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Induction and Accumulation of Major Tuber Proteins of Potato in Stems and Petioles[1]

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ABSTRACT

A family of immunologically identical glycoproteins with apparent molecular weights of approximately 40,000 are among the major tuber proteins of potato (*Solanum tuberosum* L.). These proteins, as purified by ion-exchange and affinity chromatography, have been given the trivial name 'patatin.' To determine if patatin can be used as a biochemical marker to study the process of tuberization, its amount was measured in a variety of tissues by rocket immunoelectrophoresis and by enzyme-linked immunosorbent assay (ELISA).

Patatin comprises 40 to 45% of the soluble protein in tubers regardless of whether they are formed on underground stolons or from axillary buds of stem cuttings. Under normal conditions, patatin is present in only trace amounts, if at all, in leaves, stems, or roots of plants which are either actively forming tubers or which have been grown under long days to prevent tuberization. However, if tubers and axillary buds are removed, patatin can accumulate in stems and petioles. This accumulation occurred without any obvious tuber-like swelling and would occur even under long days. In all tissues containing large amounts of patatin, the other tuber proteins were also found as well as large amounts of starch.

A potato tuber is a stem which has become specialized as a storage organ (1). Despite a large number of studies on the effect of photoperiod, hormones, heat stress, and other stimuli (reviewed in 4 and 5), little is known about the biochemical mechanisms involved in tuberization. One reason for this has been the lack of a discrete biochemical marker for storage tissue differentiation which could be studied in a detailed and definitive manner. Here we present evidence that a family of immunologically identical glycoproteins with apparent mol wt of approximately 40,000 is a good candidate for such a biochemical marker.

This family of proteins was purified by Racusen and Foote (11) by chromatography on DEAE cellulose and Con A[3] Sepharose and given the trivial name 'patatin.' These investigators found that patatin was present in all of the 31 commercial and experimental potato cultivars they examined. While patatin gave only one protein band of constant hexosamine content during sucrose gradient ultracentrifugation and gel filtration on Sephadex G-200 (11), it could often be resolved into a number of bands by gel electrophoresis or by isoelectric focusing (9, 11). Despite this heterogeneity, immunoelectrophoresis and immunodiffusion have shown that all of the isoforms of patatin are immunologically identical both within a cultivar as well as between cultivars (9). A high degree of homology between the isoforms of patatin was also indicated by NH_2-terminal amino acid sequence analysis (9). Patatin appears to be the same as a family of the major tuber proteins isolated by Kosier and Desborough using solubility fractionation and HPLC (6). This family of proteins, referred to by these workers as 'tuberin,' has very high nutritional value (in one genotype, it had an essential amino acid index of 100) and enzyme activity in the general class of hydrolase (or esterase) (Kosier and Desborough, personal communication[4]).

In the work described here, we have used SDS-PAGE, rocket immunoelectrophoresis, and ELISA to measure the distribution of patatin in potato plants. We have found that patatin is normally present in large amounts only in tubers. Under certain conditions, however, patatin can be induced to accumulate in stems and petioles. In all cases, tissues which contained large amounts of patatin also contained the other major tuber proteins as well as large amounts of starch.

MATERIALS AND METHODS

Potatoes (*Solanum tuberosum* L. cv Superior) were obtained from Tatro Potato Seeds Farms, Inc., Antigo, WI. For some of the work, the highly photoperiod-dependent selection 77-5-1 from the Purdue potato breeding program was used.

For work with controlled photoperiod, plants were grown in a greenhouse as described previously (8) under 18 h light at 25°C and 6 h dark at 20°C until flowering. In some cases, plants were then induced to tuberize by placing under short photoperiod (8 h light).

To produce above-ground axillary bud tubers, plants (cv Superior) were grown and induced to tuberize as described above except that stolons and tubers were removed from around the crown as they formed.

Stem cuttings bearing a single leaf from plants growing under long or short photoperiod were placed in a moist mixture of perlite and vermiculite (3:1 by volume) under 18 h or 8 h photoperiod, respectively. In some cases, the axillary bud was removed with a razor blade. After harvest, the samples were frozen in liquid N_2 and stored at −70°C.

Patatin was purified by the method of Racusen and Foote (11) except that 0.1 g polyvinylpolypyrrolidone/g tuber was added before grinding. Recovery of total soluble protein and patatin was similar to that reported by these authors. For production of

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[3] Abbreviations: Con A, concanavalin A; PAGE, polyacrylamide gel electrophoresis; ELISA, enzyme-linked immunosorbent assay.

[4] Communicated to NCR-84 Potato Genetics Technical Committee, December 8–9, 1981.

