam. cv. Kokei No. 14) were grown at the Nagoya University Experimental Farm. Axenic plants of the same sweete potato cultivar were obtained from Verdi Co. (Toyohashi, Japan) and maintained by subculturing apical or lateral buds on MS-agar medium [14] containing 3% sucrose. Tobacco (*Nicotiana tabacum* cv. Xanthi) plants used for *Agrobacterium*-mediated transformation were grown in a greenhouse.

Analyses of sporamin

Soluble proteins were extracted from various tissues with 2 to 3 volumes of 50 mM Tris-HCl (pH 8.0) containing 5 mM EDTA and 1% (w/v) sodium ascorbate and centrifuged at 15000 × *g* for 10 min. The supernatant was passed through Sephadex G-25 spin column equilibrated with the extraction buffer. SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotting were carried out as described previously [3] except that horseradish peroxidate (HRP)-linked protein A was used instead of ^{125}I-protein A. Color development for HRP activity was carried out with 4-chloro-1-naphtol as a substrate. Sporamin was quantitated by rocket immunoelectrophoresis as described [11]. Protein content in the extract was determined after precipitation of proteins with 10% trichloroacetic acid by the method of Lowry *et al.* [10] with bovine serum albumin as standard.

RNA isolation and in vitro *translation*

Isolation of poly(A)$^+$ RNA from the tissue, *in vitro* translation with wheat germ extract and immunoprecipitation with anti-sporamin A serum [11] were carried out as described previously [3]. Two-dimensional PAGE of the immunoprecipitated products was carried out by the method of O'Farrel [15].

Vector construction

The sporamin promoter-CAT fusion gene was constructed by inserting approximately an 1 kb fragment of the 5′ upstream region of gSPO-A1 [4] in front of the CAT coding region in pGA492 Ti-plasmid binary vector [1] as follows: an *Alu* I fragment covering from position − 970 to + 40 of gSPO-A1 (see Fig. 2 in [4]) was first cloned into the *Eco* RV site of Bluescript KS(−) vector in the direction to locate the 5′ end of the insert at the *Xba* I side and the 3′ end at the *Cla* I side of the Bluescript multiple cloning site. Then the insert was cut out with *Xba* I and *Cla* I and recloned into *Xba* I/ *Cla* I sites of the multiple cloning site of pGA492, which located the promoter in front of the CAT coding region. Deletion derivatives were created by digestion with exonucleases III and VII.

The cauliflower mosaic virus 35S promoter-CAT fusion gene was constructed by replacing the *Bgl* II-*Eco* RI fragment of pGA492 the *Bgl* II site in the multiple cloning site and the *Eco* RI site at the N-terminal part of the CAT coding region) with approximately 700 bp *Eco* RI/*Bam* HI fragment of modified pCaMVCAT [2], in which the *Bam* HI site was recreated in front of the CAT coding region.

Plant transformation

The Ti-plasmid binary vector carrying the sporamin promoter-CAT or CaMV 35S promoter-CAT fusion gene was mobilized from *Escherichia coli* HB101 to disarmed *Agrobacterium tumefacience* LBA4404 [6] by triparental mating. *Agrobacterium*-mediated transformation of tobacco (*Nicotiana tabacum* cv. Xanthi) was carried out by the leaf disk method [7]. Selection of transformants was carried out in the presence of 150 mg/l of kanamycin. Regenerated plants were maintained on MS-agar medium containing 3% sucrose, B5 vitamins, 50 mg/l kanamycin and 500 mg/l carbenicillin. Activities of CAT and NPTII were measured by the methods described in An [1] and McDonnell *et al.* [12], respectively.

Results

Organ distribution of sporamin in field-grown and axenically cultured sweet potato plants

Maeshima *et al.* [11] previously reported the organ distribution of sporamin in field-grown sweet potato plants. Besides tuberous roots, where sporamin accounts for about 80% of total soluble protein, a small amount of sporamin was also detected in stems, but not in petioles and leaves. Sporamin could not be detected in seeds as well [3]. We confirmed the presence of sporamin in stems of the field-grown plants. The amount of sporamin in stems, as assayed by rocket immunoelectrophoresis, varied from plant to plant and different parts of stems showed variation in sporamin content. Although it is usually about 1% or less of total soluble protein of the tissue, this value could reach about 4.5%. Furthermore, small amounts of sporamin, less than 1% of the total protein, could also be detected in some of the soluble protein preparations from leaves (data not shown). These results indicate that sporamin is not a tuberous root-specific protein.

