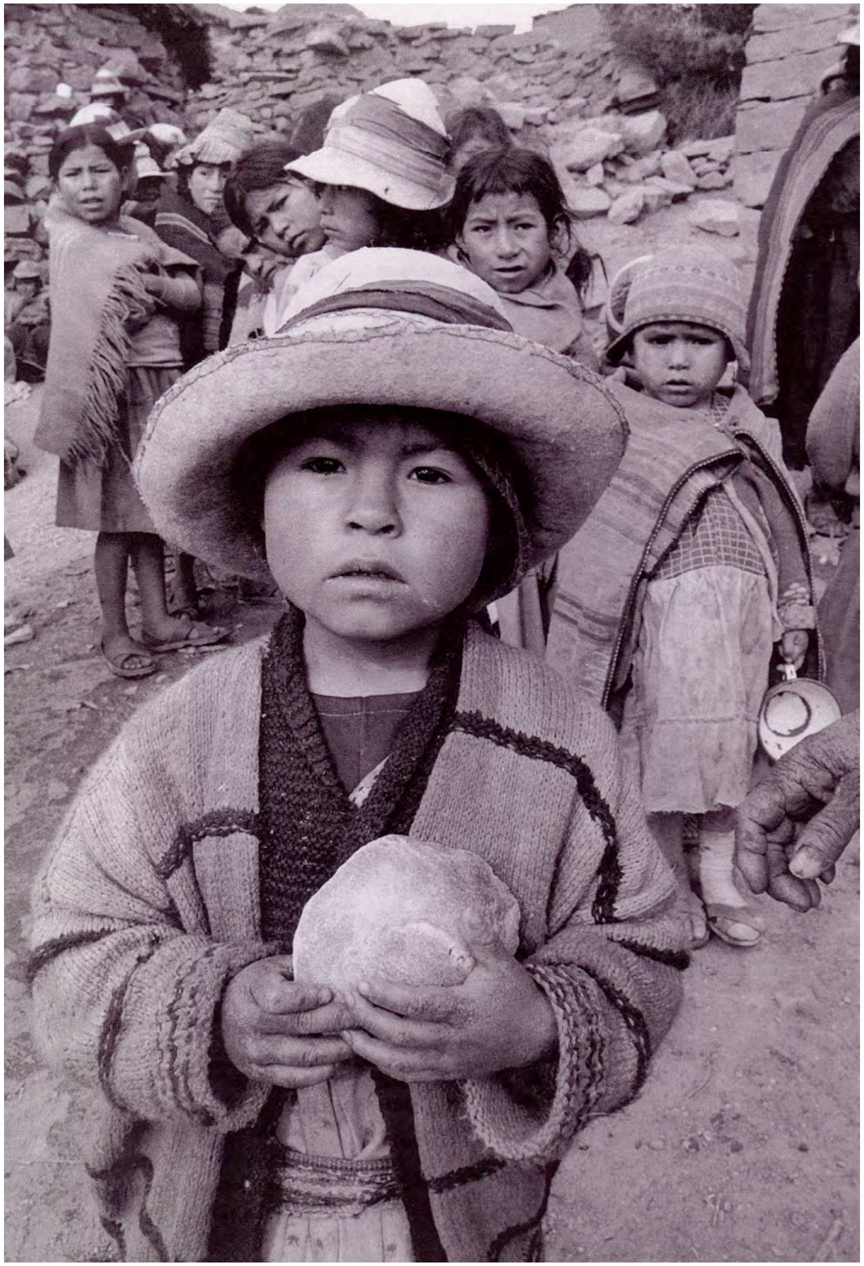
Biotechnology

Impact on the World



Improving Plant Quality

Proteins Designed for Enhanced Nutritional Value

We Were First to Demonstrate

- Expression of Designed Genes in Plants
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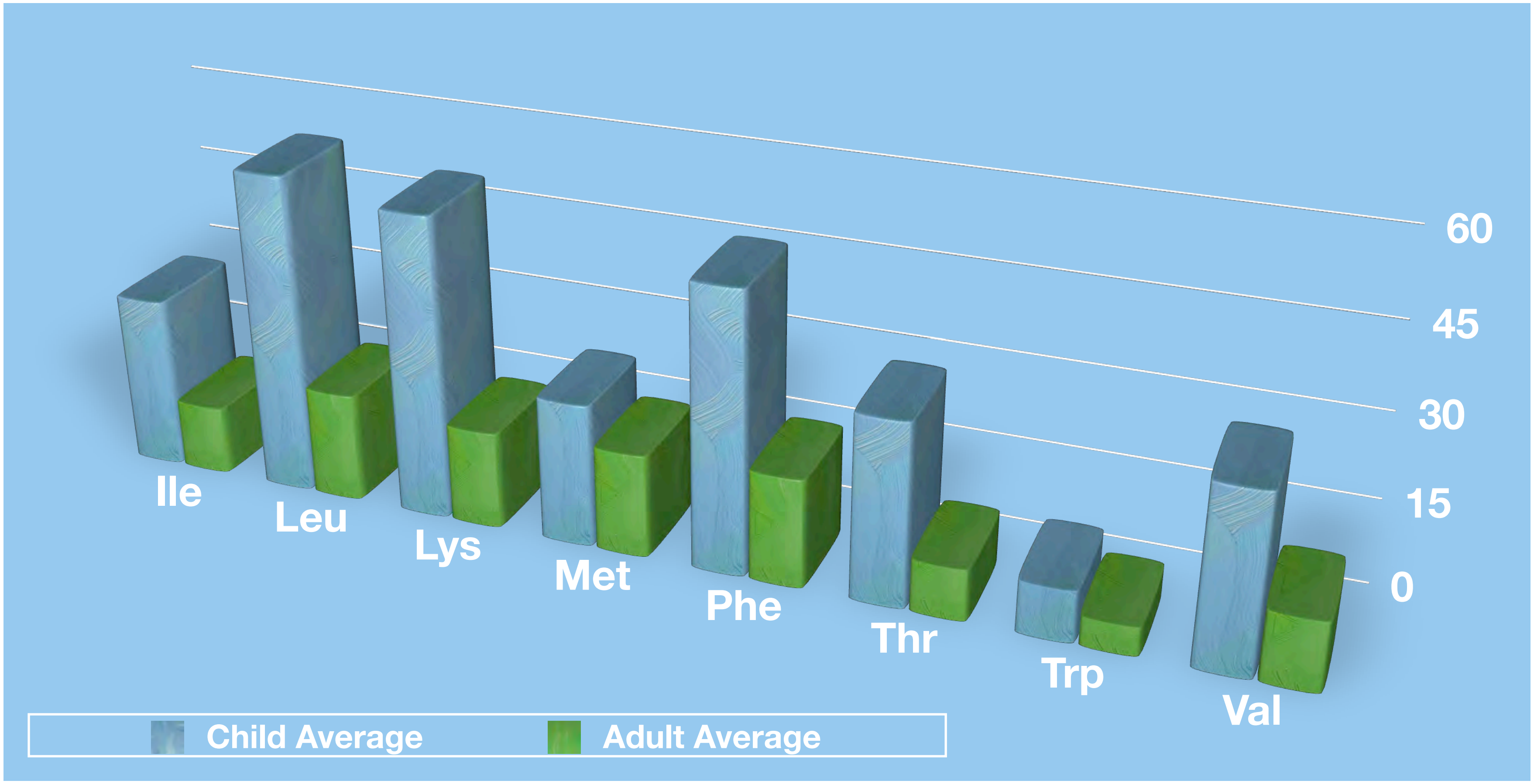
  - Yang MS, NO Espinoza, JH Dodds, and JM Jaynes. (1989) Expression of a Synthetic Gene for Improved Protein Quality in Transformed Potato Plants. *Plant Science.* 64:99-111.
  - Jaynes JM. (1992) Upgrading Plant Proteins. *The World and I*. 300-307.
  - Kim JH and JM Jaynes. (1992) Enhancing the Nutritional Qualities of Crop Plants. *Molecular Approaches to Improving Food Quality and Safety*. 1-36
  - Jaynes JM. (1994) De Novo Designed Synthetic Plant Storage Proteins: Enhancing Protein Quality of Plants for Improved Human and Animal Nutrition. In and the Feed Industry, 129-154. *Proceedings of Alltech's Tenth Annual Symposium*.
  - Zhang P., Jaynes JM, Potrykus I, Gruissem, W., Puonti-Kaerlas J. Transfer and Expression of an Artificial Storage Protein (ASP1) Gene in Cassava (Manihot esculenta Crantz). (2003). *Transgenic Research* 12: 243-250.
  - Jaynes, JM. Improving Protein Quality in Transgenic Plants. (2004) Accepted in *Encyclopedia of Plant and Crop Science*. Editor Robert M. Goodman. Marcel Dekker
  - Egnin M, Prakash C, Jaynes JM. Enhanced Essential Amino Acid Level and Increased Nutritive Value of Transgenic Sweet Potato Expressing a Synthetic Storage Protein Gene. (2009). In Preparation.

Essential Amino Acids

- Humans can synthesize 12 of the 20 protein amino acids
- The other eight are essential ingredients in the diet of humans
- Other animals possess their own unique essential amino acid requirements

Essential Amino Acids

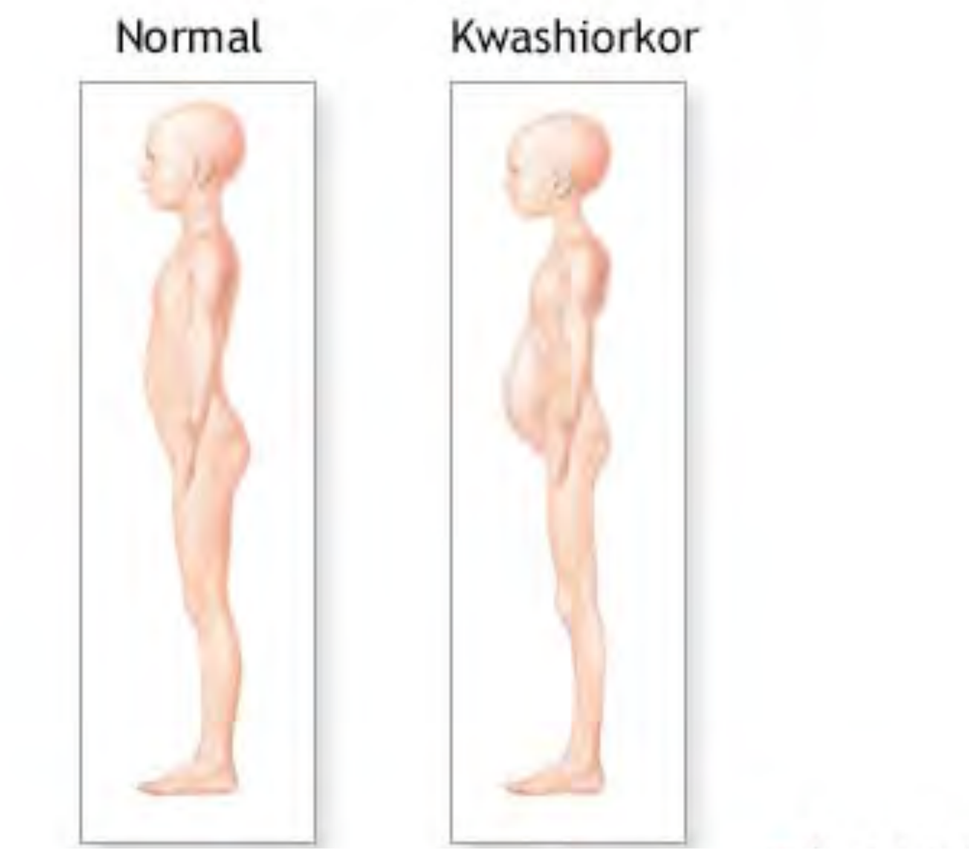


Protein Malnutrition

- People get their protein where they can. However, it is estimated that as many as 40,000 children die each day in the World for lack of high quality dietary protein. They must consume plant-derived proteins because animal products are not available.

Protein Malnutrition: Kwashiorkor

Kwashiorkor (language of Ghana)--means "displaced child", i.e., child displaced from the mother's breast by a newborn sibling. Severe malnutrition in infants and children that is caused by a diet high in carbohydrates and low in protein.

