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Root Protein Quantity and Quality in a Seedling Population of Sweet Potatoes

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Additional index words. *Ipomoea batatas*, trypsin inhibitors, nonprotein nitrogen

Abstract. **A population of 100 sweet potato seedlings from 7 parent clones was grown for one season in order to evaluate root protein quantity and quality. Protein content of the 100 seedlings ranged from 4.38% to 8.98% with a mean of 6.29%; the 7 parents ranged from 4.96% to 6.53% with a mean of 5.72%. The mean of the seedlings was not significantly different from that of the parents. The 10 seedlings with highest protein (7.40% to 8.98%) were selected for further study of protein quality. Levels of nonprotein nitrogen (NPN) in these high protein selections were not significantly different from those of the parents. The correlation between the percentage of protein and the percentage of NPN was low (r = 0.30). The amino acid pattern in the high protein selections differed significantly from the parents with lower levels of valine, cysteine, methionine, tyrosine, and phenylalanine. Trypsin inhibitor activity (TIA) levels in the selected seedlings did not vary significantly from the parents. TIA and the percentage of protein were not significantly correlated (r = 0.15). The results indicate it is possible to obtain high protein cultivars without increasing the percentages of NPN and TIA. With the exception of valine, the aromatic and sulfur-containing amino acids, the overall protein quality was not changed in the seedlings with increased protein content.**

Sweet potato is consumed as a staple in many protein-poor countries thoughout the tropics, subtropics, and at least half the temperate zone (19). The consumption of low protein foods such as sweet potato seems to be one of the most important factors contributing to protein malnutrition in developing countries (4).

Although sweet potato protein content (quantity) can be increased through the use of various cultural management practices (3, 8, 12, 24), the most consistent means of increasing plant protein content may be through breeding and selection of high protein cultivars. Genetic variability for protein content seems to exist in sweet potato. The protein content ranges from 1.7% to 11.8% on a dry weight basis (DWB) (13). Similarly, Li (7) reported a range from 1.27% to 10.07% in varius Chinese cultivars.

Li (9) indicated that a mass selection technique would be effective in increasing protein and maintaining high yield. If the measurement of total protein in sweet potatoes in based on nitrogen content, however, it is necessary to distinguish between protein nitrogen and nonprotein-nitrogen (NPN). Levels as high as 40% of the total nitrogen have been found to be NPN (14, 15). The NPN fraction can contain as much as 83.4% amino acid, composed of asparagine (61%), aspartic acid (11%), glutamic acid (4%), serine (4%), and threonine (39%) (14).

In addition to protein quantity, the nutritional value (or quality) of the protein also should be considered. One important quality factor of sweet potato is the amino acid composition. Sweet potato contains an excess of all essential amino acids except tryptophan and total sulfur amino acids, which are limiting by comparison with the Food and Agricultural Organization of the United Nations (FAO) reference protein (13). Genetic variability in tryptophan content has been reported, however, so breeding and selection may improve this quality factor (13).

The presence of trypsin inhibitors in sweet potato (11, 16, 21) may contribute to decreased protein quality. Trypsin inhibitors can affect protein digestion adversely by inhibiting proteolysis. It has been suggested that the presence of these inhibitors in sweet potato is partially responsible for the disease enteritis necroticans (EN) in man and animals (5, 6). Significant variability in trypsin inhibitors has been found in sweet potato (11). Therefore, the level of trypsin inhibitors should be considered in potentially high protein cultivars which might be utilized in areas where EN and protein malnutrition is a problem.

The objectives of this study were: 1) to screen parents and the 1st generation seedling sweet potato population for protein content: and 2) to select the top 10% for further examination of protein quality as related to protein quantity.

One hundred open-pollinated seeds from seven parents were germinated and increased asexually to 9 plants per seedling. These 9 clonal plants were arranged in 3 replications (3 plants per plot) and grown in the field in a randomized complete block design. The 7 parents were replicated 4 times in a separate field, with 10 plants per plot. The plants were grown following standard cultural practices (23) at the Horticultural Crops Research Station at Clinton, N.C., for 102 days.