We examined the presence of sporamin in various organs of sweet potato plants grown *in vitro*. These plants were derived from a meristem clone of the same sweet potato cultivar used in the field and maintained in axenic culture on MS agar medium containing 3% sucrose. Unlike the case for axenically cultured potato plants, where tuber formation can be induced under certain conditions such as increased sucrose concentration in the medium [8], these sweet potato plants do not develop any tuberous swelling of the root. Figure 1 shows SDS-PAGE profiles of soluble proteins and immunoblot detection of sporamin in leaves, stems, and roots of these axenically cultured sweet potato plants. Surprisingly, the predominant protein in the stem was found to be sporamin (Fig. 1, lane S). The amount of sporamin in the stem, which could reach to 70% of the total soluble protein of the tissue, varied from plant to plant, and stems from the older plants showed a tendency to contain more sporamin than those of

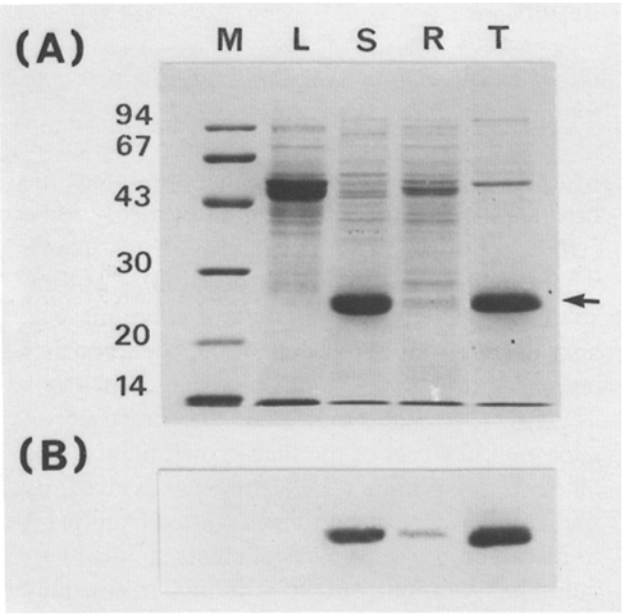

Fig. 1. Polycarylamide gel electrophoretic analysis of soluble proteins in leaves (L), stems (S) and roots (R) of axenically cultured sweet potato plants, and tuberous roots (T) harvested in the field. (A) Proteins were stained with Coomassie brilliant blue. (B) Sporamin was detected by immunoblotting using anti-sporamin serum. Molecular masses of the size markers (M) are indicated in kDa. The arrow indicates the position of sporamin.

the younger plants (data not shown). We also noticed that the lower part of the stem contained more sporamin than the upper part. Roots from these axenically cultured plants also contained a small amount of sporamin (Fig. 1, lane R). In contrast, leaves (Fig. 1, lane L) and petioles (data not shown) usually did not contain any detectable sporamin.

The accumulation of a large amount of sporamin in the stem is considered to be a phenomenon specific for plants cultured on sucrose medium, since when these plants were transferred to soil and grown to maturity, the level of sporamin in the stem decreased to the level seen in the field-grown plants (data not shown).

Comparison of sporamin gene family members expressed in the stem of axenically cultured plants and the tuberous root of field-grown plants

Sporamin is a mixture of closely related polypeptides encoded by a multigene family, which can be classified into two major subfamilies, A and B, based on their nucleotide sequence homologies [5, 13]. From genomic Southern blot analyses, the gene copy numbers for sporamin A and B subfamilies are estimated to be about 6 and 4 copies per haploid genome, respectively, or a total of about 60 sporamin gene copies in the hexaploid genome of the sweete potato (Y. Kowyama, T. Hattori, T. Kumashiro and K. Nakamura, manuscript in preparation). In order to examine whether or not members of the sporamin gene family expressed in stems of axenically cultured plants are the same as those expressed in the tuberous root of the field-grown plant, we compared sporamin precursor components translated *in vitro* from poly(A)$^+$ RNA of these two organs by two-dimensional gel electrophoresis.