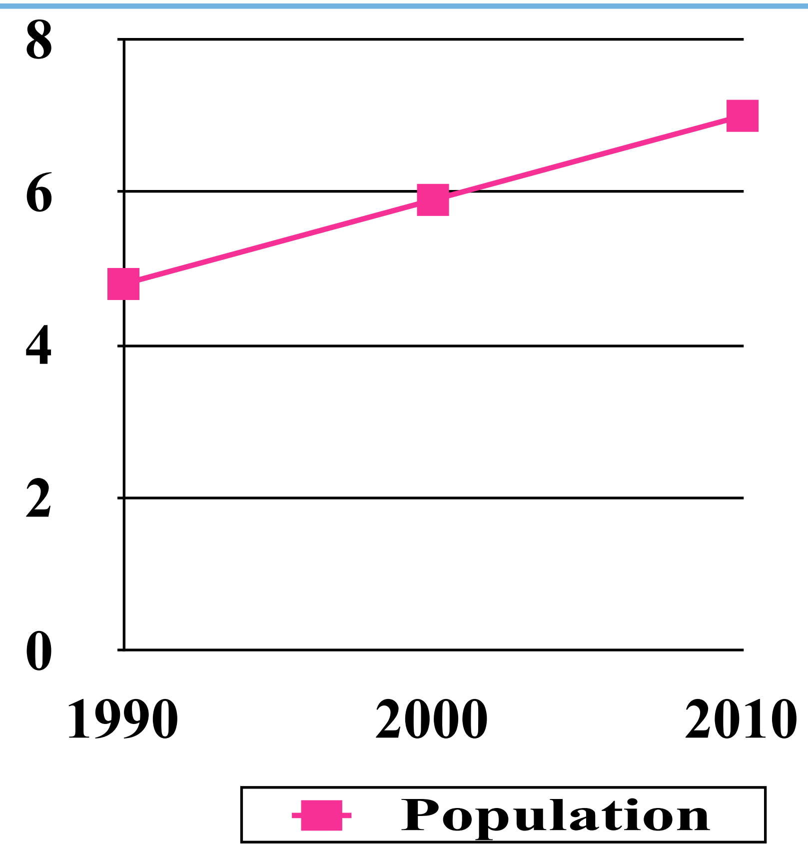


Dependence Upon Plants

Despite the "Green Revolution" the poor quality (low content of EAA) of plant-derived proteins is still the primary reason for malnourishment in the World today.

The Need Continues to Rise

The global population growth is slowing---but each year, there is still about an additional 85,000,000 people to feed



Developing World Food Needs

Developing world food needs rising

- 3/4 of a billion people today are chronically malnourished
- By the year 2020, an extra 2.5 billion people in the Developing World will require food and > 1.4 billion of them will be undernourished*

* Source: Conway, G. (1998). The Doubly Green Revolution. Ithaca: Cornell Univ. Press

Preventable Outcomes of Hunger

Malnutrition

↓

Starvation

↓

Disease

↓

Death

Food Needs in the Developing World

Problem: Low protein and disease-ridden crops are common in the Developing World

Solution: Bioengineered high protein staple crops that are disease resistant

Food Needs in the Developing World

Problem: Food needs are on the rise and economic development is struggling

Solution: Supply the technology; train regional people to implement the technology, helping to foster economic development

Best and Worst Sources of EAA

- Best--Animal Proteins
- Worst--Plant Proteins

    - When one or more of the essential amino acids is missing in an animal's diet, a constraint or limit is imposed on the bioavailability of those that are remaining.

Nutritionally Enhanced Foods and Feeds

- Necessary required consumption is reduced
- Improve human and animal health
- Less foreign exchange required to purchase high quality protein

Designed Proteins

Our Nutritional Proteins were designed to meet the specific essential amino acid requirements of the consumer, be it, human or animal.

Natural Plant Storage Proteins (NPSP's)

- Albumins, globulins, prolamins
- 100 million tons of cereal storage proteins are consumed worldwide every year
- Accounts for 50-60% of the total worldwide protein intake of humans and animals

Natural Plant Storage Proteins (NPSP)

- Multimeric
- Relatively insoluble with low bioavailability
- Maximal packing achieved in a protein body

Nutritional Limitations of NPSP’s

- Low in most EAA’s
- Particularly deficient in isoleucine, lysine, methionine, threonine, and tryptophan
- Inherently low bioavailability

Engineering of NPSP’s

- Introduction of modified protein sequences into existing natural plant storage protein
- Combine heterologous plant storage protein genes

Limitations of Engineered NPSP's

- Nutritional value far from optimum
- Value of introduced sequence is limited
- Modified storage protein is unstable
- Low expression level

Designed Nutritional Proteins

Believe it or not: if you know a few structural rules, it is easier to invent a new high essential amino acid containing protein rather than to modify an existing plant storage protein

Our Nutritional Proteins

- First de novo designed plant storage proteins
- Contain balanced composition of EAA's
- Bioavailability approaching 100%
- Specially designed for humans and various animal species

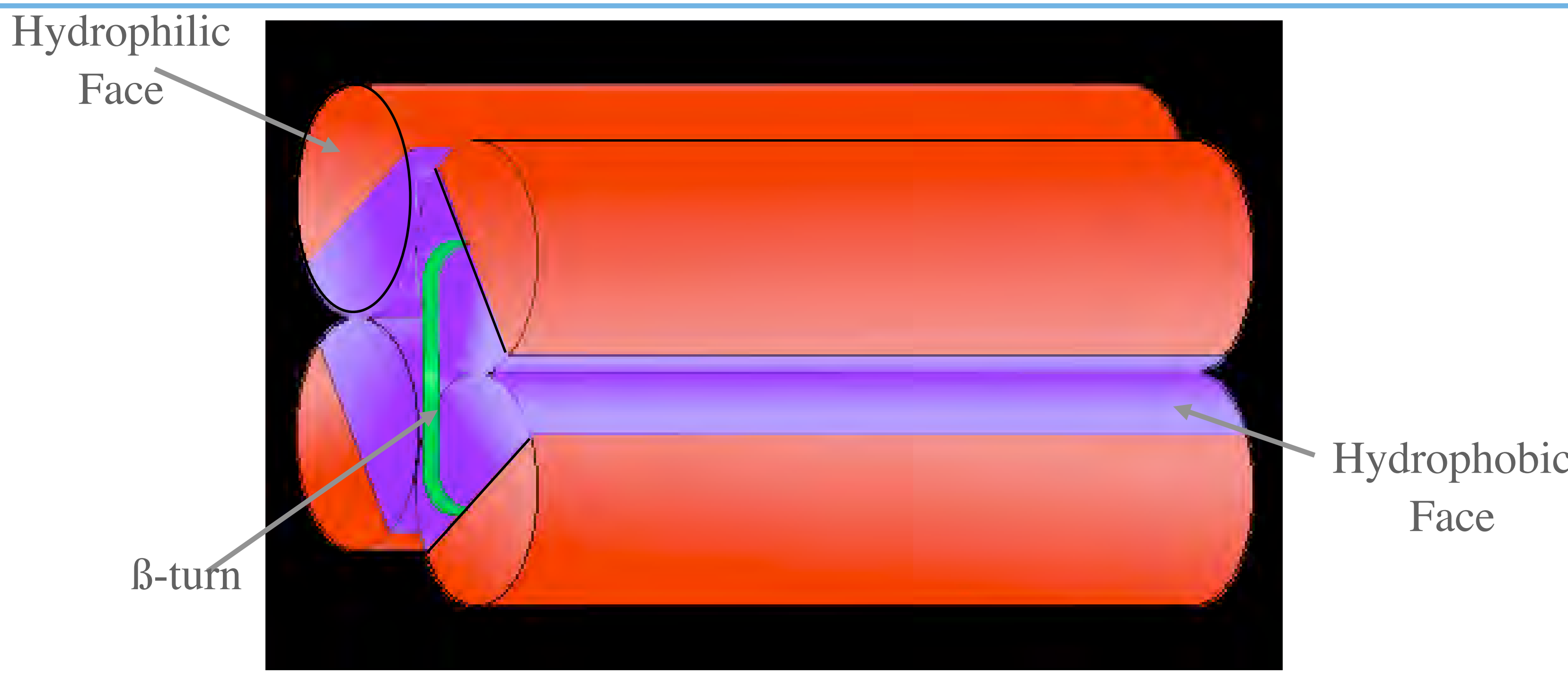
Our Designed Nutritional Proteins (DNP's)

- DNP's were designed to mimic the natural plant storage protein structure
- Composition tailored to meet the specific EAA needs of humans and animals
- Complement the EAA deficiencies of major crop plants
- Enhanced bioavailability

Novel Design Features of DNP's

- EAA content not diluted by heterologous sequences
- Amphipathy strengthens secondary structure and causes aggregation
- Further stabilized by ionic bridges
- Ultimate structure very much like natural plant storage proteins

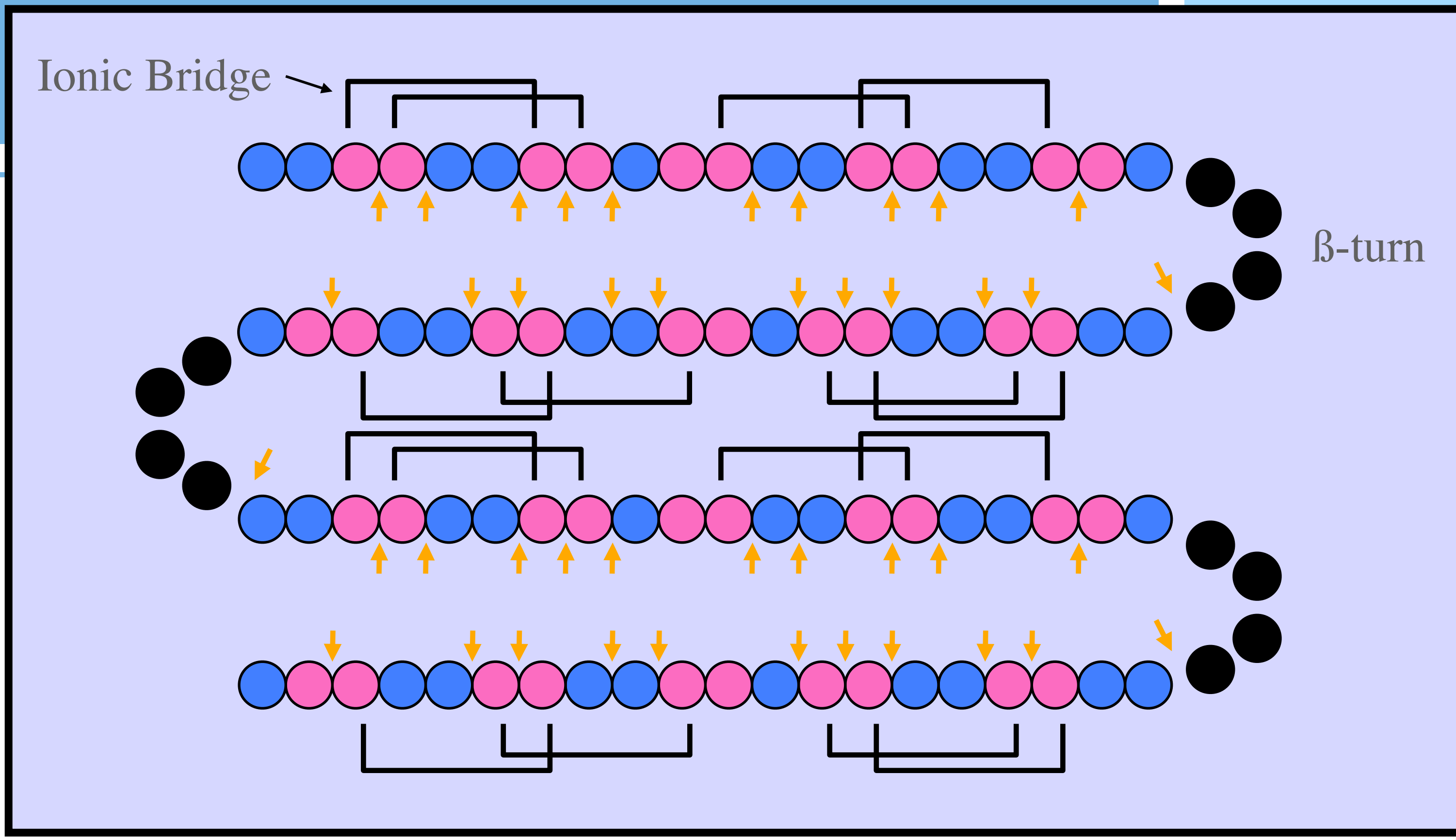
Artificial Storage Protein 1 Tetramer (ASP1)