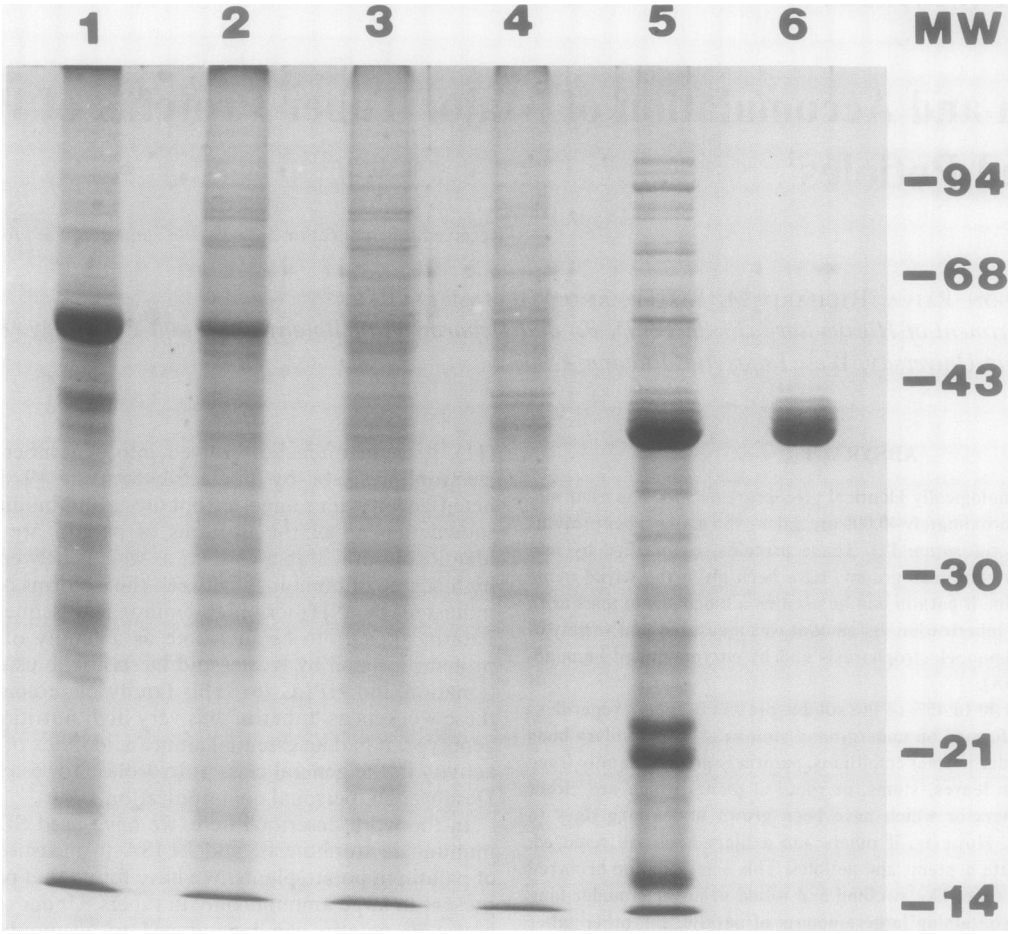

FIG. 1. SDS-PAGE of total SDS extractable protein from different tissues of cv Superior. Leaves (lane 1), stems (lane 2), and roots (lane 3) were from field-grown plants actively forming tubers. Stolon tips (lane 4) were from noninduced plants. Also shown are total extractable protein from mature tubers (lane 5), purified patatin (lane 6), and mol wt markers.

antibody, 1.5 mg patatin was emulsified with an equal volume of complete Freund's adjuvant and was injected into two sites in each thigh muscle of a New Zealand White rabbit. A second injection was given 2 weeks later using incomplete Freund's adjuvant. Serum was collected 4 to 6 weeks after the second injection.

Patatin was measured in tissue extracts either by rocket immunoelectrophoresis or ELISA. Tissues were homogenized in 3 volumes of 0.14 M NaCl, 1.4 mM KH_2PO_4, 8.0 mM $Na_2H\ PO_4$, 2.7 mM KCl, 0.02% (w/v) sodium azide, 0.05% (v/v) Tween-20, 2% (w/v) PVP-40. After squeezing through two layers of cheesecloth, the extracts were clarified by centrifugation for 2 min at 13,000*g* in a microcentrifuge. For rocket immunoelectrophoresis (7), samples were electrophoresed for 6 h at 5 v/cm in a 1-mm thick horizontal 1% agarose gel in 25 mM Tricine (pH 8.6) containing 0.02% sodium azide and a 1:150 dilution of antiserum.

For ELISA, alkaline phosphatase and *p*-nitrophenyl phosphate were used as conjugating enzyme and substrate, respectively. ELISA was by the double antibody sandwich method as described by Clark and Adams (3), except that Gilford EIA 'Cuvette-pack' plates were used, and the substrate reactions were measured with a Gilford PR-50 processor (Gilford Instrument Laboratory, Inc., Oberlin, OH). Coating and conjugated immunoglobulins, concentrated from the antiserum to patatin by two successive precipitations in half-saturated sodium sulfate, were used at 2.5 and 5.0 μg/ml, respectively. Substrate hydrolysis after 30 min was estimated colorimetrically (A_{405nm}) to compare patatin concentrations in various tissue extracts with a purified patatin standard. Dilution curves for the standard were plotted to test linearity of reaction with patatin concentration.

Table I. *Patatin Content of Tissues of Noninduced and Induced Potato Plants, cv Superior*

Noninduced plants were grown under LD (18-h photoperiod) to prevent tuberization. Induced plants were grown under field conditions and were actively forming tubers.