As shown in Fig. 2, the *in vitro* translation products of the tuberous root poly(A)$^+$ RNA, after immunoprecipitation with anti-sporamin serum, gave about 12 spots with different intensities. Sporamins migrate anomalously in SDS-PAGE when samples are treated with SDS by heating in the absence of reducing agent or without heating in SDS [3, 11]. These anomalous behavior of sporamins probably explain the appearance of multiple spots with different mobilities in the second dimension of the electrophoresis. An almost identical pattern of spots of sporamin precursors was obtained with poly(A)$^+$ RNA from the stem of axenically cultured plants (Fig. 2 (S)), although some spots showed slight quantitative differences between the two. Mixing of immunoprecipitates from two translation products did not increase the number of spots (Fig. 2 (T + S)). These results indicate that essentially an identical set of sporamin precursors are expressed in these two organs, and strongly suggest that the same sporamin multigene family members expressed in the tuberous root of field-grown

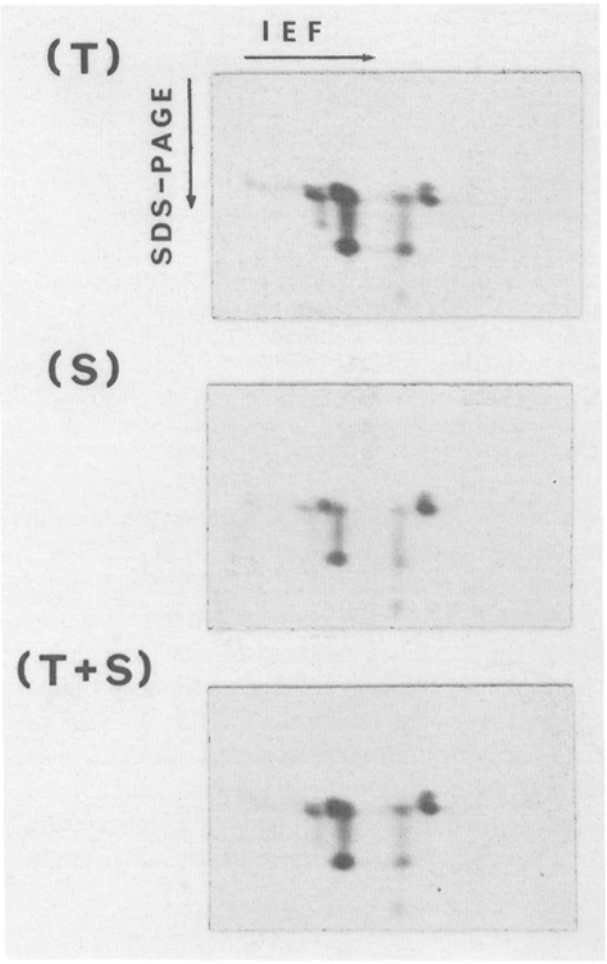

Fig. 2. Two-dimensional gel electrophoresis of sporamin precursors synthesized *in vitro*. Poly(A)$^+$ RNAs prepared from the tuberous roots (T) and the stems of axenically cultured plants (S) were translated *in vitro* in a wheat germ cell free system in the presence of ^{35}S-methionine. The ^{35}S-labeled polypeptides immuno-precipitated with anti-sporamin serum were subjected to two-dimensional gel electrophoresis, and fluorographies were taken. A mixture of immunoprecipitated materials with approximately equal radioactivity of the translation products of the tuberous root and the stem poly(A)$^+$ RNAs were analyzed in panel (T + S). Horizontal and vertical arrows indicate the directions of first-dimensional isoelectric focusing (IEF) and second-dimensional SDS-PAGE, respectively.

plants are expressed in the stem of axenically cultured plants.

