Selection of Sweetpotato Lines with High Protein Content and/or Low Trypsin Inhibitor Activity

Jun Toyama^{*1,2}, Makoto Yoshimoto¹ and Osamu Yamakawa^{1,3}

¹) National Agricultural Research Center for Kyushu Okinawa Region, 6651-2 Yokoichi, Miyakonojo, Miyazaki 885-0091, Japan

²) Research Fellow of Cooperative System for Supporting Priority Research

³) Present address: National Agricultural Research Center for Kyushu Okinawa Region, 2421 Suya, Nishigoshi, Kumamoto 861-1192, Japan

Low content of protein and the presence of an anti-nutritional factor, ‘trypsin inhibitor (TI)’ reduce the nutritional value of sweetpotato (*Ipomoea batatas* (L.) Lam.) when used as animal feed. Attempts have been made to develop sweetpotato varieties with a high protein content and/or low trypsin inhibitor activity (TIA). Using approx. 800 breeding lines of sweetpotato, the crude protein content and TIA were determined during 6 seasons from 1998 to 2003. Fifteen lines with a high protein content (27.8–44.6 mg/g DW) and 7 lines with a low TIA (11–137 U/mg DW) were selected, when compared to the standard variety ‘Shiroyutaka’ (crude protein content: 19.9 mg/g DW, TIA: 186 U/mg DW). Among the lines used, there was a strong positive correlation between TIA and the crude protein content ($r=0.871^{**}$). However, lines, ‘Q94128-1’ (crude protein content: 28.9 mg/g DW, TIA: 21 U/mg DW) and ‘KNF94225-13’ (crude protein content: 35.5 mg/g DW, TIA: 63 U/mg DW) showed a high protein content and a low TIA. Electrophoretic analysis and activity staining of TIs extracted from these two lines revealed a considerably lower amount of trypsin inhibitory bands compared to that in ‘Shiroyutaka’. Partially purified TIs from ‘Shiroyutaka’ were estimated to correspond to sporamins, sweetpotato major storage root proteins.

Key Words: *Ipomoea batatas*, sweetpotato, high protein content, low TIA, trypsin inhibitor, feed.

Introduction

About 41% of the world annual sweetpotato (*Ipomoea batatas* (L.) Lam.) production is used as animal feed (FAO 2002). Storage roots of sweetpotato are rich in nutrients, such as starch, sugars, vitamins and minerals (Ravindran *et al.* 1995, Okuno *et al.* 1998). Compounds such as polyphenolics, β -carotene, anthocyanins, vitamins and fiber in sweetpotato exhibit many beneficial dietary functions (Yoshimoto 2001). From the nutritional point, however, some shortcomings reduce the nutritional value of sweetpotato as animal feed, including the low content of protein (normally 4.5–7.0% on a dry matter basis) (Purcell *et al.* 1972), and the presence of trypsin inhibitors (TIs), which decreases the protein digestibility in uncooked storage roots (Yeh and Bouwkamp 1985).

Cultivars differ both in the protein content (Purcell *et al.* 1972, Dickey *et al.* 1984, Ravindran *et al.* 1995, Takahata *et al.* 1995) and in the trypsin inhibitor activity (TIA) (Bradbury *et al.* 1985, Dickey and Collins 1984, Ravindran *et al.* 1995, Zhang *et al.* 1998, Toyama *et al.* 2005). Since the level of TIA is positively correlated with the crude and soluble protein contents (Lin and Chen 1980), the development

of sweetpotato varieties with a high protein content and low TIA is a major objective in breeding programs.

The storage roots of sweetpotato contain large quantities of two proteins, sporamins A and B, that account for 80% of the total soluble protein content (Maeshima *et al.* 1985). They are monomeric forms with a similar molecular weight (25 kDa), and can be separated from each other by SDS-PAGE at positions corresponding to the apparent molecular size of 31 kDa (sporamin A) and 22 kDa (sporamin B) under non-reducing conditions (Maeshima *et al.* 1985). Sporamins are encoded by a multigene family. Based on nucleotide homology, sporamins can be grouped into two subfamilies, which correspond to the polypeptide classes sporamins A or B (Hattori *et al.* 1989). Moreover, sporamin has been found to be a form of TI in sweetpotato, based on recombinant DNA experiments (Yeh *et al.* 1997).