Tissue	Noninduced	Induced
	mg patatin/g fresh wt	
Leaf	<0.001	<0.001
Stem	<0.001–0.01	<0.001–0.01
Root	<0.001	<0.001
Stolon	<0.002	0.02–0.9[a]
Tuber		4.0

[a] Patatin level in stolon depends on location in the stolon and stage of growth.

Starch was measured by the method of Snyder *et al.* (12). Soluble protein concentrations in tissue extracts obtained as described above were determined by the method of Bradford (2) after precipitation with 8 volumes of cold 10% (w/v) TCA. BSA was used as a standard. SDS-PAGE was performed in a 12.5% acrylamide-0.33% Bis slab gel, as described previously (10).

RESULTS

When stolon tips differentiated to form tubers, they accumulated large amounts of several protein species (Fig. 1). By SDS-

PAGE, the major tuber proteins did not appear to accumulate in large amounts in leaves, stems, or roots of plants forming tubers.

To determine if the major tuber proteins are always found only in tubers, we used rocket gels and ELISA to measure the distribution of patatin in plants forming tubers and also in plants grown under LD to prevent tuberization. Mature (>200 g) field-grown tubers contained 4.0 ± 0.12 mg/g patatin (40–45% of the total soluble protein) (Table I). Significant amounts of patatin were also seen in stolons attached to developing tubers (Table I; Fig. 2B). Stolons attached to small rapidly growing tubers could contain up to 0.9 mg/g patatin in the region of the stolon immediately adjacent to the tubers. As tubers increased in size and their growth slowed, patatin levels in the stolons dropped.

By rocket immunoelectrophoresis, patatin normally was not detected in leaves, stems, or roots from either induced or noninduced plants, indicating that it made up less than 0.1% of the

