GENETIC VARIABILITY OF "AVAILABLE" METHIONINE TOTAL PROTEIN, SPECIFIC GRAVITY AND OTHER TRAITS IN TETRAPLOID POTATOES

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ABSTRACT

GENETIC VARIABILITY OF "AVAILABLE" METHIONINE, TOTAL PROTEIN, SPECIFIC GRAVITY AND OTHER TRAITS IN TETRAPLOID POTATOES

By

Robert Luescher

Parental cultivars of potatoes (Solanum tuberosum L. and S. tuberosum - S. stoloniferum hybrids) and 320 segregating offspring representing 8 crosses were analysed in duplicate for total protein, "available" methionine and specific gravity.

Significant differences were found for all three traits among parents and families. Heritability, mainly in the narrow sense, varied from 79 to 100% for "available" methionine, from 10 to 28% for total protein and from 35 to 99% for specific gravity.

Total protein in the offspring varied from 7.5 to 18.8 mg/100 mg dry matter, "available" methionine varied from 0.9 to 2.2 mg/16 mg N and total dry matter ranged from 12.7 to 28% of the fresh weight.

"Available" methionine, total protein and specific

gravity were all positively correlated with chip color (r= 0.14, 0.13 and 0.44 respectively).

No definite relationship between "available" methionine content and rest period could be observed.

From the 320 segregating offspring, 16 clones were selected according to their total protein and "available" methionine content. These samples were analysed for protein and nonprotein nitrogen, free methionine and free cysteine. The BV of the total protein and the nonprotein nitrogen fraction was assessed by means of a microbiological method using Streptococcus zymogenes.

In this study free methionine (mg/16 mg N) ranged from 0.34 to 0.97% and was highly correlated with "available" methionine (r= 0.96). Free methionine provided between 12 and 62% of all methionine present in the total protein. Free methionine and "available" methionine seemed to be independent of the total protein level.

No measurable amounts of free cysteine could be detected. The BV of the total protein was negatively correlated with the total protein level (r= -0.55). The BV of the nonprotein N fraction was very dependent upon % nonprotein N and the total protein level (r= -0.96 and -0.69 respectively).

Tuberin, tuberinin, globulin II, prolamin and glutelin were isolated from the dry matter of three advanced seedlings by means of conventional extracting procedure.

Tuberin, tuberinin and prolamin of the cultivars 58 and 322-6 contained similar amounts of methionine and cystine,

whereas the sum of methionine and cystine of the same proteins of 709 were considerably lower.

Electrophoretic analyses showed that tuberin is composed of at least three major protein bands. The relative quantity of these bands varied between genotypes and could thus cause some variation in the content of the sulfur containing amino acids. The bands of tuberinin were almost identical to the bands of tuberin, but in different proportions.

Selection criteria to develop a potato high in the sulfur amino acids were discussed.

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LIST OF DEFINITIONS

"available" methionine: methionine determined microbiologically in an enzymatic digest of the total protein.

b: regression coefficient.

BV: biological value of protein.

Cov: covariance.

cystine: cystine determined microbiologically in an acid hydrolysate of the total protein.

EAA - Index: essential amino acid index.

free cysteine: cysteine determined microbiologically in the nonprotein nitrogen fraction.

free methionine: methionine determined microbiologically in the nonprotein nitrogen fraction.

methionine: methionine determined microbiologically in an acid hydrolysate of the total protein.

NPU: net protein utilization.

P: probability of error.

r: correlation coefficient.

total protein: (Kjeldahl N of potato flour) x 6.25.

V: variance.

INTRODUCTION

Plant geneticists have bred plants to alter specific characters such as color, size, shape, disease resistance and yield. Biologists studying plants and animals have shown that specific proteins are characteristic of certain families (Dalby and Lillevik, 1969; Boulter and Thruman, 1968). The nutritional implications of this type of work are obvious, namely, that quantitative variation in protein occurs with genetic variation, and can be further enhanced by making suitable crosses.

With the increasing world population and the ensuing food shortage which threatens to affect even the economically developed countries, scientists have begun to look seriously at many hitherto unexplored areas for protein rich foods. Emphasis has been put on plant sources as primary providers of both protein and calories.

Algae and leaves are being examined as possible protein sources for humans. Although such food can be used in animal feeds or make interesting academic studies, acceptability is an important factor in human nutrition. Most of these unusual sources of food need either to be processed suitably or require tremendous effort to educate people to accept these unusual foods.

Protein deficiency is a major nutritional and health problem in the world today. Kwashiorkor, a protein deficiency disease, is common in many developing countries. Since population growth may increase more rapidly than food supply, the problem of protein deficiency is likely to become even more acute. This problem can be averted only by a combination of population control, improved agricultural practices, food preservation and economical development.

The available animal protein is insufficient to balance the world diet. Two thirds of the protein for present world consumption comes from cereals (Borgstrom, 1967). Cereals, however, do not provide balanced proteins. In addition, the primary aim of past breeding practices was based on the misconception that quantity alone can feed the world. Most of the cereals first cultivated contained 12 to 15% protein. In contrast, the present high yielding soft wheat varieties contain 10% or less of protein.

If the dietary protein is significantly less than 8% of the calories provided by human milk, it is not possible to feed an infant enough food to meet the protein requirement. If proteins of lower quality than those of human milk are fed, the intake should be proportionally higher, but efforts should be made to provide the infant with the highest possible quality of protein. Future agricultural research must not overlook the improvement of the nutritional quality of food crops.

For centuries the potato (Solanum tuberosum L.) has

been a reliable food source for man and animal. The "Incas" built a civilization around the potato and the Irish, before the blight catastrophes of the 1840's, existed on potatoes with a small amount of animal protein. Although the potato is considered primarily starch, it can supply more than 1000 lbs of protein per acre. In addition, the nutritive value of its protein is far superior to that of corn and wheat. Kofranyi and Jekat (1967) reported the daily protein requirement for man to be an average of 0.55g per kg body weight when potato was the only source of protein. That is almost equal to the nutritional value of whole egg protein and better than beef, tuna, whole milk, wheat flour, corn, rice, soybean and kidney bean protein.

In its fresh state, the potato has only an average of 2% total protein, but can range from 1.5% up to 4.0%. However, on a dry weight basis the total protein content of potatoes is not different from that of wheat and can amount to more than 17%. One hectare of land under potato cultivation can supply the protein requirement for 9.5 people, while the protein of wheat from the same land can satisfy only 6.3 people (Borgstrom, 1969). The figures in Table 1 clearly demonstrate that the potato can easily outyield the two main crops of the U. S. A. .

Recent calcultaions of the composition of average national diets, based on FAO information, concluded that lysine rarely appears to be the limiting amino acid in characteristic regional diets; it is usually the sulphur-containing amino

acids (Auret et al., 1968). Miller and Donoso (1963) reported similar results after feeding regional diets to rats.

Table 1: Net protein production of corn, wheat and potatoes in the U. S. A.

	Corn[+++]	Wheat[+++]	Potatoes
Yield[+] tons/ha	4.5	2.1	24.4
Dry matter tons/ha	3.3	1.8	4.9
% protein content[++]	8.5	12	2
NPU[++]	50	60	70
Net protein[++] kg/ha	193	161	340

[+]Production Yearbook 1970

[++]Kuppuswamy et al., 1958

[+++]yield of grain

$$\text{kg net protein/ha} = \frac{\text{yield (tons/ha)} \times \%\text{protein} \times \text{NPU}}{10}$$

Amino acid analysis and studies with animal and human adults fully agree that the sulfur containing amino acids are the first limiting in potato protein (Schuhpan, 1958; Rios et al., 1972; Kies and Metzfox, 1972). Kies and Metzfox (1972) proved in their study with human beings that the protein value of dehydrated potato flakes can be improved by

adding methionine to the diet. To obtain a potato diet higher in methionine, various theoretical approaches may be taken. These include genetic selection of potato tubers having a higher methionine content, addition of purified methionine in the industrial processing of dehydrated potato flakes or education of consumers in usage of desirable food combinations.

The attempt to improve the biological value of potato protein by increasing its methionine content may result in an additional benefit. Methionine appears to be a precursor of some flavor compounds in potatoes (Gumbmann and Burr, 1964). By increasing the methionine content and thus possibly increasing the flavor, it may be possible to enhance the "taste appeal" of this food. This could become an important consideration in determing whether consumers will purchase and use potatoes in their daily diet.

Recent studies indicate that in some plant tissues methionine is a natural precursor of the plant hormone ethylene (Lieberman et al., 1966; Burg and Clagett, 1967). Poabst et al. (1968) showed that potato tubers contain endogenous ethylene. Since gibberellic acid is known to stimulate potato sprouting, and ethylene and gibberellic acid interact with each other, the possibility that methionine could indirectly be involved in controlling the rest period should not be excluded in future studies.

The object of this research was to investigate the genetic variability of methionine in potato protein. To faciliate

eventual breeding work in this area, it is necessary to have more information about the components responsible for such a genetic variation. Attention will also be given to possible side effects of methionine content on agronomic factors and quality factors of the potato.

REVIEW OF LITERATURE

Nitrogen containing constituents of potatoes

Nitrogen has been found bound in free amino acids and amides, in protein soluble in various extracting solutions, in an insoluble protein residue and in trace quantities of numerous constituents. These include nucleic acids, alkaloids, choline, enzymes and some vitamins.

The proteins present in the potatoes are:

Tuberinin: (Albumin) is soluble in water. It is heterogeneous in composition and the role ascribed to it is mainly enzymic.

Tuberin: (Globulin) is insoluble or sparingly soluble in water, but its solubility is greatly enhanced by the addition of neutral salts like sodium chloride. In the potato, the

quantitative distribution of tuberinin and tuberin is pH dependent. At a pH of 6, tuberinin is present in small amounts whereas at a pH of 3 more tuberinin can be obtained at the expense of tuberin (Jirgensons, 1946). Jirgensons postulated that tuberin when exposed to an acid environment is converted to tuberinin plus a very insoluble casein-like protein. This conversion was reversible in an alkaline environment. Tuberin can easily be extracted with 2% NaCl solution. Its molecular weight is estimated to be between 295,000 and 330,000 (Hoelzl and Bancher, 1961).

Tuberin and tuberinin are present in a dissolved form in the cell sap of the potato. If once the cell wall is ruptured, these two proteins can easily be extracted with water (Hoelzl and Bancher, 1961).

At a pH of 6.8 both proteins have a negative isoelectric point (Groot et al., 1947). Together they account for 30 to 60% of the total protein.

Protein crystals: have been observed in the protoplasm of the outer layers of potato cells. Depending upon varieties, these crystals can also be synthesized in other tissues of the potato tuber (Hoelzl and Bancher, 1959). These crystals, hardly exceeding the size of 1 cubic μ, are assumed to be of a globular protein type and represent only a small fraction of the total protein.

Prolamins: are soluble in 70 vol.% ethanol.

Glutelins: are soluble in a 0.2% NaCl solution made up in

60 vol.% ethanol.

