JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

J. Agric. Food Chem. **2002,** *50,* 1939−1944 **1939**

Characterization of the Major Proteins of Tubers of Yam Bean (*Pachyrhizus ahipa*)

JANE L. FORSYTH AND PETER R. SHEWRY*

IACR Long Ashton Research Station, Department of Agricultural Sciences, University of Bristol, Long Ashton, Bristol BS41 9AF, U.K.

Tubers of six accessions of ahipa (*Pachyrhizus ahipa*) contained between 0.77 and 1.34% nitrogen on a dry weight basis. This corresponds to 4.8 to 8.4% crude protein based on a nitrogen to protein conversion factor of 6.25; but detailed analysis of AC230 showed that although 93% of the total N was extracted with buffer containing 1.0 M NaCl, about a third of this was lost on dialysis. It was calculated, therefore, that salt-soluble proteins comprise about 60% of the total tuber nitrogen, with low-molecular-mass nitrogenous components comprising a further 30%. Electophoretic analysis of the salt-soluble proteins showed similar patterns of components in the six accessions, with none being present in amounts sufficiently high to suggest a role as storage proteins. Furthermore, light microscopy failed to show significant deposits of protein within the tuber cells. Five "major" protein bands, which together accounted for about 19% of the total salt-soluble protein fraction were purified and subjected to *N*-terminal amino acid sequencing. Comparison of these with sequences in protein databases revealed similarities to α-amylases, chitinases and chitin binding proteins, cysteine proteinases (including major components from *P. erosus* tubers), a tuberization-specific protein from potato, and proteins induced in soybean and pea by stress or the plant hormone abscisic acid, respectively. It was concluded that the primary roles of these proteins are probably in aspects of tuber metabolism and development and/or conferring protection to pests and pathogens, and that true storage proteins are not present. The absence of storage proteins is consistent with the biological role of the tubers as storage organs for carbohydrates (cf cassava tuberous roots) rather than as propagules (cf yam and potato tubers).

KEYWORDS: ***Pachyrhizus*; yam bean**

INTRODUCTION

Tuber crops are widely grown, particularly in the tropics where cassava (*Manihot esculenta*, Euphorbiaceae), sweet potato (*Ipomoea batatas*, Convolvulaceae), and yams (*Dioscorea* spp, Dioscoreaceae) are the most important species. Similarly, potato (*Solanum tuberosum*, Solanaceae) and sugar beet (*Beta vulgaris*, Chenopodiaceae) are important in temperate countries. Despite the fact that tuberous organs may have different botanical origins (roots, stems, or hypocotyls) and the crop species are not closely related taxonomically, all are characterized by storing high levels of carbohydrates (starch and/or sugars) with low protein. Hence, they must be combined with more protein-rich foods (e.g., legume seeds or animal protein) to provide the protein requirements of humans or livestock.

Pachyrhizus is a genus of legumes, native to South and Central America, with several species being cultivated for their tuberous roots rather than seeds. These are *P. tuberosus*, which is cultivated in Bolivia, Peru, Equado, and Brazil; *P. erosus*, which is cultivated in Central America and the Caribbean; and *P. ahipa* (ahipa), which is native to the Andean valleys of Bolivia and Northern Argentina (*1*, *2*). Although the tubers of these species are rich in carbohydrates, they have also been reported to contain between about 5 and 15% crude protein (*2*−*6*), which is between 1.5 and 5 times the protein levels reported for other tuberous crops (*7*).

We have therefore reinvestigated the protein composition of *P. ahipa* tubers to determine the total amount and identify major components which could contribute to its nutritional quality or be exploited in the food industry.

MATERIALS AND METHODS

Plant Cultivation. Seeds of AC230 were sown in 10-cm pots (1 L) containing a mixture of a peat-based compost (Levington M2) and a slow release fertilizer (Osmocote, 4.1 g/L). The plants were kept at 25−30 °C with a 16-hour day. Once established (about 6 true leaves), the plants were transferred to 20-cm pots (5 L) containing supports and watered automatically via capillary matting. Reproductive pruning was carried out to ensure tuberization (*8*) and to minimize the effects of day length.

Localization of Tuber Protein. To establish the distribution of the protein within the tubers, tissue prints of both longitudinal and transverse

* To whom correspondence should be addressed [telephone +44 (0) 1275 392181; fax +44 (0) 1275 394299; e-mail peter.shewry@bbsrc.ac.uk].

10.1021/jf011017j CCC: $22.00
Published on Web 02/26/2002

sections were taken. The cut surface of the tuber was blotted dry with tissue to prevent smudging and to remove excess starch, and then pressed firmly onto nitro-cellulose membrane presoaked in 10% C-TBST-75. The membrane was placed in a total protein stain (60% (v/v) ethanol, 20% (v/v) acetic acid, 20% (v/v) distilled water, and 1 mg of Coomassie BB R250/cm^3), and then destained (60% (v/v) ethanol, 20% (v/v) acetic acid, and 20% (v/v) distilled water).