Expression of sporamin-CAT fusion gene in transgenic tobacco plants cultured on sucrose medium

We questined whether the preferential expression of sporamin genes in the stem of axenically cultured plants can be observed even in heterologous plants which normally do not produce tuberous storage organs. To test this possibility, we made a CAT fusion gene in which approximately 1 kb (− 970 to + 40 relative to the transcription start site) of 5′ upstream region of gSPO-A1, a sporamin A gene (see Fig. 2 in [4]), is fused to a CAT gene in a 'promoter probing' Ti-plasmid binary vector pGA492 [1] (Fig. 3), and introduced it into tobacco genome by *Agrobacterium* -mediated transformation. The kanamycin-resistant tobacco plants obtained were grown under the same axenic culture conditions as used for the growth of sweet potato plants except that the medium contained kanamycin and carbenicillin. The integration of the sporamin-CAT fusion gene in tobacco chromosomal DNA was examined by Southern blot hybridization of leaf DNA with probes derived from sporamin promoter region and CAT coding region (data not shown). Using plants which showed the presence of the sporamin-CAT fusion gene, CAT activities in leaves, stems, and roots were assayed. The vector, pGA492, carries nopaline synthase (*nos*) promoter-neomycin phosphotransferase II fusion gene (NOS-NPTII) as a selectable maker. We also measured NPTII activity driven by *nos* promoter using the same tissue extracts as used for CAT assay as control.

All of the transgenic tobacco plants with the sporamin promoter-CAT fusion gene preferentially exhibited high CAT activity in their stems. Figure 4 shows typical results for 4 independent plants. Some transformants showed weak expression of CAT activity in roots. Preferential expression of CAT activity in stems was consistently observed after repeated subculture of apical or lateral buds of these transgenic plants. The expression spectra of NPTII activities driven by the *nos* promoter in various organs was different from that of CAT activities, although variation in the level of NPTII activities was seen among tans-

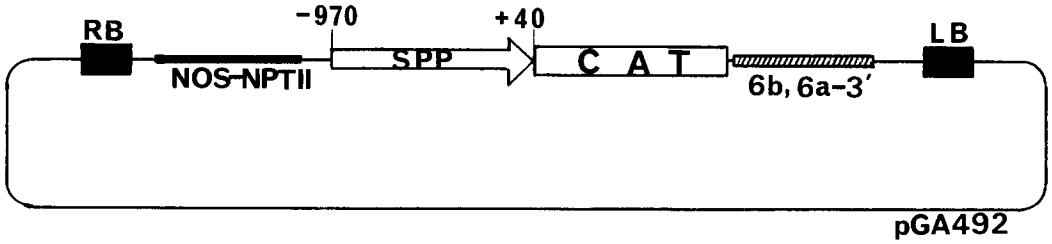

Fig. 3. Illustration of sporamin promoter-CAT fusion gene constructed in Ti plasmid binary vector pGA492. SPP represents the 5′ upstream region of gSPO-A1. 6b, 6a-3′ indicates the 3′ downstream region of transcripts 6b and 6a of octopine-type Ti plasmid A6 which provides the fusion gene with poly(A) addition signal and transcription terminator. LB and RB represent T-DNA left and right borders, respectively.

formants and among organs. Furthermore, in contrast to sporamin-CAT fusion gene, transgenic plants with CaMV 35S promoter-CAT fusion gene showed roughly equal CAT activities in leaves, stems and roots (Fig. 4, panel 5).

These results suggest that the sporamin promoter-CAT fusion gene can be expressed in tobacco and that its expression pattern among organs of axenically cultured tobacco plant is similar to the expression pattern of sporamin genes in axenically cultured sweet potato.

The 5′ detection analysis of the sporamin promoter

We have constructed two kinds of 5′ deletion mutants from the 5′ upstream region of gSPO-A1, one to − 305 and the other to − 94, in sporamin promoter-CAT fusion gene in order to roughly locate the sequence required for its preferential expression in stems of transgenic tobacco plants cultured axenically (Fig. 5A). The analysis of CAT activities in these deletion mutants was carried out together with NPTII assays for at least 4 independent transgenic plants of axenic culture for each construct, and these plants consistently