Sclereoproteins: are found in the residue which can not be extracted with the above mentioned extracting solutions. This fraction can be 10% of the total protein.

The nonprotein nitrogen fraction is composed mainly of free amino acids and amides. The principle amides are asparagine and glutamine. The free amino acids and the amides represent the amino acid pool of the plant and are involved in many different physiological activities of the plant. Together they can contain between 35 and 65% of the total nitrogen.

Amino acid composition and nutritive value of the various nitrogen containing constituents

Lindner et al. (1960) measured the relative amounts of different proteins in the potato and found that of the total protein, tuberin accounted for 76.4%, globulin II 1.4%, tuberinin (albumin) 4%, prolamin 1.8%, glutelin 5.5%, and the insoluble residue 11%.

The essential amino acids in the nonprotein nitrogen fraction are present at a much lower level than in the protein fractions. Frequently no free tryptophan or no free cysteine could be detected (Woodward and Talley, 1953). The difficulties involved in the proper recovery of cysteine may partially explain these variations. Furthermore one has to consider that the potato tuber is a metabolically active unit, even

when stored at low temperature. Therefore the chemical composition of the tuber may vary if analysed at different physiological stages.

The nonprotein nitrogen alone cannot promote growth of weanling rats (Slack, 1948; Chick and Cutting, 1943). The same investigators have shown that the nitrogen of the intact potato supports growth at least as well as tuberin alone. The complementary nutritive effect between the tuberin and the nonprotein nitrogen fraction of the press juice could not be explained in terms of their amino acid contents. A paper by Rose et al. (1948) contains a description of experiments in which growth of weanling rats was definitely stimulated by the addition of 2% glutamic acid to a diet in which the nitrogen, adequate in amount, was supplied as a mixture of the ten "essential" amino acids. It may be possible that some nutritional significance is attached to the relatively large amounts of glutamine present in the potato tuber.

The insoluble scleroproteins are found mainly in the skin and outer cortex. When these layers were removed, the apparent digestibility was raised from 74 to 79% (Chick and Slack, 1949) resulting in better growth of the rats.

In nitrogen balance studies, using human adults as test individuals, potato protein proved to have the best nutritive value of all analysed plant proteins (wheat flour, corn, rice, algae, soybean and kidney bean protein). Jekat and Kofranyi (1970) demonstrated by means of human bio-assays

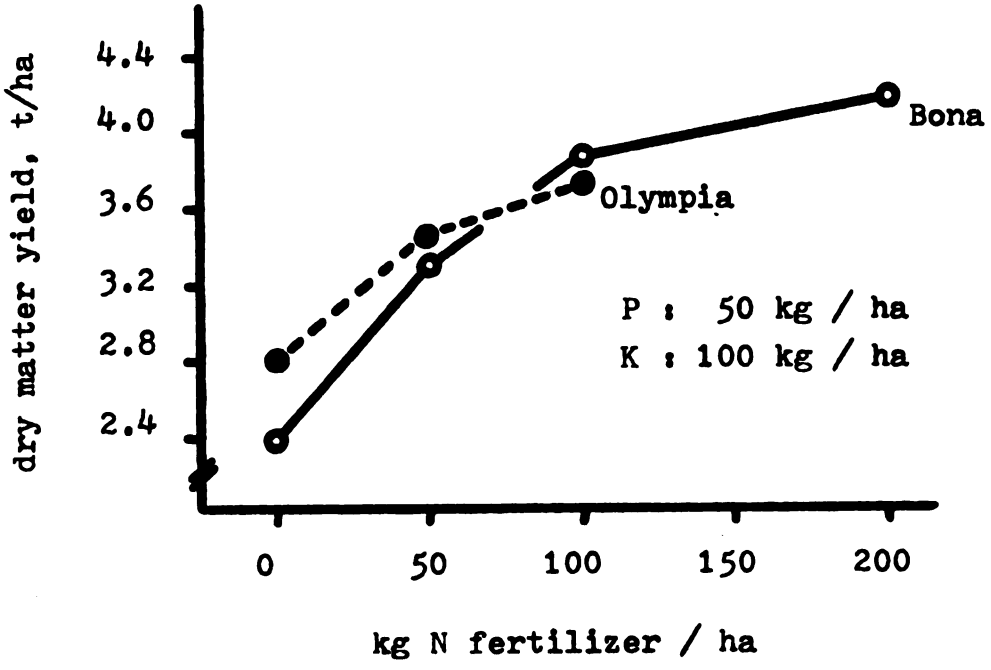
complementary effects of potato protein with egg, soybean, algae, rice, corn, beans and wheat protein. The lowest ever reported minimal protein requirement per kg body weight was obtained with a mixture of 36% whole egg protein and 64% potato protein (Kofranyi and Jekat, 1967).

In other studies using a similar technique as Jekat and Kofranyi (1970), potato diets were supplemented with leucine, phenylalanine and methionine. In comparison with the regular potato diet, only the human adults whose diets were supplemented with methionine showed significantly improved utilization of the potato protein. This is another proof that methionine is the first limiting amino acid in potato protein (Kies and Metzfox, 1972).

Environmental influences on the composition of the nitrogen containing constituents of the potato

One of the most important factors in this respect is the level of soil fertility and nutrient availability. This factor influences the nonprotein nitrogen fraction of the potato, because this chemically mobile part is actively involved in the metabolic processes of the plant.

Figure 1: Relationship between levels of nitrogen fertilization and yield of 2 potato varieties+.



+ Schuhpan, 1970

Table 2 demonstrates the detrimental effect of excessive nitrogen fertilization on potato protein quality. However, attention must be given to other factors which are influenced by fertilization. Figure 1 shows the dependence of total dry matter production upon nitrogen fertilization and Table 2 reflects the response of applied nitrogen fertilizer to net protein content.

Table 2: Influence of fertilizer level on the net protein content[+++]

level of N fertilizer kg/ha	[+]BV	net protein content[++] relative/ha
0	76	100
50	82	144
100	74	184
200	71	201

[+] BV, determined on the growing pig

[++] net protein content = $\frac{\%N \times 6.25 \times \text{true absorption} \times BV}{1000}$

[+++] Brune, 1968/69

Most of these experiments used just one potato variety. However, the extent of response in amino acid composition as influenced by nitrogen fertilizer may be dependent upon the genotype. Schuhpan (1970), for instance, reported that when 120kg/ha nitrogen fertilizer was applied, the variety Bona had an EAA-Index of 64 and the variety Olympia 86. In addition, the EAA-Index of the variety Olympia decreased only slightly with increasing amounts of nitrogen, whereas that of Bona dropped from a high of 95 to a low of 60.

The role of methionine in the potato

Methionine as a precursor is involved in the synthesis

of ethylene and flavor compounds.

Burton (1957) reviewed earlier work on the effects of ethylene and carbon dioxide on sprouting. Brief treatments with ethylene at intervals stimulated sprouting, while treatments of longer duration suppressed it. Since conditions leading to ethylene accumulation in storage would also lead to carbon dioxide accumulation, Burton suggested that sprout suppression attributed to high CO_2 was, in fact, caused by ethylene. His own work suggested that carbon dioxide stimulated sprouting when ethylene was removed. Poabst et al. (1968) proved chemically that potato tubers contain endogenous ethylene, and treatment with gibberellic acid increased the ethylene content. This result conflicts with Burton's hypothesis, since gibberellic acid stimulates potato sprouting. It may be that the influence of gibberellic acid is strong enough to overcome any ethylene produced as a result of its application.

Ethylene has been recognized as an endogenously produced hormone which initiates fruit ripening and regulates many aspects of plant growth. It has been reported to be produced nonenzymically from methionine and its analogues, mediated either by a Cu^{++} -ascorbate -H_2O_2 (Lieberman et al., (1965) or by a FMN -light system (Yang et al., 1966; Yang et al., 1967). The findings that methionine stimulates ethylene production and is readily converted to ethylene in fruit and vegetative tissues (Lieberman et al., 1966; Burg and Clagett, 1967) indicate that this amino acid is a natural

precursor of ethylene in plant tissues.

The importance of sulfur-containing compounds to flavor lies in their extremely low odor thresholds. From all sulfur-containing volatiles produced during the cooking process, methylmercaptan and dimethyl-disulfide make up 90% of the mixture, with ethyl-mercaptan and methyl-sulfide making up most of the remainder (Gumbmann and Burr, 1964).

Although the pathways and intermediates of the sulfur metabolism in plants and animals represent an area largely unexplored, there is sufficient information available to account for the appearance of these compounds in food products. Both, primary and secondary mechanisms may be regarded as responsible for the production of volatile compounds during cooking. For example, the breakdown of the sulfur amino acids is thought to be the primary source for simple organic sulfur compounds. Pathways through which methionine, cystine and cysteine could be converted into flavor compounds are reported by Neukom (1967).

Relations among specific gravity, total nitrogen and quality factors of potatoes

Certain aspects of the quality of fried potato products are apparently associated with high starch content. Thus potatoes of high specific gravity, in general, give french fries that are crisper than those prepared from tubers of low specific

gravity (Kirkpatrick et al., 1956). In selecting potatoes for processing into chips, it is important that tubers of high specific gravity or dry matter content are chosen. Smith (1951) showed that for every increase in specific gravity of 0.005, there is approximately an increase of one percent in yield of chips. Pope et al. (1971) found an inverse relation between specific gravity and potato chip oil content and yellowness of potato chips.

Houghland (1966) observed that in potatoes having 13.3% total solids, about 56.5% of this dry matter consisted of starch. At a total solids content of 32,2%, however, he showed that starch content to be 79.0% of the dry matter. On a fresh basis there was an increase of only 1% in the non-starch fraction of the tuber, and an increase of 18.9% in the solids or an average of 0.053% non-starch for each 1% increase in total solids between these extrems. Thus, any effort to increase the protein content of potatoes by breeding for an increase in dry matter content would show little promise of success. Fitzpatrick et al.,(1969) confirmed these findings.

Toxic constituents of potatoes

Protease inhibitors: The crystallization of two thermolabile trypsin inhibitors from the potato was described by Sohonie and Ambe (1955). The potato may contain a number of other

protease inhibitors which, because of incomplete characterization may or may not be identical. These include also a potent inhibitor of chymotrypsin (Ryan and Balls, 1962). The molecular weight of this inhibitor is approximately 22,000. The inhibitor was devoid of carbohydrate. It rather unexpectedly contained no cystine but rather 4 residues of cysteic acid and 1 residue of methionine sulfoxide. If the latter should be true, then, unlike most of the other protease inhibitors which have been characterized, the chymotrypsin inhibitor is apparently devoid of disulfide bridges (Balls and Ryan, 1962, 1963). Ryan (1966) points out that the chymotrypsin inhibitor of the potato is quickly destroyed by heating in the intact potato even through the purified inhibitor is quite stable.

