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Selection of sweetpotato with high protein and/or low trypsin inhibitor activity

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Abstract. Low content of protein and the presence of anti-nutritional factor 'trypsin inhibitor' diminish the nutritional value of sweetpotato (*Ipomoea batatas* L.) as animal feed. Efforts have been made to develop sweetpotato with high content of protein and/or low activity of trypsin inhibitor (TIA). Crude protein content and TIA of more than 800 breeding lines of sweetpotato were measured during 5 seasons from 1998 to 2002. Seven lines were selected as low TIA (14 to 120 TUI/mg DW) and 15 lines as high protein (26.1 to 46.6 mg/g DW), when compared to a standard variety 'Shiroyutaka' (TIA: 148 TUI/mg DW, protein content: 17.4 mg/g DW). There was a strong positive correlation between protein content and TIA (r=0.856**). However, Q94128-1 (TIA: 6 TUI/mg DW, protein content: 26.8 mg/g DW) and KNF94225-13 (TIA: 59 TUI/mg DW, protein content: 32.0 mg/g DW) had high contents of protein but low TIA. Activity staining of trypsin inhibitor in the proteins extracted from these two lines on polyacrylamide gel revealed lack or less activity of trypsin inhibitor.

Introduction

About 44% of the world's annual sweetpotato production is used as animal feed (FAO, 2001). It is rich in nutrients, such as starch, sugars, vitamins and minerals (Ravindran *et al.*, 1995, Okuno *et al.*, 1998). The compounds such as polyphenolics, β-carotene, anthocyanins, vitamins, fibre and jalapin in sweetpotato have many beneficial dietary functions (Yoshimoto, 2001). From the nutritional point, sweetpotato may also be a good material for animals. However, some shortcomings diminish its nutritional value as animal feed. One of these is the low protein content, normally 4.5-7 % of dry matter (Purcell *et al.*, 1972). The other is the trypsin inhibitor (TI), which reduces protein digestibility in uncooked roots (Yeh and Bouwkamp, 1985).

The major sweetpotato protein in roots, sporamin, accounts for 80% of total soluble protein (Maeshima *et al.*, 1985). Sporamin is one form of TI in sweetpotato confirmed by recombinant DNA experiments (Yeh *et al.*, 1997). The level of trypsin inhibitor activity (TIA) positively correlates with soluble protein (Bouwkamp *et al.*, 1985) and crude protein content (Zhang *et al.*, 1998). Therefore, development of a sweetpotato variety with high protein and low TIA is a big challenge.

There are cultivar differences both in protein contents (Purcell *et al.*, 1972) and TIA (Wang and Yeoh., 1996, Zhang *et al.*, 1998, Yeoh *et al.*, 2000). The rapid method of estimating TIA (Yeoh *et al.*, 2000) helps to select a good number of breeding lines with low TIA. High protein content, low TIA, as well as a high dry matter yield will make sweetpotato more attractive as a source of animal feed. In this report, we present some sweetpotato lines with high protein content and / or low TIA, which were selected from more than 800 breeding lines during 1998-2002. TIs from high and low lines were compared using SDS-PAGE. Partially purified TIs were used as standards.

Materials and Methods

Plant materials. More than 800 breeding lines or cultivars of sweetpotato were used for the analysis between 1998-2002. These breeding lines were obtained after crossing more than 150 breeding lines or cultivars.

Experimental design. Twenty plants for each lines were planted in two rows at a density of 38100 plants/ha. NPK fertilizer was applied (600 kg/ha) (1998-1999: N-P_2O_5-K_2O=6-8-12, 2000-2002: 8-12-20). The crop was allowed to grow for 5 months. Twenty-two lines selected until 2001 were tested again in 2002.

Preparation of sweetpotato flour and crude extract. Six freshly harvested roots for one line were washed, shredded and mixed. A 100g sample was lyophilized and the dried sample ground in a blender for 1 min. to pass through a 212 µm sieve. The resulting flour (50mg) was extracted in 2 ml 50 mM Tris-HCl buffer (pH8) in 2 ml micro-centrifuge tubes. Samples were vigorously shaken for 10 min. followed by centrifugation at 13,000 rpm at 4 ÚC for 15 min. The supernatant was then used for trypsin inhibitor assay and soluble protein determination.

TIA estimation. TIA was measured according to Yeoh *et al.* (2000). TIA was expressed as TUI (trypsin unit inhibited) per 1 mg dry sample (Zhang *et al.*, 1998). Trypsin units were defined as an A_{410} increase of 0.01 under the assay conditions where trypsin and substrate solution were mixed.