Protein Extraction. Tubers of the six *P. ahipa* varieties (AC102, 229, 230, 251, 254, 526) and *P. erosus* (*cv.* Catalina) were peeled, cut into 2-cm^3 pieces, and freeze-dried. The dry pieces were ground to flour which was sampled for total N analysis (Kjeldahl method, *9*). The remainder of the flour was used for extraction. A 100-g portion of flour was mixed with 1 L of extraction buffer (0.05 M sodium phosphate buffer, pH 7.4, 100 mM PMSF, 1 M NaCl) and stirred continuously for 2.5 h at 4 °C. The mixture was then filtered through two layers of muslin. The solid residue was retained, and the filtrate was centrifuged at 10,000 rpm (4 °C) for 25 min. The supernatant was collected and stored at 4 °C. The pellet was combined with the residue from the muslin filtration, re-suspended in 1 L of extraction buffer, and stirred at 4 °C for 2.5 h. This process was repeated three times. The supernatants from the three extractions were pooled and labeled the salt soluble fraction. An aliquot of this fraction was retained for total N analysis and SDS-PAGE using 12% Tris/glycine gels (*10*). The gel separations were quantified using the Bio-Rad Geldoc 1000 system.

Proteins remaining after extraction of the soluble fraction were extracted from the residue using 1 L of sodium phosphate buffer (pH 7.4, containing 1% (w/v) SDS and 2% (v/v) 2-mercaptoethanol). The solution was stirred at 4 °C for 2.5 h, followed by centrifugation (10,000 rpm, 4 °C, 25 min). The supernatant (**insoluble fraction**) was retained, dialyzed against distilled water overnight, and freeze-dried for total N analysis and SDS-PAGE. The residue was retained for total N analysis to determine the efficiency of the extraction process.

The **soluble fraction** was further fractionated using ammonium sulfate precipitation at 30, 50, and 90% saturation. Following centrifugation at 10,000 rpm (4 °C), the pellets were re-suspended in 0.05 M sodium phosphate buffer, pH 7.4, and dialyzed (using a low cut-off membrane M_r 3,500) against distilled water overnight. The three fractions were then freeze-dried, and a sample of each was separated by SDS-PAGE. To purify the individual proteins, a sample of each fraction was re-suspended in 0.05 M Tris/HCl buffer, pH 7.5, and separated on an anion exchange column (DEAE Sepharose). Bound proteins were eluted with a 0−0.5 M linear gradient of NaCl. The fractions from peaks containing protein were pooled, dialyzed against water, freeze-dried, and separated by SDS-PAGE.

Five fractions were selected for *N*-terminal amino acid sequence analysis. These samples were separated on 10% Tris/Tricine gels (*11*) and blotted onto Pro Blot PVDF membrane, and stained for protein, and the bands of interest were excised for sequencing. Automated Edman degradation was carried out using a pulsed liquid mode PVDF program on an Applied Biosystems model 491 procise sequencer.

Western Blotting. The tuber salt-soluble proteins and total seed proteins (0.25 mg of crushed seed in 1 mL of sample buffer; 0.0625 M Tris/HCl, pH 6.8, 2% (w/v) SDS, 10% (v/v) glycerol, 5% (v/v) 2-mercaptoethanol, and 0.002% (w/v) bromophenol blue) were separated by SDS-PAGE using 12% Tris/glycine gels (*10*). The separated proteins were blotted onto nitro-cellulose membrane and incubated with polyclonal antibodies raised against pea globulin proteins (legumin, vicilin, and convicilin) (1:100 dilution) followed by a goat anti-rabbit alkaline phosphatase conjugate secondary antibody (1:6250). Bands were visualized using BCIP/NBT alkaline phosphatase color development solution.

Total Nitrogen Analysis. The total N content was calculated using Kjeldahl analysis (*9*). A typical sample size of 0.5−0.7 g dry weight was used and analyzed using KB8S Kjedatherm digestion system (Gerardt).

RESULTS

Comparison of the Protein Content and Composition of *P. ahipa* Accessions and *P. erosus*. Six accessions of *P. ahipa* were grown in a replicate random block experiment in the glasshouse at IACR-Long Ashton with *P. erosus* cv. Catalina being grown under similar conditions. Samples of peeled tubers from the five replicate plants were bulked, and their dry weights and nitrogen contents were determined. The *P. ahipa* tubers contained about 75−80% water with Kjeldahl N contents ranging from about 0.77 to 1.34% dry weight (**Table 1**). Assuming a conversion factor of 6.25, this corresponds to between 4.8 and 8.4% protein on a dry weight basis and 1.3 to 2.3% on a fresh weight basis. In contrast, the *P. erosus* tubers contained more water (88.6%) and less protein, 2.7 and 0.35% on dry and fresh weight bases, respectively (**Table 1**).