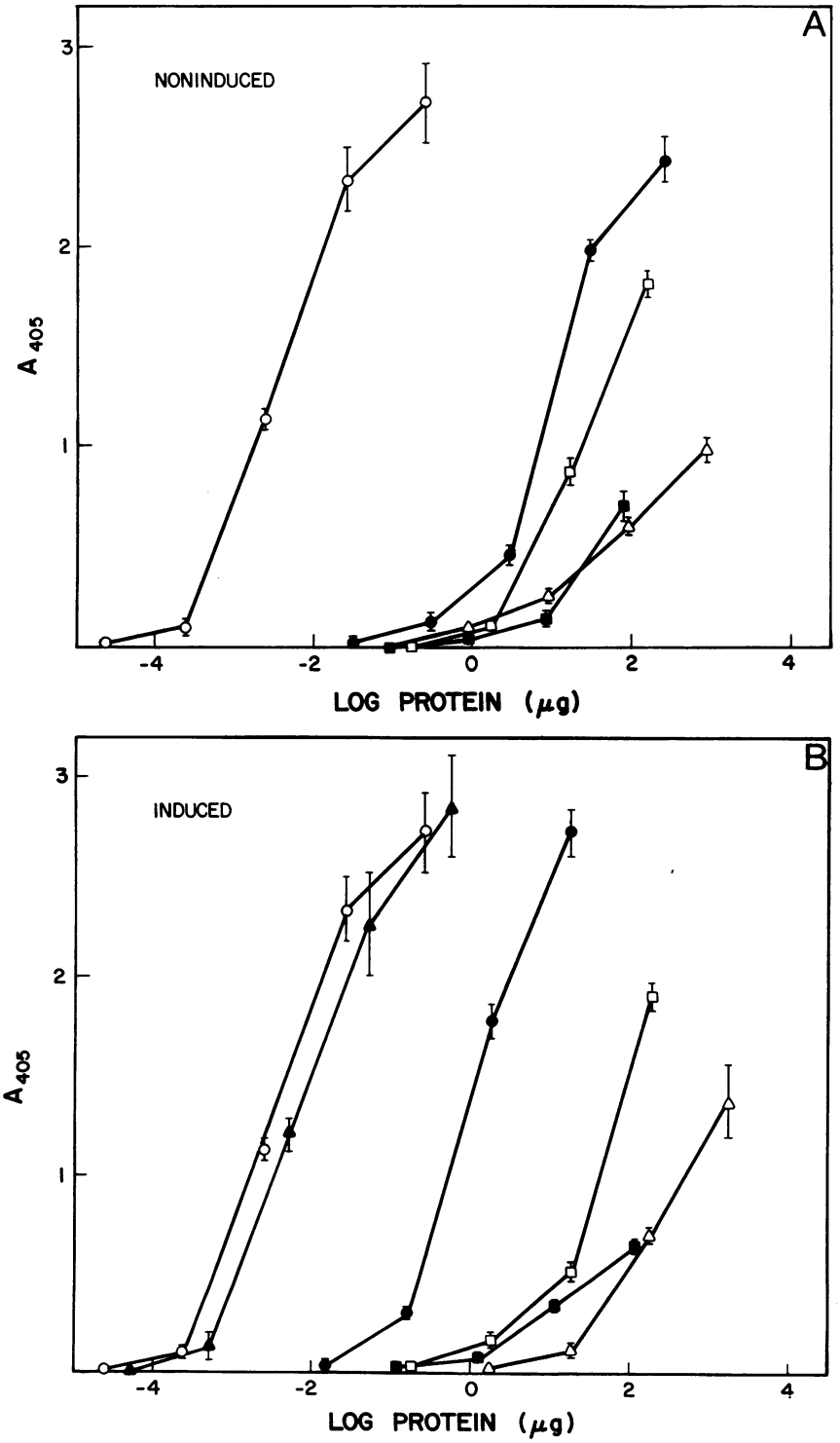

FIG. 2. ELISA assay of patatin in various dilutions of tissues from plants grown under 18-h photoperiod to prevent tuberization (A) or in field-grown plants actively forming tubers (B). Leaves (△), stems (□), stolons (●), roots (■), and tubers (▲) were assayed as described in "Materials and Methods." Also shown are the results obtained with purified patatin (○). Note linearity of reaction with patatin dilution.

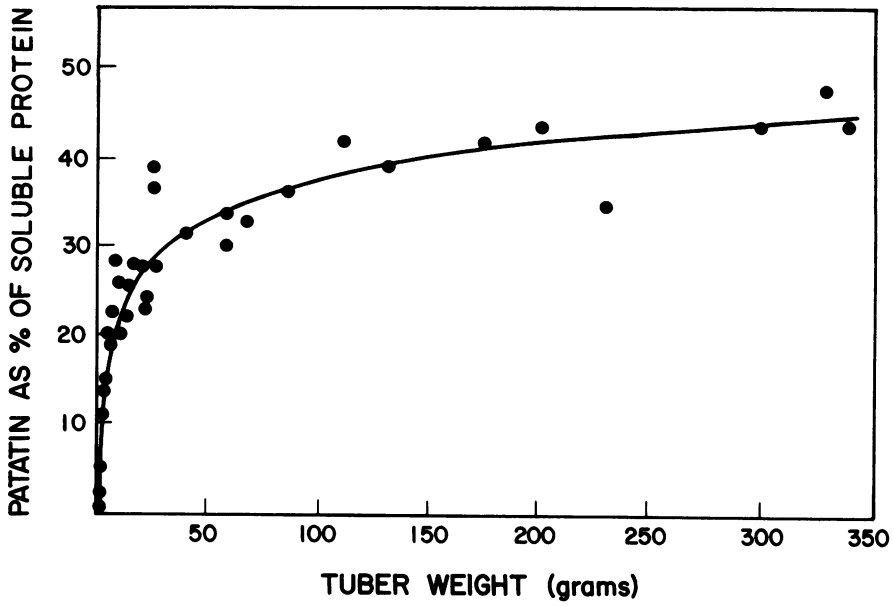

FIG. 3. Patatin content of tubers (cv Superior) of different weights. Tubers were harvested at different times during development and tubers of mean size or larger were assayed.

FIG. 4. Potato plants (cv Superior) forming above-ground tubers. Plants were grown under 8-h photoperiod until flowering, and were then induced to form above-ground axillary tubers by removing stolons and tubers from around the crown as they formed.

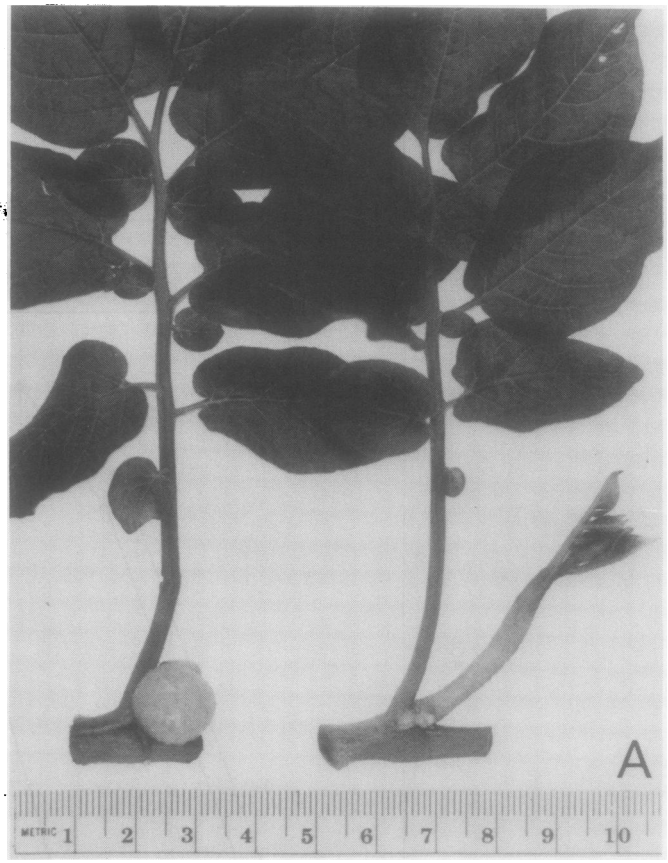

FIG. 5A. Development of axillary buds from stem cuttings of selection 77-5-1 grown under SD or LD. In stem cuttings grown under SD (left), the axillary buds developed as small tubers. Under LD (right), the axillary buds developed as shoots.

protein. Also, the slight reactions obtained by the more sensitive ELISA tests of extracts of stems, leaves, and roots could be due to nonspecific background reactions, and they were similar for both induced and noninduced plants (Fig. 2, A and B).