Cholinesterase inhibitors: The only cholinesterase inhibitor identified is the glycoside, solanine, which is present in highest concentration in the sprouts and skin (especially when green) of the potato. Although human fatalities due to the consumption of green potatoes have been reported from time to time (Hanson, 1925; Hams and Cockburn, 1918), proof that solanine was "the" causative agent is largely indirect. However, it is significant that solanine is not destroyed by cooking (Baker et al., 1955), and the poisoning of livestock has sometimes been observed even with cooked potatoes (Kingsbury, 1964).

PART I

INHERITANCE OF "AVAILABLE" METHIONINE, TOTAL PROTEIN AND SPECIFIG GRAVITY IN TETRAPLOID POTATOES

INHERITANCE OF "AVAILABLE" METHIONINE, TOTAL PROTEIN AND SPECIFIC GRAVITY IN TETRAPLOID POTATOES

This portion of research was undertaken to study the inheritance pattern of methionine, total protein and specific gravity. Also, the possible relationships among these three traits and potato chip color, fresh weight, total dry matter and rest period were investigated.

MATERIALS AND METHODS

Five thousand segregating progenies (1 tuber each) representing 8 crosses and the parental clones were planted on the Montcalm Experimental Farm in 1971. Plant spacings were 45 cm and row spacings 86.4 cm. The soil, a uniform Montcalm sandy loam, was fertilized with 185 kg N, 110 kg P_2O_5 and 110 kg K_2O per hectare.

At harvest, 7 hills of each parent and 40 cultivars from each cross were randomly selected and stored at room temperature until analysed.

To determine the specific gravity, the potatoes from each hill were weighed in air and in water.

From each first year seedling 3 tubers were taken and cut longitudinally into halves. From one half of each of

three tubers, 5 slices were taken for chipping. From each of the other three halves, 7 to 10 two mm thick slices were removed and immediately frozen on a layer of dry ice. After freeze drying the samples were ground in a Wiley mill through a sixty mesh screen. Most of the peel was removed in this process.

From each parent, 7 hills were chosen at random for analysis. Two 3 tuber samples were taken from each hill and prepared as outlined above.

The freeze dried samples were used for the analysis of total protein and "available" methionine (Luescher and Thompson, 1972).

For chipping, the potato slices were rinsed in cold water and fried at 190 C until water evaporation ceased. The potato chip color was estimated using the standard color chart of the National Potato Chip Institute.

After chipping the remaining potatoes were stored at 4.5 C and checked monthly for sprouts. When half of the tubers of a seedling showed visible sprouts (2 mm), it was considered to have broken the rest period.

The female parents in population I originated from a breeding program in which S. stoloniferum was backcrossed several times with S. tuberosum L. and the progenies selected for specific gravity. All the other parental cultivars were strictly of the S. tuberosum type.

The following variance and covariance components (Table 3) were estimated for "available" methionine, total

Table 3: Composition of variance and covariance components of half-sib families in a tetraploid+.

Source of variance	Estimate	Composition
Among half-sib family (offspring) means	V_0	$1/4\sigma^2_A + 1/36\sigma^2_D$
Within half-sib families (offspring)	V_W	$3/4\sigma^2_A + 35/36\sigma^2_D + \sigma^2_T + \sigma^2_F + \sigma^2_E$ ++
Among mid-parents	V_{MP}	$1/2\sigma^2_A + 1/2\,\sigma^2_D + 1/2\sigma^2_T + 1/2\sigma^2_F$
Family (offspring) mean: mid-parents covariance	Cov	$1/2\sigma^2_A + 1/6\,\sigma^2_D$

where σ^2_A = additive genetic variance

σ^2_D, σ^2_T, σ^2_F = interaction variances of 2, 3, and 4 alleles

Heritability mainly in the narrow sense was calculated in two ways:

1. $4\,V_0/(V_0+V_W) = \dfrac{\sigma^2_A + 4/36\sigma^2_D}{\sigma^2_A + \sigma^2_D + \sigma^2_T + \sigma^2_F + \sigma^2_E}$

2. $2\,\mathrm{Cov}/(2V_{MP}+V_E) = \dfrac{\sigma^2_A + 2/6\,\sigma^2_D}{\sigma^2_A + \sigma^2_D + \sigma^2_T + \sigma^2_F + \sigma^2_E}$

++ in this variance estimate the environmental variance is included because no replications were available from the first year seedlings

+ Kempthorne, 1955

protein and specific gravity and interpreted genetically assuming autotetraploid inheritance and absence of inbreeding in the ancestries of the parental clones (Kempthorne, 1955).

In both cases the numerator contains some variance due to the interactions of 2 alleles which is analogous to dominance deviations in the diploid case. The variance of half-sib family means contains the least of this interaction variance. Falconer (1960) points out that the half-sib correlation and the regression of offspring on the male parent give the most reliable estimation of heritability.

RESULTS AND DISCUSSION

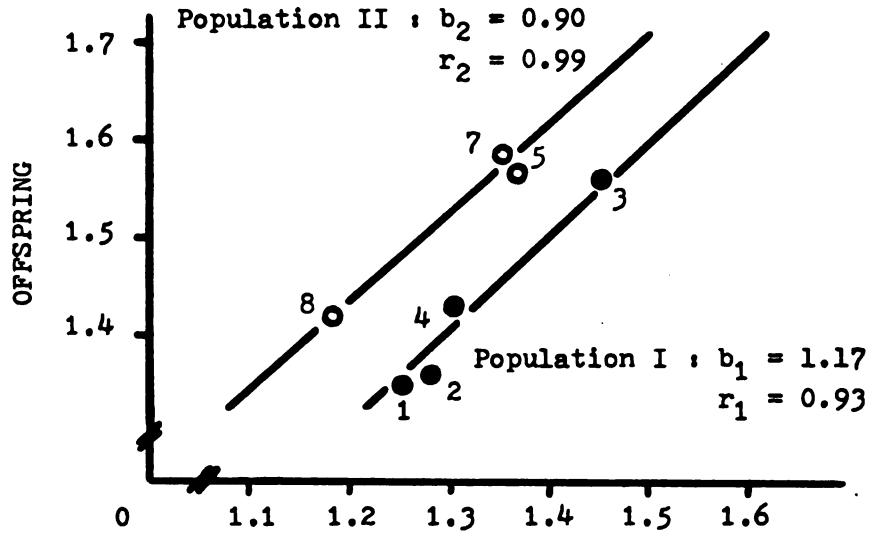
There is limited information on the genetic variability of methionine in potatoes. From the total phenotypic variance in the parental nursery the variance between the two 3 tuber samples approached zero (P=0.89). This indicates that one 3 tuber sample per hill (first year seedling) is a representative sample for methionine analysis.

In population II the offspring family means of methionine were somewhat higher (but not significant) than those in population I (Figure 2, Table 4). In population II, total protein averaged 9% higher than in population I. This difference and the positive correlation between total protein

Table 4: Parent and offspring family means for "available" methionine (mg/16mg N), total protein (mg/100mg dry matter) and specific gravity.

Population	Cross Parentage ♀	♂	Crossnumber	Methionine ♀	♂	Midparent	Offspring	Total Protein ♀	♂	Midparent	Offspring	Specific gravity ♀	♂	Midparent	Offspring
I	320- 6x709		1	1.04	1.45	1.25	1.35	10.76	15.03	12.85	11.52	1.1090	1.0759	1.092	1.0745
	321-38x709		2	1.10	1.45	1.28	1.36	15.30	15.03	15.17	12.41	1.0740	1.0759	1.075	1.0784
	321-65x709		3	1.45	1.45	1.45	1.58	9.00	15.03	12.02	11.11	1.1110	1.0759	1.093	1.0854
	321-70x709		4	1.15	1.45	1.30	1.43	14.70	15.03	14.87	11.84	1.0959	1.0759	1.086	1.0780
II	706-34x709		5	1.24	1.45	1.35	1.58	14.44	15.03	14.76	12.83	1.0646	1.0759	1.070	1.0726
	706-34x706-32		6	1.24	-	-	1.32	14.44	-	-	12.99	1.0646	-	-	1.0704
	706-34x711- 8		7	1.24	1.48	1.36	1.57	14.44	16.36	15.40	13.40	1.0646	1.0673	1.066	1.0669
	706-34x735- 1		8	1.24	1.11	1.18	1.42	14.44	10.61	12.53	12.04	1.0646	1.0733	1.069	1.0677

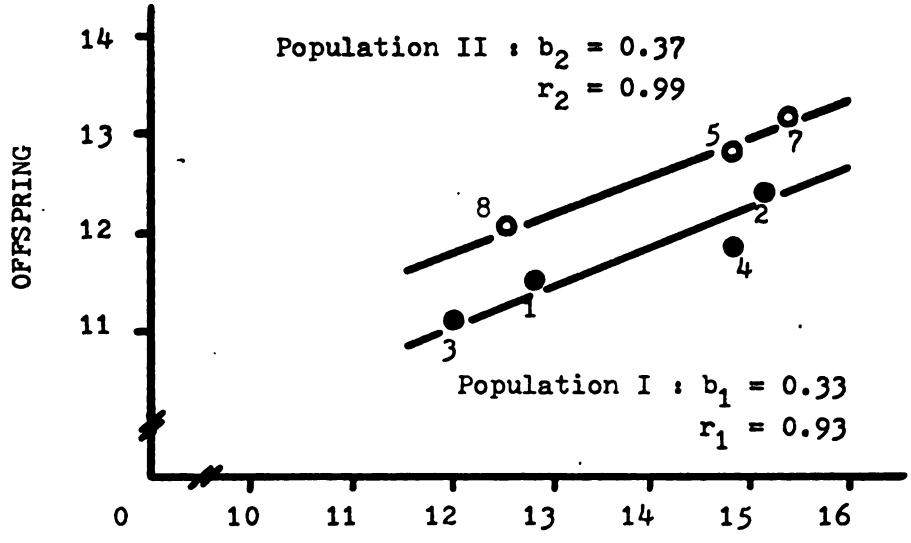
Figure 2 : Offspring : Mid-parent regression for "available" methionine



