Genotype and Fertilization Effects on Trypsin Inhibitor Activity in Sweetpotato

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Additional index words. animal feed, cultivar, crude protein, fertilizer, *Ipomoea batatas*, proteinase, soybean, *Glycine max*

Abstract. Sweetpotato [*Ipomoea batatas* (L.) Lam.] is intensively used as an animal feed in many developing countries. Information about trypsin inhibitor activity (TIA), an antinutritional component in this crop, will be useful for breeding sweetpotato as animal feed. Nine sweetpotato lines were grown at two locations and fertilized or nonfertilized conditions at each location. Samples were analyzed for TIA using a substrate-specific colorimetric method. Soybean [*Glycine max* (L.) Merr.] seeds were used to compare the levels of TIA in sweetpotato and soybean. Activity in roots ranged from 29.5 to 55.0 units in the nine lines. The mean TIA in roots was 40.7 units averaged over lines and environments, which was $\approx 28\%$ of the mean for the five soybean cultivars. Activity in sweetpotato vines was only $\approx 14.6\%$ of that in the roots, and TIA in fertilized plots was 150% and 67% higher than that in nonfertilized plots in the two locations, respectively. There was a small but significant positive correlation between TIA and crude protein in roots. These results suggested that TIA in sweetpotato storage roots may be high enough to have a substantial nutritional impact on animals, whereas TIA in vines is very low and should be of less nutritional concern.

Annually, $\approx 35\%$ to 40% of the world's annual sweetpotato production is used as animal feed (International Potato Center, 1992). Sweetpotato roots are usually used fresh, sun-dried, or as silage to feed monogastric and ruminant animals under subsistence farming systems, but they are used only as a partial substitute for cereal feed ingredients, particularly maize (Yeh and Bouwkamp, 1985). Poor protein digestibility because of trypsin inhibitors was thought to be partially responsible for unsatisfactory feeding efficiency when uncooked roots were used as swine feed (Chien and Lee, 1980; Yeh and Bouwkamp, 1985). Trypsin is a major digestive-tract enzyme that hydrolyzes proteins. In sweetpotato and a number of other species, several polypeptides and proteins are potent inhibitors of these proteolytic enzymes, i.e., they impede metabolism of the proteins and, as a consequence, are nutritionally highly undesirable. Trypsin inhibitors are one group of these proteinase inhibitors. In chickens and rats, trypsin inhibitors increased pancreatic secretions and hypertrophy of the pancreas (Birk, 1989). As pancreatic secretions are rich in sulfur-containing amino acids, which are usually defi-

cient in the diet, animals may also become deficient in these amino acids, resulting in a further depression in growth (Birk, 1989). Batterham et al. (1993) found that a growing pig can tolerate at least $4.7 \text{ mg} \cdot \text{g}^{-1}$ of trypsin inhibitors in the diet. This threshold level is unlikely to be exceeded in most conventional diets containing grain legumes, except for raw soybean, in which a much higher level of trypsin and chymotrypsin inhibitors ($8\text{--}20 \text{ mg} \cdot \text{g}^{-1}$) was reported (Saini, 1989).

Levels of trypsin inhibitor activity (TIA) in sweetpotato cultivars have been reported (Bradbury et al., 1985; Dickey et al., 1984; Lin and Chen, 1980). Because of the lack of uniform assay methods and different inhibition "units" used for TIA, the level of trypsin inhibitor in sweetpotato cannot be generalized, nor are the reported TIA levels comparable to those of other crops, such as soybean. Thus, whether the amount of trypsin inhibitor in sweetpotato is high enough to be of nutritional concern is not clear.

Various environmental effects, such as growing season (Bouwkamp et al., 1985; Lin, 1989), accumulated rainfall (Lin, 1989), and location (Bradbury et al., 1985) have been reported to affect TIA. Nitrogen fertilization significantly affects protein content in sweetpotato (Andrade, 1994; Collins and Walter, 1982; Purcell et al., 1982); whether trypsin inhibitor is also affected is not known.

Sweetpotato vines are used almost exclusively as animal feed in many subsistence farming systems. Sweetpotato vines are inferior to roots in energy content but superior in quantity and quality of protein. The average crude protein (CP) in sweetpotato vines is $\approx 20\%$ on a dry-mass basis (dmb) (Li, 1974). Usually, vines are used directly as feed without precooking. Sweetpotato vines appear to be easily digested by ruminant animals (Woolfe, 1992). Some adverse effects have been reported in monogastric animals, such as swine (Lee and Lee, 1979; Lee and Yang, 1979), and these were attributed to high fiber content, lower digestible energy (Yeh and Bouwkamp, 1985), and presence of trypsin inhibitors (Lee and Lee, 1979). The level of TIA in sweetpotato vines has not been reported.