Although the amount of patatin in stolon tips from noninduced

plants was very low, it increased rapidly at the very first stages of tuberization (Fig. 3). There was a linear relationship between patatin as a percentage of total soluble protein and the logarithm of tuber weight from 0.3 to 300 g.

Table II. *Patatin Content of Plants with Above-Ground Tubers*

Plants, cv Superior, were induced to form above-ground tubers by removing stolons and tubers from the crown of the plant as they formed. Control plants were not disturbed and formed normal underground tubers.

Tissue	Plants with Developing Above-Ground Axillary Tubers[a]	Control Plants
	mg patatin/g fresh wt	
Leaf	<0.001	<0.001
Upper stem	0.05	<0.01
Lower stem	0.30	<0.01
Root	<0.001	<0.001
Underground tubers		4.0
Above-ground tubers	1.0–4.0	

[a] Patatin level in stems of plants with fully developed axillary tubers were similar to control plants.

Although tubers are normally formed on underground stolons, they can also be formed above-ground from axillary buds (Fig. 4). This condition is sometimes seen in the field as a result of injury or disease, but can also be produced by removing stolons and underground tubers as they form. Unlike normal plants, plants which were forming above-ground tubers contained elevated levels of patatin in their stems (Table II). This was particularly evident in the lower stems where most of the axillary bud tubers were forming. Fused rocket immunoelectrophoresis indicated that the patatin in stems was immunologically identical with that isolated from tubers. When axillary tuber growth stopped, the amount of patatin in stems declined (Table II). The formation of above-ground tubers did not cause an increase in patatin in leaves or roots (Table II).

To investigate further the control of patatin synthesis, we also used single-node stem cuttings of selection 77-5-1, a late season selection which is very photoperiod dependent. When stem cuttings were taken from plants grown under SD (8-h photoperiod) and placed in moist vermiculite under SD, the axillary buds grew out as small tubers (Fig. 5A). When similar stem cuttings taken