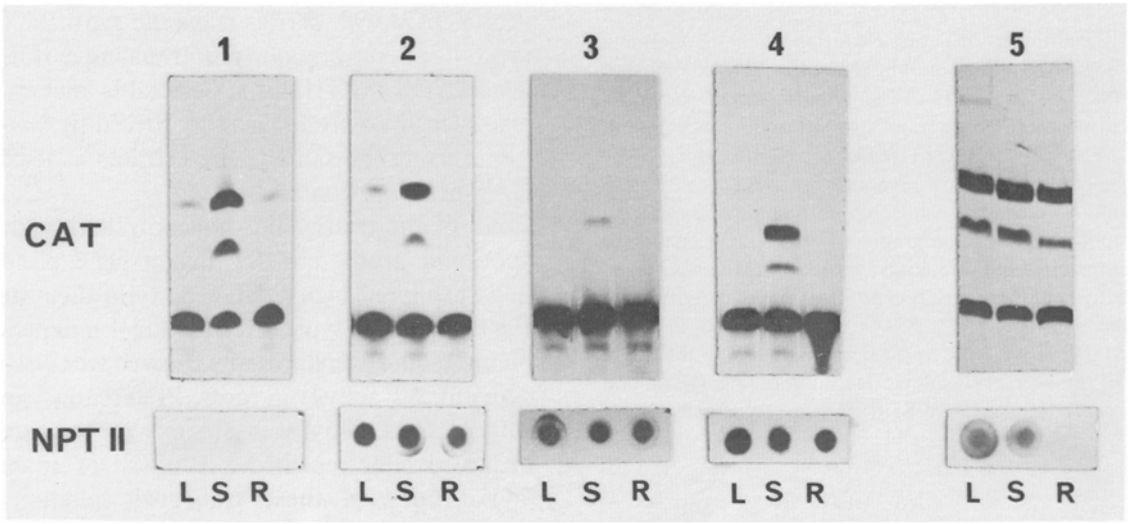

Fig. 4. CAT and NPT II assays with leaf (L), stem (S) and root (R) extracts of transgenic tobacco plants cultured axenically on sucrose containing MS-agar medium. Panels 1–4 and 5 show the results with four independent transformants carrying sporamin promoter-CAT fusion gene shown in Fig. 3, and CaMV 35S promoter-CAT fusion gene, respectively. For each CAT and NPT II assay 60 and 20 μg of protein were used respectively.