Table 1. Contents of Water, Nitrogen, and Total Protein (N × 6.25) in Tubers of Accessions of *P. ahipa* and *P. erosus* cv. Catalina

species	accession/ cultivar	H_2O % FW	N % DW	protein % FW	protein % DW
P. ahipa	102	79.0	1.34	2.22	8.4
P. ahipa	229	77.5	1.02	1.85	6.4
P. ahipa	230	78.8	0.77	1.28	4.8
P. ahipa	521	78.0	0.77	1.36	4.8
P. ahipa	524	80.0	1.23	1.92	7.7
P. ahipa	526	76.0	1.18	2.33	7.4
P. erosus	*Catalina*	88.6	0.43	0.35	2.7

Preliminary studies with freeze-dried tubers of *P. ahipa* AC230 showed that 93% of the total tuber nitrogen was extracted with 0.05 M phosphate buffer, pH 7.4, containing 1.0 M NaCl and PMSF to inhibit endogenous proteinases. Of this, about 31% was lost on dialysis with a low cut-off (M_r 3500) membrane, demonstrating that about 62% of the total tuber nitrogen consisted of salt-soluble proteins and about 31% low M_r nitrogenous compounds (presumably peptides, amino acids, etc.). A further 3.3% of the nitrogen was recovered by further extraction with the same buffer containing 1% (w/v) SDS and 2% (v/v) 2-mercaptoethanol (with 1.5% being lost on dialysis) leaving about 2% of the total tuber nitrogen in the insoluble residue. It was, therefore, concluded that the bulk of the tuber nitrogen comprised salt-soluble proteins, and further studies were focused on this fraction.

SDS-PAGE of the salt-soluble proteins from all six *P. ahipa* accessions showed that each contained multiple components with no apparent differences between them (**Figure 1**). In particular, all contained five "major" bands of M_r about 44,000, 30,000, 27,000, 22,000, and 17,000 (labeled P1−5 in **Figure 1**, track g) which were calculated by image analysis of gel separations of AC230 to account for about 2.1, 5.4, 6.4, 2.4, and 2.8%, respectively, of the total fraction. Therefore, the five proteins together accounted for about 19% of the total fraction. Because the salt-soluble proteins accounted for 62% of the total nitrogen in AC230, it can be calculated that P1−P5 together accounted for about 12% of the total nitrogen in this accession.

No bands corresponding to the major seed storage proteins of *P. ahipa* (see below) were observed in the salt-soluble protein fractions from *P. ahipa* or *P. erosus* tubers and no reactions were observed when western blotting was carried out with antibodies to the seed storage globulins (legumin, vicilin, and convicilin) of pea (results not shown). This contrasts with the results obtained when *P. ahipa* seed proteins were blotted (see below) and indicates that the tubers do not contain typical legume seed-storage globulins.

Purification of P1−P5 from Tubers of *P. ahipa* AC230. The total salt-soluble proteins were initially fractionated by precipitation with ammonium sulfate (to 30, 50, and 90% saturation), and the three precipitates were separated by ion

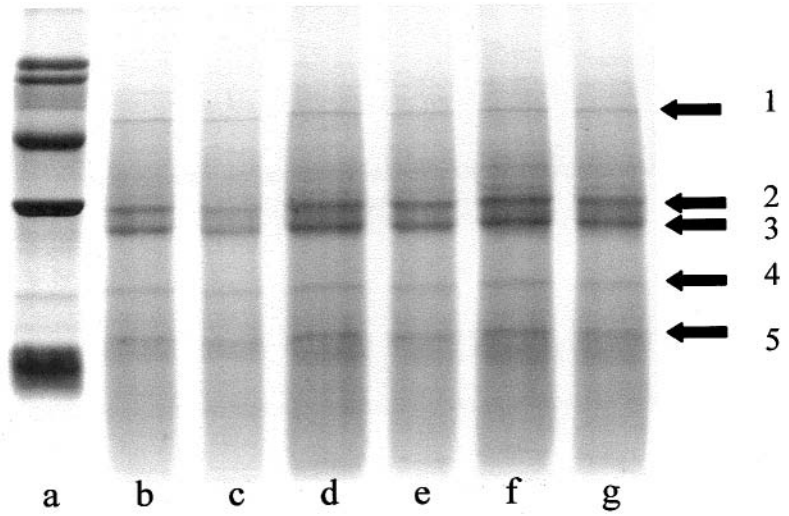

Figure 1. SDS-PAGE of total protein fractions extracted from *P. ahipa* tuber samples: (a) marker proteins of M_r 76,000, 66,000, 42,000, 30,000, and 17,000; (b–g) fractions from AC 102, 229, 230, 521, 524, and 526, respectively. The arrows indicate the five "major" proteins discussed in the text.