Amino Acid Sequence of ASP1

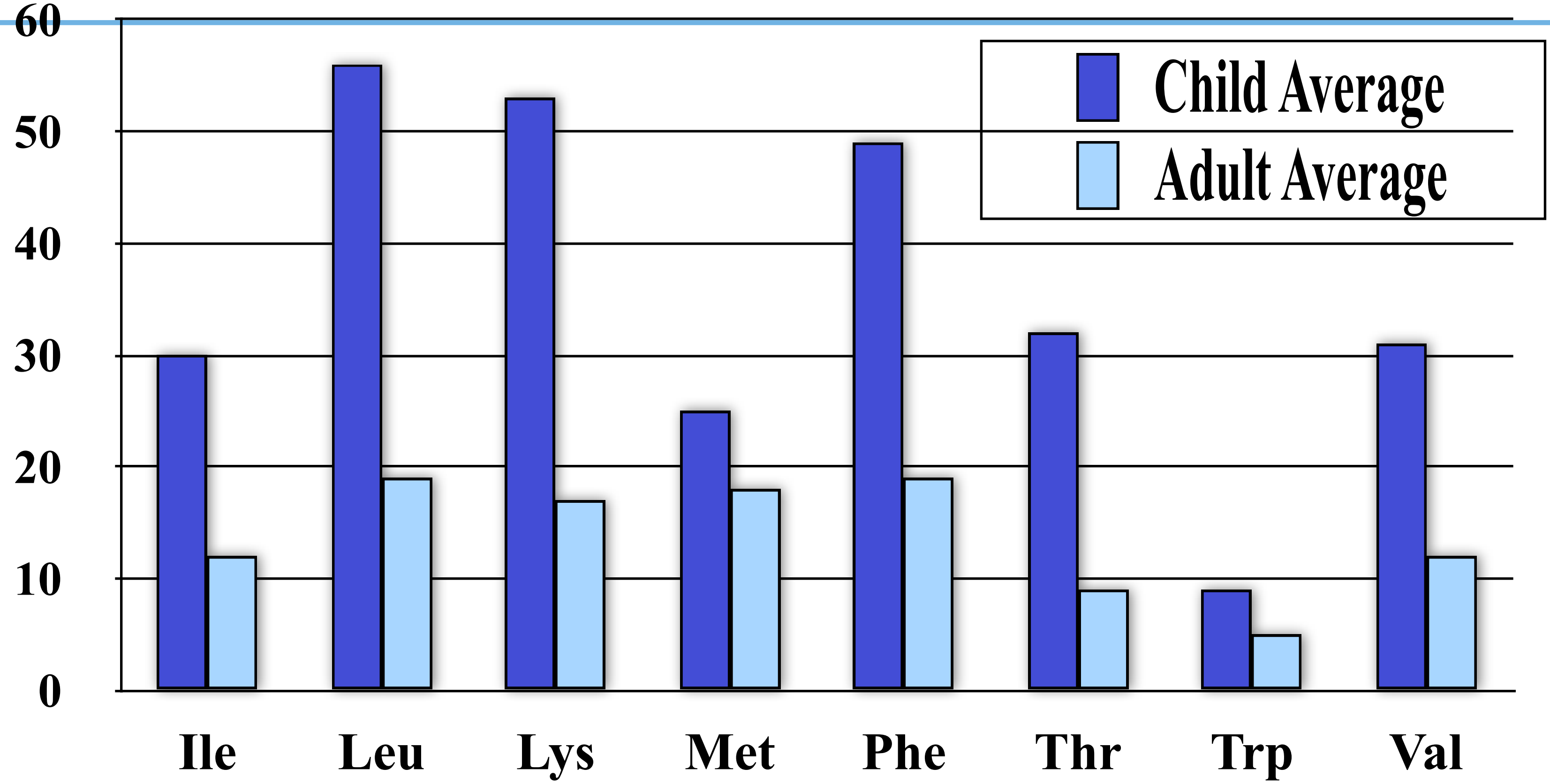
MLEELFKKMTEWIEKVIKTM
gpgrMLEELFKKMTEWIEKV
IKTMgpgrMLEELFKKMTEW
IEKVIKTMgpgrMLEELFKK
MTEWIEKVIKTM

Physical Nature of ASP1

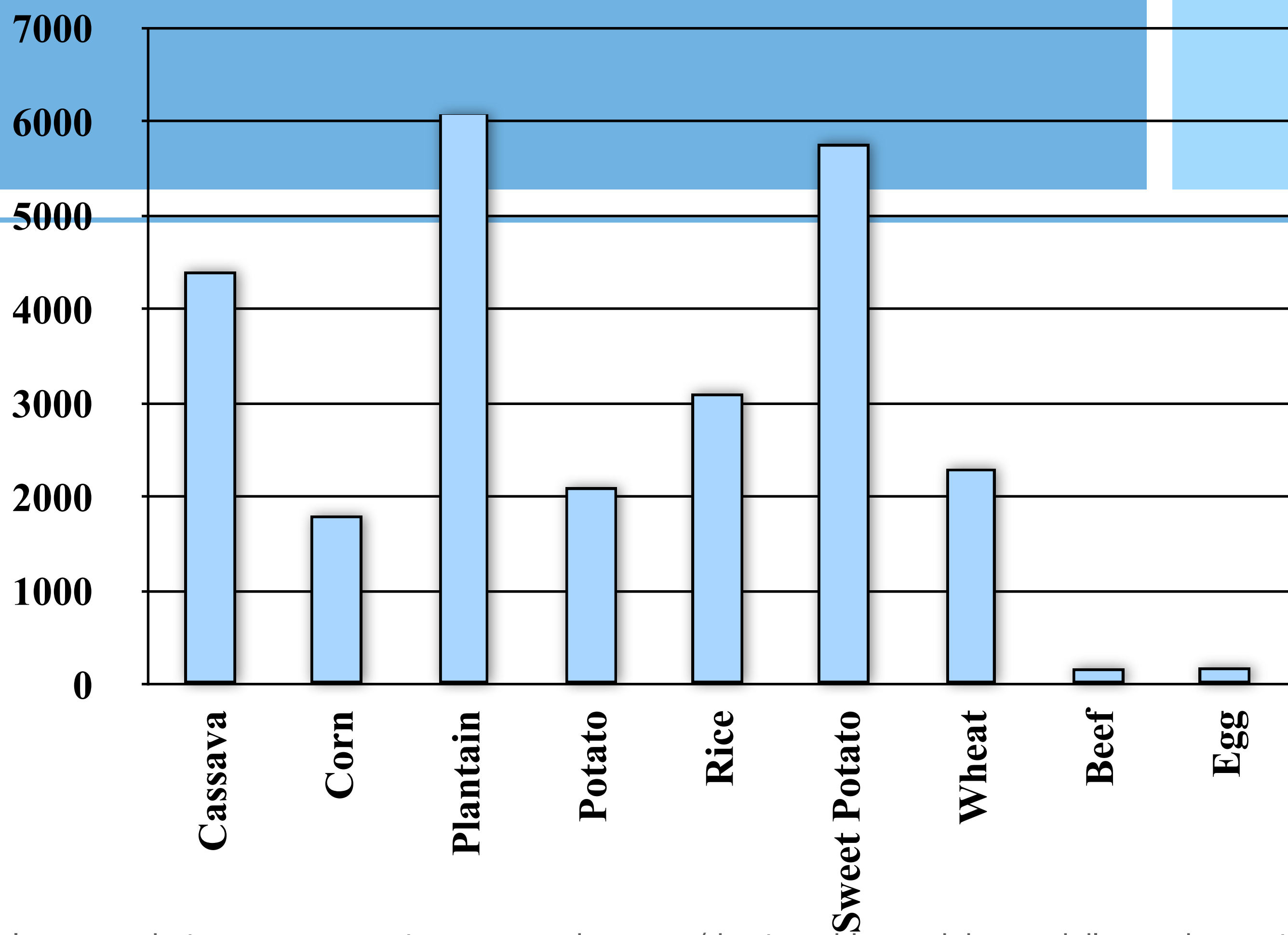


*Yellow arrows denote the sites of hydrolysis for the three most common gut enymes in animals--ASP1 was designed for stability and digestibility

Essential Amino Acid Needs of Humans



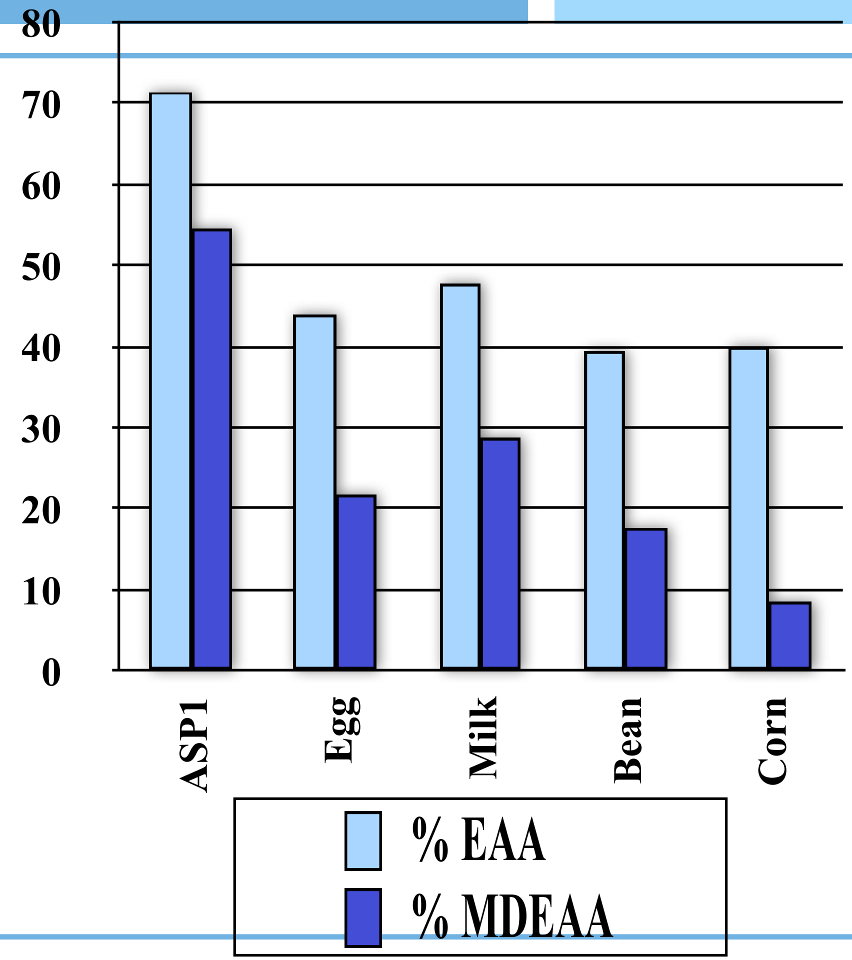
Consumption Necessary



*The values are what are necessary to consume in grams/day to achieve minimum daily requirement for all essential amino acids for a 10 year old child. This assumes that the protein, in each foodstuff, is 100% bioavailable, and we know it is not, so, these numbers should be increased to an even higher level.

EAA Comparison

- ASP1 is more than twice the composition of other primary foodstuffs in the essential amino acids: isoleucine, lysine, methionine, threonine, and tryptophan



Transgenic Sweet Potato Plants

<u>Collaborators:</u>
Dr. CS Prakash
Dr. Marceline Egnin
Mgavi Braithwaite
Michon Walker
Tuskegee University

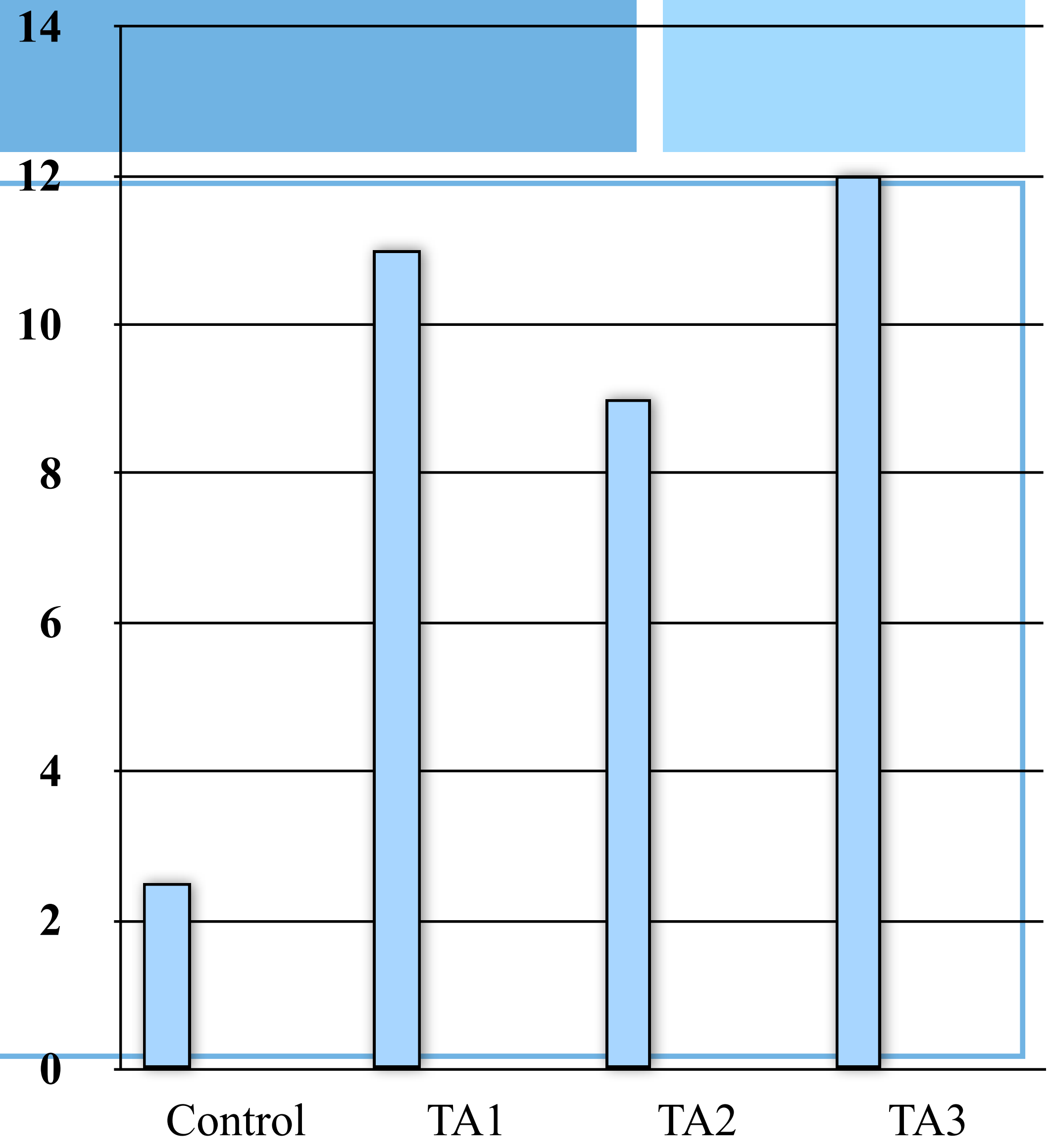
Dr. Kenzo Nakamura
Nagoya University

Nutrient Dense Sweet Potato

All daily EAA requirements, for children and adults, may be met by eating a moderate amount of this modified sweet potato