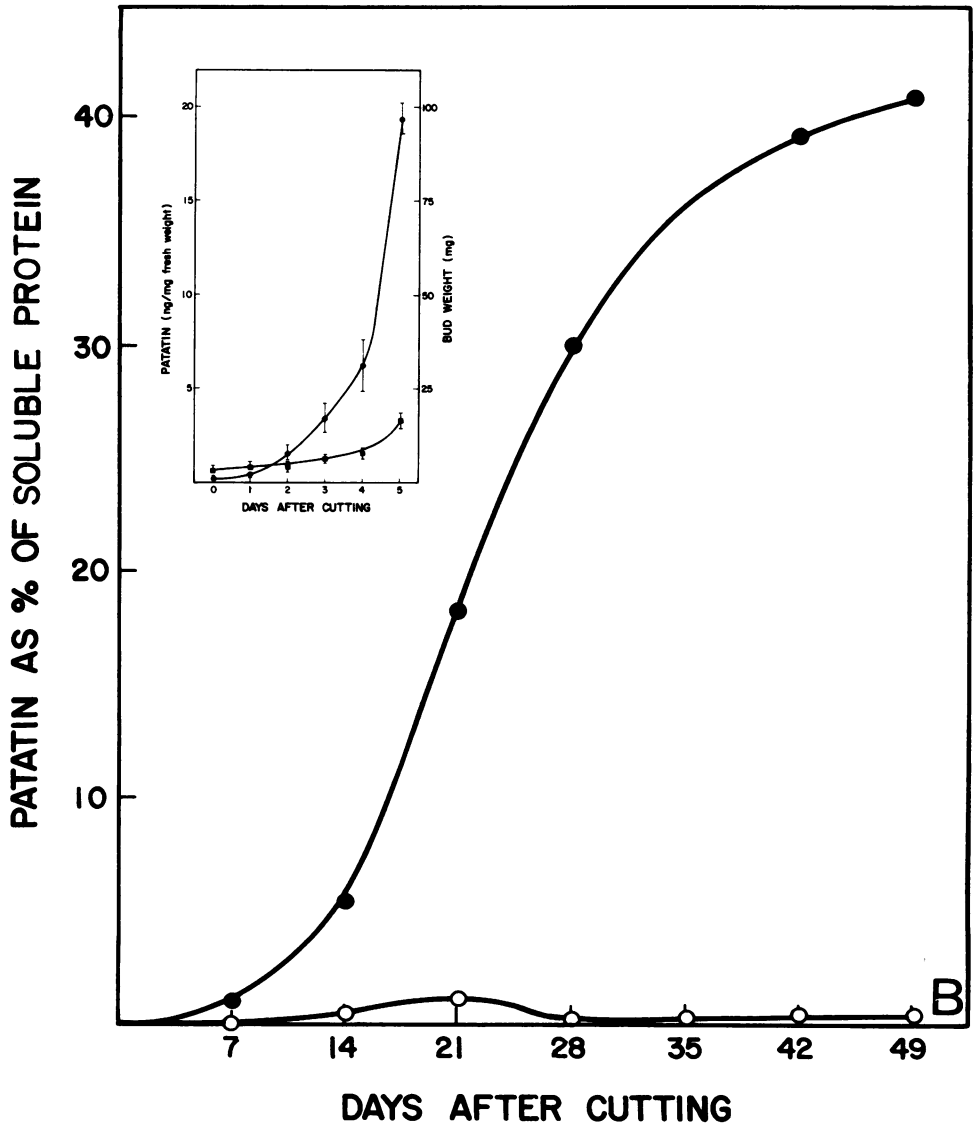

FIG. 5B. Patatin content of axillary buds developing as tubers (●) or shoots (○) as assayed by rocket immunoelectrophoresis. Patatin content measured by ELISA during the first 5 d of development as tubers is plotted against fresh weight of the buds (■) on an enlarged scale in the inset.

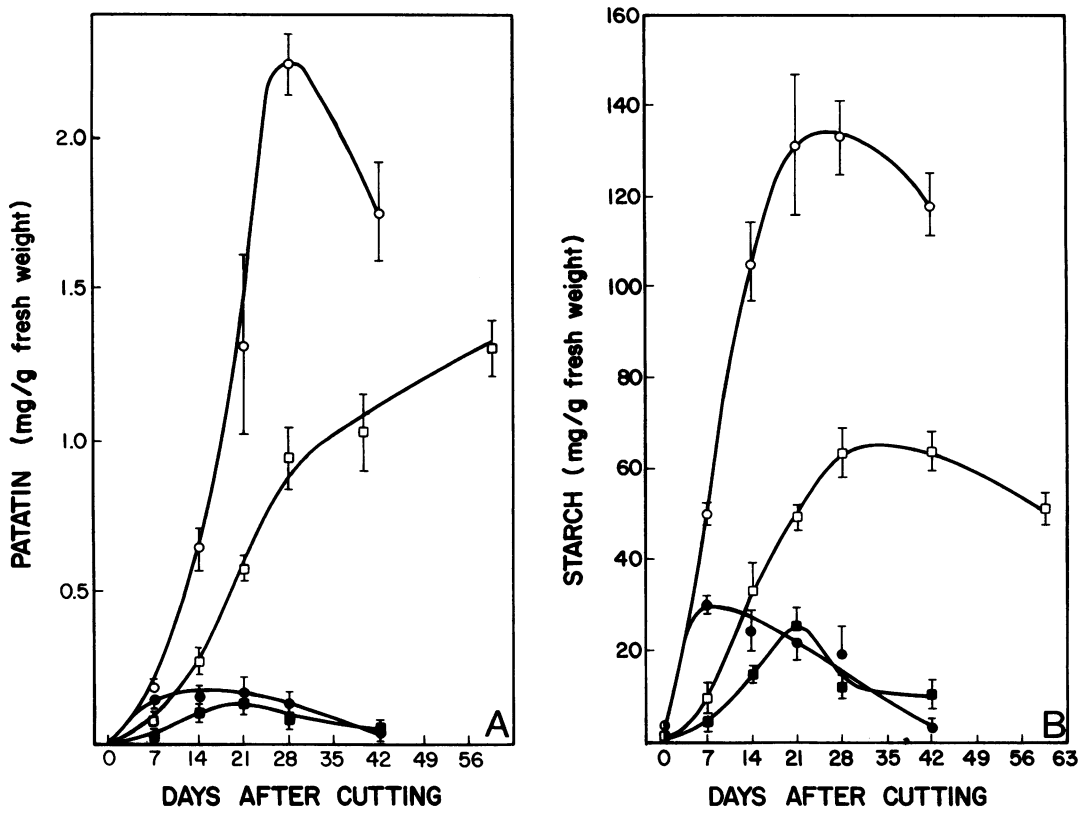

FIG. 6. Patatin and starch content of petioles from stem cuttings cultured with (●, ■) or without axillary buds (○, □). Single-leaf stem cuttings from the selection 77-5-1 were grown under SD (●, ○) or LD (■, □).

from plants grown under LD (18 h) were placed under LD, the buds grew out as shoots. In buds developing as tubers, patatin rapidly accumulated (Fig. 5B). After 42 d, patatin accounted for 40% of the total soluble tuber protein. As shown in the inset of Figure 5B, the patatin level increased substantially within 48 h—well before there was any significant increase in the fresh weight of the buds or change in their gross morphology. In contrast, when buds developed as shoots they slowly accumulated only a small amount of patatin, which decreased as the shoots began rapid expansion. In the petioles of stem cuttings in which the buds were developing as either tubers or shoots there was a small amount of patatin (approximately 0.2 mg/g) that accumulated transiently (lower portion of Fig. 6A). The level of patatin in these petioles and its transient nature paralleled the results for the stems of plants with above-ground tubers described above (Table II).