Table 2. *N*-terminal Sequences and M_r Determined for Proteins P1 to P5 from Tubers of *P. ahipa* Compared with Those of Related Proteins Identified in the SWISS–PROT Database

species	protein	sequence	M_r	reference
P. ahipa	P1	[1]AL(L)F(Q)(G)(F)(N)[8]	44,000	this study
wheat	α-amylase	[2]QI LFQGFNWE[11]	45,370	*12*
rice	α-amylase	[3]QI LFQGFNWD[12]	48,872	*32*
P. ahipa	P2	[1]DDLPDYVDDR[10]	30,000	this study
P. erosus	cysteine proteinase YGB1	[1]DDLPDYVDXR[10]	28,000	*13*
P. erosus	cysteine proteinase YGB2	[1]DDLPDYVDWR[10]	26,000	*13*
P. ahipa	P3	[1]AEPEPVVDKQ[10]	27,000	this study
potato	tuberisation specifc protein	[56]EEPAPVVEKE[65]*	22, 638	*18*
P. ahipa	P4	[1]EQ(C)GRQAGGK[10]	22, 000	this study
rubber	hevein	[1]EQCGRQAGGK[10]	4695	*15*
arabidopsis	chitinase	[1]EQCGRQAGGA[10]	34,609	17
bean	chitinase	[1]EQCGRQAGGA[10]	35,444	16
P. ahipa	P5	[1]GVFVFSDETS[10]	17,000	this study
soybean	stress response protein	[2]GVFTFEDEIN[11]	16,772	19
pea	ABA-response protein	[2]GVFVFDDEYV[11]	16,629	20

* *N*-terminus not known.

exchange chromatography on DEAE-Sephacel. SDS-PAGE showed that individual fractions from the ion exchange separations were enriched in bands P1−5, which were blotted from the SDS-PAGE gels onto Pro-Blot membrane for *N*-terminal sequencing. The sequences obtained for the first 10 amino acid residues of P1−P5 are compared with those of related proteins identified in the SWISS−PROT database in **Table 2**.

The sequence determined for P1 showed homology with the *N*-terminal sequences of cereal α-amylases and this identification is also consistent with the M_r determined for P1 by SDS-PAGE: approximately 44,000 compared with 45,000 for α-amylases (*12*). Similarly, P2 of *P. ahipa* (M_r approximately 30,000) is clearly homologous with two cysteine proteinases purified from tubers of *P. erosus* (M_r 26,000 and 28,000) by Gomes et al. (*13*) and with a developmentally regulated cysteine proteinase from the slime mold *Dictyostelium* (*14*) (sequence not shown). The sequence of P4 is related to the chitin-binding domains of basic (class I) chitinases (including the bean and *Arabidopsis* proteins shown in **Table 2**) and other chitin-binding proteins including lectins (not shown) and hevein from rubber latex (also shown in **Table 2**) (*15*). These chitin binding domains are at the *N*-termini of the mature hevein and chitinase proteins, as in P4. The M_r of P4 (approximately 22,000) differs from those of the mature herein polypeptide (4695) and those of basic chitinases (the bean and *Arabidopsis* enzymes having M_r of approximately 35,000 and 34,000, respectively (*16, 17*)).

P. ahipa proteins P3 and P5 also showed homologies with proteins in the SWISS-PROT database, although the functions of these proteins can only be inferred from their regulatory properties. Thus, P3 showed homology with an internal sequence in a protein encoded by a tuberization-specific gene from potato (Accession number P33191, entry name TUB8_SOLTU) (*18*), whereas P5 showed homology with proteins encoded by mRNAs induced by stress (including possibly osmotic stress) in soybean (*19*) and the hormone abscisic acid (ABA) in pea (*20*). The Mr of P5 (≃ 17,000) was also similar to those of the soybean (16,772) and pea (16,629) proteins. As ABA is itself induced by water stress (*20*), it can be suggested that P5 plays a role in the response of the tuber to water availability.

Localization of Protein in *P. ahipa* AC230 Tubers. The homologies demonstrated for P1−P5 are consistent with these proteins playing roles in the development and metabolism of the tuber, in defense against pests and pathogens, or in resistance to abiotic stresses, rather than as storage proteins. We therefore carried out analyses to determine whether protein deposits were present in the mature tubers. Initial studies by tissue printing (**Figure 2**) showed that protein was uniformly distributed across the tuber. Blocks of tissue from within the tuber were, therefore, sectioned and stained for protein for light microscopy. The staining was largely restricted to the cell walls and cytoplasm with no evidence for protein accumulation in the vacuole as reported for storage proteins in a range of systems including storage tubers of yam (*Dioscorea rotundata*) (*21, 22*) and sweet potato (*23*), and seeds of many species including legumes (*24*). Similarly, there was no evidence for accumulation of protein elsewhere in the cell (results not shown). It was concluded therefore, that tubers of *P. ahipa* do not accumulate deposits of specialist storage proteins.