Six freshly harvested roots from each plot of the parents and seedlings were thoroughly washed, shredded, mixed, and a 180-g subsample was taken. Samples were frozen and

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Table 1. Amino acid and NH_4 content (g/100 g protein), percentage of NPN, percentage of protein, and TIA (units/g dry wt.) of 7 sweet potato parental clones.

	Clone designation							Waller Duncan
	171	196	308	320	323	343	719	LSD
Essential amino acids								
Threonine	4.37	4.47	4.93	4.81	3.81	4.93	4.71	0.51
Valine	6.29	7.62	8.08	7.45	6.57	7.85	7.44	1.54
Methionine	2.27	2.00	2.11	1.97	1.68	1.90	2.05	0.00
Isoleucine	3.58	4.08	4.09	4.13	3.36	3.99	3.85	0.37
Leucine	6.34	6.47	6.16	6.63	5.93	6.58	6.48	0.00
Phenylalanine	5.42	5.49	5.81	5.98	4.65	5.87	5.60	0.67
Lysine	4.16	4.88	5.21	4.63	4.52	4.57	4.74	0.45
Tryptophan	0.27	0.26	0.30	0.32	0.33	0.31	0.27	0.03
Histidine	4.81	6.29	6.38	6.27	6.02	6.09	6.40	0.77
Arginine	1.56	1.95	1.98	1.87	1.88	1.91	2.08	0.46
Nonessential amino acids								
Aspartic acid	24.21	16.48	19.16	21.74	21.45	21.48	18.24	1.91
Serine	5.62	5.67	6.11	6.33	5.50	6.30	5.67	0.68
Glutamic acid	11.24	9.48	11.96	10.11	10.10	10.16	10.34	0.98
Half-cystine	1.85	1.57	2.09	1.84	1.93	1.85	1.95	0.36
Proline	3.55	3.13	3.10	2.55	2.57	2.63	2.72	0.00
Glycine	4.10	4.08	4.60	4.10	3.65	3.38	4.19	0.67
Alanine	4.30	4.32	5.60	4.82	4.43	4.56	4.58	0.00
Tyrosine	4.04	4.37	4.66	4.50	3.83	4.52	4.44	0.57
NH_4	2.79	1.95	2.07	2.36	2.33	2.10	2.16	0.28
NPN (%)	31.18	16.64	22.01	30.36	25.96	33.69	20.80	4.06
Protein (%)	5.85	6.53	4.96	5.73	5.66	5.70	5.67	0.70
TIA	119	775	518	112	846	122	144	79.51

Table 2. Amino acid and NH_4 content (g/100 g protein), percentage of NPN, percentage of protein, and TIA (units/g dry wt.) of high protein sweet potato selections.