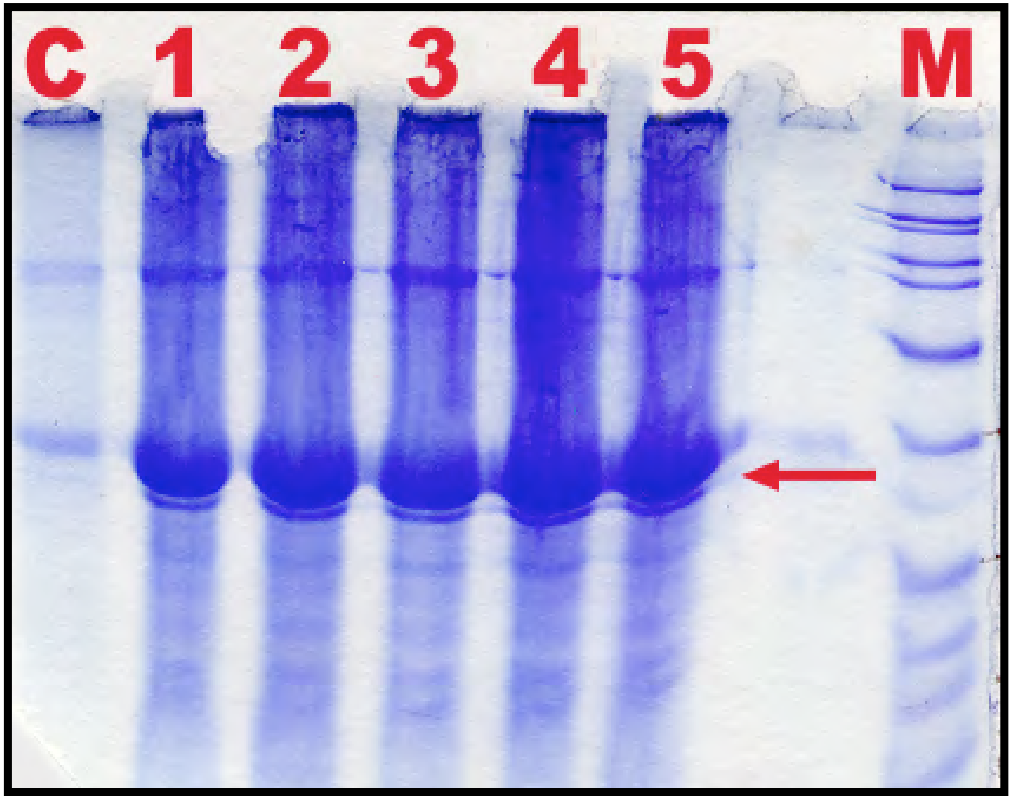
Increase in Protein

Sweet potato plants engineered with the ASP1 gene showed a 3 to 5 fold increase in protein (dry weight basis) content over that observed in the control.

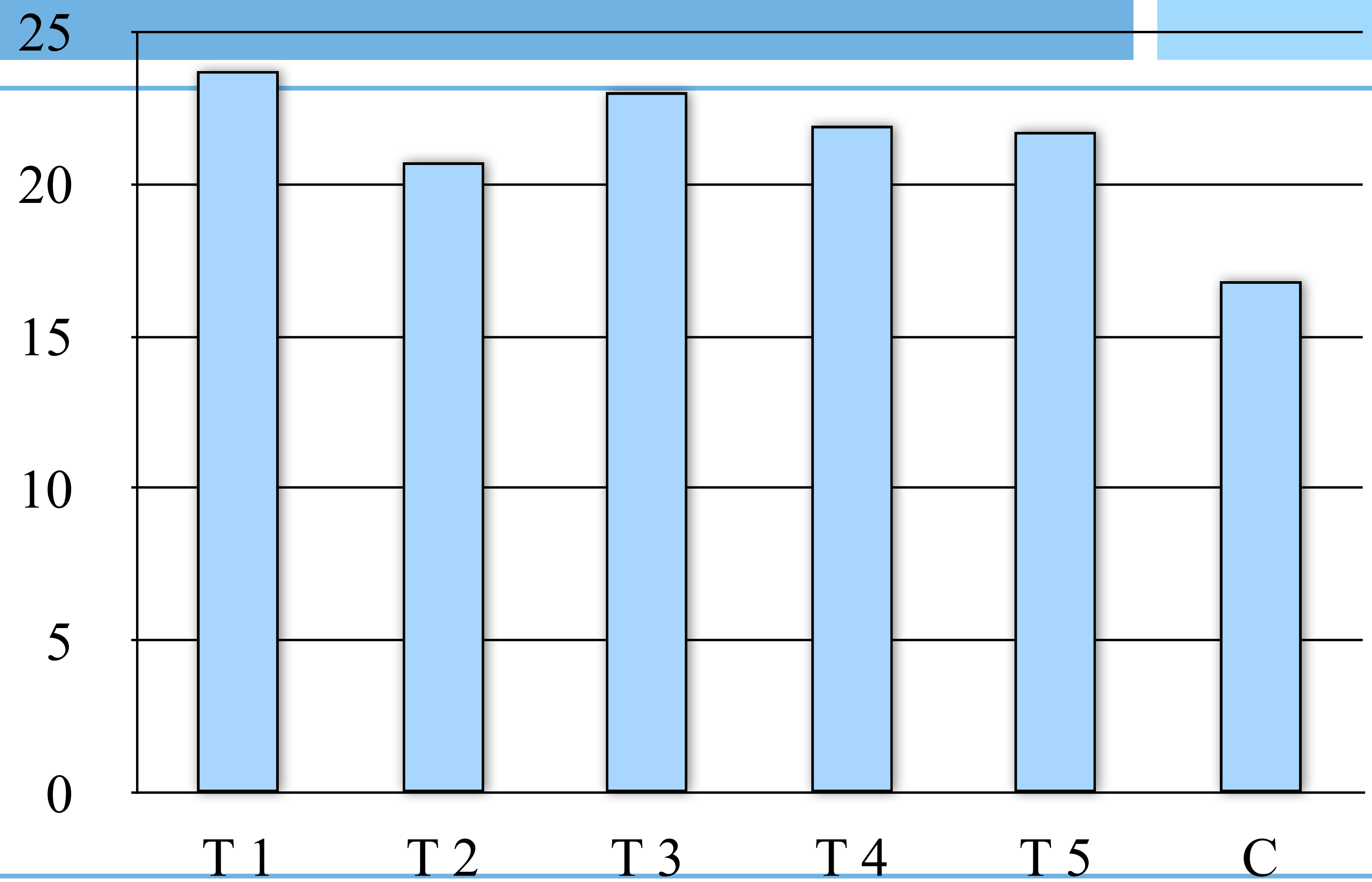


Gel Electrophoresis of Protein

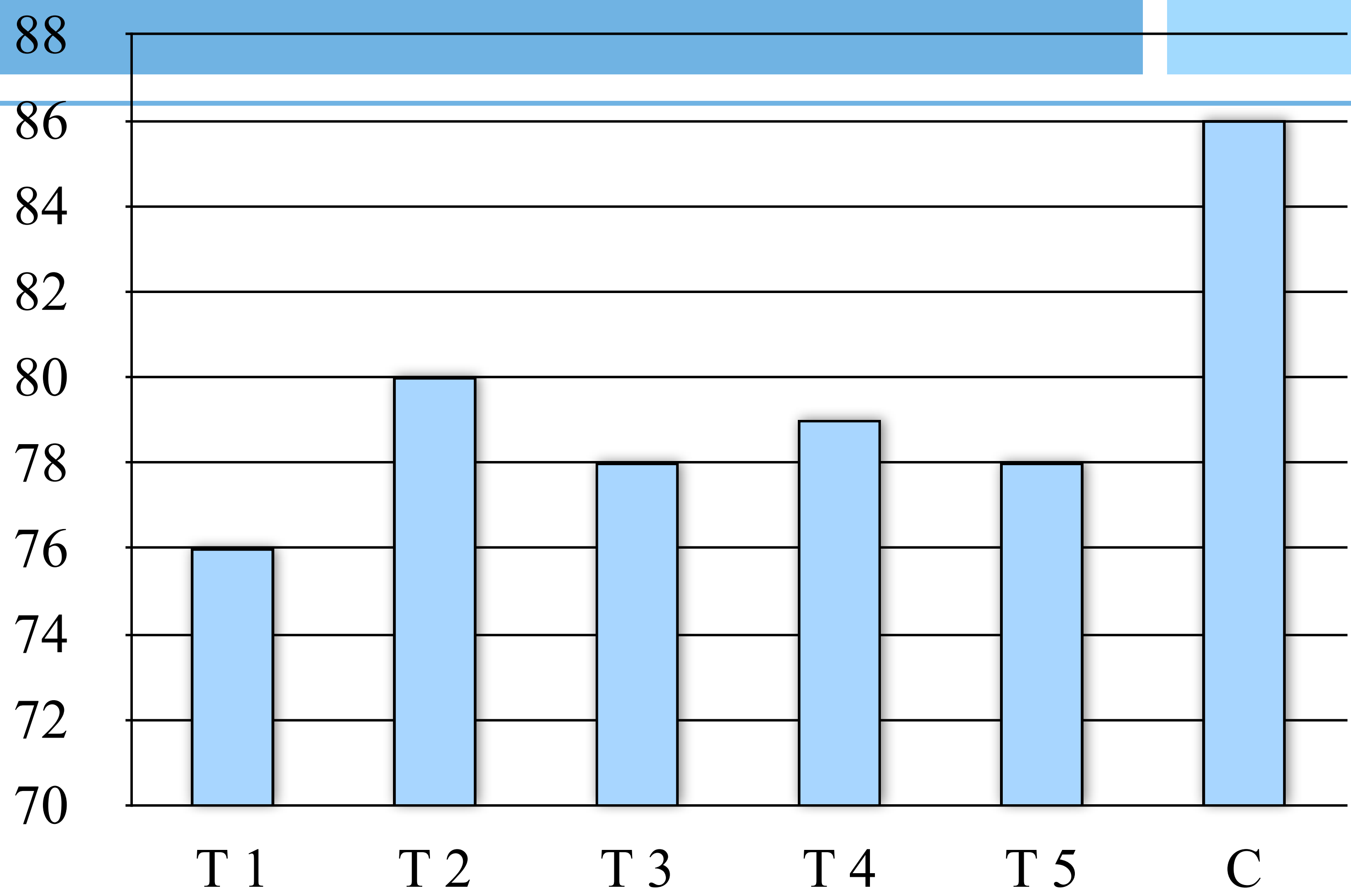
Gel electrophoresis and coomasie blue staining of transgenic sweet potato (1-5) with control (C) shows the difference in overall protein content in the storage roots. protein content is higher in transgenics over the control