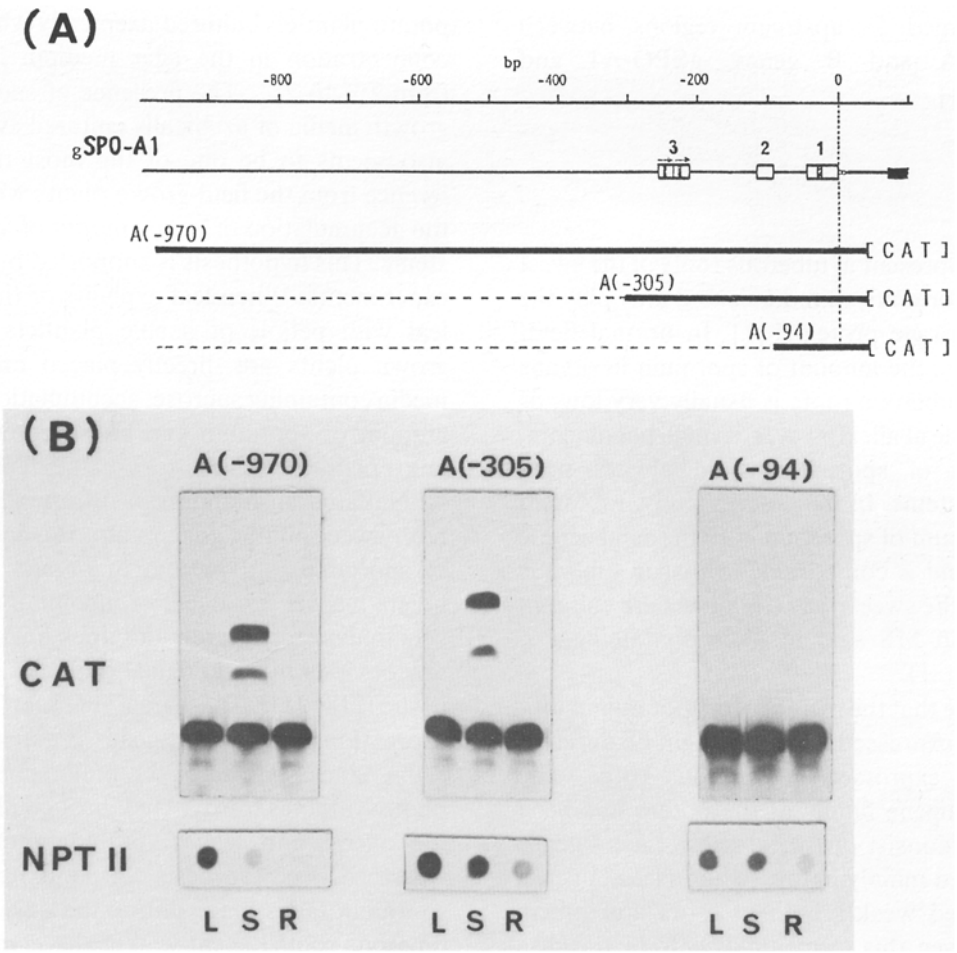

Fig. 5. 5′deletion analysis of sporamin promoter in transgenic tobacco plants cultured axenically on sucrose containing MS-agar medium. (A) Deleted portion from the original construct – A(– 970) – shown in Fig. 3. A(– 305) and A(– 94) represent constructs with deletion end points at nucelotide positions – 305 and – 94, respectively. Above the three constructs, positions of sequence boxes 1, 2 and 3 which show conservation between the 5′ upstream regions of gSPO-A1 and gSPO-B1 (see text and Fig. 4 in ref. (4)) are indicated. (B) CAT and NPT II activities were assayed with leaf (L), stem (S) and root (R) extracts of axenically cultured transgenic tobacco plants transformed with constructs A(– 970), A(– 305) and A(– 94). For each CAT and NPT II assays, 60 and 20 μg of protein were used, respectively.

exhibited the same pattern of expression. Typical results are shown in Fig. 5B.

Transgenic tobacco plants harboring the – 305 (A – 305) construct exhibited preferential expression of CAT activity in stems as seen for the original construct (A – 970). However, transgenic tobacco plants with the – 94 (A – 94) construct showed very little CAT activity in any of the organs analyzed. Although variations in CAT activities were observed from transformant to transformant, no significant difference in the level of CAT activities was observed between plants having the original and – 305 constructs. The expression pattern of NPTII activity was again found to be different from that of CAT activities. These results indicate that the 211 bp sequence from – 305 to – 94 of the 5′ upstream region of gSPO-A1 contains information necessary for driving the reporter gene to be expressed preferentially in stems of axenically cultured plants. Interestingly, this 211 bp region contains two kinds of sequence blocks which show conservation in the

highly diverged 5′ upstream regions between sporamin A and B genes, gSPO-A1 and gSPO-B1 [4].

Discussion

Sporamin is present in tuberous roots of the sweet potato in enormous abundance and may play the role of a storage protein [11]. In normal field-grown plants, the amount of sporamin in organs other than tuberous roots is usually very low, or not detectable at all. However, a small but detectable amount of sporamin could always been detected in stems. In the present study, we found that the amount of sporamin in stems can become very high, and is comparable to that in tuberous roots when the sweet potato plantlets are cultured axenically on MS agar medium containing 3% sucrose (Fig. 1).

It could be that the members of sporamin multigene family expressed in stems might be different from those expressed in tuberous roots. The patatin multigene family of the potato has been reported to consist of two classes; class I genes are expressed mainly in tubers and class II genes are expressed weakly both in roots and tubers [16]. However, this seems unlikely to be the case for sporamin, since the results presented in Fig. 2 suggest that an almost identical set of the members of sporamin multigene family are expressed in both of these organs. However, since this kind of analysis may not detect all of the products of active members of sporamin gene family, we cannot rule out the possibility that some family members may be regulated in a different manner.