Analysis of *P. ahipa* Seed Proteins. Comparative studies were also carried out on seeds of the six accessions of *P. ahipa*. Their water contents varied from about 5.1 to 5.6%, with protein contents calculated (as N × 6.25) to range from 42.9 to 48.6% dry weight.

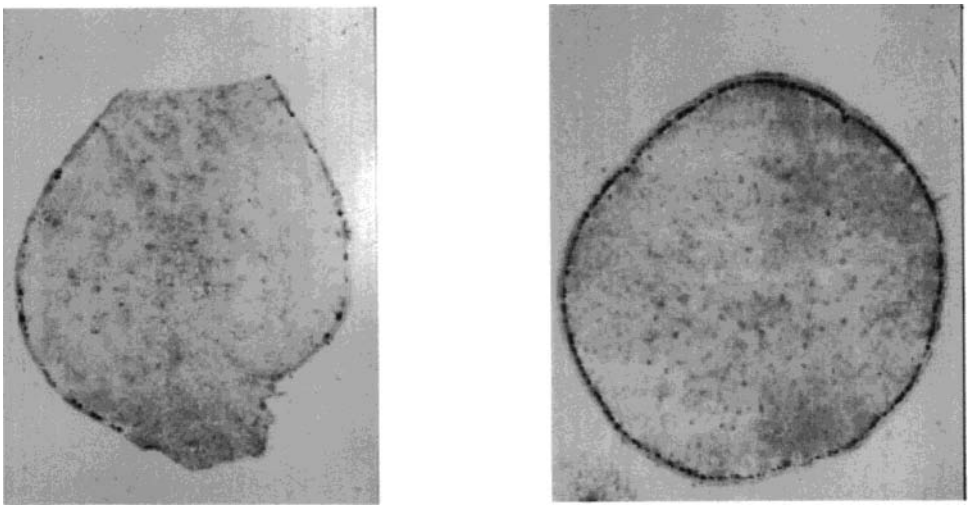

Figure 2. Longitudinal and transverse sections of a tuber of *P. ahipa* (AC230) stained for total protein.

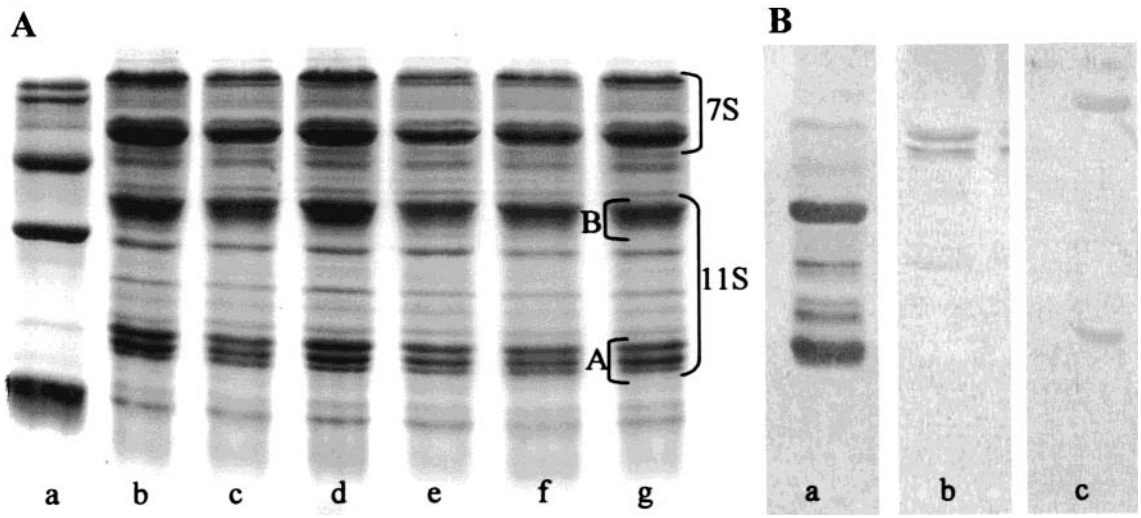

Figure 3. SDS-PAGE of total protein fractions extracted from seeds of *P. ahipa*. (A) Gel stained with Coomassie BBR250: (a) marker proteins of M_r 76,000, 66,000, 42,000, 30,000, and 17,000; (b–g) fractions from AC102, 229, 230, 521, 524, and 526, respectively. The major groups of 11S and 7S globulin subunits are indicated in track g. (B) Western blots of the fraction from AC230 reacted with antibodies to (a) 11S legumin; (b) 7S vicilin; and (c) 7S convcillin proteins of pea.