"available" methionine, mg met / 16mg N

MID - PARENTS

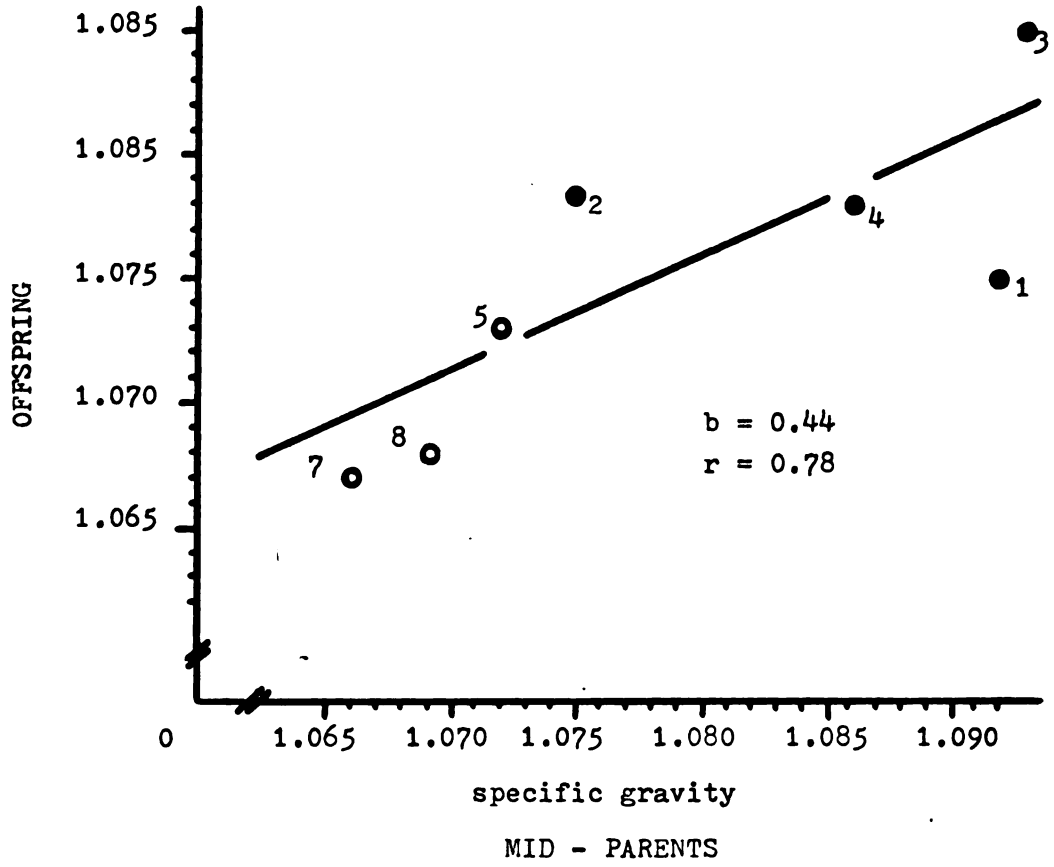
Figure 3 : Offspring : Mid-parent regression for total protein



total protein, mg Nx6.25 / 100mg dry matter

MID - PARENTS

Figure 4 : Offspring : Mid-parent regression for specific gravity



and "available" methionine ($r=0.16$, $P< 0.01$) may be partially responsible for the higher methionine values in population II. Another cause of the difference could arise from ancestral dissimilarities of the parents. All female parents in population I originated from a program in which S. stoloniferum was backcrossed several times with S. tuberosum and the progenies selected for specific gravity (see Figure 4). The possibility of maternal biases in population I should not be excluded "a priori".

The heritability figures are presented in Table 5. For "available" methionine, heritability ranged from 79 to 100%. From a biochemical point of view these figures are surprisingly high. But it must be remembered that about half of the total nitrogen is made up from free amino acids and amides. From the total "available" methionine 20 to 60% can be present as free methionine. Hence, total "available" methionine can vary without any quantitative or structural changes in the proteins. For a more detailed discussion about the possible cause of variation in "available" methionine see Part II of this thesis. "Available" methionine ranged from 0.9 to 2.2 mg met/16 mg N.

From the point of view of practical potato breeding, the heritability estimates obtained from the offspring: mid-parent regression are probably more meaningful. In this study heritability figures calculated from the offspring: mid-parent regression varied less from population I to II than those computed from half-sib family variance components.

Table 5: Narrow sense heritability estimates for "available" methionine, total protein and specific gravity

Type of estimates	"Available" methionine			Total protein			Specific gravity		
	Pop.I	Pop.II	Av.	Pop.I	Pop.II	Av.	Pop.I	Pop.II	Av.
Half-sib family V components	79	100	90	23	10	11	99	39	69
Offspring: Mid-parent regression	94	87	90	25	28	26		35	35

In the case of total protein, from the total phenotypic variance of parental clones, the variance due to environment made up 24%. Although "available" methionine is expressed in % of total protein, its environmental variance component is not even half that of the total protein, suggesting that the genetic expression of methionine is coupled, at least to a certain extent, with that of total nitrogen. The heritability for total protein ranged from 10 to 28%. Sanford *et al.* (1971) reported similar results.

The analysis of variance of the parental clones for total protein showed that there was a significant difference ($P < 0.01$) between 3 tuber samples taken from each hill. The mean total protein of population II was 0.95% greater than that of population I (Figure 3, Table 4). This difference, however, was not significant ($P = 0.05$). Population I was considerably higher in average specific gravity than population II. In addition there existed a highly significant correlation

($r = -0.24$, $P < 0.01$) between specific gravity and total protein. These two factors combined could be partially responsible for the differences between the two population means. Total protein ranged from 7.5 to 18.8 g (N x 6.25)/ 100 g dry matter.

The environmental variance of specific gravity amounted to 16% of the total parental phenotypic variance. The high heritability of 99% in population I was due to a relatively large variance component of family means (Table 5). In population II differences among family means were much smaller and therefore gave only 39% heritability. The within family variance component was almost identical in both populations.

The effect of previous selection for specific gravity in the female parentage of population I was clearly reflected in higher mid-parent and progeny family means (Figure 4, Table 4).

Specific gravity ranged from 1.041 to 1.115 or from 12.7 to 28% dry matter.

The F-test (variance among families + variance within families / variance within families) was applied to the rest period, chip color and total dry matter data and was in all cases highly significant ($P < 0.01$); thus genetical differences existed for these traits. Duncan's Multiple Range was used to separate the family means (Table 6).

The correlation between chip color and specific gravity was positive. High specific gravity was primarily associated with a bright chip color and a low specific gravity with a

dark chip color. The same is true for the correlations of total protein and "available" methionine with chip color.

Table 6 : Means and ranges of chip color, rest period and total dry matter of 320 seedlings representing 8 crosses

Cross #	Chip color+			Rest period++			Total dry matter+++		
	Mean	Range low	Range high	Mean	Range low	Range high	Mean	Range low	Range high
1	6.2 ab	1	9	1.8 a	1	3	175 e	17	404
2	4.6 bc	1	10	1.9 a	1	4	192 de	86	342
3	4.1 c	1	10	3.1 b	1	5	220 b	84	468
4	5.2 abc	2	10	3.0 b	1	5	198 cd	56	507
5	5.2 abc	1	9	3.0 b	1	5	214 bc	85	432
6	6.7 a	1	10	3.3 b	1	5	220 b	72	434
7	6.1 ab	1	8	3.4 b	1	5	200 cd	88	422
8	5.7 abc	3	9	3.5 b	2	5	247 a	115	442

means were separated with Duncan's multiple Range Test, P=0.01

\+ Chip color according to the chart of the National Potato Chip Institute

++ 1: visible sprouting started in December
2: visible sprouting started in January

+++ (g fresh weight per hill) x (dry matter content), in g

Thus, by selecting for higher "available" methionine, higher specific gravity and higher total protein, one enhances the improvement of chip color. However, too great a selection

pressure cannot be put on specific gravity and total protein at the same time, because a negative correlation exists between the two ($r = -0.24$, $P < 0.01$). Other than that, no negative side effects should appear when selecting for higher "available" methionine, higher total protein and higher specific gravity.

Table 7: Correlations among "available" methionine, total protein, specific gravity, fresh weight, total dry matter, rest period and chip color (data from 320 seedlings).

	Rest period	Fresh wt.	Spec. gravity	Total protein	Av. methionine	Chip color	Total dry matter
Rest period	1.						
Fresh wt.	0.09	1.					
Spec.grav.	-0.09	-0.27[b]	1.				
Total prot.	-0.09	-0.05	-0.24[b]	1.			
Av.methionine	0.11	0.05	0.05	0.17[b]	1.		
Chip color	-0.07	-0.05	0.44[b]	0.13[a]	0.14[a]	1.	
Total dry matter	0.06	0.97[b]	-0.06	-0.09	0.07	0.05	1.

a: significant at P= 0.05

b: significant at P= 0.01

No significant correlation was observed between the rest period and "available" methionine, thus the level of "available" methionine does not, to a measurable extent, appear to interfere with the duration of the rest period.

The positive correlation ($r = 0.17$, $P < 0.01$) between "available" methionine and total protein is rather surprising, considering that this potato crop received 185 kg/ha of nitrogen fertilizer. Mulder and Bakema (1956) indicate that the amino acid composition of the potato proteins was not affected by the amount of the nitrogen fertilization. But the relative amounts of some essential amino acids (among them methionine) decreased with increasing rates of nitrogen fertilization. This points out the importance of making selections under conditions similar to those of commercially grown crops.

SUMMARY

Significant differences in "available" methionine, total protein and specific gravity were found among parents and families. Heritability, mainly in the narrow sense, varied from 79 to 100% of "available" methionine, from 10 to 28% of total protein and from 35 to 99% of specific gravity. "Available" methionine, total protein and specific gravity are all positively correlated with chip color ($r=0.14$, 0.13 and 0.44 respectively). No definite relationship between "available" methionine content and rest period could be

observed.

No negative correlations could be detected between "available" methionine and fresh weight, specific gravity, total protein and total dry matter production.

PART II

CAUSE OF VARIATION IN "AVAILABLE" METHIONINE IN POTATO PROTEIN

CAUSE OF VARIATION IN "AVAILABLE" METHIONINE IN POTATO PROTEIN

In the potato, nitrogen is found in the form of free amino acids and amides, in proteins soluble in various extracting solutions, in an insoluble protein residue, in nucleic acids, in choline and some vitamins.

Methionine can be found in the form of free methionine or bound in different proteins and in the insoluble protein residue.

Contradictory results have been reported about the presence of free cysteine. Mulder and Bakema (1956) recovered some whereas Hoff *et al.* (1971) did not. Methods of analysis, environmental conditions and/or genetic differences of the analysed potatoes may be responsible for this disagreement.

The nonprotein nitrogen could contain a considerable percentage of all methionine present in the total protein.

The structural proteins can be split up into at least 6 different fractions or proteins: tuberin, tuberinin, globulin II, prolamin, glutelin and a residue (Lindner *et al.*, 1960). Some of these proteins differ in their quantity and their methionine content (Lindner *et al.*, 1960).

The purpose of this study was to investigate the extent to which free methionine was responsible for variation in "available" methionine content.

MATERIALS AND METHODS

From 320 segregating progenies representing 8 crosses, 16 were selected to form 4 groups: a low total protein - low methionine, a low total protein - high methionine, a high total protein - low methionine and a high total protein - high methionine group.

Growing conditions and preparation of the samples for analysis were outlined previously (Part I of this thesis).

"Available" methionine, methionine and cysteine were analysed according to Luescher and Thompson (1972).

The "biological" value of total protein and nonprotein nitrogen was assessed microbiologically (Ford, 1960). In this method the total growth of Streptococcus zymogenes on a given amount of sample protein has been expressed in percent of the growth on the same amount of casein protein. To assess the BV of total protein, the same digests were used as for the analysis of "available" methionine (Luescher and Thompson, 1972). The samples containing nonprotein nitrogen did not need enzymatic digestion.