We still need to justify altering TIA in sweetpotato, either through genetic modification, cultivation practice, or processing. Both determining whether the amount of trypsin inhibitor in sweetpotato storage roots and vines would exceed the tolerance threshold of animals, such as growing pigs, and an understanding of the major sources of variation in TIA in sweetpotato, so that an appropriate approach can be taken to alter trypsin inhibitor levels, are necessary.

The objectives of this research were to 1) compare TIA in sweetpotato with that in soybean; 2) study genotype, production location, and fertilization effects on TIA in storage roots; 3) clarify the relationship between TIA and CP in storage roots; and 4) measure TIA in sweetpotato vines.

Materials and Methods

Field experiment. Nine sweetpotato lines or advanced clones (Table 1) were grown at the Castle Hayne and Clinton Horticultural Crops Research Stations in North Carolina in

Table 1. Mean trypsin inhibitor activity (TUI/mg dry mass) of nine sweetpotato lines in four environments, North Carolina, 1991.

Lines	Storage roots				Vines	
	Clinton		Castle Hayne		Clinton	
	Fertilized	Nonfertilized	Fertilized	Nonfertilized	Mean	Fertilized
Jewel	40.6	22.4	51.4	17.6	33.0	5.8
A-21	49.4	34.7	78.3	24.6	46.7	4.4
White Delite	45.4	13.2	44.9	17.1	30.1	7.2
NC PR 198	64.4	35.7	85.6	34.3	55.0	12.3
Carolina Nugget	55.8	39.6	76.6	28.5	50.1	9.6
MD 88-26	53.4	42.9	43.9	22.3	40.6	10.3
Cordner	42.8	15.0	39.0	21.2	29.5	5.9
MD 810	47.9	39.7	71.9	30.3	47.4	3.7
Beauregard	43.7	24.2	48.8	19.9	34.2	5.7
Mean	49.3	29.7	60.0	24.0	40.7	7.2

^aTUI = trypsin units inhibited. One trypsin unit = an increase of 0.01 in absorbance at A_{410} .

Received for publication 18 Dec. 1996. Accepted for publication 16 June 1997. We thank William M. Walter and Julia Wright for their critical review and Bill Hardy for his editing of this manuscript. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked advertisement solely to indicate this fact.

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1991. The soil type in both locations is sandy loam. Transplants were produced from bedded roots at the Horticultural Crops Research Station, Clinton, and transplanted in early June, following corn in the rotation.

At each of the two locations, fertilizer was applied in one field, but not in the other adjacent field. In the fertilized field, 240 kg·ha⁻¹ of 15N-0P-14K was applied to each plot as a preplant application. Sixty days after transplanting, 650 kg·ha⁻¹ of 8N-0P-24K was applied as a sidedressing. Within each field, a randomized complete-block design with four replications was used. Each single-row plot consisted of 10 plants spaced 0.3 m apart in the row. Replications were separated by a 1-m buffer zone. 'White Delite' sweetpotato plants were grown in the rows adjacent to the test. Irrigation and other cultivation practices followed Wilson et al. (1980) and were applied uniformly in both locations. Roots were harvested 135–140 d after transplanting.

Sample preparation. Six freshly harvested U.S. no. 1 storage roots from each plot were washed, shredded, and mixed. A 100-g sample was freeze-dried and the dried sample was ground in a blender for 40 s to pass through a 425- μ m sieve (35-mesh). The resulting "flour" was stored at 4 °C and used in subsequent assays.

The vine samples were taken from the fertilized field in Clinton only. Five full-length vines from each plot were cut at 20 cm above the soil surface, chopped into small pieces, and mixed. A subsample of 200 g was freeze-dried and the samples from four replications were mixed thoroughly; thus, each sample representing one clone was averaged over four replications. The samples were then ground into powders in a blender.

Five soybean cultivars (Table 2) were used. The soybean seeds were provided by North Carolina Foundation Seed Producers, Raleigh. About 10 g of soybean seeds were ground and passed through a 425- μ m sieve (35-mesh). The resulting flour was used for the TIA assay. Three replicated samples were taken for each cultivar.