The use of a rapid method of estimating TIA enables to screen a large number of sweetpotato samples (Toyama *et al.* 2005). High protein content, low TIA, as well as a high storage root yield and high dry matter content may enhance the value of sweetpotato as a source of animal feed.

In the present paper, the selection of sweetpotato lines with a high protein content and/or low TIA, from approx. 800 breeding lines is reported. Root proteins extracted from the lines with high and low TIA were compared using SDS-PAGE.

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*Corresponding author (e-mail: juntoyamajp@yahoo.co.jp)

Materials and Methods

Plant materials

Approximately 800 breeding lines or cultivars of sweetpotato (*Ipomoea batatas* (L.) Lam.) obtained from more than 300 crosses, using about 150 breeding lines or cultivars were examined in the present study.

Field trials

The experiments were carried out in an experimental field of the National Agricultural Research Center for Kyushu Okinawa Region, Miyazaki, Japan during the period of 1998–2003. A preliminary selection was conducted during the period of 1998–2001 for the screening of TIA and the crude protein content, as well as storage root yield and dry matter content of sweetpotato breeding lines. Twenty-two lines selected from the trials were tested during the period of 2002–2003 to evaluate these characters. Twenty cuttings for each line were planted in a plot (1.5 m × 3.5 m) with two rows, with 3 replications for the standard variety ‘Shiroyutaka’, or without replications for the other lines. Fertilizer application amounted to 600 kg/ha (1998–1999: N-P₂O₅-K₂O=6-8-12, 2000–2003: 8-12-20). Planting period extended over a period of about 5 months (May–Oct.) for all the years.

Preparation of sweetpotato flour and crude extract

Six freshly harvested storage roots for each line were washed, shredded and mixed. A portion (100 g) of the mixed sample was lyophilized and ground in a blender for 1 min to pass through a 212 µm sieve. Crude proteins were extracted from the flour (50 mg) with 2 ml of 50 mM Tris-HCl buffer (pH 8.0) in 2 ml micro-centrifuge tubes. The samples were vigorously shaken for 10 min, followed by centrifugation at 13,000 g at 4°C for 10 min. The supernatant was then used as crude extract for the TI assay and soluble protein analysis.

TIA measurement

Porcine pancreatic trypsin (Wako, Osaka, Japan) and Benzoyl-DL-arginine-p-nitroanilide (BAPA, Wako, Osaka, Japan) were basically used as enzyme and substrate, respectively, for the TIA assay (Kakade *et al.* 1969). An improved colorimetric method (Zhang *et al.* 1998) was modified to become suitable for microplate analysis for the assay. The crude extract (20 µl) was incubated with 20 µl of a trypsin solution (160 µg/ml in 5 mM HCl and 5 mM CaCl₂) for 2 min at 37°C. An aliquot of this mixture (20 µl) was then assayed in 200 µl of a freshly prepared substrate solution [(100 mM Tris-HCl, 100 mM CaCl₂, pH 8.0) : (10 mg BAPA/1 ml DMSO)=19:1]. The reaction mixture was incubated for 10 min at 37°C and the reaction was terminated by the addition of 100 µl of 10% acetic acid. For the blank (to correct for background absorbance), a 20 µl mixture of extract and trypsin solution was added to the assay mixture containing acetic acid. For reference, an uninhibited reaction was also run by replacing the extract with 20 µl of 50 mM Tris-HCl buffer

(pH 8.0). The resultant color formed was read at 410 nm (A_{410r}: reference, A_{410s}: sample) and the activity was expressed as U (inhibited trypsin activity unit) per 1 mg of dry sample. Trypsin unit was defined as follows: A₄₁₀ increase of 0.01 under the conditions of the assay where trypsin and the substrate solution were mixed. TIA was calculated by the formula:

$$\text{TIA (U/mg DW)} = (A_{410r} - A_{410s}) \times 100/\text{mg DW}$$

Protein analysis

The crude protein content was estimated from the total nitrogen content analysed by the Kjeldahl method using 2 g of sweetpotato flour. The soluble protein content was determined by using the Bio-Rad protein assay reagent (Bio-Rad, CA, USA) with BSA as a standard.