The procedure used in this study to precipitate the protein was as follows: samples containing 50 mg (N x 6.25) were weighed out into 50 ml centrifuge tubes. Then 30 ml of buffer (1 g of trisodium citrate and 6 mg of sodium cyanide were dissolved in one l distilled water and the pH adjusted to 7.0) at 80 C were added to each tube. The tubes were shaken vigorously and placed into a water bath at 80 C for

5 minutes (Neuberger and Sanger, 1942). After cooling to room temperature, the tubes were centrifuged (12,100 g, 10 min.). The residue was washed once with 20 ml buffer at 80 C, cooled to room temperature and centrifuged as described above. After adjusting the pH to 7.0, the combined supernatant was made up to 50 ml. Two aliqouts of each sample were analysed for nitrogen using the technique of the Hengar Company[1]. For the assessment of free methionine 2 ml portions and free cysteine 0.5 ml portions were taken from the above prepared samples and microbiologically analysed (Luescher and Thompson, 1972).

RESULTS AND DISCUSSION

Analysis of free amino acids by automatic ion exchange chromatograph, usually uses picric acid to precipitate the protein (Toepfer, 1965). Kaldy (1971) precipitated the proteins of 7 potato varieties by means of picric acid and trichloroacetic acid. Picric acid precipitated 1.5 to 2.5 times more nitrogen than trichloroacetic acid. Thus amino acid analyses performed on samples of which proteins were precipitated by picric acid, must be interpreted with some caution.

1 Hengar Company
6825 Greenway Avenue
Philadelphia, Pa. 19142

Figure 5 : Regression of "available" methionine on free methionine

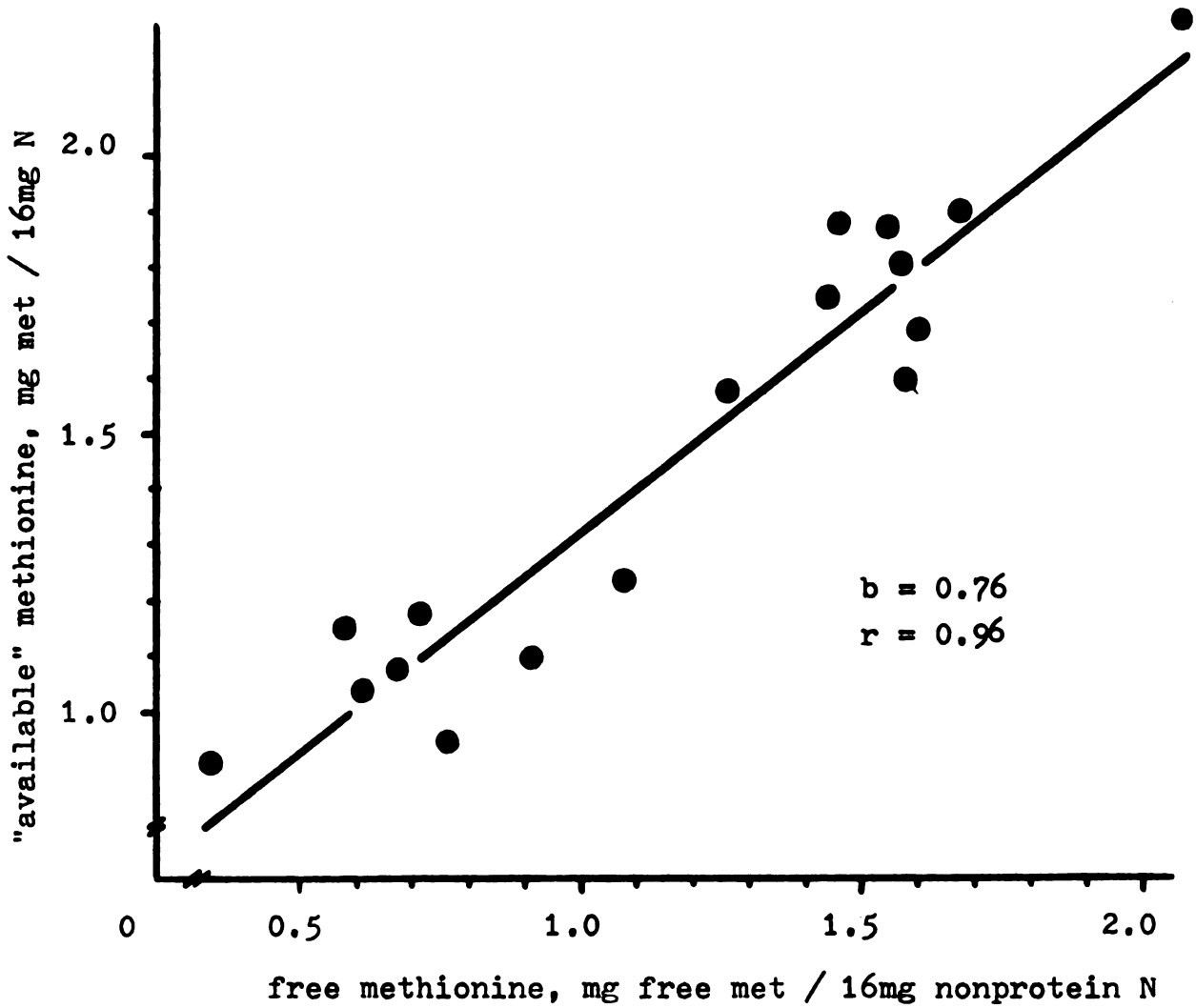


Table 8: Means of two analyses for total protein, "available" methionine, methionine, free methionine, nonprotein N, BV of nonprotein N, BV of total protein and contribution of free methionine to methionine of total protein.

Clone No.	Total protein mg/100mg dry matter	"Available" methionine mg/16mg N	methionine mg/16mg N	free methionine mg/16mg nonprotein N	Nonprotein N mg/100mg total N	BV of nonprotein N	BV of nonprotein N	%contribution of free methionine to methionine of total protein
5-10	16.4	1.08	1.13	0.67	63.9	61	77	38
6- 8	18.8	1.24	1.11	1.08	62.6	62	78	61
6-28	16.3	0.95	1.18	0.76	56.8	68	77	36
6-34	15.9	1.1	1.16	0.91	52.8	70	74	41
Av.	16.87	1.09	1.14	0.85 b	59.0 a	66 b	77 b	44
1-34	9.2	1.15	1.55	0.58	44.4	95	85	16
1-40	10.8	0.91	1.33	0.34	47.9	83	80	12
2-92	8.6	1.18	1.38	0.71	52.3	77	81	27
6- 6	9.0	1.04	1.31	0.61	39.1	101	78	18
Av.	9.39	1.06	1.40	0.59 b	45.9 b	90 a	81 ab	18
1-38	14.8	1.69	1.60	1.60	53.3	76	81	53
5-22	16.5	1.60	1.51	1.58	55.5	70	77	58
5-33	15.2	1.58	1.40	1.26	68.8	54	74	62
4-197	13.7	1.88	1.75	1.46	65.4	61	80	54
Av.	15.04	1.69	1.57	1.47 a	60.7 a	66 b	78 b	57
3-22	11.6	1.81	1.89	1.57	48.8	91	88	40
3-24	11.7	1.90	1.86	1.67	56.8	71	81	51
5-14	10.5	2.25	2.01	2.07	54.1	76	83	56
7- 6	10.0	1.75	1.71	1.44	56.8	71	81	47
Av.	10.95	1.93	1.87	1.69 a	54.1 ab	78 ab	84 a	49

group averages were separated with Duncan's Multiple Range Test, P= 0.05

Table 9: Correlations among total protein, "available" methionine, methionine, cystine, nonprotein N, free methionine, BV of total protein and BV of nonprotein N (data from 16 seedlings analysed in 2 replications each.

	total protein	"available" methionine	methionine	cystine	free N	free methionine	BV of total N
total protein (%of dry matter)	1.00						
"available" methionine (mg met/16 mg N)	-0.11	1.00					
methionine (mg met/16 mg N)	-0.49[a]	0.86[b]	1.00				
cystine (mg cys/16 mg N)	-0.08	-0.39	-0.20	1.00			
free N (% of total N)	0.63[b]	0.26	-0.09	-0.03	1.00		
free methionine (mg free met/ 16 mg free N)	0.09	0.96[b]	0.76[b]	-0.45	0.30	1.00	
BV of total protein	-0.55[a]	0.40	0.62[b]	0.00	-0.41	0.24	1.00
BV of non-protein N	-0.69[b]	-0.15	0.21	-0.02	-0.96[b]	-0.24	0.60[a]

a: significant at P= 0.05

b: significant at P= 0.01

Neuberger and Sanger (1942) compared heat coagulation, precipitation with trichloroacetic acid and filtering through a membrane with an average pore size of 7 mμ. All three methods gave comparable results.

Only traces of free cysteine could be detected in the 16 analysed cultivars.

The regression of "available" methionine (mg met/16 mg N) on free methionine (mg free met/16 mg nonprotein N) amounted to 0.76 and accounted for 93% of the total variation in "available" methionine (Figure 5). Although total protein was highly correlated with nonprotein N, it had no influence on free methionine (Table 8).

In the low total protein - low methionine group, free methionine provided 18% of all the methionine present in the total protein, whereas in the high protein - high methionine group, free methionine accounted for 57% (Table 8).

From the nutritional point of view an increase in methionine or cystine or both would be desirable. In the clones analysed however, cystine was present exclusively in the protein fractions. The various proteins could theoretically differ in their quantity and in their cystine content and thus cause variation in overall cystine content (Part III of this thesis).

Variation in methionine is composed of variation in free methionine and in methionine present in the proteins. The latter could be explained as for cystine. According to this study, however, free methionine is responsible for 93%

of the variation in "available" methionine.

Free methionine ranged from 0.34 to 2.07 mg/16 mg nonprotein N. Whether or not there exists an upper limit for free methionine could not be appraised. It must be remembered, however, that these potato cultivars received 185 kg of N fertilizer per hectare during their growth. Mulder and Bakema (1956) reported that free methionine dropped from 1.9% when fertilized with 33 kg N/ha to 1.0% when fertilized with 150 kg N/ha. If this observation is taken into account, the highest free methionine content of 2.07% is remarkable where 185 kg N/ha were applied.

The fact that growing conditions have a great influence on free methionine is a major drawback. If the genotype x environment interactions are reasonably small, the genetical gain in free methionine would be actual.

A potato high in free methionine must be cooked and processed carefully to avoid a loss of nonprotein nitrogen in cooking or rinsing water. The high free methionine content could enhance the formation of flavor compounds.

S. zymogenes has an absolute requirement for exogenous leucine, methionine, tryptophan, arginine, histidine, isoleucine, valine and glutamic acid. Thus the amount of these amino acids determines the BV obtained by this microbiological assay.

The BV of the nonprotein nitrogen was very dependent upon the level of the nonprotein nitrogen and total protein ($r = -0.96$ and -0.69 respectively). Even the BV of the total

protein was negatively correlated (Table 9) with these two factors; however, the regression coefficients were not significant. At the 0.01% level, no group means of the BV results could be separated with Duncan's Multiple Range Test (Table 8).

High and low total protein cultivars were found with a high "available" methionine content. The high "available" methionine content was proportional to the amount of non-protein nitrogen.