Assay of TIA and crude protein. An improved colorimetric method (Liu and Markakis, 1989) was used to determine TIA. One gram of lyophilized flour sample was homogenized in 20 mL of 0.9% NaCl (4 °C) using a polytron (Fisher Scientific, Hampton, N.H.) for 1 min. The sample was then centrifuged for 20 min at 27,000 g. The supernatants were filtered through Whatman no. 2 paper. The final sample solution was obtained by adding 1–10 mL of

tris buffer (50 mM, pH 8.2) to 50 μ L of supernatant to give 30% to 70% inhibition of trypsin activity.

Ten milligrams of crystalline porcine trypsin (type IX; Sigma Chemical, St. Louis) were dissolved in 50 mL of 1 mM HCl, containing 2.5 mM CaCl₂, to make a stock solution. A working trypsin solution was prepared by diluting the stock solution 12.5 times with 1 mM HCl. A synthetic trypsin-specific substrate, BAPNA (benzoyl-DL-arginine-p-nitroanilide hydrochloride), was used. A stock BAPNA solution was prepared by dissolving 400 mg of BAPNA (Sigma Chemical) in 10 mL of dimethyl sulfoxide. Immediately before determining TIA, the stock BAPNA solution was diluted 100 times with 50 mM of Tris buffer (prewarmed at 37 °C).

One mL of sample solution was mixed with 2 mL of BAPNA and incubated for 5 min at 37 °C. To start the reaction, 0.5 mL of trypsin solution was added. After 10 min, the reaction was stopped by injecting 0.5 mL of 30% acetic acid. The absorbance at 410 nm (sample reading, A_{410s}) was a measure of the trypsin activity in the presence of the sample inhibitors. An uninhibited reaction was also run by replacing the sample with 1 mL of Tris buffer. The absorbance for this reaction (reference reading, A_{410r}) measures the uninhibited trypsin activity. Trypsin inhibitor activity is expressed in trypsin units inhibited (TUI) per milligram of dry sample. Trypsin units were defined as an A_{410} increase of 0.01 under the conditions of the assay; TIA was calculated as follows:

$$\text{TUI per mg of dry sample} = \frac{(A_{410r} - A_{410s}) \times 100}{\text{mg dry sample}}$$

Total N was determined using a semi-micro-Kjeldahl method (Association of Official Analytical Chemists, 1975). Crude protein concentration was then estimated by using 6.25 as the conversion factor.

Statistical analysis. Within each environment (location $\times$ fertilizer), a two-way analysis of variance (ANOVA) model with block and line as the main effects was used for analysis. Variance homogeneity across the four environments was tested using Bartlett's test (Steele and Torrie, 1980). A combined analysis was then completed for the four environments. Both line and environment were considered fixed (Cochran and Cox, 1957).

Pearson's correlation between TIA in storage roots and vines was calculated in the

fertilized field of Clinton station. Pearson's correlation between TIA and crude protein in storage roots was calculated among 36 samples (nine cultivars $\times$ four replications) within each of the four environments.

Analysis of variance, the general linear model (GLM), and PROC CORR procedure of SAS (SAS Institute, 1992) were used for computation.

Results and Discussion

TIA in storage roots. TIA in storage roots differed significantly among the nine lines, ranging from 29.5 to 55.0, with a mean of 40.7 units (Tables 1 and 2). TIA in the five control soybean lines ranged from 123.2 to 169.6, with a mean of 143.3 units (Table 3). The mean soybean TIA level is close to the values reported by Liu and Markakis (1989), where the same assay method was used. Sweetpotato storage roots have a TIA level about one-third that of soybean seeds. Assuming an average of 10–20 mg·g⁻¹ of trypsin inhibitors in soybean seeds (Batterham et al., 1993), an average of 3.5–7 mg·g⁻¹ (dmb) of trypsin inhibitors in sweetpotato storage roots might be deduced. Bradbury et al. (1984) estimated that the percentage of trypsin inhibitors present in the total protein of sweetpotato was $\approx$ 0.03% to 2%, which would give a range of 1.2–10.2 mg·g⁻¹ of trypsin inhibitors, with a 5.08% mean crude protein content among the nine lines (Table 2).