Partial purification of total TIs

Fresh storage roots (ca. 1.5 kg) of the sweetpotato cultivar, ‘Shiroyutaka’ were washed, shredded and homogenized with two volumes (w/v) of water in a homogenizer for 100 sec. The homogenates were filtered through two layers of gauze and centrifuged at 8,000 g for 10 min to remove the starch. The supernatant was lyophilized and stored at –80°C until use. For further purification of the proteins, 75 g of the lyophilized powder was dissolved in 1.5 l of 100 mM Tris-HCl buffer (pH 7.9). The precipitates of this solution formed in the range between 30 and 60% ammonium sulfate saturation were dissolved in 100 ml of 100 mM Tris-HCl buffer (pH 7.9). After dialysis against the same buffer, they were loaded onto a trypsin affinity column prepared according to the method of Koshikawa and Miyazaki (1996). The active portion was collected according to the method of Hou and Lin (1997) and was dialyzed against distilled water and lyophilized.

Protein and TI activity staining on SDS-PAGE gels

The crude extract was concentrated with Ultrafree-MC 5000NMWL (nominal molecular weight limit) (Millipore, Bedford, MA, USA) and used for SDS-PAGE analysis. SDS-PAGE was carried out using commercially available precast gel systems (Invitrogen, Carlsbad, CA, USA). Tris-Glycine gels (gel concentration of 14%) were used with Tris-Glycine running buffer (25 mM Tris, 192 mM Glycine, 0.1% SDS, pH 8.3). Samples for this system were prepared according to the method of Hou and Lin (1998), where the reducing agent was not added to the samples, and binding of the samples with SDS was performed at room temperature overnight instead of heating. Reduced samples were prepared by the addition of 50 mM of dithiothreitol (DTT) and heating at 70°C for 10 min. TI activity staining of the non-reduced TI bands was carried out according to the method of Hou and Lin (1998). NuPAGE gels (gel concentration of 10%) were used with NuPAGE MES (2-(N-morpholino) ethane sulfonic acid) running buffer (50 mM MES, 50 mM Tris, 3.5 mM SDS, 1 mM EDTA, pH 7.3). Samples for this system were prepared (65% sample, 25% 4×NuPAGE LDS

(Lithium Dodecyl Sulfate) sample buffer, 10% NuPAGE reducing agent (0.5M DTT), heated at 70°C for 10 min, and then separated on NuPAGE gels. Separation of the protein bands was more sensitive on the NuPAGE system than on the Tris-Glycine system. Protein bands both on the Tris-Glycine gels and NuPAGE gels were stained with Coomassie brilliant blue R250. Soluble protein contents of the samples for SDS-PAGE analysis were determined by the Bio-Rad protein assay.

Pepstatin treatment

Forty μ M (final concentration) of Pepstatin A (Wako, Osaka, Japan), an aspartic type protease inhibitor, was dissolved in a small volume of DMSO and added to the extraction buffer. TIA measurement and SDS-PAGE analysis were performed for the samples extracted with this buffer.

Results and Discussion

Selection of high protein and/or low TIA lines

The crude protein content ranged from 8.4 to 54.0 mg/g DW for approx. 800 sweetpotato breeding lines analysed during the period of 1998–2001, while that of a standard variety, ‘Shiroyutaka’ was in the range of 17.2–21.4 mg/g DW. Fifteen lines with a high protein content (dry matter content and storage root yield were also considered) were selected and these lines were tested during the period of 2002–2003.