Taken together these results suggested that the accumulation of patatin in stems or stem-like tissue might be due to the absence of adequate alternate sinks. To test this hypothesis, we assayed patatin in the petioles of stem cuttings from which the axillary buds had been removed (also shown in Fig. 6A). Under SD, the level of patatin in the petioles of stem cuttings without axillary buds increased to 2.2 mg/g of tissue. This level was comparable to that seen in normal underground tubers of this variety (2.3 ± 0.13 mg/g). Surprisingly, we found that if the axillary bud was removed patatin also accumulated in large amounts in petioles of cuttings grown under LD. In LD cuttings, the rate of patatin accumulation was slower, but the level increased for at least 56 d reaching a level of 1.3 ± 0.1 mg/g. Unlike cuttings from SD plants grown under SD, the LD cuttings did not senesce after 30 d.

Petioles from stem cuttings without buds also contained large amounts of starch (Fig. 6B). There was a correlation between starch and patatin levels ($r = 0.88$) in the petioles of stem cuttings with or without axillary buds, under both LD or SD, from 7 to 42 d after cutting. Even though there was no obvious tuber-like swelling (7A), the amount of both starch and patatin in the lower two-thirds of the petiole (Fig. 7B, bars A and B) was comparable to that seen in normal underground tubers. The level of starch and patatin in the section of stem was comparable to that in the lower petiole, but was more variable, perhaps due to the presence of the large wounds.

In contrast to the petiole and stem, patatin did not accumulate in the leaves of stem cuttings with or without axillary buds. Even after 28 or 60 d under either short or long photoperiod, the level of patatin in leaves was less than 0.01 mg/g of tissue.

When the starch-containing petioles were examined during development by SDS-PAGE (Fig. 8), it was found that in addition to patatin, the other major tuber proteins were also present. These other proteins arose in a coordinate fashion with patatin, as was the case during normal tuber development. Also, they were present in approximately the same relative amounts as in tubers. Similar results were obtained when the proteins in these petioles were compared with those in tubers by nondenaturing polyacrylamide gels at pH 8.6 (data not shown). Ouchterlony double immunodiffusion tests and immunoelectrophoresis indicated that the proteins in starch-containing petioles were immunologically the same as those in tubers (data not shown).

DISCUSSION

A potato tuber is a region of stem that has differentiated to form a storage organ by expanding radially and becoming filled with starch and a characteristic group of proteins. In the work described here, we have found that the major tuber protein, patatin, was present in tubers regardless of whether they were formed from axillary buds or from stolon tips. Under normal conditions, patatin was present in only trace amounts if at all in other tissues.

If alternate sinks were removed, however, patatin accumulated in large amounts in stems and petioles. This accumulation occurred without any obvious tuber-like swelling and would occur