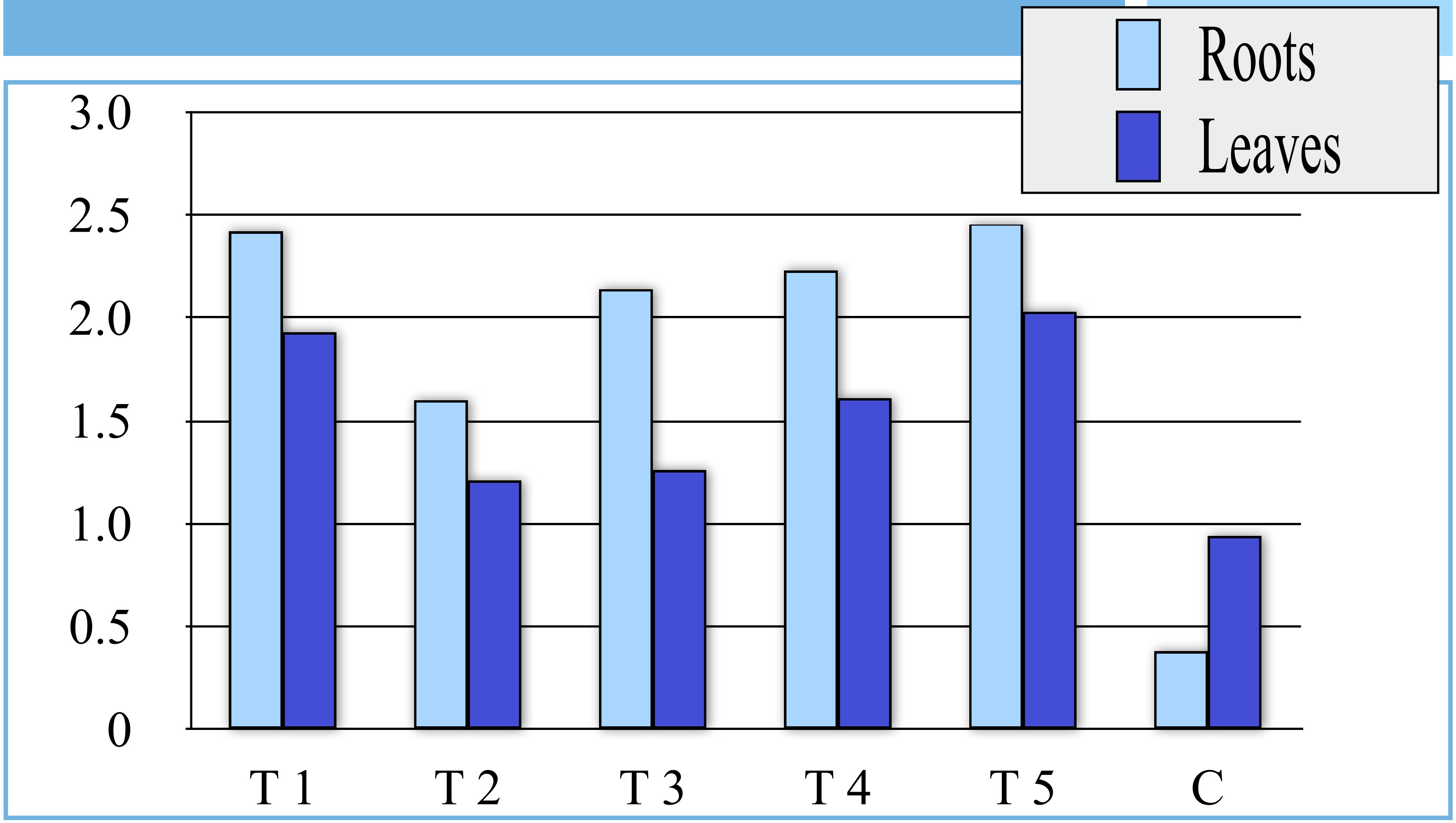
Sweet Potato: % Dry Matter



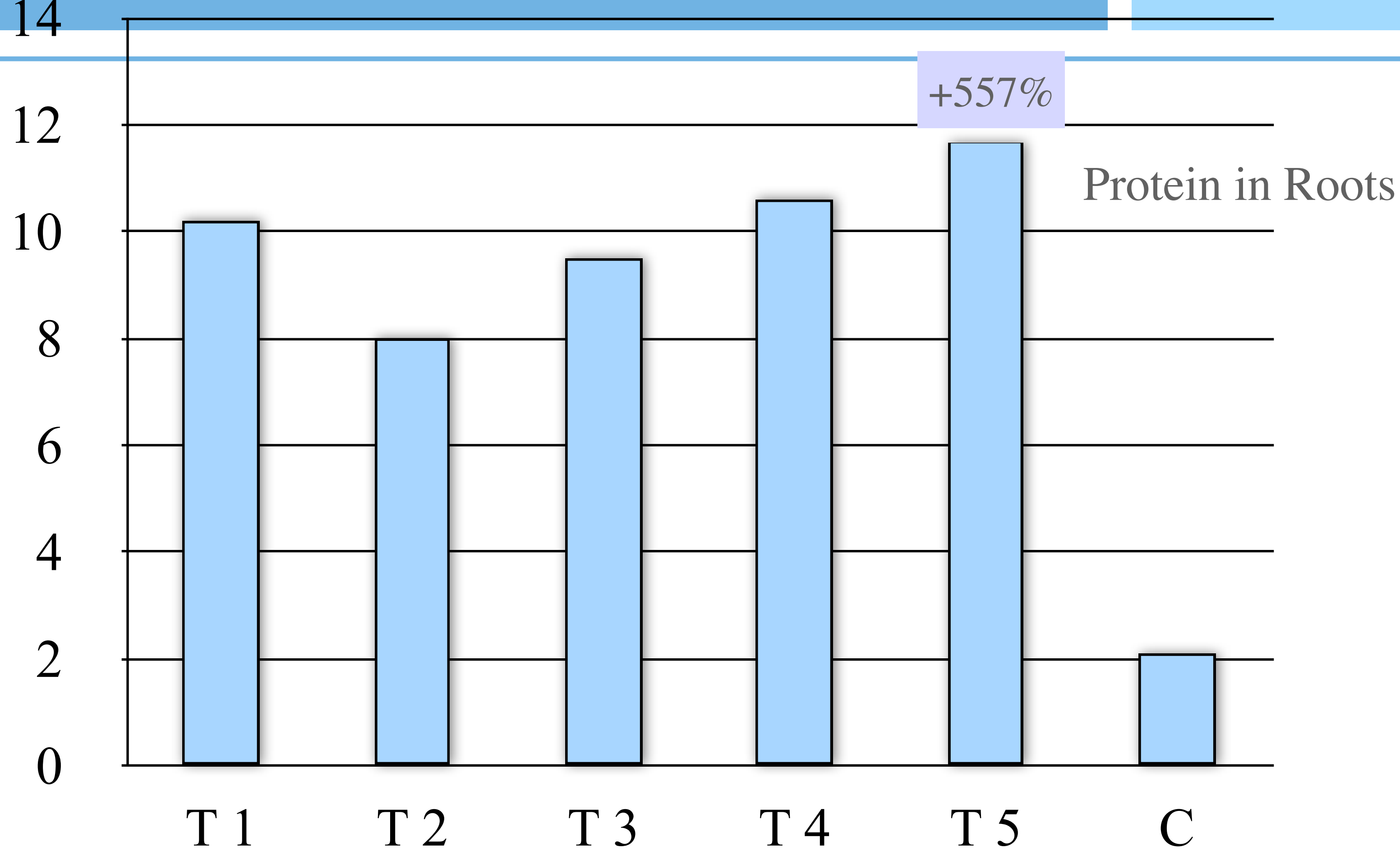
Sweet Potato: % Moisture



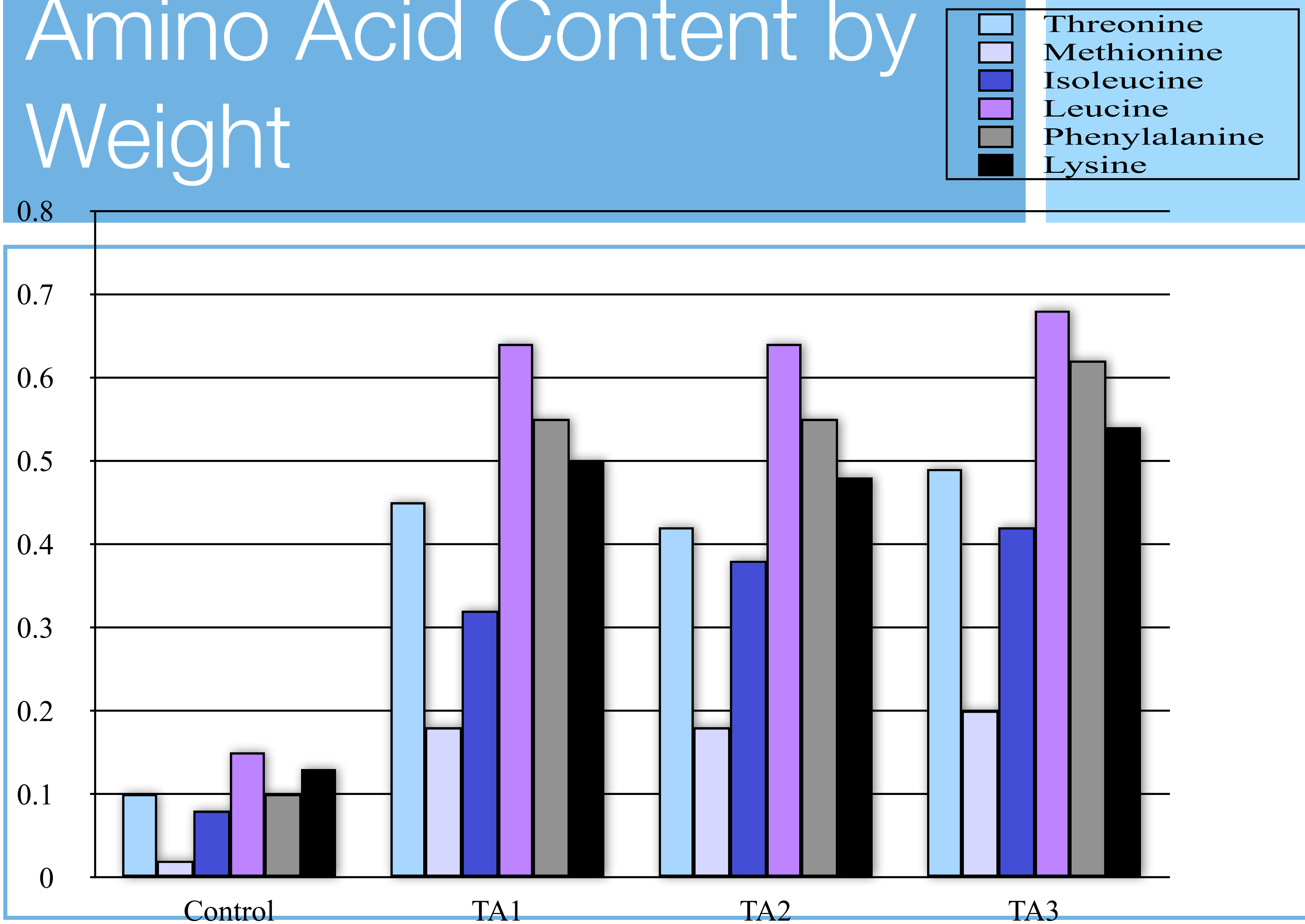
Sweet Potato: % Protein-Fresh Weight



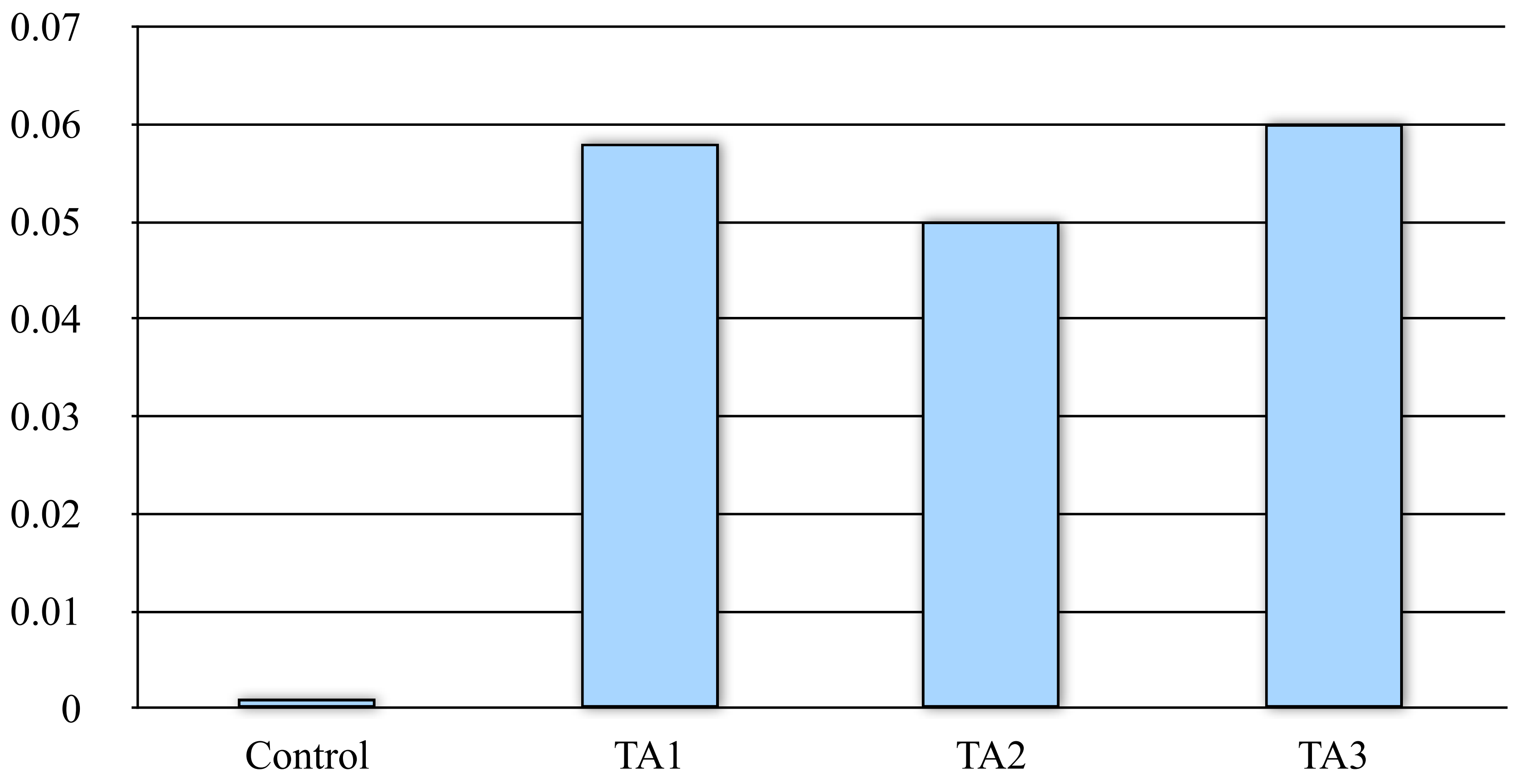
Sweet Potato: % Protein-Dry Weight



Amino Acid Content by Weight

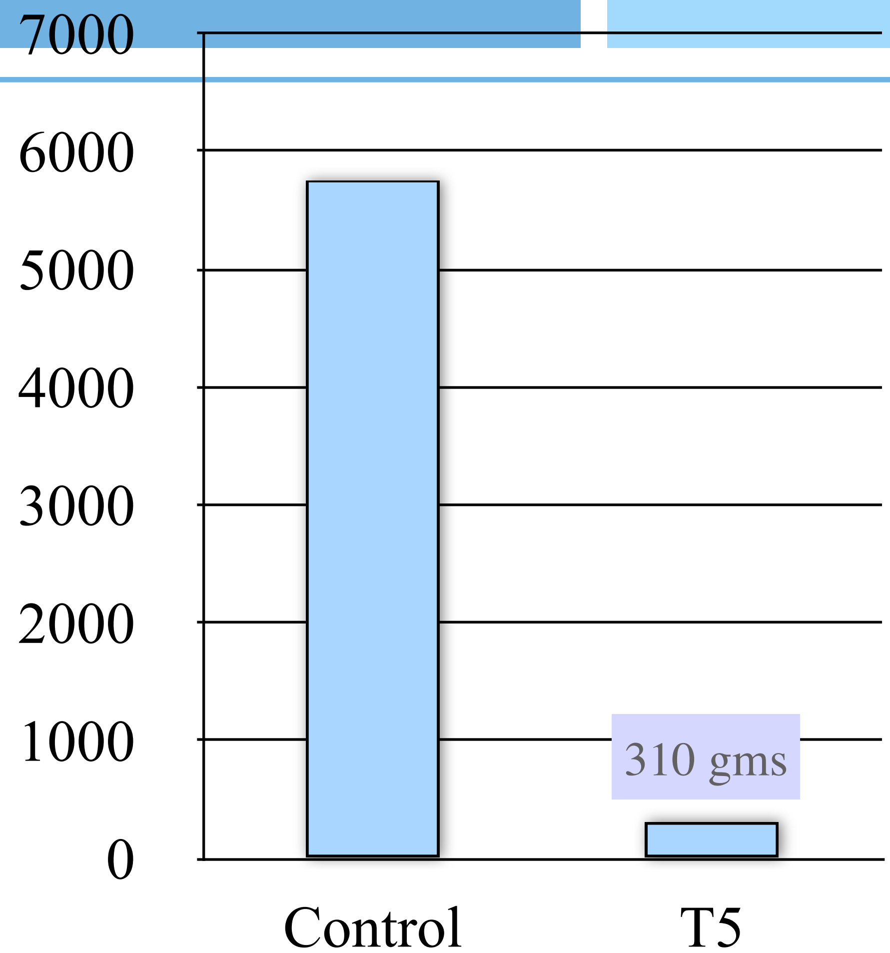


Tryptophan Content



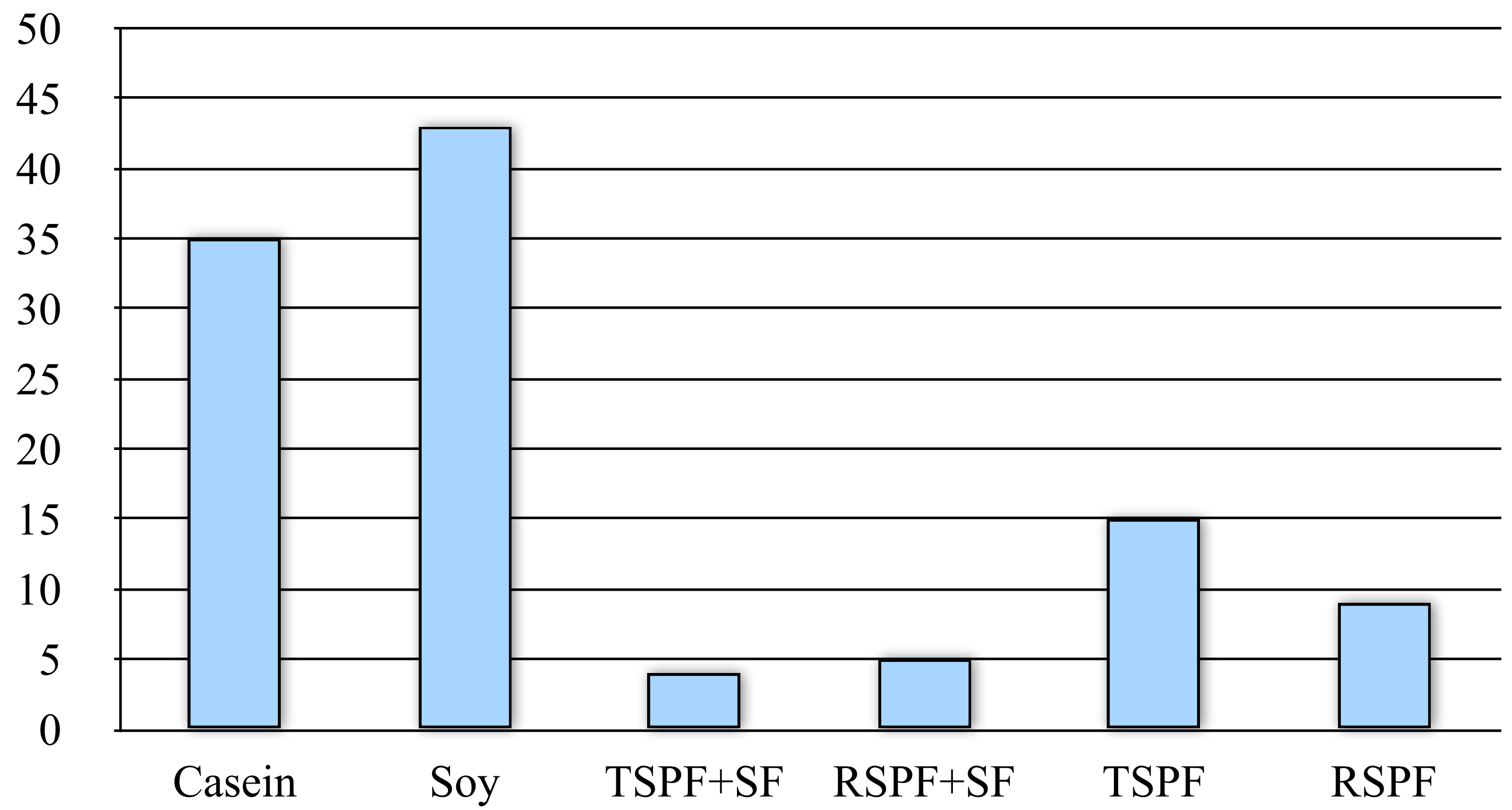
ASP1 Sweet Potato (T5)

- Protein increases 5X
- 10 year old child's requirements decrease more than 10X
- Higher level of EAA Tryptophan in T5 contributes to this phenomenon (100X)