Protein determination. Protein contents were estimated from total N analyzed by Kjeldahl method. Two g rammes of sweetpotato flour was used for the analysis. Soluble protein was determined by using the BioRad protein assay reagent.

Sweetpotato TI purification. Ten kilogrammes of 'Shiroyutaka' roots stored for six month was used for TI purification. Roots were washed, shredded and homogenized with two volumes (w/v) of water in a homogenizer for 100s. The homogenates were filtered through two layers of mesh and centrifuged for starch elimination at 8000 × g for 10 min. The resultant supernatant was lyophilized and about 500 g of the crude TI powder was obtained. They were stored at -80 ÚC until use. For further purification, 75g of the lyophilized powder were dissolved in 1.5 l of 100 mM Tris-HCl buffer (pH 7.9). The precipitates from 30-60% ammonium sulphate saturation of this solution were dissolved in 100 ml of 100 mM Tris-HCl buffer (pH 7.9). After dialysis against the same buffer they were loaded on to trypsin affinity column. The active portion was collected according to Hou and Lin (1997). They were dialyzed against water and lyophilized.

Protein and TI activity staining on SDS-PAGE gels. SDS-PAGE was carried out using commercially available precast gel systems (Invitrogen Corp., Carlsbad, CA, USA). Tris-Glycine gels (gel concentration of 14 %) and Nupage gels (gel concentration of 10 %) were used with Tris-Glycine buffer and MES buffer as the electrophoresis buffer, respectively. Coomassie brilliant blue R250 was used for protein staining. TI activity staining was carried out according to Hou and Lin (1998).

Results and Discussion

Selection of high protein and/or low TIA lines. Crude protein contents of more than 800 sweetpotato breeding lines were analyzed during 1998-2001. The protein content varied from 8.0 to 54.0 mg/g DW. The protein content of standard variety, 'Shiroyutaka' was from 17.0 to 21.4. Twenty-two lines selected so far were tested again in 2002 for protein content. Fifteen lines were finally selected as high protein (26.1 to 46.6 mg/g DW) (Table 1). The protein content of the line Q98174-11 (46.6 mg/ g DW) was the highest among the selected 15 lines, but was much lower than the protein content (91.4 mg/g DW) of varieties from North Carolina root collection (Purcell *et al.*, 1972). Generally, an increase in the rate of applied N to plantings of

sweetpotato leads to an increase in root N content (Purcell and Walter, 1982). Low protein level of our lines may be due to low N level in the breeding field. Although we found negative correlation between crude protein content and dry matter (-0.456) or dry matter yield (–0.405) (Table 2), three lines were found to have higher dry matter content (36.3 to 39.1 %) than 'Shiroyutaka' (Table 1).

Seven lines were selected as low TIA (14 to 120 TUI/mg DW). Most of the lines selected had higher dry matter (31.1 to 40.3 %) than 'Shiroyutaka', although dry matter yield of all lines were lower than 'Shiroyutaka' (Table 1). Over all, TIA showed a negative correlation with dry matter content (-0.268) and dry matter yield (–0.215) (Table 2).

The analysis of 244 breeding lines in 2001 showed that there is very high correlation (0.856) between crude protein content and TIA (Table 2). Most of the fifteen high protein lines selected were with high TIA, and seven low TIA lines selected had low protein content (Table 1). However, two lines, Q94128-1 (TIA:

Table 1: Selected sweet potato breeding lines with high protein or low TIA.

	Lines	Crude protein (mg/gDW)	TIA (TUI/mg DW)	Dry matter content (%)	Dry matter yield (t/ha)
High protein	Q90142-22	31.9	632	35.7	11.7
	Q93017-5	26.7	327	32.1	9.5
	KNF93111-4	28.7	379	28.2	10.5
	Q94128-1	26.8	6	22.9	6.5
	KNF94225-13	32.0	59	19.2	4.1
	Q97260-8	43.8	661	30.7	8.5
	Q97236-4	35.9	435	28.5	5.9
	Q98106-5	28.5	327	35.4	12.7
	Q98158-4	27.9	403	39.1	8.2
	Q98158-12	26.1	150	34.3	10.7
	Q98160-7	37.8	462	33.4	5.1
	Q98161-4	36.1	319	37.6	7.8
	Q98161-5	37.8	624	36.3	8.7
	Q98174-11	46.6	579	22.7	8.1
	Q95175ÿ2	33.5	418	36.1	9.2
Low TIA	Q94279-2	15.5	120	39.5	12.1
	Q95180-3	15.2	31	39.2	12.9
	Q97106-9	11.6	36	40.3	10.3
	Q97106-58	14.4	14	31.1	8.4
	Q98082-3	15.4	74	39.1	10.2
	Q98082-10	15.6	85	37.3	10.8
	Q98097-4	16.0	88	39.4	12.4
Standard	Shiroyutaka	17.4	148	36.2	13.6

Table 2: Correlation between some important characters for feeding purpose.