The results described in this paper indicate that the expression of sporamin genes can be induced in organs other than roots under certain conditions. Similar observations have been reported previously for the potato tuber storage proteins. Paiva *et al.* [18] reported that accumulation of large amount of patatin can be induced in petioles and stems when single-leaf stem cuttings are excised from potato plants. While our studies are in progress, Rocha-Sosa *et al.* [21] reported that patatin accumulation can be induced in leaves of potato plantlets cultured axenically when sucrose concentration in the agar medium is increaed from 2% to 7%. The presence of sucrose in the growth media of axenically cultured sweet potato also seems to be one of the most notable difference from the field-grown plants which induce the accumulation of large amount of sporamin in stems. This hypothesis is supported by our recent experiments. When leaf explants or the detached leaf with petiole of axenic plantlets and field-grown plants are directly placed on MS agar media containing sucrose, accumulation of a large amount of sporamin can be observed in leaves and petioles (T. Hattori, H. Fukumoto and K. Nakamura, manuscript in preparation). In both sweet potato and potato, the accumulation of sporamin and patatin in organs other than tubers seems to occur concomitant with the accumulation of starch and does not accompany any obvious morphological differentiation of the tissue [18, 21]. The effect of sucrose on the expression of sporamin genes is especially interesting since sucrose is the major component of photosynthates transported through phloem from the overground leaves to undergbround roots where active synthesis of both starch and sporamin takes place during the development of tuberous root. Recently, Wenzler *et al.* [23] also reported that the high-level expression of a patatin promoter-GUS (β-glucuronidase) fusion gene in leaf and stem explants from transgenic potato plants can be induced by high concentration of sucrose. It seems possible that some metabolic signals, which trigger the development of storage functions of cell or tissue, play an important role in the activation of genes coding for major tuberous organ proteins in both sweet potato and potato, and these signals may have a close link to the activation of starch synthesis [21, 23].

In order to examine whether the cellular response to metabolic signals which regulate the sporamin gene expression is a general phenomenon in a wide variety of plants, we examined the expression of a sporamin promoter-CAT fusion gene in tobacco. The results presented in Fig. 4 show that the 5′ upstream region of a sporamin A gene, gSPO-A1, can direct the expression of a

CAT reporter gene in stems of transgenic tobacco plants axenically cultured on a sucrose-containing medium as is the case for sporaminin sweet potato. Our preliminary analyses with the sporamin promoter-GUS fusion gene suggest that the histochemical pattern of GUS expression in stems of transgenic tobacco cultured axenically is similar to the pattern of starch accumulation in stems of axenically cultured sweet potato (S. Ohta, T. Hattori and K. Nakamura, unpublished results). These results suggest that the expression of sporamin genes is regulated by molecular mechanisms which may be common to various plant species rather than specific for the sweet potato.

Since reliable transformation and regeneration systems are not yet available for the sweet potato, the expression system in axenically cultured tobacco plants could be utilized for putative identification of some of *cis*-regulatory elements required for the regulation of sporamin gene expression. In fact, the results shown in Fig. 5 suggest that within the 211 bp sequence between nucleotide positions − 94 and − 305 of gSPO-A1, there exist sequence elements imporatant for expression in stems of tobacco plants cultured axenically on sucrose-containing medium. This sequence contains two kinds of sequence elements of possible importance in the regulation of sporamin gene expression that have been previously postulated because of their conservation between the highly diverged 5′ upstream regions of gSPO-A1 and gSPO-B1, sporamin A and B genes [4]. One of the elements, called box 2, is a 28 bp sequence with 75% homology. The other element is directly repeated twice in gSPO-A1, called box 3, or 4-times in gSPO-B1, called box 3′. Boxes 3 and 3′ share a common core motif sequence of AAATCA$(N)_6$TTA. The results presented in Fig. 5 further suggest that these sequences are likely to be *cis*-regulatory elements of sporamin genes.

Acknowledgements

We tanks Mr K. Matsuoka for the initial analysis of proteins from *in vitro* cultured sweet potato plantlets, Dr G. An of Washington State University for providing pGA492, Dr S. Yoshida of the Nagoya University Experimental Farm for growing the sweet potato, Verdi Co. for meristemic clone culture of the sweet potato, Japan Tobacco Co. for *Nicotiana tabacum* cv. Xanthi seeds. This work was supported in part by Grants-in-Aid for Special Project Research and for Scientific Research on Priority Areas to K.N. and for Encouragement of Young Scientists to T.H. from the Ministry of Education, Science and Culture, Japan.