Feeding of ASP1 Sweet Potato to Hamsters

Effect of Dietary Protein on Growth



Nutritional Evaluation of Sweet Potato

The effects of dietary protein on the growth of hamsters*.

Diets	Total body weight gain (g/28 days)	Corrected PER[5]
Soy protein	42.50 ± 1.67^{a}	3.72 ± 0.05^{a}
Transgenic SP	14.02 ± 2.05^{c}	3.71 ± 0.05^{a}
Control SP	8.94 ± 1.61^{c}	2.57 ± 0.05^{b}

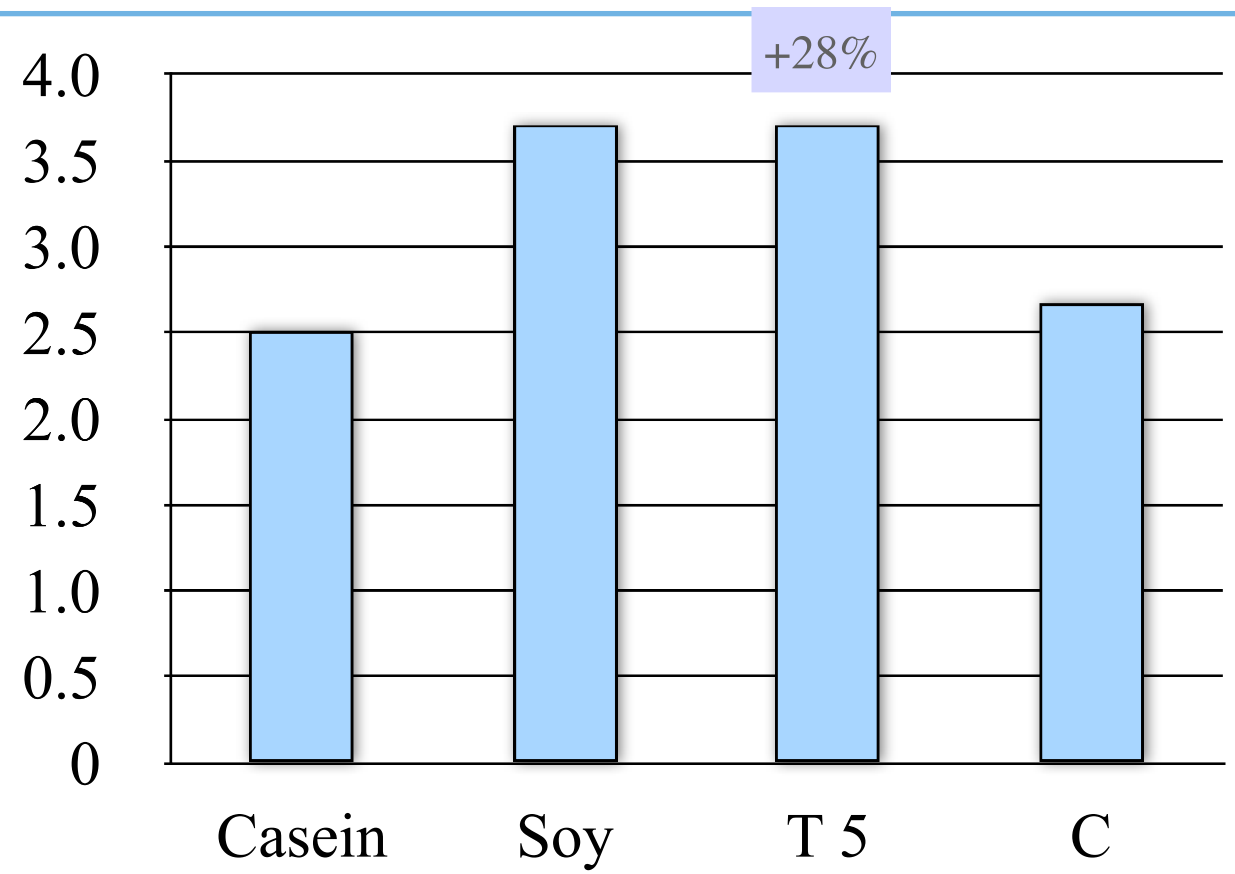
*Values in a column not sharing the same superscript letter are significantly different ($P<0.05$)

[1]Data are expressed as mean and ± S.E.