Batterham et al. (1993) studied the threshold level of trypsin and chymotrypsin inhibitors by adding meals rich in these inhibitors to the diets of growing pigs and recording responses. They found that the pigs could tolerate dietary levels of at least 4.7 and 4.5 mg·g⁻¹ (dry sample) of trypsin and chymotrypsin inhibitors, respectively. They also suggested that the threshold level should be lower for piglets because their digestive system is immature and thus more sensitive to the effects of digestive inhibitors. Therefore, the sweetpotato-based diet, if not processed, may be partially responsible for the poor feeding efficiency in previously reported animal feeding trials (Lee and Lee, 1979; Yeh and Bouwkamp, 1985). However, because the exact mechanism of trypsin inhibition in the digestive system is not fully understood, and the physiological response varies among animal species, it would be premature to generalize the effects of a sweetpotato-based diet on

Table 2. Combined analyses of variance for trypsin inhibitor activity of nine sweetpotato lines from fertilized and nonfertilized field at two locations, North Carolina, 1991.

Source of variation	DF	SS	MS	F-value	P-value
Location	1	226	226	4.26	0.0607
Fertilizer	1	27825	27825	528.01	0.0001
Location $\times$ fertilizer	1	2456	2456	46.6	0.0001
Replication					
(location $\times$ fertilizer)	12	632	53	0.69	0.7541
Line	8	11424	1428	18.81	0.0001
Line $\times$ fertilizer	8	1593	199	42.62	0.0114
Pooled error	112	8502	76		

Table 3. Trypsin inhibitor activity (TUI/mg dry mass) of five soybean cultivars, Raleigh, N.C., 1991.

Cultivar	Replication			Mean
	I	II	III	
Ciby G361	168.2	165.3	173.2	169.6
Hutcheson	174.3	166.6	169.3	168.0
Holladay	134.0	131.6	130.3	132.0
Young	143.5	135.6	138.9	139.2
Brim	125.7	122.2	122.9	123.2
Mean	149.1	144.2	146.9	146.4

^aTUI = trypsin units inhibited. One trypsin unit = an increase of 0.01 in absorbance at A_{410} .

animal feeding. Tsou and Hong (1989) reported that the poor feeding efficiency of sweetpotato might be due to its inferior starch digestibility. In vivo trials on different animals are necessary to assess the nutritional impact of sweetpotato clones with high TIA levels.

TIA in vines. Among the nine lines grown in the fertilized field at Clinton, TIA ranged from 3.7 to 12.3 units, with a mean of 7.2 units (Table 1). This is only 14.6% of the mean TIA level in the storage roots of the nine lines in the same plots, or $\approx 5\%$ of the mean level in five soybean cultivars. It is equivalent to 0.5–1 mg·g⁻¹ of trypsin inhibitors (dmb). Such a level is far below the reported threshold for growing pigs (Batterham et al., 1993). Lee and Lee (1979) found that the hog feed ration with added sweetpotato vines had a higher TIA content and lower feed efficiency than the

other rations. They suspected that trypsin inhibitors might be one of the factors responsible. Similar results were also reported by Lee and Yang (1979). The present results suggest that TIA in sweetpotato vines is very low relative to that in storage roots; thus, it should be of less nutritional importance to animals. Other factors, such as fiber, might be more important for digestibility (Yeh and Bouwkamp, 1985).

Environmental effects on trypsin inhibitors. Fertilizer effect and line $\times$ fertilizer interaction were highly significant (Table 2). In both locations (Castle Hayne and Clinton), the mean TIA in the fertilized plot was significantly higher than that in the nonfertilized plot. No location effect was found. This result agreed with the conclusions of Bradbury et al. (1985) and Bouwkamp et al. (1985) that TIA

in sweetpotato varies widely with environment. Nitrogen fertilization significantly increases protein content in sweetpotato (Andrade, 1994; Collins and Walter, 1982). A similar result was observed in this experiment, where crude protein was doubled by fertilization (Table 4). This study is the first to demonstrate that trypsin inhibitor also responds to fertilizer. However, response in fertilized environments differed among lines. For example, TIA in 'White Delite' tripled in the fertilized plots, whereas TIA in 'MD 88-26' increased only 50% (Table 1). This difference in response explains the significance of line $\times$ fertilizer interaction.

Correlation between crude protein and TIA. Correlation coefficients between crude protein content and TIA in each of the four environments ranged from $r=0.22$ to $r=0.43$, with three r values being significant (Fig. 1). This indicates that there is a moderate positive correlation between crude protein and TIA across lines.