They all showed a higher crude protein content (27.8 to 44.6 mg/g DW) than ‘Shiroyutaka’ (19.9 mg/g DW) (Table 1). The crude protein content of the line ‘Q98174-11’ (44.6 mg/g DW) was the highest among the selected 15 lines, although it was much lower than that (91.4 mg/g DW) of the varieties with the highest content from the North Carolina root collection reported by Purcell *et al.* (1972). However, the crude protein content of ‘Shiroyutaka’ (19.9 mg/g DW) and ‘Q93017-5’ (29.1 mg/g DW) (Table 1) increased to 56.0 and 92.0 mg/g DW, respectively, when the plants were grown in a different field (data not shown). The crude protein content of ‘Shiroyutaka’ reported by Takahata *et al.* (1995) also showed a higher value (54.5 mg/g DW). The protein content of the same variety differed considerably depending on the field conditions, as reported by Purcell *et al.* (1978). Generally, a high application of N-fertilizer to plantings of sweetpotato leads to an increase in the storage root nitrogen content (Purcell *et al.* 1982). The low protein level of our lines may be due to the low nitrogen level of the soil in the experimental field.

The TIA values ranged from 27 to 944 U/mg DW for approx. 800 sweetpotato breeding lines cultivated during the period of 1998–2001. The TIA values of ‘Shiroyutaka’ ranged from 149 to 313 U/mg DW. Seven lines with a low TIA value (dry matter content and storage root yield were also considered) were selected and these lines were tested during the period of 2002–2003. They all showed lower TIA

Table 1. Some characters of sweetpotato breeding lines¹⁾ with high protein or low trypsin inhibitor activity (TIA)

	Lines	Root yield (kg/a)	Dry matter (%)	Crude protein (mg/g DW)	TIA (U/mg DW)
High protein (and low TIA) (and low TIA)	Q90142-22	286 $\pm$ 58 ²⁾	34.5 $\pm$ 1.6	34.1 $\pm$ 3.0	578 $\pm$ 76
	Q93017-5	275 $\pm$ 27	31.6 $\pm$ 0.7	29.1 $\pm$ 3.4	372 $\pm$ 64
	KNF93111-4	360 $\pm$ 15	28.2 $\pm$ 0.0	32.2 $\pm$ 4.9	515 $\pm$ 192
	Q97260-8	246 $\pm$ 45	31.1 $\pm$ 0.5	42.9 $\pm$ 1.2	649 $\pm$ 17
	Q97236-4	184 $\pm$ 31	28.4 $\pm$ 0.2	35.2 $\pm$ 1.0	417 $\pm$ 26
	Q98106-5	309 $\pm$ 72	35.6 $\pm$ 0.3	29.9 $\pm$ 2.0	353 $\pm$ 37
	Q98158-4	171 $\pm$ 56	37.8 $\pm$ 1.9	30.9 $\pm$ 4.3	449 $\pm$ 64
	Q98158-12	223 $\pm$ 126	33.9 $\pm$ 0.5	28.9 $\pm$ 4.0	183 $\pm$ 46
	Q98160-7	124 $\pm$ 40	33.5 $\pm$ 0.1	38.9 $\pm$ 1.6	443 $\pm$ 27
	Q98161-4	216 $\pm$ 12	32.9 $\pm$ 6.6	32.9 $\pm$ 4.5	337 $\pm$ 25
	Q98161-5	215 $\pm$ 34	35.1 $\pm$ 1.7	36.9 $\pm$ 1.3	554 $\pm$ 100
	Q98174-11	291 $\pm$ 94	23.1 $\pm$ 0.6	44.6 $\pm$ 2.8	510 $\pm$ 97
	Q95175-2	229 $\pm$ 37	35.7 $\pm$ 0.6	27.8 $\pm$ 8.1	259 $\pm$ 224
	Q94128-1	265 $\pm$ 27	22.5 $\pm$ 0.6	28.9 $\pm$ 3.0	21 $\pm$ 21
	KNF94225-13	193 $\pm$ 31	18.7 $\pm$ 0.7	35.5 $\pm$ 5.0	63 $\pm$ 6
Low TIA	Q94279-2	301 $\pm$ 9	38.8 $\pm$ 1.0	17.3 $\pm$ 2.5	137 $\pm$ 23
	Q95180-3	330 $\pm$ 0	38.2 $\pm$ 1.4	16.7 $\pm$ 2.1	58 $\pm$ 38
	Q97106-9	271 $\pm$ 23	40.4 $\pm$ 0.1	13.1 $\pm$ 2.1	46 $\pm$ 14
	Q97106-58	293 $\pm$ 34	32.4 $\pm$ 1.8	14.1 $\pm$ 0.4	11 $\pm$ 4
	Q98082-3	294 $\pm$ 45	38.9 $\pm$ 0.3	18.4 $\pm$ 4.3	130 $\pm$ 79
	Q98082-10	268 $\pm$ 31	37.1 $\pm$ 0.3	16.5 $\pm$ 1.3	87 $\pm$ 2
	Q98097-4	298 $\pm$ 24	39.1 $\pm$ 0.4	16.2 $\pm$ 0.3	98 $\pm$ 14
Standard	Shiroyutaka	315 $\pm$ 85	35.5 $\pm$ 1.0	19.9 $\pm$ 3.5	186 $\pm$ 54