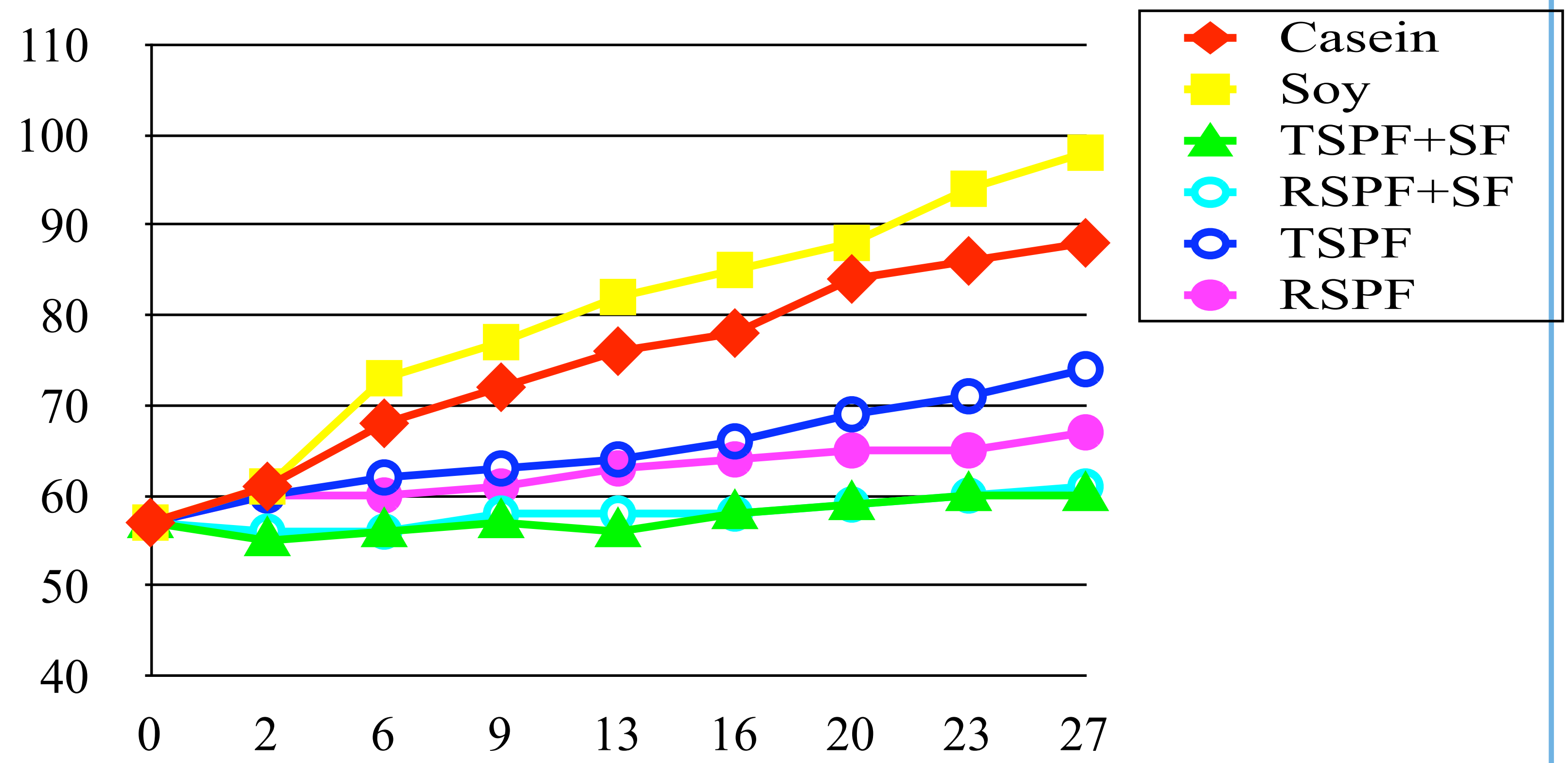
[5]Corrected PER-Experimental PER of sample X 2.50/Experimental PER of casein

Sweet Potato: Protein Efficiency Ratio

Fed to Hamsters



Effects of Dietary Protein on Growth

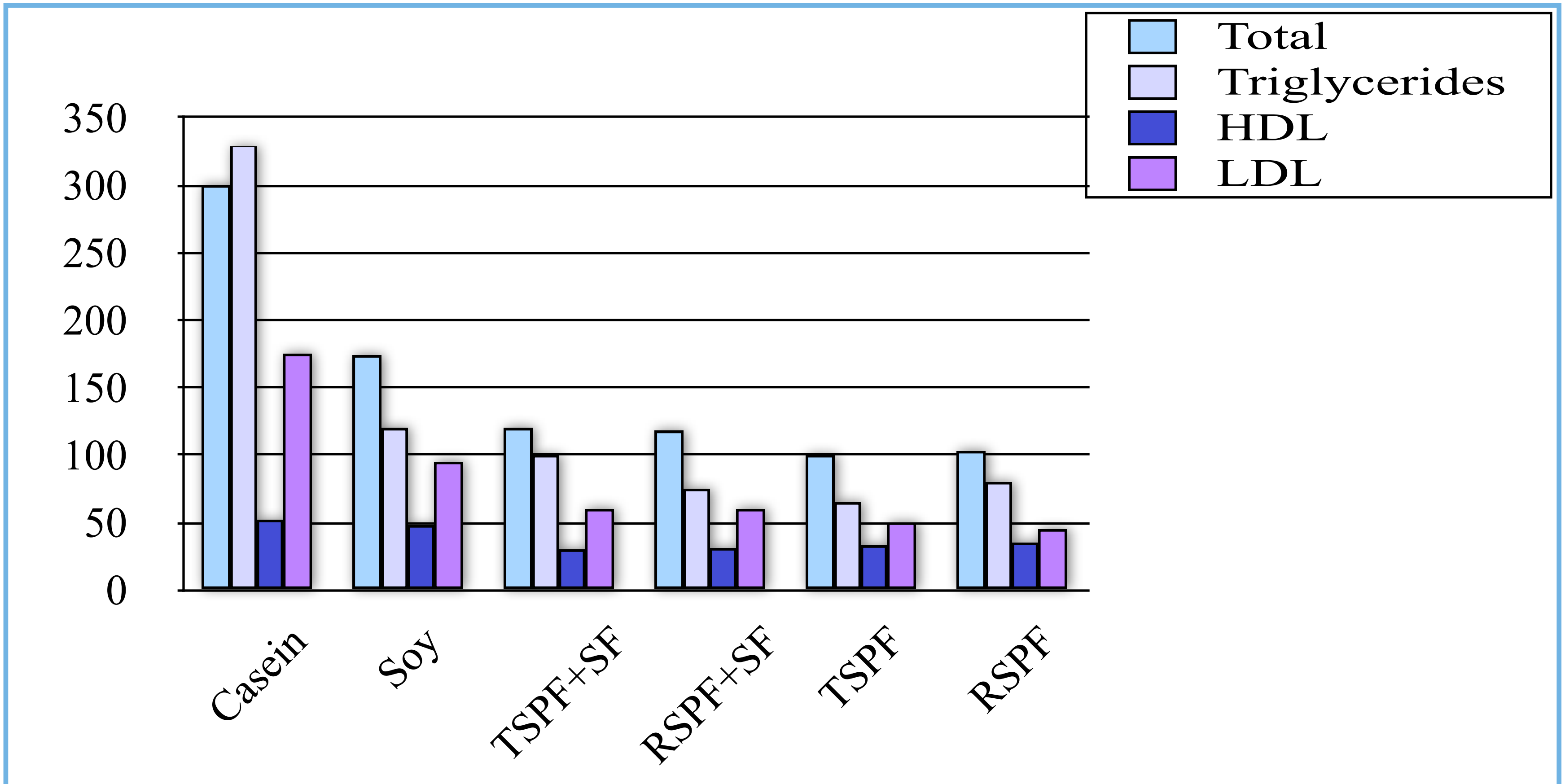


ASP1's Effect on Sugar Content in Sweetpotato

	Sucrose	Glucose	Fructose
Control	0.54	0.06	0.06
Transgenic 4	0.95	0.06	0.06
Transgenic 5	1.57	0.15	0.13

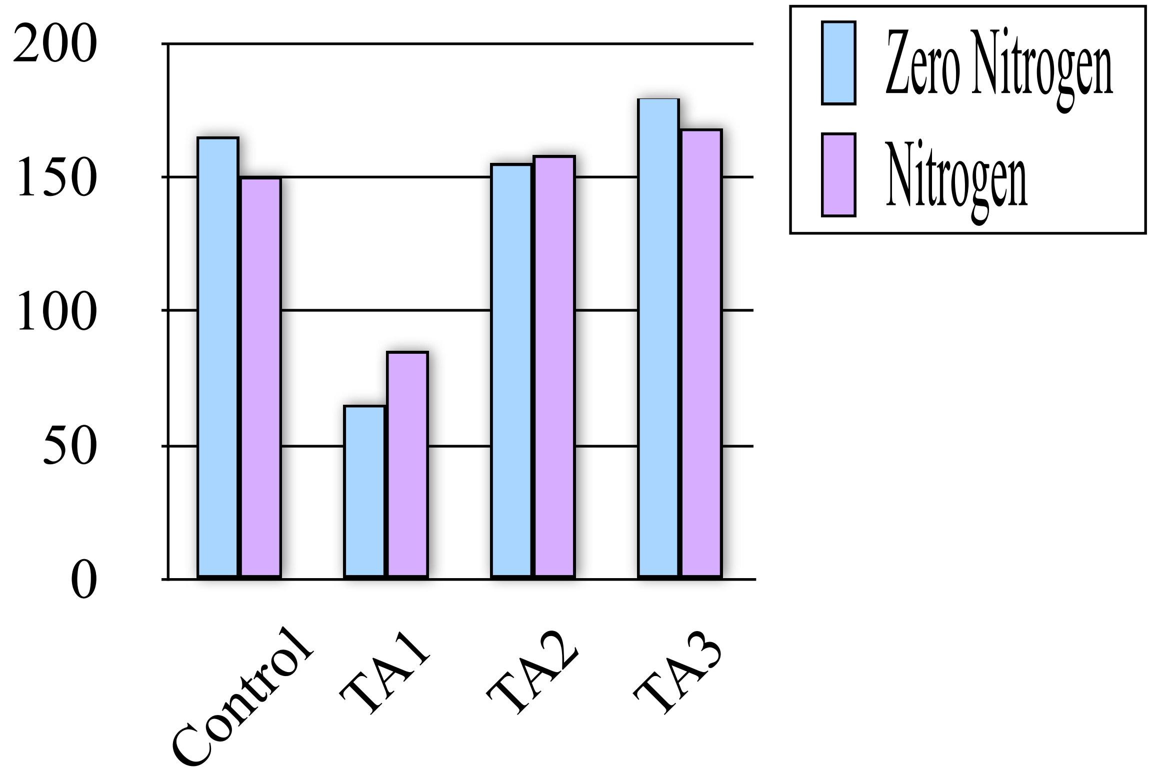
*Numbers are percent of total weight of the root

Plasma Lipid Levels in Hamsters



Yield Comparison

- Transgenic sweet potatoes were grown under field conditions with or without nitrogen fertilizer (32 kg/acre). Two transgenic lines with the highest protein content showed no significant difference in their storage root yield. Further, nitrogen fertilizer did not contribute to yield differences.



Field Test

In a field study, transgenic sweet potato lines grew normally with no apparent abnormalities or yield penalty. Storage roots of transgenic plants appeared normal.

ASP-1 Transgenic Sweetpotato

- Exhibit Very High Levels of Total Protein (~500% Increase!)
- Contained Higher Dry Matter
- Extraordinary Levels of Five Most Limiting Amino Acids
  - Lysine, Tryptophan, Methionine, Threonin and Isoleucine
- Normal Phenotype But Slower Root Development

Rice Research

Collaborator:
Dr. Ingo Potrykus

Nutritionally optimized rice is becoming a scientific reality.
Variety development and de-regulation will take five years.

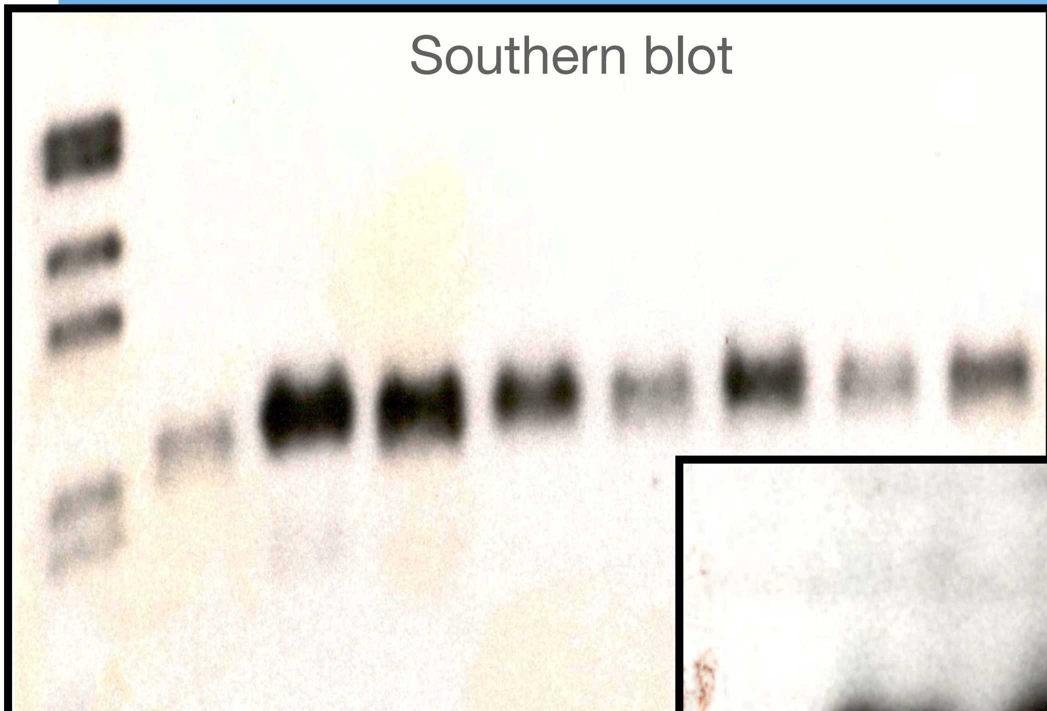
1 seed produces in 5 generations food for 100 Million poor and carries technology to prevent blindness of thousands of children.