SDS-PAGE of the total seed proteins showed multiple bands with M_r ranging from below 20,000 to above 80,000 (**Figure 3A**). Western blotting with antibodies to 11S (legumin) (**Figure 3B** track a) and 7S (vicilin and convicilin) (**Figure 3B** tracks b and c) storage globulins of pea revealed the presence of related subunits in *P. ahipa* seeds. Groups of components of M_r about 22,000 and 35,000 (brackets A and B in **Figure 3A** track g) clearly corresponded to the major acidic and basic chains, respectively, of 11S globulin subunits and were estimated to account for about 14% and 14.5%, respectively, of the total fraction. However, the western blotting showed the presence of additional immunoreactive bands of intermediate M_r. Bands of about M_r 25,000, 50,000, and 70,000 reacted with the antibodies to the pea 7S globulins vicilin and convicilin.

DISCUSSION

Our results show that tubers of *P. ahipa* contain between about 0.8 and 1.3% nitrogen on a dry weight basis. Although multiplication by a standard factor of 6.25 would indicate that this represents about 5−8% protein, analyses of one accession (AC230) indicate that only 60−70% of the total nitrogen actually derives from protein. The true values are, therefore, likely to range from about 3 to 5% crude protein. This value is certainly higher than the protein contents reported for cassava (1−2% dry weight, (*25*)) and yams (1−3% dry weight, (*26*)) and is within the range reported for sweet potato (1−10% dry weight, (*27*)) and potato (calculated as about 3−6% dry weight based on data in Burton (*28*)). However, it is below the values reported for *P. ahipa* by others, 5−11% by Gruneberg et al. (*4*), 5−10% by Grum et al. (*5*), about 7% by Tadera et al. (*3*), and about 8−20% by Sørensen et al. (*2*). Similarly, the value of over 9% protein reported for yam bean (presumably *P. erosus*) by Barnes and Gomes (*7*) is considerably higher than that determined for *P. erosus* here (2.7%). The protein contents of tuberous crops can be expected to vary with growth conditions, but the levels in field grown tubers are unlikely to exceed those of tubers grown under more optimal conditions in the glasshouse. It can be concluded, therefore, that the protein content of *P. ahipa* tubers is sufficiently high to contribute significantly to its nutritional properties but not high enough for it to be grown as a specialized protein crop.

Over 60% of the total nitrogen in *P. ahipa* tubers was present in nondialyzable salt-soluble components (presumably proteins) and these accounted for about 90% of the total protein in the

tuber. SDS-PAGE of the salt-soluble proteins showed a range of components but none appeared to be present in sufficient quantities to suggest a storage function. Similarly, light microscopy of tubers failed to show any protein deposits within the tuber parenchyma cells.

Previous studies of other storage tubers have shown that their storage protein components appear to be derived from different protein families, and may have secondary or residual activities. Thus, patatin from potato exhibits activity as an esterase and lipid acyl hydrolase (*29*, *30*), sporamin from sweet potato is related to Kunitz-type proteinase inhibitors (*23*), and dioscorin from yam is related to carbonic anhydrase (*22*, *31*). Typical seed storage proteins have not been reported to occur in these tubers, and the 7S and 11S seed storage globulins which are typical of legume seeds were similarly not detected in tubers of *P. ahipa*, although they were clearly major components in the seeds.

The apparent lack of storage proteins in *P. ahipa* tubers resembles the situation in cassava and may relate to the biological role of the storage roots in these two species. Both cassava and *Pachyrhizus* are perennial crops in which the tuberous roots act as storage organs for carbohydrates rather than as propagules. In contrast, the tubers of yam, sweet potato, and potato act as propagules and contain storage proteins for mobilization during sprouting and plant regrowth.

Although no individual components accounted for high proportions of the total protein in *P. ahipa* tubers, five bands were clearly distinguishable and were partially purified for *N*-terminal amino acid sequencing. Three of these (P1, P2, P4) were related to proteins of known function which can be proposed to be involved in starch metaboloism (α-amylase) and defense against pests and pathogens (cysteine proteinases (*33*) and chitin-binding proteins). However, only one major cysteine proteinase of M_r about 30,000 was identified in *P. ahipa*, compared to two of M_r about 26,000 and 30,000 reported by Gomes et al. (*13*) for *P. erosus*. The other two major proteins, P3 and P5, showed homology with proteins encoded by mRNAs associated with tuberization in potato (P3) and induced by stress or ABA in soybean and pea, respectively (P5). These proteins may, therefore, be involved in the regulation of tuber metabolism, in response to either endogenous developmental signals or exogenously applied stresses. It can be concluded therefore, that ahipa tubers do not contain specialized storage proteins which are present in sufficient amounts to affect their nutritional quality. Nevertheless, the high total protein content of the tubers compared with that of most other tuberous crops could be significant if used for feeding livestock.