¹⁾ Lines were selected from approx. 800 breeding lines, cultivated during the period of 1998–2001.

²⁾ Data are presented as Mean $\pm$ SD of 2002 and 2003.

values (11–137 U/mg DW) than ‘Shiroyutaka’ (186 U/mg DW) (Table 1).

A significant positive correlation ($r = 0.871^{**}$) was found between TIA and the crude protein content among 207 breeding lines in 2001 (Table 2). Therefore, most of the fifteen high-protein lines selected showed a high TIA, and seven low TIA lines selected showed a low content of crude protein (Table 1). However, only two lines, ‘Q94128-1’ (crude protein content: 28.9 mg/g DW, TIA: 21 U/mg DW) and ‘KNF94225-13’ (crude protein content: 35.5 mg/g DW, TIA: 63 U/mg DW) displayed a high protein content and low TIA (Table 1). A similar correlation was observed for cultivars in Taiwan (Lin and Chen 1980). In contrast, no significant correlation between TIA and the protein content was found in either North America (Dickey *et al.* 1984) or Papua New Guinea (Bradbury *et al.* 1984) samples.

Significant negative correlations were found between the crude protein content and the dry matter content ($r = -0.472^{**}$) or storage root yield ($r = -0.233^{**}$) (Table 2). However, three lines showed a higher dry matter content (35.6–37.8%) than ‘Shiroyutaka’ (35.5%) in the high-protein selections (Table 1). Most of the lines selected with a low TIA showed a higher dry matter content (37.1–40.4%) than that of ‘Shiroyutaka’, although the storage root yield of most lines was lower than that of ‘Shiroyutaka’ (Table 1). Over all, TIA was negatively correlated with the dry matter content ($r = -0.285^{**}$) and not with the storage root yield (Table 2).

Partial purification of TIs in trypsin affinity column

A summary of TI purification from sweetpotato storage roots is shown in Table 3. As a final product, 66 mg of TI was obtained from 1.5 kg of fresh storage roots of ‘Shiroyutaka’. Figure 1 shows the protein staining (A) and TI activity staining (B) of the purified TIs (lane 1) and crude extract (lane 2) from storage roots on SDS-PAGE gel. There were at least 2 major bands of 33 kDa and 22–21 kDa in the purified TIs (lane 1) as well as the crude extract (lane 2), both corresponding to TI high activity bands, indicating that the TI proteins are the main components of storage root proteins. TIs under reduced conditions showed a single 25 kDa band (Fig. 2). These patterns of purified TIs on SDS-PAGE were similar to the pattern of sporamins, which are the major storage root proteins of sweetpotato reported by Maeshima *et al.* (1985). They showed that the molecular size of the

Table 2. Correlation between some important characters of sweetpotato storage roots for feeding purpose

	TIA (dry basis)	Dry matter	Yield
Crude protein content (dry basis)	0.871**	−0.472**	−0.233**
TIA (dry basis)	—	−0.285**	−0.115

** : Significant at $p < 0.01$.

Data from 207 breeding lines were used in 2001.

sporamins was 31 kDa (sporamin A) and 22 kDa (sporamin B) when the compounds were separated without reduction of the disulfide bonds with DTT, and 25 kDa under reduced conditions. Moreover, it was shown that sporamin was one form of TI in sweetpotato, based on recombinant DNA experiments (Yeh *et al.* 1997). Recently, Hou *et al.* (2004) have reported that the first 15 amino acids in the N-terminal region of 33 kDa TI were identical with those of the deduced sequence of sporamins A and B. Based on our data and previous reports (Maeshima *et al.* 1985, Hou *et al.* 2004), the 33 kDa and 22–21 kDa bands of the purified TIs were estimated to correspond to sporamin A and sporamin B, respectively.