SUMMARY

Sixteen cultivars selected for their total protein and "available" methionine contents were studied.

Free methionine ranged from 0.34 to 2.07 mg/16 mg nonprotein N and was highly correlated with "available" methionine (r= 0.96). Free methionine contributed from 12 to 62% of all methionine present in the total protein. Free methionine and "available" methionine were independent of the total protein content.

No measurable amounts of free cysteine could be detected. Advantages and disadvantages of a potato high in "available" methionine are discussed.

The BV of the total protein determined by microbiological assay was negatively correlated with total protein

content and positively with methionine. However, the group means could not be separated with Duncan's Multiple Range Test at P= 0.01.

PART III

ELECTROPHORESIS AND ANALYSIS OF THE SULFUR AMINO ACIDS OF VARIOUS POTATO PROTEINS

ELECTROPHORESIS AND ANALYSES OF THE SULFUR AMINO ACIDS OF VARIOUS POTATP PROTEINS

Variation in amino acid composition can be due to variation in the nonprotein fraction and/or variation in the protein fractions of the total protein in potatoes. In Part II of this thesis variability in "available" methionine could be explained mainly by the variability in free methionine.

Lindner et al. (1960) isolated five different proteins and analysed three of them for methionine but not for cystine. Only limited information is available about the uniformity of these extracted proteins.

This research was designed to investigate the variability of methionine and cystine in the classical proteins of three potato cultivars.

MATERIALS AND METHODS

Random samples, 4 kg each, of the experimental cultivars Nos. 58, 709 and 322-6 were used for this study. All three cultivars have a completely different pedigree: Number 58 is an inbred Merrimack, 322-6 originates from a breeding program in which S. stoloniferum was backcrossed several times with S. tuberosum, and 709 is S. tuberosum.

All three cultivars were grown on a Montcalm sandy loam and fertilized with 220 kg N, 150 kg P_2O_5 and 110 kg K_2O per hectare. The tubers were cut into 2 mm thick slices and immediately frozen on a layer of dry ice. After freeze drying the samples were ground in a Wiley mill to pass through a sixty mesh screen. Most of the peel was removed in this process.

Methionine, cystine and total protein were determined according to Luescher and Thompson (1972).

Free methionine and nonprotein nitrogen were assayed as outlined in Part II of this thesis.

To isolate the various proteins, the procedure outlined by Lindner et al. (1957) was applied (Figure 6).

The potato flour together with the extracting solutions were in all cases blended for four minutes at room temperature.

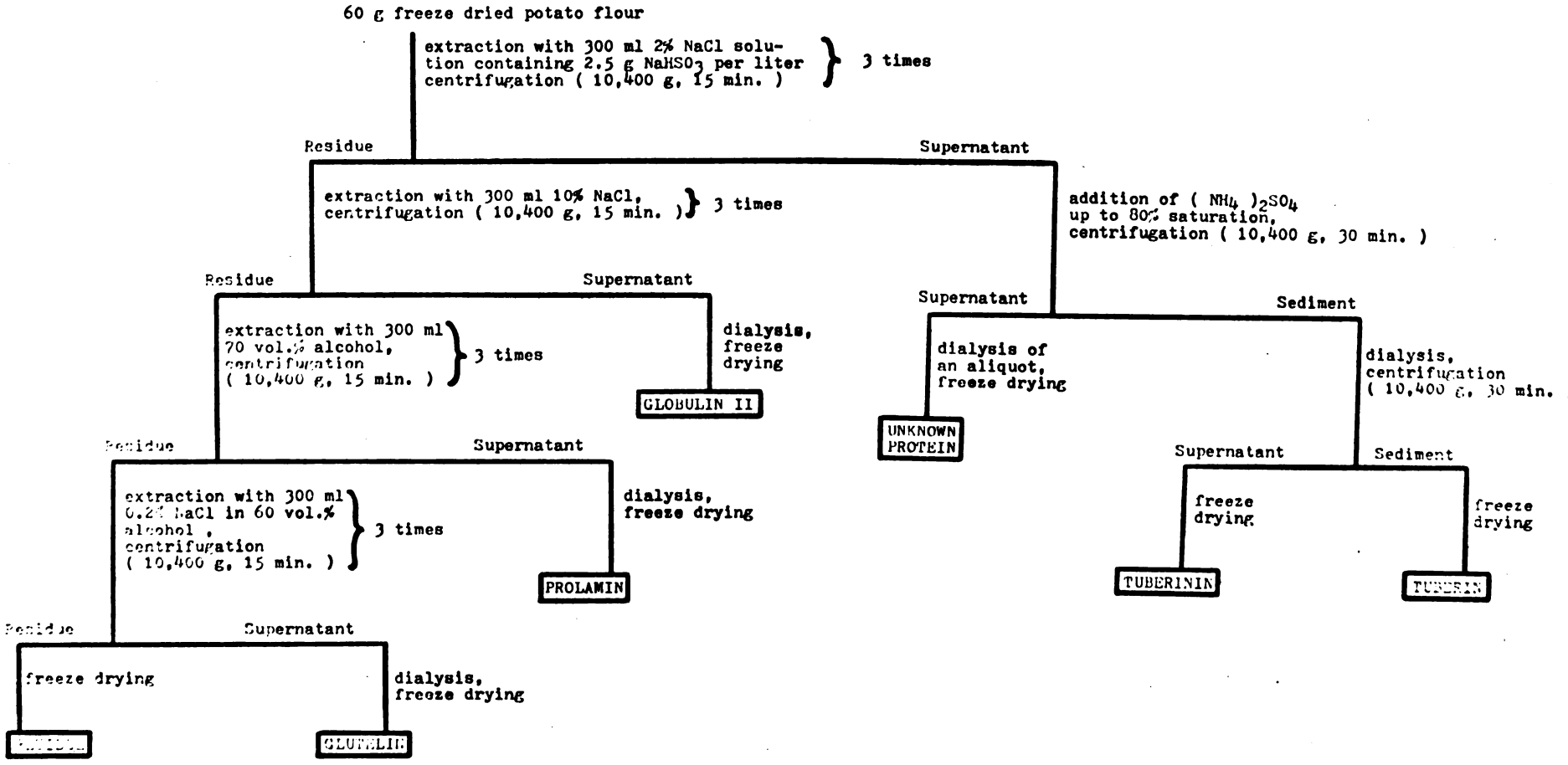
The cellophane bags[1] were dialized against 100 times its volume of distilled water for 48 hours at 4 C. During dialysis the water was changed 4-5 times.

After the first complete isolation, tuberin, tuberinin, globulin II and prolamin were dissolved in their corresponding extracting solutions followed by centrifugation, dialysis, centrifugation and freeze drying. In addition tuberin was washed 4 times with distilled water.

Electrophoretic separations of the proteins were performed according to Davis (1964). Instead of preparing a sample gel, the sample was mixed with a sucrose solution

[1] Number 27/100 was obtained from Union Carbide

Figure 8 : Scheme for fractionation of potato proteins (Lindner et al.,1957)



and an aliquot portion was layered on top of the spacer gel.

The gels were stained for 15 minutes with Coomassie blue, 0.125 g in a solution of 5 parts methanol, 5 parts distilled water and 1 part acetic acid respectively. For destaining the gels were soaked in a solution containing 5% methanol and 7% acetic acid at 50 C for 24 hours. The destaining solution was changed twice.

Protein extracts from the potato flour were prepared in the following manner: a sample containing 50 mg of total protein was extracted with 4 ml of a 2% NaCl solution containing 500 mg $NaHSO_3$ per 100 ml, then centrifuged (10,000g, 10 min.) and the supernatant was saved. The supernatant of a second extraction with 4 ml of the same solution was added to the first one and the volume was made up to 8 ml. For the electrophoretic analysis the extract was diluted 1:1 with a 40% sucrose solution. From this mixture 10 µl portions were analysed.

One mg each of tuberinin and tuberin was dissolved in 1 ml of a 2% NaCl solution and then diluted 1:1 with a 40% sucrose solution.

RESULTS AND DISCUSSION

Tuberin and the nonprotein nitrogen fraction contained 86 to 92% of the total nitrogen. The amount of nonprotein

Table 10: Distribution of total nitrogen in 60 g dry matter of 3 cultivars.

	58		709		322-6	
	mg	%	mg	%	mg	%
Protein fractions						
Tuberin	2059	28.8	1769	33.5	2082	35.4
Globulin II	41	0.6	20	0.4	16	0.3
Tuberinin	77	1.1	64	1.2	56	1.0
Prolamin	42	0.6	32	0.6	49	0.8
Glutelin	6	0.1	5	0.1	6	0.1
unknown nitrogen compounds	60	0.8	32	0.6	128	2.2
Residue	482	6.7	267	5.1	579	9.8
Total	2767	38.7	2189	41.4	2916	49.6
Nonprotein N	4379	61.3	3093	58.6	2968	50.4
Total protein	7146	100.0	5282	100.0	5884	100.0
nonprotein N obtained by heat coagulation		31.6		45.6		55.6

Table 11: Methionine and cystine contents of tuberin, tuberinin, prolamin and the nonprotein N fraction of 3 cultivars.

	58			709			322-6		
	met+	cys+	total	met+	cys+	total	met+	cys+	total
Tuberin	2.9	1.2	4.1	2.0	1.4	3.4	2.7	1.1	3.8
Tuberinin	2.0	1.0	3.0	1.5	1.5	3.0	2.0	1.2	3.2
Prolamin	0.3	3.7	4.0	0.3	3.1	3.4	0.3	3.9	4.2
Nonprotein N fraction	0.8	trace	0.8	0.7	trace	0.7	0.3	trace	0.3