Most previous studies agree that there is significant positive correlation between protein content and trypsin inhibitor in sweetpotato; where an environment favors protein content, it also favors trypsin inhibitors (Bouwkamp et al., 1985; Bradbury et al., 1985; Lin and Chen, 1980). However, the correlation among genotypes in the same environment has been controversial. A significant positive correlation between water-soluble protein and TIA was found for cultivars planted in Taiwan in a single season (Lin and Chen,

Table 4. Mean crude protein content (g/100 g dry matter) of nine sweetpotato lines in four environments, North Carolina, 1991.

Lines	Environment				Mean
	Clinton		Castle Hayne		
	Fertilized	Nonfertilized	Fertilized	Nonfertilized	
Jewel	6.35	2.66	6.71	3.33	4.76
A-21	7.06	2.11	8.02	3.52	5.18
White Delite	5.99	3.20	5.94	2.87	4.50
NC PR 198	7.23	3.71	8.53	3.72	5.80
Carolina Nugget	6.51	2.82	8.17	3.37	5.22
MD 88-26	5.97	2.49	7.66	3.76	4.97
Cordner	5.56	2.36	6.84	3.34	4.53
MD 810	5.51	4.07	6.85	3.54	4.99
Beauregard	7.20	2.94	9.11	3.93	5.80
Mean	6.37	2.93	7.54	3.49	5.08

Fig. 1. Relation between trypsin inhibitor activity and crude protein in sweetpotato storage roots from fertilized and nonfertilized fields in two locations, North Carolina, 1991.

1980) and in different seasons (Bouwkamp et al., 1985). In contrast, no correlation between TIA and crude protein was found in cultivars from either North America (Dickey et al., 1984) or Papua New Guinea (Bradbury et al., 1985). The disagreement might be attributable to the difference in the way protein was quantified. Estimation of crude protein, which is converted from total N ($Kjedahl\ N \times 6.25$), gives inflated values in sweetpotato. Crude protein of sweetpotato includes all nitrogenous compounds present in the hydrolysate. Sweetpotatoes at harvest contain up to 35% nonprotein N and the proportion varies among cultivars (Walter et al., 1984). Therefore, correlation between trypsin inhibitor and true protein would be a better measurement of this relationship.

Based on the present study, we draw the following conclusions:

Sweetpotato storage roots contain a relatively high level of trypsin inhibitors, equivalent to 28% of the mean TIA level in five soybean cultivars. This level may be sufficiently high to exceed the tolerance threshold of certain animals when uncooked sweetpotato roots are used as feed.

Sweetpotato vines have $\approx 14.6\%$ the TIA of storage roots (dmb), which is roughly equivalent to $0.5\text{--}1\text{ mg}\cdot\text{g}^{-1}$ of trypsin inhibitors. The low TIA in vines should not have a significant nutritional impact on animal feeding.

Environment and line $\times$ environment interaction significantly affect TIA in sweetpotato. Fertilization significantly increased TIA. When environmental conditions change, TIA and crude protein changes parallel one another. Within each environment, crude protein and TIA were statistically correlated among lines.