Hattori *et al.* (1989) reported that amino acid sequence similarities between sporamins A and B ranged from 82 to 84%, and that four Cys residues were conserved at the corresponding positions of both sporamins A and B to form intra-chain disulfide bonds in a similar manner to that in the Kunitz-type protease inhibitors. Therefore, further studies should be carried out to elucidate the differences in the behavior of sporamins A and B when separated under non-reducing conditions.

One of the two major bands of purified TIs (corresponding to sporamin B) showed 2 bands (22 and 21 kDa), although the 22–21 kDa band from the crude extract was not clearly separated (Fig. 1A). Nakamura *et al.* (1993) reported the post-translational processing of a sporamin precursor into two smaller-molecular-weight forms. Moreover, sporamins are encoded by a multigene family and the sequence similarity was in the range of 94–98% within the subfamily (Hattori *et al.* 1989). Therefore, it is considered that the small-size polymorphisms may be formed within subfamilies.

Table 3. Purification steps of trypsin inhibitors (TIs) in sweetpotato cultivar, ‘Shiroyutaka’¹⁾

Procedure	Total protein ²⁾ (mg)	Total activity (× 1,000 U)	Specific activity (× 1,000 U/mg protein)	Yield (%)	Specific activity ratio (-fold)
Crude extraction	2447	36300	14.8	100	1.0
Ammonium sulfate precipitation (30–60%)	1343	30100	22.4	83	1.5
Trypsin affinity column chromatography	94	17080	180.9	47	12.2
Dialysis	66	14560	221.1	40	14.9

¹⁾ Starting material: 1.5 kg of fresh storage roots.

²⁾ Soluble protein content (determined by Bio-Rad protein assay with BSA as a standard).

Fig. 1. Protein staining (A) and trypsin inhibitor (TI) activity staining (B) of purified TIs (lane 1) and crude extract (lane 2) of sweetpotato storage roots on 14% SDS-PAGE gels (samples were not reduced and not heated). Four μ g of protein for the purified TIs and 30 μ g of protein for the crude extract were loaded.

Fig. 2. Protein staining of purified sweetpotato trypsin inhibitors (TIs) on 14% SDS-PAGE gels. Samples were reduced and heated at 70°C for 10 min. Four μ g of protein was loaded.

Varietal differences of TIs analysed on SDS-PAGE

Figure 3 detects the TI activity staining of the crude extract from storage roots of some selected lines (Table 1), with a high TIA (510–649 U/mg DW) and a low TIA (11–137 U/mg DW). High TIA lines (lanes 2–5) displayed 2 forms of strong TI bands comparable to those of ‘Shiroyutaka’ (lane 10), that corresponded to the 33 kDa and 22–21 kDa bands of the purified TIs (lane 1), while the low TIA lines (lanes 6–9) did not show any detectable TI band or showed weak TI bands compared to those of ‘Shiroyutaka’ (lane 10). ‘Q94128-1’ (high protein and low TIA line) did not show any detectable TI band (lane 6). The intensity of the two TI

Fig. 3. Trypsin inhibitory activity in storage root extracts from some lines of sweetpotato with high (lanes 2–5) and low (lanes 6–9) trypsin inhibitor activity (TIA) analysed on 14% SDS-PAGE gels. Lane 1: Purified TIs, lane 2: Q90142-22, lane 3: Q97260-8, lane 4: Q98161-5, lane 5: Q98174-11, lane 6: Q94128-1, lane 7: Q94279-2, lane 8: Q95180-3, lane 9: Q97106-58, lane 10: Shiroyutaka. Four μ g of protein for the purified TIs and 30 μ g of protein for the crude extract were loaded.

bands in the activity staining was positively related to the TIA measured (Table 1).