„Golden Rice“

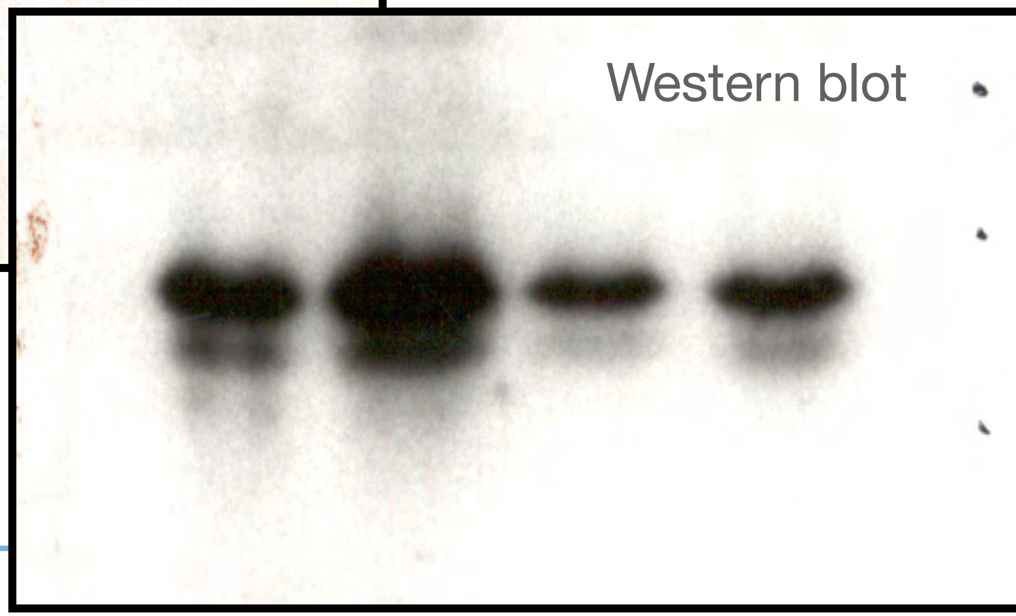
These seeds not only look like gems, they are.

High Quality Protein Rice

Asp-1 transgenic TP 309



Preliminary data from the first seeds of a collection of 85 independent transgenic lines.



Initial Analysis of Protein Enhanced Rice in 2 Transgenic Lines

	C	**#1**	**#2**
EAA Infants	26.68	23.79	32.23
% above C	0.00	-10.83	20.84
EAA Ch-Ad	21.09	18.63	24.22
% above C	0.00	-11.65	14.86
Total	54.14	46.66	64.16
% above C	0.00	-13.80	18.52

Why High Quality Protein Rice?

- A diet of 300 g of rice provides less than 10% of the required essential aminoacids histidine, isoleucine, leucine, lysine, methionine, thyrosine, threonine, tryptophan, and valine (EAA). High quality protein ideally contains a balanced mixture of all EAA‘s. EAA‘s are involved in numerous cellular functions. Their deficiency heavily impairs the development of children.
- The transgenic concept: transfer under endosperm-specific regulation for synthesis and storage, the genetic information for a balanced deposition of all eight EAA‘s.

Cassava Research

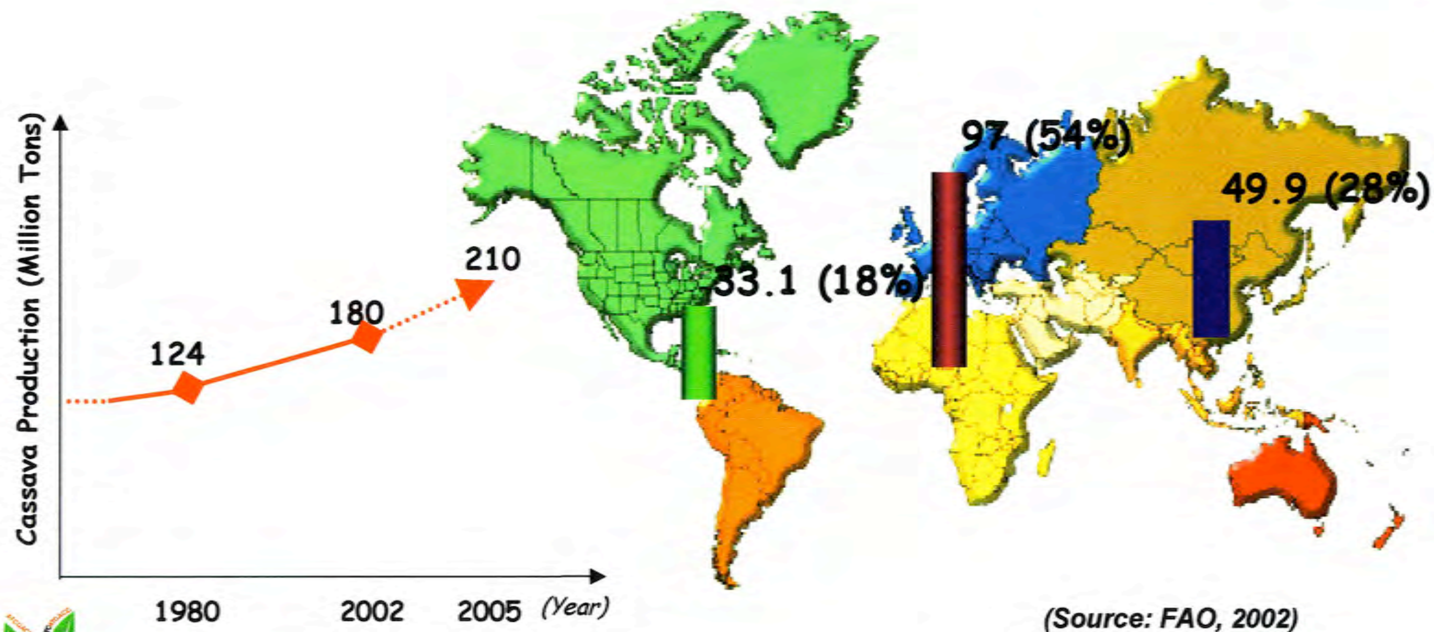
Collaborators:
Dr. Ingo Potrykus
Dr. Tom Zimmerman

Control

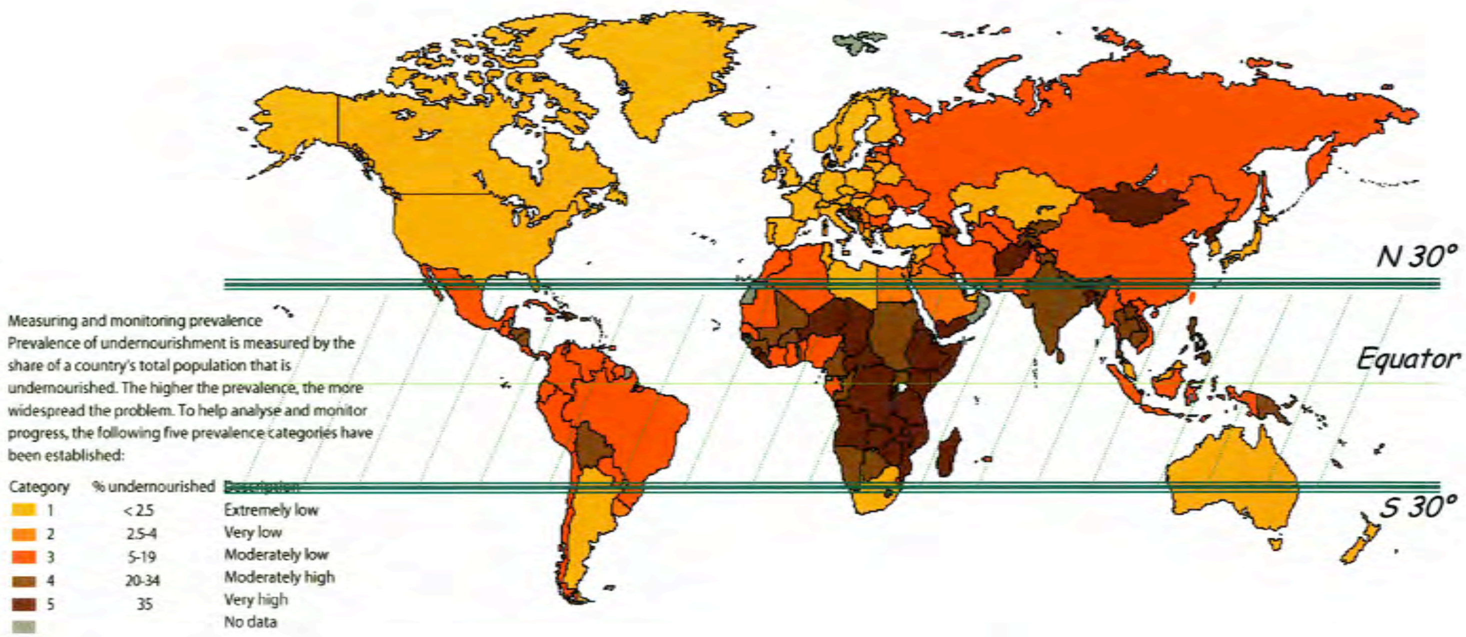
ASP1 Transgenic

Cassava
(*Manihot esculenta* Crantz)

- Third most important calorie source in the tropics
- Food for >600 million people worldwide



Cassava Distribution and Global Prevalence of Undernourishment



- *A key role in food security of developing countries*

(Source: FAO)

Cassava
(*Manihot esculenta* Crantz)

Advantages:

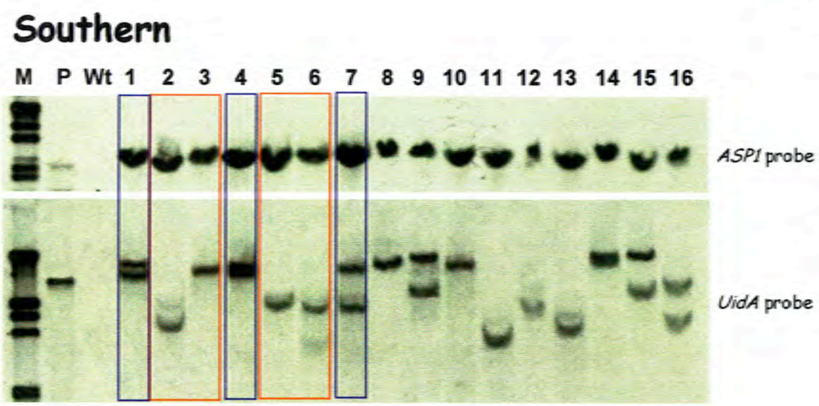
- Cheapest dietary carbohydrate source
- Tolerant of unfavourable ecological conditions
- Reliable yield and flexible harvesting time
- Vegetative propagation mode

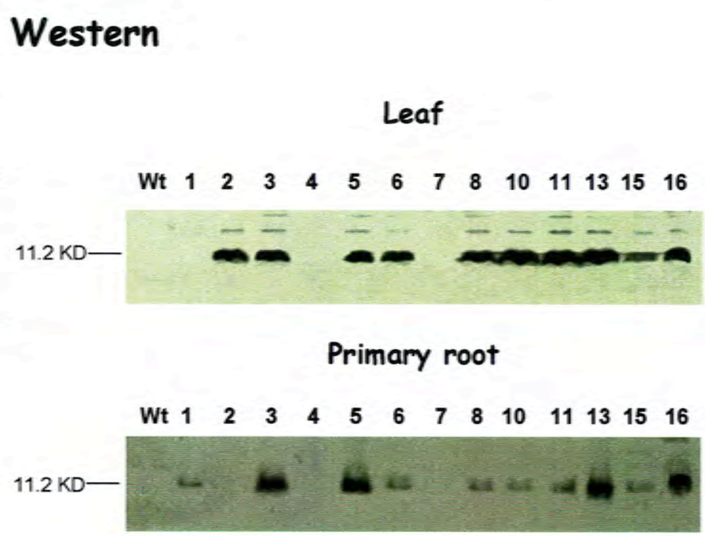
Bottleneck problems:

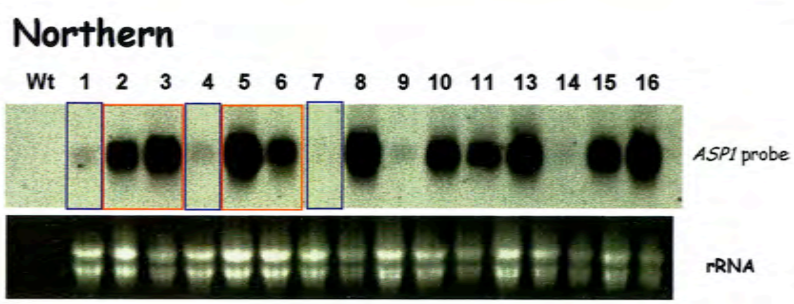
- Plants can be attacked by various insect pests and virus diseases
- Storage roots are rich in starch but low in protein
- Mature leaves contain appreciable quantities of protein and vitamins but have a short life
- Storage roots suffer a rapid post-harvest deterioration
- Traditional breeding is difficult and time consuming

Gruissem/CBN VI

Integration and expression of *ASP1* (Poster #61)







Zhang et al. (2003) Transgenic Research, 12: 243-250

Gruissem/CBN VI