ACKNOWLEDGMENT

We thank Dr. Rod Casey (John Innes Centre, Norwich, UK) for providing antibodies to pea globulins and Mr. Bo Ørting (Department of Botany, Dendrology and Forest Genetics, The Royal Veterinary and Agricultural University (KVL), Denmark) and Dr. Felicia Hosein (Department of Plant Sciences, University of the West Indies, St Augustine, Trinidad and Tobago, West Indies) for providing seeds of *P. ahipa* and *P. erosus*, respectively. *N*-terminal amino acid sequencing was carried out by Dr. M. Naldrett at the John Innes Centre, Norwich, UK.

LITERATURE CITED

(1) Sørensen, M. *Yam Bean (Pachyrhizus DC.)*. Institute of Plant Genetics and Crop Plant Research, Gatersleben/International Plant Genetic Resources Institute: Rome, 1996; pp 141−145.

(2) Sørensen, M.; Døygaards, S.; Estella, J. E.; Kvist, L. P.; Nielsen, P. E. Status of the South American tuberous legume *Pachyrhizus tuberosus* (Lam.) Spreng. *Biodiversity and Conservation* **1997**, *6*, 1581−1625.

(3) Tadera, K.; Taniguchi, T.; Teramoto, M.; Arima, M.; Yagi, F.; Kobayshi, A.; Nagahama, T.; Ishihata, K. Protein and starch in tubers of winged bean, *Psophocarpus tetragonolobus* and yam bean (*Pachyrhizus erosus* L. Urban). *Memoirs of Faculty Agriculture Kagoshima University* **1984**, *20*, 73−81.

(4) Gruneberg, W. J.; Buttner, G.; Delgado-Licon, E. Eucarpia International Symposium on Breeding of Protein and Oil crops, Pontevedra, Spain. 1988; pp 95−97.

(5) Grum, M.; Halafihi, M.; Stolen, O.; Sørensen, M. Yield performance of yam bean in Tonga, South Pacific. *Exp. Agric*. **1994**, *30*, 67−75.

(6) Fernandez, M. V.; Warid, W. A.; Loaiza, J. M.; Montiel, A. C. Development patterns of jicama (*Pachyrhizus erosus* L. Urban) plant and the chemical constituents of roots grown in Sonra, Mexico. *Plant Foods Hum. Nutr*. **1997**, *50*, 279−286.

(7) Barnes, J. A.; Gomes, A. V. The yam bean: a plant of promise. *Biologist (London)* **1998**, *45*, 221−224.

(8) Nielsen, P. E.; Sørensen, M. Reproductive pruning in *Pachyrhizus ahipa* (Wedd.) Parodi. 2nd International Tuberous Legume Symposium, 1996; pp 245−251.

(9) *Approved Methods of the AACC.* American Association of Cereal Chemists: St. Paul, MN, 1987; Vol. II.

(10) Laemmli, U. K. Cleavage of structural protein during the assembly of the head of bacterophage T4. *Nature* **1970**, *227*, 680.

(11) Schagger, H.; von Jagow, G. Tricine-sodium dodecyl sulphate-polyacrylamide gel electrophoresis for the separation of proteins in the range from 1 to 100 kDa. *Anal. Biochem.* **1987**, *166*, 368−379.

(12) Baulcombe, D. C.; Huttly, A. K.; Martienssen, R. A.; Barker, R. F.; Jarvis, M. G. A novel wheat α-amylase gene. *Mol. Gen. Genet*. **1987**, *209*, 33−40.

(13) Gomes, A. V.; Grace, S.; Barnes, J. A. Major proteins of yam bean tubers. *Phytochemistry* **1997**, *46*, 185−193.

(14) Williams, J. G.; North, M. J.; Mahbubani, H. A developmentally regulated cysteine proteinase in *Dictyostelium discoideum*. *EMBO J*. **1985**, *4*, 999−1006.

(15) Soedjanaatmadja, U. M. S.; Hofsteenge, J.; Jeronimus-Stratingh, C. M.; Bruins, A. P.; Beintema, J. J. Demonstation by mass spectrometry that pseudo-hevein and hevein have ragged *C*-terminal sequences. *Biochim. Biophys. Acta* **1994**, *1209*, 144−148.

(16) Lucas, J.; Henschen, A.; Lottspeich, F.; Voegeli, U.; Boller, T. Amino-terminal sequence of ethylene induced bean leaf chitinase reveals similarities to sugar binding domains of wheat germ agglutination. *FEBS Lett*. **1985**, *193*, 208−210.