	Selection designation										Waller Duncan
	10	11	16	41	48	50	61	71	82	93	LSD
Essential amino acids											
Threonine	4.37	3.89	4.39	4.57	4.41	4.26	4.74	4.30	3.50	3.36	0.40
Valine	4.55	5.22	6.14	6.05	6.91	5.75	5.97	7.27	5.70	4.73	0.94
Methionine	1.37	1.19	1.10	1.33	1.94	0.95	1.46	2.03	1.58	0.77	0.44
Isoleucine	3.27	3.34	3.92	3.69	3.67	4.02	3.77	3.93	3.92	3.07	0.26
Leucine	5.43	5.47	5.89	6.05	6.02	6.43	5.95	6.22	4.68	5.01	0.80
Phenylalanine	5.12	4.50	5.42	5.37	5.20	5.17	5.00	5.42	3.91	4.24	0.36
Lysine	3.86	5.31	4.78	4.56	4.51	5.11	4.55	4.57	3.86	4.10	0.53
Tryptophan	0.24	0.31	0.25	0.23	0.23	0.31	0.23	0.28	0.33	0.30	0.05
Histidine	4.28	5.46	4.58	4.62	5.60	5.62	4.53	5.08	4.27	6.32	1.45
Arginine	2.22	2.09	2.54	2.75	2.33	2.12	1.91	2.17	1.48	1.27	0.50
Nonessential amino acids											
Aspartic acid	23.55	22.20	23.27	20.93	20.02	22.52	26.11	17.98	25.24	24.50	3.22
Serine	5.12	5.56	5.70	5.53	5.47	5.93	5.53	5.73	4.73	4.69	0.64
Glutamic acid	10.93	10.27	11.77	9.99	9.84	10.65	11.28	9.63	9.71	8.48	1.40
Half-cystine	1.29	1.33	1.65	1.51	1.62	1.48	1.38	1.55	1.32	1.44	0.51
Proline	2.42	2.45	2.83	2.63	2.98	2.92	2.72	3.46	2.62	2.20	0.64
Glycine	3.24	3.60	3.85	3.65	4.01	4.38	4.10	3.76	3.15	3.33	0.59
Alanine	3.54	4.60	3.93	3.60	4.66	4.99	5.25	3.22	4.19	4.48	1.51
Tyrosine	3.79	3.60	4.05	4.01	3.91	3.96	3.92	4.25	3.16	3.40	0.34
NH_4	2.28	2.34	2.22	1.82	2.19	2.62	2.85	1.85	2.57	2.46	0.38
NPN (%)	33.49	34.19	26.27	24.43	24.34	27.23	32.31	22.10	33.75	37.74	5.29
Protein (%)	8.44	8.55	7.95	7.87	7.68	8.04	7.40	7.80	8.98	8.55	1.72
TIA	165	649	178	192	676	803	734	189	246	716	90.30

subsequently freeze-dried. The percentage of dry matter was determined after freeze-drying, and samples were ground with a Wiley Mill to pass a 40-mesh screen. The resulting powder was used in all subsequent analyses.

Total root protein content of all parents and seedlings was measured by determining nitrogen content using a semi-micro Kjeldahl method (2). Protein then was estimated by using 6.25 as the conversion factor.

Root nonprotein-nitrogen was measured in the 7 parents and the 10% of the seedlings which had the highest total protein content. Nonprotein was estimated from 80% ethanol soluble nitrogen (10).

Root amino acid composition of each high protein selection and the 7 parents was determined on the freeze-dried powder by automated amino acid analysis, a modification of Spackman et al. (17). Samples of 300 mg were hydrolyzed in 20 ml of 6 N HCl for 2 hr at 145°C in Klimax culture tubes with teflon caps (25 × 150 mm; Kimble #45066-A) flushed with N_2 gas. After hydrolysis, samples were adjusted to pH 2.2 and brought up to 100 ml with citrate buffer, pH 2.2. Subsamples were centrifuged at 10,000 g for 5 min and 20 µl aliquot of the supernatant was injected into a Durram D-500 Amino Acid Analyzer (Dionex Corp., Sunnyvale, Calif.). Tryptophan was determined colorimetrically (1) substituting 20% N-bromosuccinimide for the dioxane-butyric acid after enzymatic hydrolysis (13, 18, 22). A further test on trytophan, to ascertain if any interference might result from the 80% ethanol soluble NPN and carbohydrate fraction, was determined by measuring tryptophan in selection 16 before and after the 80% ethanol soluble fraction was removed. An additional analysis (not replicated) of amino acid content was made the following year to determine variability over years and over storage on one parental line. Roots were analyzed by the methods previously described at harvest in the fall of 1981 and after 69 days of storage without humidity control at 13°C after curing for 5 days at 30°C and 90% to 95% relative humidity.

Four grams of freeze-dried sample in 20 ml of 0.9% NaCl (4°C) were homogenized using a Polytron for 20 sec and were centrifuged for 20 min at 27,000 g (4°). Dilutions were made with 0.1 M phosphate buffer (pH 8.0). Samples were assayed for trypsin inhibitor activity (TIA) by incubation with trypsin and subsequent measurement of proteolytic action on casein as given by Sugiura et al. (21).