0	TIA	Dry matter content	Dry matter yield
Crude protein	0.856**	-0.456**	-0.405**
TIA	-	-0.268**	-0.215**

**: Significant at p<0.01 %. Data used were from 244 breeding lines in 2001.

6 TUI/mg DW, protein content: 26.8 mg/g DW) and KNF94225-13 (TIA: 59 TUI/mg DW, protein content: 32.0 mg/g DW) had high content of protein and low TIA (Table 1).

Partial purification of TIs by trypsin affinity column. The purification step is summarized in Table 3. There were 3 major bands of 33 kDa, 22 kDa and 21 kDa in purified TIs as well as the crude extract, corresponding to trypsin inhibitor bands. However, the TI activity spots for 22 kDa and 21 kDa bands could not be distinguished.

Cultivar differences of TIs analyzed on SDS-PAGE. High TIA lines showed 2 forms of strong trypsin inhibitor bands compared to 'Shiroyutaka', corresponding to purified 33 kDa and 21-22 kDa bands, while low TIA lines lacked or had weak trypsin inhibitor bands compared to 'Shiroyutaka'. Q94128-1 (high protein and low TIA line) completely lacked trypsin inhibitor bands. The intensities of the two trypsin inhibitor bands showed positive correlation with TIA measured. Wang and Yeh (1996) also detected 2 forms of trypsin inhibitor bands in their experiments.

Effect of pepstatin. Hou *et al* (2002) reported that sweetpotato proteinaceous TIs were degraded by an endogenous aspartic type protease. To determine whether there was any influence from endogenous proteases during sample preparation, measurements were repeated on four high protein lines with high TIA (Q90142-22, Q93017-5) or with low TIA (Q94128-1, KNF94225-13) and one middle TIA line 'Shiroyutaka', with and without pepstatin A (an aspartic type protease inhibitor). The levels of TIA as well as soluble protein contents measured did not show any difference in all the 5 lines, with and without pepstatin (Table 4). This was further confirmed on SDS-PAGE (reduced and heated condition) analyzed by Nupage system where separation and detection of protein bands were more sensitive than Tris-glycine system. The molecular weight of purified TIs was 23 kDa on this system. Protein profiles including 23 kDa TI which were extracted with 40 μM,

Table 3: Purification of sweetpotato TIs procedure.

Procedure	Total protein (mg)	Total activity (TUI)	Specific activity (TUI/mg protein)	Yield (%)	Ratio
Crude extraction	2447	36300000	14838	100	1.0
Ammonium sulphate precipitation (30-60%)	1343	30100000	22421	83	1.5
Trypsin affinity column chromatography	94	17080000	180910	47	12.2
Dialysis	66	14560000	221109	40	14.9

Starting material was 75 g of dried sweetpotato extract (equivalent to about 1.5 kg of raw material).

Table 4: TIA and soluble protein contents of crude extract containing 0, 40 and 400 μM of pepstatin.

Lines	TIA level	TIA (TUI/mg DW)			Soluble protein (mg/gDW)		
		Pepstatin (μM)			Pepstatin (μM)		
		0	40	400	0	40	400
Q90142-22	High	454	491	-	11.3	11.4	-
Q93017-5	High	301	311	-	9.2	9.3	-
Q94128-1	Low	5	10	25	11.1	11.3	11.5
KNF94225-13	Low	57	63	-	12.6	11.9	-
Shiroyutaka	Middle	144	147	-	6.4	7.2	-

400 µM pepsatatin or without pepstatin did not show any difference. These results showed that endogenous protease degradation of TIs and other soluble proteins at least by aspartic type did not occur during the sample preparation. Instead of 23 kDa TI, a lot of bands of relatively high molecular weight were seen in Q94128-1 and Q94225-13, while 23 kDa TI were predominant in 'Shiroyutaka' and Q90142-22. In conclusion, we were able to select Q94128-1 and Q94225-13 as lines with high protein content and very low TIA after screening more than 800 lines. These lines could be further developed into a good source material for animal feed as well as human consumption.

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