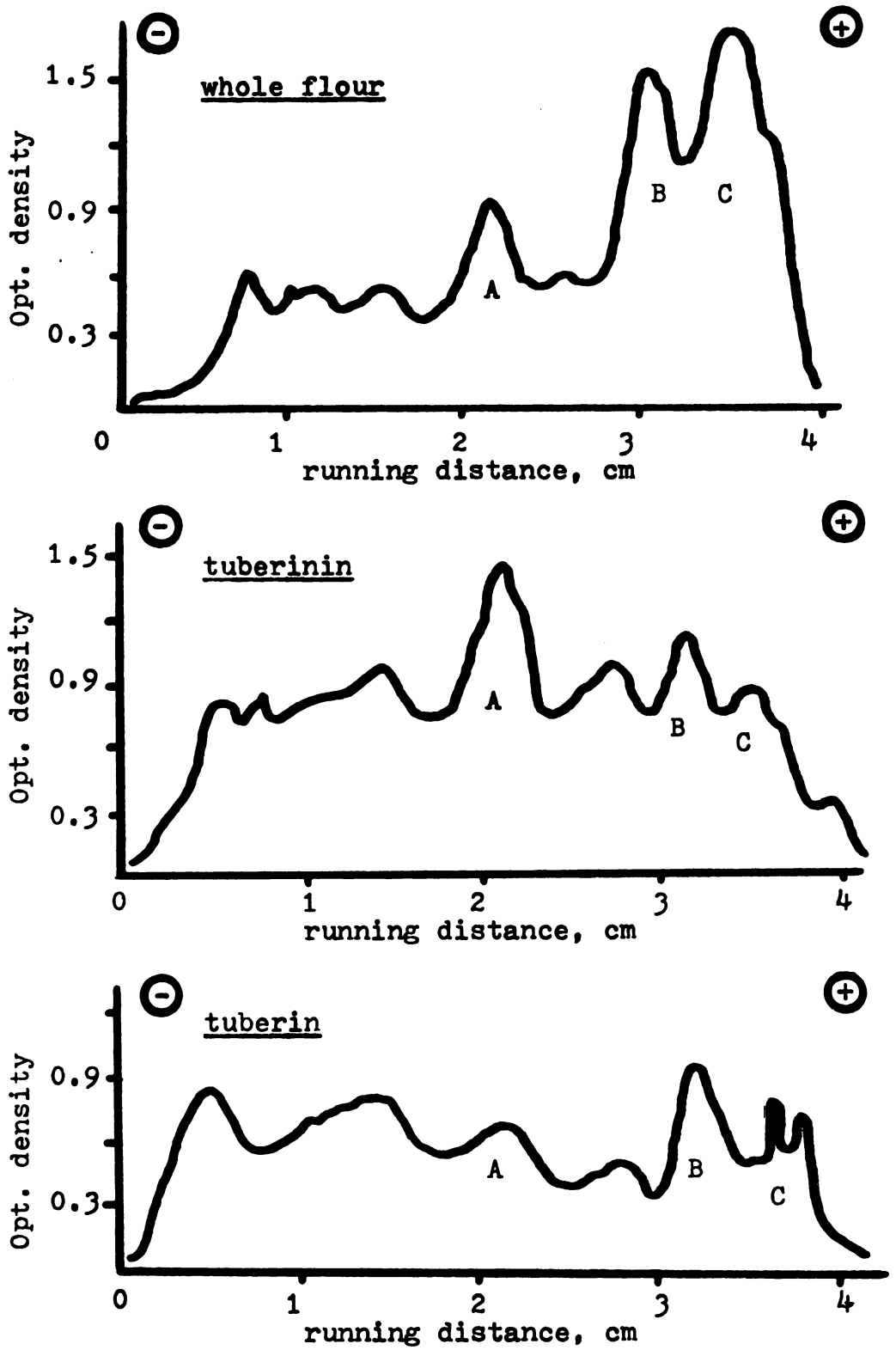
+ amino acids are expressed in mg/16 mg N; means of two independent analyses

nitrogen obtained by heat coagulation and by the extraction procedure agreed reasonably well considering the large number of nitrogen analyses involved in the extraction procedure (Table 10). Ammonium sulfate (at 80% saturation) did not precipitate all nitrogen present in larger molecules of the 2% NaCl solution. In Table 10 this residue left after precipitation with $(NH_4)_2\ SO_4$ was called "unknown nitrogen compounds".

The four major nitrogen containing constituents, tuberin, tuberinin, prolamin and the nonprotein fraction were analysed for methionine and cystine (Table 11). Tuberin and tuberinin of the cultivars 58 and 322-6 contained about twice as much methionine as cystine. Cultivar 709 had considerably less methionine in both the tuberin and tuberinin fraction. On the other hand both of these protein fractions contained more cystine than 58 and 322-6. In all three cultivars the sum of methionine and cystine was smaller in tuberinin than in tuberin suggesting that these two proteins differ more than by an atom of hydrogen as indicated by Jirgensons (1946). Tuberin, the main protein fraction, contained amounts of sulfur amino acids similar to milk but only about 75% of the methionine and 50% of the cystine present in the protein of whole egg (Block, 1951). Some variability can be expected in the amino acid composition of tuberin, however, to what extent was not determined from this study.

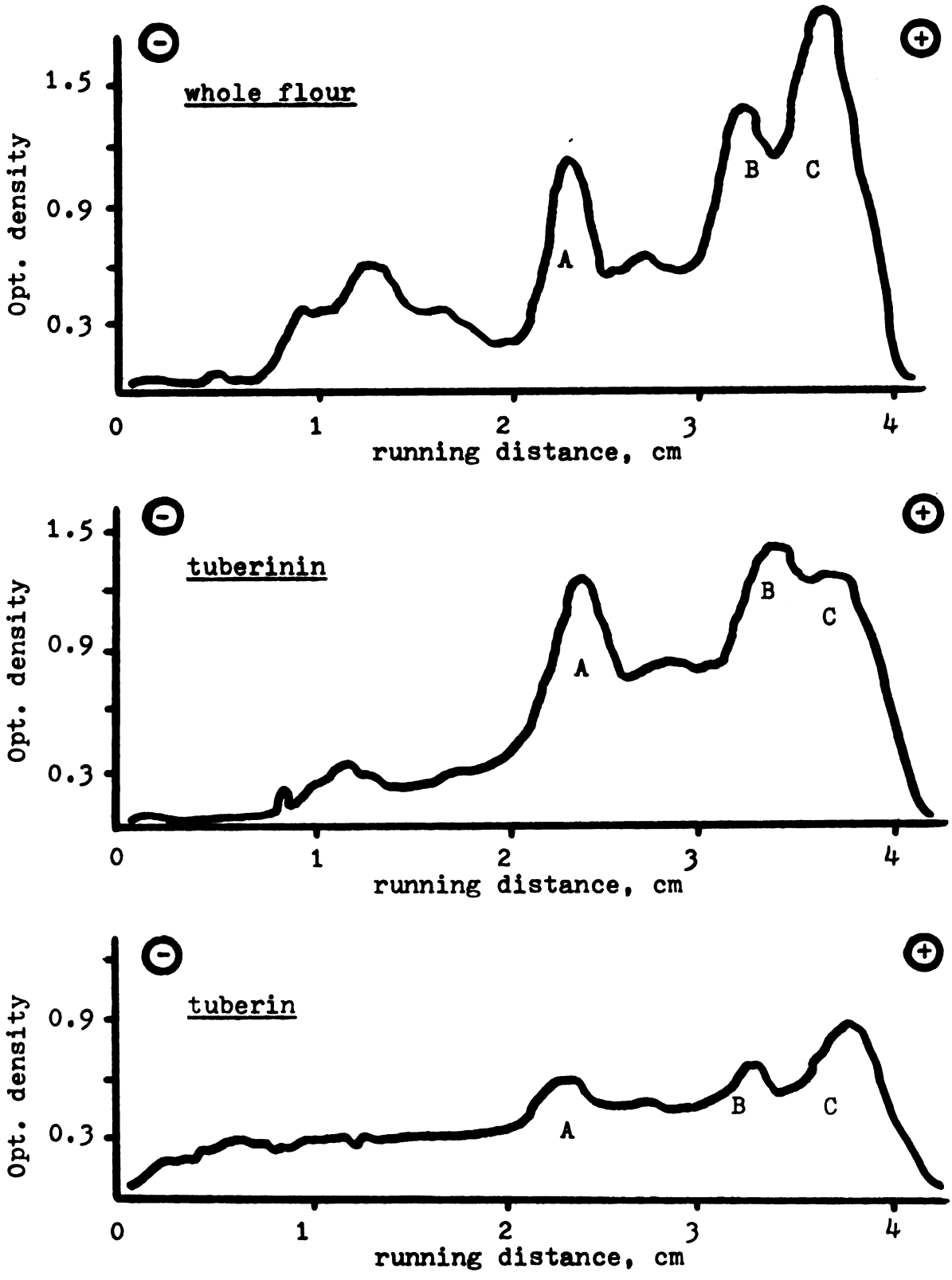
Prolamin is an excellent source of cystine. However,

Figure 7: Densitometrical readings of the gels of 58



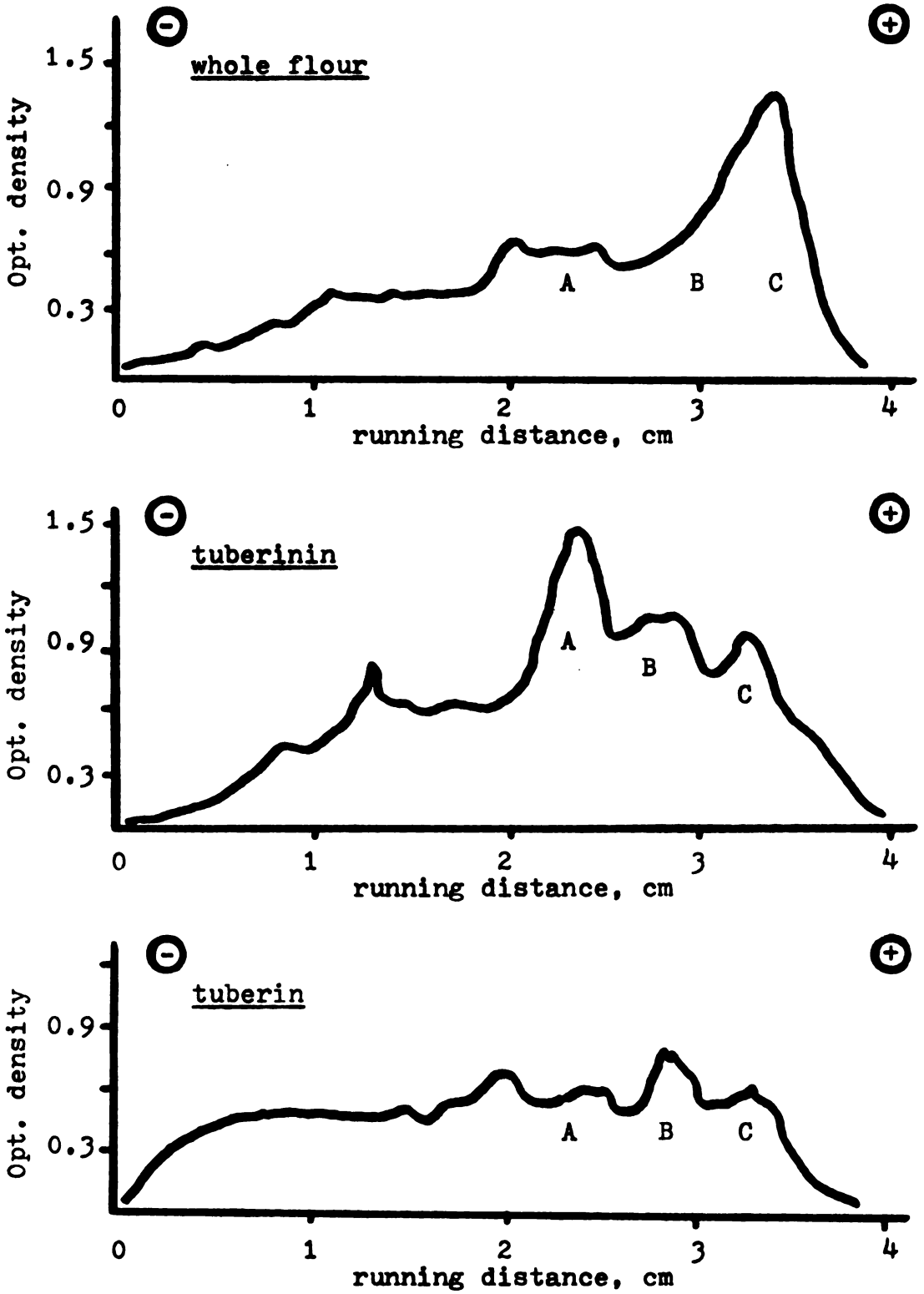
A,B,C : major protein bands

Figure 8 : Densitometrical readings of the gels of 322-6



A,B,C : major protein bands

Figure 9 : Densitometrical readings of the gels of 709



A,B,C : major protein bands

the sum of cystine and methionine was not greater than in tuberin.