Protein content of the 100 seedlings ranged from 4.38% to 8.98% with a mean of 6.29% (dry weight basis) (Fig. 1). The 7 parents ranged from 4.96% to 6.53% with a mean of 5.72% (Table 1). The mean protein content of the seedling population was not significantly different ($P \leq 0.05$) from the mean protein content of the parents. The 10 seedlings with highest protein content were selected for a study of protein quality. They ranged from 7.40% to 8.98% with a mean of 8.13%

The percentage NPN fraction of the 10 seedlings with highest protein content ranged from 22.10% to 37.74% with a mean value of 29.58% (Table 2). The parents ranged from 16.64% to 33.69% with a mean of 25.80% (Table 1). The mean values of the parents and selections were not significantly different ($P \leq 0.05$). Protein content and percentage of NPN were not correlated ($r = 0.30$). Thus, selection for high protein need not result in an appreciable change in NPN. Correlations between amino acids and NPN were examined within selections and parents. There was a significant correlation ($r = 0.58$) between aspartic acid in the crude protein and NPN in the total population, supporting the statement that aspartate or asparagine is a major amino acid in the NPN fraction, as reported by Purcell and Walter (14).

The amino acid pattern between selections and parents differed significantly in valine, half-cystine, methionine, tyrosine, and phenylalanine. These amino acids were lower in the selections than in the parents ($P \leq 0.05$). This reduction in valine, the sulfur containing amino acids, and the aromatic