Effect of pepstatin

Hou *et al.* (2002) reported that sweetpotato TIs are degraded by an endogenous aspartic type protease. To estimate the influence of endogenous proteases during sample preparation of proteins, four high protein lines with a high TIA (Q90142-22, Q93017-5) or with a low TIA (Q94128-1, KNF94225-13) and one medium TIA line ‘Shiroyutaka’, were used for protein extraction with and without pepstatin A (aspartic type protease inhibitor). No significant differences were detected in the levels of TIA ($p=0.125$) as well as soluble protein contents ($p=0.697$) between the extracts with and without pepstatin treatment (Table 4), which was further confirmed on the NuPAGE system (Fig. 4). The estimated molecular weight of the purified TIs was 23 kDa in this system (Fig. 4A). Profiles of proteins including 23 kDa TIs which were extracted with 40 μ M pepstatin or without pepstatin did not show any differences (Fig. 4B). These results indicated that endogenous protease (aspartic type) degradation of TIs and other soluble proteins did not occur during sample preparation. The proportion of 23 kDa TIs to all the protein bands was lower in ‘Q94128-1’ and ‘KNF94225-13’ than in ‘Shiroyutaka’ and ‘Q90142-22’, where 23 kDa TIs predominated (Fig. 4B). Instead of the TI bands, ‘Q94128-1’ and ‘KNF94225-13’ showed a large number of protein bands. Apart from the TI bands, β -amylase (about 5% of total soluble protein), whose molecular weight for the subunit is about 52,000 (Cudney and McPherson 1993) was an other major protein in the crude extract. In Figure 4, high TI lanes showed an overall increase in the intensity for the proteins. As in the case of soybean TI, the sensitivity of the purified sweetpotato TIs to the Bio-Rad protein assay was

Table 4. Effect of pepstatin on the trypsin inhibitor activity (TIA) and soluble protein content of sweetpotato crude extracts

Lines	TIA level	TIA (U/mg DW)		Soluble protein ¹⁾ (mg/g DW)	
		Pepstatin (μM)		Pepstatin (μM)	
		0	40	0	40
Q90142-22	High	454	491	11.3	11.4
Q93017-5	High	301	311	9.2	9.3
Q94128-1	Low	5	10	11.1	11.3
KNF94225-13	Low	57	63	12.6	11.9
Shiroyutaka	Medium	144	147	6.4	7.2
Average		192 ^{n.s.2)}	204 ^{n.s}	10.1 ^{n.s}	10.2 ^{n.s}

¹⁾ Soluble protein content (determined by Bio-Rad protein assay with BSA as a standard).

²⁾ Not significant between pepstatin treatments.

Fig. 4. NuPAGE (10%) analysis of sweetpotato trypsin inhibitors (TIs). (A) Purified TIs. (B) Crude extracts from storage roots of some sweetpotato lines. Lanes 1,2: Q94128-1, lanes 3,4: KNF94225-13, lanes 5,6: Shiroyutaka, lanes 7,8: Q90142-22. Forty μM pepstatin was added to the extraction buffer for lanes 2,4,6,8, during TI extraction. Four μg of protein for the purified TIs and 30 μg of protein for the crude extract were loaded.

much lower than that of other soluble proteins. It is, therefore, possible that a larger amount of protein from ‘Shiroyutaka’ and ‘Q90142-22’ was loaded on the gel, compared to that of ‘Q94128-1’ and ‘KNF94225-13’.

We screened approx. 800 sweetpotato lines during 6 seasons, with emphasis placed on the protein content and TIA. Fifteen high-protein lines and 7 low TIA lines showed reproducible data. Among these lines, ‘Q94128-1’ and ‘KNF94225-13’ displayed both a high protein content and a low TIA, hence a considerably lower amount of TIs per protein compared to that in most of the other sweetpotato lines. Further genetic studies on the protein content of these 2 lines will be carried out for use as materials suitable for animal feed as well as human consumption.

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