Literature Cited

- Andrade, M. 1994. Physiological basis of yield stability of sweetpotato. PhD Diss., North Carolina State Univ., Raleigh.
- Association of Official Analytical Chemists. 1975. Official methods of analysis, 12th ed. Assoc. Offic. Anal. Chem., Washington, D.C.
- Batterham, E.S., S. Harpal, M.A. Lynette, and D.B. Robin. 1993. Tolerance of growing pigs to trypsin and chymotrypsin inhibitors in chickpeas (*Cicer arietinum*) and pigeonpeas (*Cajanus cajan*). J. Sci. Food Agr. 61:211–216.
- Birk, Y. 1989. Protein protease inhibitors of plant origin and their significance in nutrition, p. 83–94. In: J. Huisman, T.F. Van der Poel, and I.E. Liener (eds.). Recent advances in research in antinutritional factors in legume seeds. Proc. First Intl. Wkshp. Antinutritional Factors (ANF) in Legume Seeds. Pudoc, Wageningen, The Netherlands.
- Bouwkamp, J.C., S.C.S. Tsou, and S.S.M. Lin. 1985. Genotype and environment effects on the relationship between protein concentration and trypsin inhibitor levels in sweet potatoes. HortScience 20:886–889.
- Bradbury, J.H., J. Baines, B. Hammer, M. Anders, and J.S. Miller. 1984. Analysis of sweetpotato (*Ipomoea batatas*) from the highlands of Papua New Guinea: Relevance to the incidence of *Enteritis necroticans*. J. Agr. Food Chem. 32:469–473.
- Bradbury, J.H., B. Hammer, T. Nguyen, M. Anders, and J.S. Miller. 1985. Protein quantity and quality and trypsin inhibitor content of sweet potato cultivars from the highlands of Papua New Guinea. J. Agr. Food Chem. 33:281–285.
- Chien, S.L. and P.K. Lee. 1980. The effect of physical treatment on the available lysine and trypsin inhibitor of sweet potatoes. Taiwan Livestock Res. 13:75–84.
- Cochran, W.G. and G.M. Cox. 1957. Experimental Design. 2nd ed. Wiley, New York.
- Collins, W.W. and W.M. Walter. 1982. Potential for increasing nutritional value of sweet potatoes, p. 355–363. In: R.L. Villareal and T.D. Griggs (eds.). Sweet potato, Proc. First Intl. Symp. Asian Vegetable Research and Development Center, Shanhua, Tainan.
- Dickey, L.F., W.W. Collins, and C.T. Young. 1984. Root protein quantity and quality in a seedling population of sweet potatoes. HortScience 19:689–692.
- International Potato Center. 1992. International Potato Center 1992 Annu. Rpt. Lima, Peru.
- Lee, P.K. and M.S. Lee. 1979. Study on hog feed formula of using high protein sweet potato chips and dehydrated sweet potato vines as the main ingredient. J. Taiwan Livestock Res. 12: 49–55.
- Lee, P.K. and Y.F. Yang. 1979. Nutritive value of high protein sweet potato meal as feed ingredient for leghorn chicks. J. Agr. Assoc. China 106:71–76.
- Li, L. 1974. Variation in protein content and its relation to other characters in sweetpotatoes. J. Agr. Assoc. China. 88:17–22.
- Lin, Y.H. 1989. Relationship between trypsin-inhibitor activity and water-soluble protein and cumulative rainfall in sweetpotatoes. J. Amer. Soc. Hort. Sci. 114:814–818.
- Lin, Y.H. and H.L. Chen. 1980. Level and heat stability of trypsin inhibitor activity among sweet potato (*Ipomoea batatas* L.) lines (Chinese with English summary). Bot. Bul. Acad. Sinica 21:1–13.
- Liu, K.S. and P. Markakis. 1989. An improved colorimetric method for determining antitryptic activity in soybean products. Cereal Chem. 66:415–422.
- Purcell, A.E., W.M. Walter, W.W. Nicholaides, W.W. Collins, and H. Chancy. 1982. Nitrogen, potassium, sulfur fertilization and protein content of sweetpotato roots. J. Amer. Soc. Hort. Sci. 107:427–435.
- Saini, H.S. 1989. Activity and thermal inactivation of protease inhibitors in grain legumes, p. 249–253. In: J. Huisman, T.F. Van der Poel, and I.E. Liener (eds.). Recent advances in research in antinutritional factors in legume seeds, Proc. First Intl. Wkshp. Antinutritional Factors (ANF) in Legume Seeds. Pudoc, Wageningen, The Netherlands.
- SAS Institute. 1992. SAS/STAT users guide, vers. 6.09. 4th ed. vol. 2. SAS Inst., Cary, N.C.
- Steele, R.G. and J.H. Torrie. 1980. Principles and procedures of statistics. McGraw-Hill, New York.
- Tsou, S.C.S. and T.L. Hong. 1989. Digestibility of sweet potato starch, p. 127–136. In: Improvement of sweetpotato (*Ipomoea batatas*) in Asia. Rep. 'Workshop on sweetpotato improvement in Asia', ICAR, India, 24–28 Oct. 1988.
- Walter, W.M., W.W. Collins, and A.E. Purcell. 1984. Sweet potato protein: A review. J. Agr. Food Chem. 32:695–697.
- Wilson, L.G., C.W. Averre, J.V. Baird, E.A. Sorensen, E.O. Beasley, and W.A. Skroch. 1980. Growing and marketing quality sweetpotatoes. North Carolina State Univ. Ext. Publ. AG–09, Raleigh.
- Woolfe, J. 1992. Sweetpotato, an untapped food resource. Cambridge Univ. Press, Cambridge, England.
- Yeh, T.P. and J.C. Bouwkamp. 1985. Roots and vines as animal feed, p. 235–253. In: J.C. Bouwkamp (ed.). Sweet potato products: A natural resource for the tropics. CRC Press, Boca Raton, Fla.