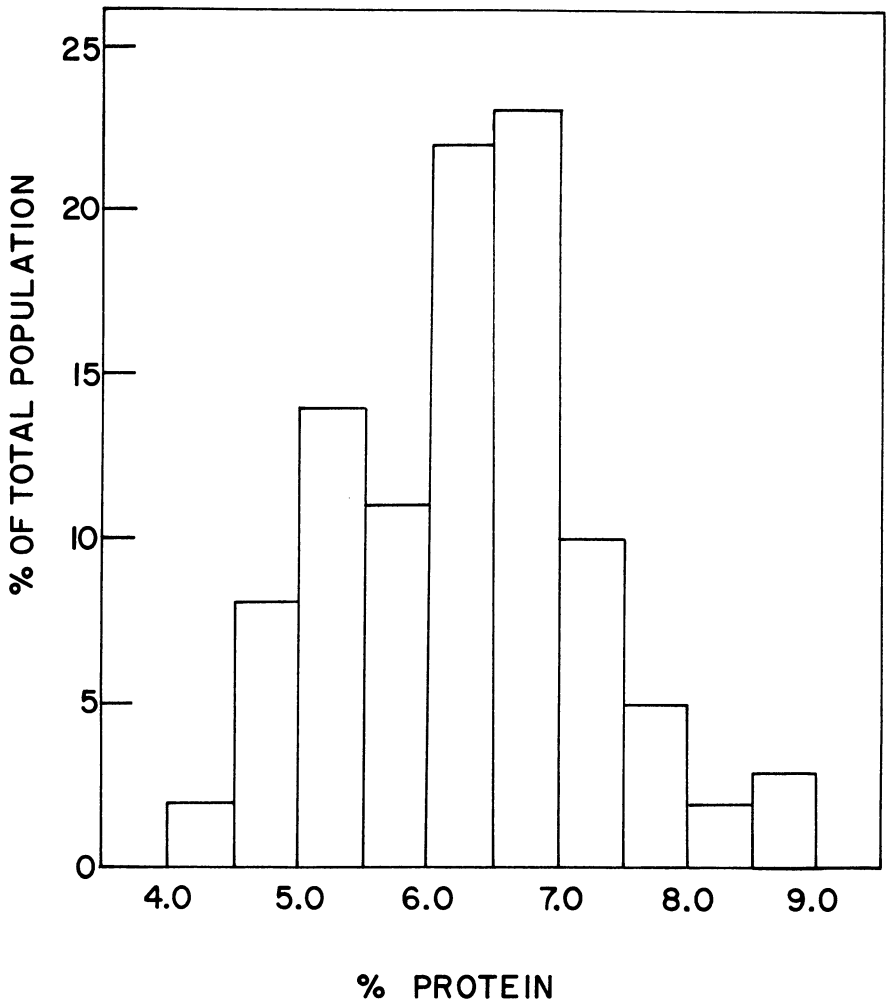

Fig. 1. Frequency distribution of protein content (dry weight basis) in 100 sweet potato seedlings from 7 parental clones.

amino acids needs to be confirmed in the next generation before definitive conclusions can be made. Except for half-cystine and alanine, there was significant variation ($P \leq 0.05$) for amino acids among the high protein selections. Thus, genetic variability in amino acid composition still remains for selection in the following generation. Parental variation was significant ($P \leq 0.05$) except for leucine, methionine, serine, proline, alanine, and arginine.

The histidine content found in these selections and parents was higher than levels reported previously (13). The mean histidine of selections and parents was 5.54 g/100 g protein as compared to the average amount of 1.86 g/100 g protein found in 6 varieties at harvest by Purcell et al. (13). When one of the parental clones, NC 196, was analyzed at harvest in 1981, the histidine contient of 5.80 g/100 g protein was very similar to the histidine content of 6.29 g/100 in 1980 at harvest, suggesting low year-to-year variability. Histidine content did diminish by 39% after 69 days of storage (from 5.80 to 3.56 g/100 g protein). These data were not from replicated trials and further study is needed to confirm this reduction during storage. Cultivar differences and perhaps changes in histidine levels during storage may be responsible for the reduced levels reported previously (13).

Tryptophan content in the high protein selections and parents average 0.28 g/100 g protein. This tryptophan level was significantly lower than the tryptophan content reported previously (1.22 g/100 g protein which is close to the FAO reference protein requirement of 1.4 g/100 g) (13). The tryptophan levels of the roots in this study approached those found by Splittstoesser and Martin (20) of 0.1 g/100 g protein. When the parental clone, NC 196, was analyzed in 1981,tryptophan measured 0.24 g/100 g protein, the same as in 1980 at harvest. After 69 days of storage, tryptophan measured 0.25 g/100 g protein (again from nonreplicated tests). Thus, tryptophan does not seem to vary appreciably between years and during storage. When the 80% EtOH soluble fraction was removed in selection 16 in order to assess any possible interferences, the tryptophan level was 0.25 g/100 g protein. There was no apparent interference in the tryptophan assay by the ethanol soluble NPN and carbohydrate fraction.

Trypsin inhibitor activity in the high protein selections ranged from 165 to 803 TIA units/g (dry weight basis) with a mean of 455 (Table 2). The parents ranged from 112 to 846 TIA units/g with a mean of 374 (Table 1). The difference between the mean of the parents and selections was not significant. Overall, trypsin inhibitor activity levels did not change significantly in the selections. Also, no significant ($P \leq 0.05$) correlation ($r = 0.15$) was found between protein quantity and TIA, in contrast to data given by Lin and Chen (11), who found a significant correlation. The findings in this study suggest high protein is not associated with high levels of trypsin inhibitors.

The possible changes in amino acid composition should be examined in future generations in order to determine if this quality factor is at least maintained. Otherwise, the results indicate that it is possible to obtain high protein cultivars with lower NPN and TIA. With the possible exception of valine, the aromatic and sulfur containing amino acids, results of this study indicate that overall sweet potato protein quality can be maintained in high protein selections.