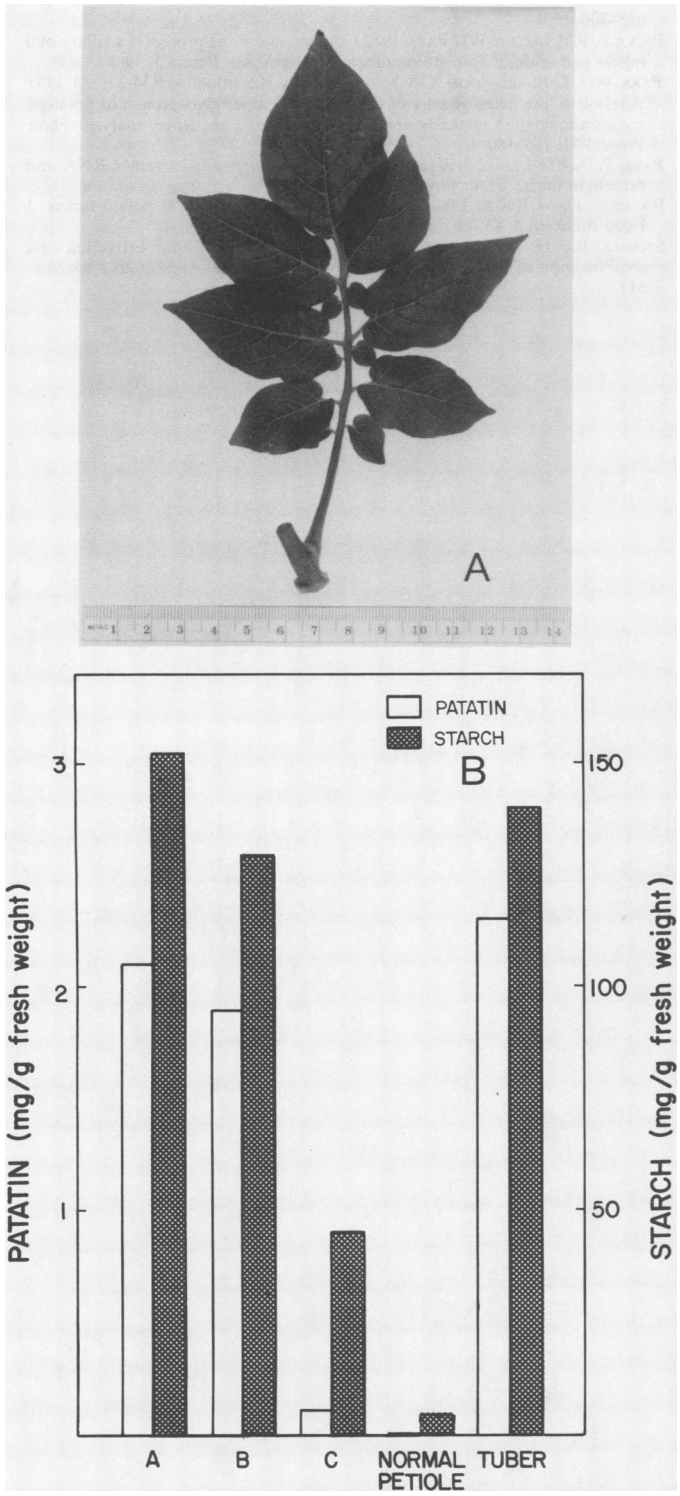

FIG. 7. Single-leaf stem cutting (A) with the axillary bud removed, kept under 8-h photoperiod for 28 d. Patatin and starch content (B) of petiole of the cutting shown in A. Bars A–C refer to the lower, middle, and upper thirds of the petiole. Also shown (B) are results from an underground ('normal') tuber and the lower third of a normal petiole from an intact plant under SD.

even under LD. In all cases, tissue which contained large amounts of patatin also contained the other major tuber proteins as well as large amounts of starch.

These data suggest that the biochemical differentiation of somatic storage tissue can occur independently of the morphological

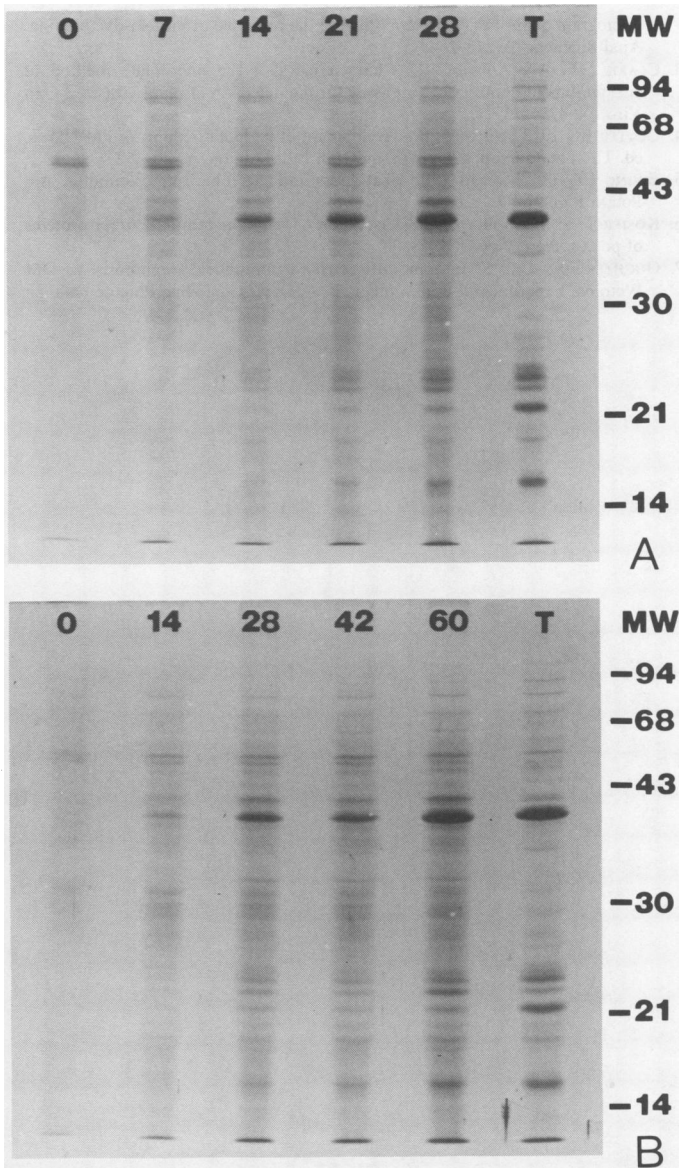

FIG. 8. SDS-PAGE of total soluble protein in petioles of selection 77-5-1 cuttings without axillary buds during growth under SD (A) or LD (B). The numbers above the lanes indicate the number of days after cutting. Also shown is the SDS profile of normal underground tubers of this selection (T).

changes normally associated with tuberization. This differentiation apparently can occur in any stem or petiole, but we have no evidence of its occurrence in other tissues such as roots or leaves.

The data also suggest that patatin may be a very useful biochemical tool to study the process of somatic storage tissue differentiation. Patatin is very easy to measure by rocket immunoelectrophoresis or ELISA and can also be studied at the nucleic acid level. It will be interesting to examine how patatin synthesis is affected by photoperiod, hormones, and other stimuli as well as to compare the regulation of patatin synthesis in potato cultivars which differ in characteristics such as photoperiod responsiveness, heat resistance, and starch and protein content.

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