References

1. An G: Development of plant promoter expression vectors and their use for analysis of differential activity of nopaline synthase promoter in transformed tobacco cell. Plant Physiol 81: 86–91 (1986).
2. Fromm M, Taylor LP, Walbot V: Expression of genes transferred into monocot and dicot plant cells by electroporation. Proc Natl Acad Sci USA 82: 5824–5828 (1985).
3. Hattori T, Nakagawa T, Maeshima M, Nakamura K, Asahi T: Molecular cloning and nucleotide sequence of cDNA for sporamin, the major soluble protein of sweet potato tuberous roots. Plants Mol Biol 5: 313–320 (1985).
4. Hattori T, Nakamura K: Genes coding for the major tuberous root protein of sweet potato: Identification of putative regulatory sequences in the 5′ upstream region. Plant Mol Biol 11: 417–426 (1988).
5. Hattori T, Yoshida N, Nakamura K: Structural relationship among the members of a multigene family coding for the sweet potato tuberous root storage protein. Plant Mol Biol 13: 563–572 (1989).
6. Hoekema A, Hirsch PR, Hooykaas PJJ, Schilperoort RA: A binary plant vector strategy based on separation of the Vir and T-region of the *Agrobacterium tumefacience* Ti-plasmid. Nature 103: 179–180 (1983).
7. Horsch RB, Fry JE, Hoffmann NL, Eichholtz D, Rogers SG, Fraley RT: A simple and general method for transferring genes into plants. Science 227: 1229–1231 (1985).
8. Hussey G, Stacey NH: Factors affecting the formation of in vitro tubers of potato (*Solanum tuberosum* L.). Ann Bot 53: 565–578 (1984).
9. Jones DB, Gersdorff CEF: Ipomoein, a globulin from sweet potatoes, *Ipomoea batatas*. Isolation of a secondary protein derived from ipomoein by enzymic action. J Biol Chem 93: 119–126 (1931).
10. Lowry OH, Rosenbrough NJ, Farr AL, Randall RJ: Protein measurement with the Folin phenol reagent. J Biol Chem 193: 265–275 (1951).

11. Maeshima M, Sasaki T, Asahi T: Characterization of major proteins in sweet potato tuberous roots. Phytochemistry 24: 1899–1902 (1985).
12. McDonnell RE, Clark RD, Smith WA, Hinchee MA: A simplified method for the detection of neomycin phosphotransferase II activity in transformed plant tissues. Plant Mol Biol Rep 5: 380–386 (1985).
13. Murakami S, Hattori T, Nakamura K: Structural differences in full-length cDNAs for two classes of sporamin, the major soluble protein of sweet potato tuberous roots. Plant Mol Biol 7: 343–355 (1986).
14. Murashige T, Skoog F: A revised medium for rapid growth and bioassay with tobacco tissue cultures. Physiol Plant 15: 473–497 (1962).
15. O'Farrell PH: High resolution two-dimensional electrophoresis of proteins. J Biol Chem 250: 4007–4021 (1975).
16. Pikaard CS, Brusca JS, Hannapel DJ, Park WD: The two classes of genes for the major tuber protein, patatin, are differentially expressed in tubers and roots. Nucleic Acids Res 15: 1979–1994 (1987).
17. Racusen D, Foote M: A major soluble glycoprotein of potato tubers. J Food Biochem 4: 43–52 (1980).
18. Paiva E, Lister RM, Park WD: Induction and accumulation of major tuber proteins of potato in stems and petioles. Plant Physiol 71: 161–168 (1983).
19. Park WD: Tuber proteins of potato – A new and surprising molecular system. Plant Mol Biol Rep 1: 61–66 (1983).
20. Park WD, Blackwood C, Mignery GA, Hermodson MA, Lister RM: Analysis of the heterogeneity of the 40,000 molecular weight tuber glycoprotein of potatoes by immunological methods and by NH_2-terminal sequence analysis. Plant Physiol 71: 156–160 (1983).
21. Rocha-Sosa M, Sonnewald U, Frommer W, Stratmann M, Schell J, Willmitzer L: Both developmental and metabolic signals activate the promoter of a class I patatin gene. EMBO J 8: 23–29 (1989).
22. Twell D, Ooms G: The 5′ flanking DNA of a patatin gene directs tuber specific expression of a chimaeric gene in potato. Plant Mol Biol 9: 365–375 (1987).
23. Wenzler HC, Mignery GA, Fisher LM, Park WD: Analysis of a chimeric class-I patatin-GUS gene in transgenic potato plants: High level expression in tubers and sucrose-inducible expression in cultured leaf and stem explant. Plant Mol Bil 41–50 (1989).