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Delayed Harvest Reduces Yield of Dry Red Chile in Southern New Mexico

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Additional index words. *Capsicum*, chili, chilli

Abstract. **Mature red chile fruit [*Capsicum annuum* (L.)] were harvested over 3 years at 2 locations in southern New Mexico to determine the effects of harvest date on yield and color. Yields peaked in late October or early November and then declined linearly through December or January. Declines were correlated highly with fewer marketable pods harvested due to detachment or discoloration. The detachment of mature red pods over the test period was affected differentially by cultivar. Color (in ASTA units) varied from good commercial levels to substandard ones between years, but the color of late-harvested pods was normally equal to or better than that from earlier-harvested fruit.**

Mildly pungent chile produced for dry red powder is an important crop for southern New Mexico growers. Harvesting usually begins after 15 Sept. and may continue into January. Delayed harvesting results in natural fruit drying, reducing the fossil fuel energy consumed in artificial drying. Delayed harvest also spreads out the demand for harvesting labor and reduces capital costs for processing and harvesting equipment. Few quantative data are available on yield and quality effects of delayed harvest in arid climates. Leyendecker (4) reported molds proliferated in pods following a hard freeze, which adversely affected quality of New Mexico chile. Palevitch et al. (6), working in Israel, reported that dry fruit yields were not affected by delaying harvest 28 days, and the color intensity increased during late-summer field drying. However, Kanner et al. (2) showed that color of stored powder deteriorated more rapidly when produced from fruits allowed to dry on the plant. In New Mexico, it commonly is assumed that yield and quality decline as harvest is delayed after pod maturity. This report summarizes data collected over 3 years at 2 locations to determine effects of harvesting date on dry red fruit yields and color.

Uniform stands with mature plants were selected at 2 sites in southern New Mexico (Las Cruces and Roswell). For experiments conducted in Las Cruces, harvested plots of 'New Mexico No. 6–4' or 'NuMex R. Naky' consisted of a single row, 1.01 m wide and 3.1 m long. At Roswell, plots were established in fields planted with 'California Mild'. The plots were 1.01 m wide and 0.92 or 1.0 m long in 1980 and 1981, respectively. Cultural management procedures were normal commercial practices.

All fully red, nonblemished pods were harvested from the plants by the same person

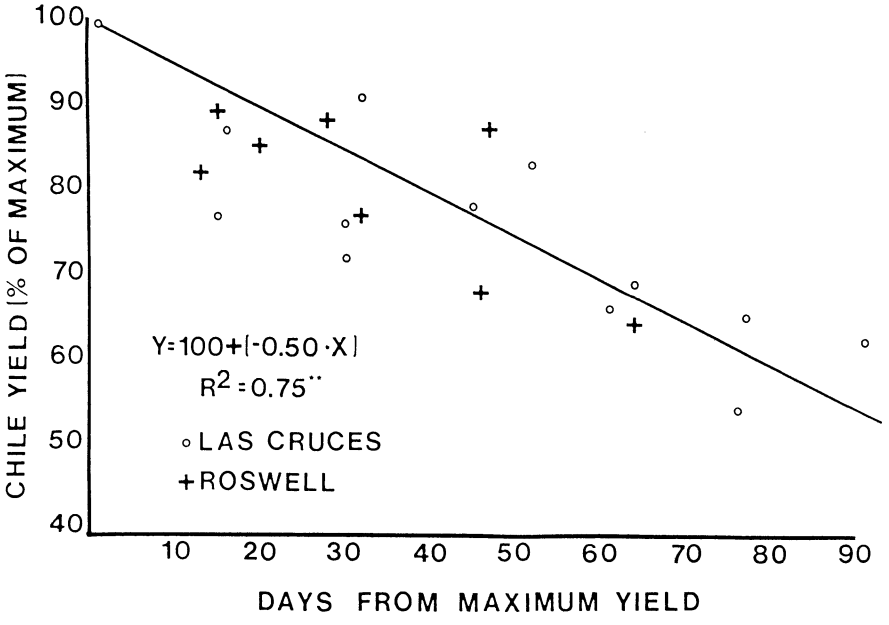

Fig. 1. The regression of delayed harvest on percentage of maximum dry red chile yield for 6 tests conducted at 2 locations over 3 years.

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