(17) Samac, D. A.; Hironaka, C. M.; Yallaly, P. E.; Shah, D. M. Isolation and characterisation of the genes encoding basic and acidic chitinase in *Arabidopsis thalina*. *Plant Physiol*. **1990**, *93*, 907−914.

(18) Taylor, M. A.; Mad Arif, S. A.; Kumar, A.; Davies, H. V.; Scobie, L. A.; Pearce, S. R.; Flavell, A. J. Expression and sequence analysis of cDNA induced during early stages of tuberization in different organs of the potato plant (*Solanum tuberosum* L.). *Plant Mol. Biol.* **1992**, *20*, 641−651.

(19) Crowell, D.; John, M. E.; Russell, D.; Amasino, R. M. Characterization of a stress-induced, developmentally regulated gene family from soybean. *Plant Mol. Biol*. **1992**, *18*, 459−466.

(20) Iturriaga, E. A.; Leech, M. J.; Barratt, D. H.; Wang, T. L. Two ABA-responsive proteins from pea (*Pisum sativum* L.) are closely related to intracellular pathogenesis-related proteins. *Plant Mol. Biol*. **1994**, *24*, 235−240.

(21) Harvey, P. J.; Boulter, D. Isolation and characterization of the storage protein of yam tubers (*Dioscores rotundata*). *Phytochemistry* **1983**, *22*, 1687−1693.

(22) Conlan, S.; Griffiths, L. A.; Turner, M.; Fido, R.; Tatham, A.; Ainsworth, C.; Shewry, P. R. Characterization of the yam tuber storage protein dioscorin. *J. Plant Physiol*. **1988**, *153*, 25−31.
(23) Yeh, K. W.; Chen, J. C.; Lin, M. I.; Chen, Y. M.; Lin, C. Y. Functional activity of sporamin from Sweet potato (*Ipomoea batatas* Lam): a tuber storage protein with trypsin inhibitory activity. *Plant Mol. Biol*. **1997**, *33*, 565−570.
(24) Kermode, A. R.; Bewley, J. D. Synthesis, processing and deposition of seed proteins: The pathway of protein synthesis and deposition in the cell. In *Seed Proteins*; Shewry, P. R., Casey, R., Eds.; Kluwer Academic Publishers: Dordrecht, The Netherlands, 1999; pp 807−841.
(25) Clowes, A. E. E.; Tatham, A. S.; Beeching, J. R.; Shewry, P. R. Characterization of cassava root proteins. In *Proceedings of the Second International Scientific Meeting of the Cassava Biotechnology Network* Bogor, Indonesia, 22−26 August, CIAT, Cali, Colombia, Vol II, 1995; pp 716−728.
(26) Coursey, D. G. Yams: An account of the nature, origins, cultivation and utilization of the useful members of the Dioscoreaceae. Longman: London, 1995.
(27) Walter, A. M, Jr.; Collins, W. W.; Purcell, A. E. Sweet potato protein: A review. *J. Agric. Food Chem*. **1984**, *32*, 695−699.
(28) Burton, W. G. *The Potato*. Longman: Harlow, U.K., 1989.
(29) Racusen, D. Lipid acyl hydrolase of patatin. *Can. J. Bot*. **1984**, *62*, 1640−1644.
(30) Racusen, D. Esterase specificity of patatin from two potato cultivars. *Can. J. Bot*. **1986**, *64*, 2104−2106.
(31) Hou, W. C.; Liu, J. S.; Chen, H. J.; Chen, T. E.; Chang, C. F.; Lin, Y. H. Dioscorin, the major tuber storage protein of yam (*Dioscorea batatas* Decne) with carbonic anhydrase and trypsin inhibitor activities. *J. Agric. Food Chem*. **1999**, *47*, 2168−2172.
(32) Sutliff, T. D.; Huang, N.; Litts, J. C.; Rodriguez, R. L. Characterization of an α-amylase multigene cluster in rice. *Plant Mol. Biol*. **1991**, *16*, 579−591.
(33) Pechan, T.; Ye, L.; Chang, Y.; Mitra, A.; Lin, L.; Davis, F. M.; Williams, P. W.; Luthe, D. S. A unique 33-kD cysteine proteinase accumulates in response to larval feeding in maize genotypes resistant to fall armyworm and other lepidoptera. *Plant Cell* **2000**, *12*, 1031−1040.

Received for review July 31, 2001. Revised manuscript received December 4, 2001. Accepted December 18, 2001. IACR receives grant-aided support from the Biotechnology and Biological Sciences Research Council of the United Kingdom. This work was supported by European Union FAIR Grant PL98-4297: Ahipa: Exploring the potential of a sustainable crop as an alternative nonfood source.

JF011017J