The recordings of the densitometer of the gels are presented in the Figures 7, 8 and 9. The gels of the whole potato flour of 322-6 and 58 had 3 major bands (A, B, and C). In 709 band C was greatest, although the other two bands were present as can be seen from the tuberin fraction. Most of the bands in all three cultivars could be found in tuberin as well as in tuberinin. However their relative quantities seemed to differ. For instance band A was most abundant in the tuberinin of all three cultivars whereas bands B and C predominated in the whole flour and in the tuberin fractions.

Not only was the sulfur amino acid composition of tuberin, tuberinin and prolamin similar for the cultivars 58 and 322-6, but also the densitometrical recordings of their gels had some similarities.

A few major bands make up most of the proteins defined in the classical terms. The relative portions of these bands can vary from genotype to genotype. The fact that the same proteins of the three cultivars differed in methionine and cystine leads to the conclusion that the protein bands must vary in the amounts of sulfur amino acids.

In a breeding program for increased sulfur containing amino acids, production practices must be considered.

Depending upon the growing practices two different approaches have to be chosen. The deciding factor in this respect is the extent of applied nitrogen fertilizer. High

Table 12: Upper limits in sulfur amino acid contents of potatoes grown with low and high nitrogen fertilization+

	Growing conditions			
	No or only limited nitrogen fertilization		Intensive nitrogen fertilization	
	Tuberin	Nonprotein fraction	Tuberin	Nonprotein fraction
% N of total N	80	20	40	60
% met	3	1.0	3	2.5
% cys	1.5	-	1.5	-
% met of total protein		2.6		2.7
% cys of total protein		1.2		0.6
cys + met of total protein		3.8		3.3
% of the sulfur amino acids present in the whole egg protein		63%		55%

+ The methionine and cystine values are the highest values obtained from analysis of 700 segregating cultivars for "available" methionine.

rates of nitrogen cause an increase in the nonprotein nitrogen fraction at the expense of the protein fractions (Mulder and Bakema, 1956).

In areas where little or no nitrogen fertilizer is applied (e.g. in developing countries) a potato with a high tuberin content should be sought. The ratio of protein nitrogen / nonprotein nitrogen would be the selection criteria in this case. The composition in sulfur amino acids which could be expected is presented in Table 3.

In this country, another approach must be taken. Nitrogen fertilization of 100 to 200 kg per hectare is common. Thus it would be difficult to find potato clones in which total proteins would contain 80% tuberin. Thus the ideal potato to select should be high in free methionine and tuberin and should account for 40 to 50% of the total protein. The possible presence of free cysteine should be kept in mind. Here, methionine content would be the most important selection criteria but in a final evaluation cystine and free cysteine should be considered.

SUMMARY

From the dry matter of three cultivars, tuberin, tuberinin, globulin II, prolamin and glütelin were isolated. Of the total nitrogen, tuberin accounted for 29 to 35%, the nonprotein nitrogen fraction for 50 to 61%, the residue for

5 to 10% and all the other fractions for less than 2.5%. Tuberin, tuberinin and prolamin of the cultivars 58 and 322-6 contained similar amounts of methionine and cystine, whereas the sums of methionine and cystine of the corresponding proteins of 709 were considerably lower.

The electrophoretic separation showed that tuberin is composed of at least 3 major bands. The proportion of these 3 bands was dependent upon the genotype and could possibly be responsible for the differences in the content of the sulfur amino acids. Tuberinin contained primarily the same protein bands as tuberin, however, their relative portions were different.

Different section criteria applicable to breeding a potato high in the sulfur amino acids are discussed.

SUMMARY AND CONCLUSIONS

SUMMARY AND CONCLUSIONS

In this study the high heritability of the amino acid methionine was established. With this information it should be possible to breed a potato variety containing two or more mg met/16 mg N. Seventy g of such a potato protein would provide 1.4 g methionine or more than the daily minimal requirement of 1.1 g for a human adult.

Based on this study one hectare of land planted with 26,000 hills of the potato clone 7-34 would produce 11,200 kg of dry matter which would contain 1,900 kg of total protein. The methionine content of this clone was 1.6 mg/16 mg N. This example demonstrates the tremendous potential of the potato to produce large quantities of proteins as well as carbohydrates. Human nutritionists and food technologists should give more attention to the potato as a source of protein.

It is known that methionine is a precursor of flavor compounds. It would be very worthwhile to know whether the formation of these flavor compounds is proportional to the content of methionine in potatoes. This knowledge would help the potato breeder to direct his future research.

This study indicated that the mostly abundant protein, the tuberin, is composed of at least 3 separate proteins. It would be of practical interest to know whether these proteins contain different amounts of sulfur containing amino acids. This information would help the plant breeder to obtain the

highest overall content in sulfur amino acids.

From the data presented it can be inferred that:

1. "Available" methionine is highly heritable and is independent upon the level of total protein.
2. Total protein and specific gravity are moderately heritable.
3. No negative correlations could be observed between "available" methionine and rest period, fresh weight, specific gravity, total protein, chip color and total dry matter production.
4. Ninty-three percent of the variation in "available" methionine was due to variation in free methionine.
5. Tuberin is composed of at least three major protein bands. The relative presence of these bands seems to vary from genotype to genotype and could therefore cause some variation in the content of the sulfur amino acids.
6. Tuberinin contained almost the same bands as tuberin, but in different proportions.
7. The cultural practices dictate the selection criteria to be applied in order to breed a potato high in sulfur amino acids.

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APPENDIX

A. DATA OF 320 OFFSPRING REPRESENTING 8 CROSSES

Legend:

total protein	: mg (N x 6.25)/100mg dry matter
"available" methionine	: mg "av." met/16mg N
chip color	: according to the color chart of the National Potato Chip Institute 1 = very bright 10 = very dark
rest period	: 1 = rest p. broken in December 2 = rest p. broken in January 3 = rest p. broken in February 4 = rest p. broken in March 5 = rest p. broken in April 6 = rest p. broken in May
fresh weight	: total weight of one hill, in g
specific gravity	: (wt. in air)/(wt. in air-wt. in water
total dry matter	: calculated from specific garavity and fresh weight, in g

Cross #	Clone #	Total prot.	"av."methionine	Chip color	Rest p.	Fresh wt.	Spec. grav.	Total d.m.
1	2	13.67	1.77	5.5	1	420	1.077	84
1	5	9.48	1.40	3.5	1	970	1.072	187
1	6	10.82	1.40	3.5	3	1070	1.075	214
1	7	11.56	1.47	5.5	3	1065	1.081	226
1	8	8.70	0.90	5.5	1	1060	1.076	214
1	9	13.67	1.17	5.5	2	465	1.069	87
1	10	12.29	1.80	6.5	1	855	1.069	160
1	11	13.45	1.65	9.0	1	595	1.062	102
1	12	10.96	1.60	7.0	1	1115	1.070	211
1	14	12.14	1.45	1.5	1	1180	1.078	243
1	15	7.69	1.40	5.5	1	930	1.075	186

Cross #	Clone #	Total prot.	"av."methionine	Chip color	Rest p.	Fresh wt.	Spec. grav.	Total d.m.
1	16	11.23	0.95	5.5	3	645	1.066	116
1	17	9.42	1.37	6.0	1	380	1.070	72
1	18	12.19	1.47	5.0	2	1215	1.085	269
1	19	10.17	1.02	5.5	1	680	1.079	142
1	20	10.16	1.52	9.5	2	945	1.074	187
1	21	10.25	0.95	7.5	1	970	1.066	175
1	22	14.29	1.27	7.5	2	1180	1.063	205
1	23	11.20	1.25	9.5	2	395	1.082	85
1	24	9.68	1.35	7.5	3	350	1.077	71
1	25	8.91	1.10	5.5	1	1515	1.063	263
1	26	12.79	1.55	7.5	2	1370	1.083	297
1	29	14.75	1.60	7.5	2	805	1.073	157
1	30	12.33	1.75	5.0	1	600	1.081	128
1	31	13.42	1.35	5.5	2	460	1.082	99
1	32	10.70	1.80	4.0	2	1115	1.082	239
1	34	9.16	1.02	7.5	1	570	1.075	114
1	36	16.91	1.15	7.5	1	520	1.072	100
1	37	12.82	1.22	7.5	2	1315	1.069	245
1	38	14.79	1.90	5.0	1	1560	1.072	301
1	39	11.60	1.07	7.5	2	2140	1.070	404
1	40	10.76	0.90	5.0	3	640	1.076	129
1	41	8.85	1.27	7.5	2	465	1.081	99
1	42	10.01	1.30	7.5	2	805	1.073	157
1	43	9.94	1.40	7.5	2	545	1.079	113
1	44	11.56	1.15	7.5	3	350	1.077	71
1	46	10.16	1.30	7.5	2	1395	1.077	284
1	47	9.82	1.35	7.5	3	530	1.071	101
1	48	17.57	1.37	3.5	1	660	1.073	129
1	49	10.64	1.47	3.5	2	1520	1.086	339
2	26	14.23	1.52	1.5	2	690	1.087	156
2	31	12.51	1.15	4.5	1	835	1.084	183
2	32	12.38	1.37	3.5	3	790	1.082	170
2	36	11.81	1.57	5.5	2	1095	1.079	228
2	37	11.71	1.60	5.5	3	1660	1.078	342
2	38	15.53	1.42	5.5	2	1720	1.068	317
2	39	12.13	1.30	1.5	4	540	1.091	126
2	42	14.45	1.40	7.5	1	870	1.081	185
2	43	12.47	1.25	3.5	3	1365	1.079	284
2	50	12.32	1.42	5.5	3	640	1.076	129
2	51	11.31	1.13	5.5	3	565	1.076	114
2	53	12.48	1.30	1.5	1	630	1.086	141
2	59	13.84	1.30	3.5	1	530	1.093	126
2	60	10.18	1.30	3.5	1	1215	1.090	282
2	62	9.83	1.15	3.5	2	600	1.091	140
2	64	10.46	1.17	3.5	1	1260	1.082	270
2	65	15.52	1.45	9.5	2	620	1.069	116
2	71	12.73	1.52	7.5	1	1430	1.071	273
2	72	13.60	1.17	5.5	2	685	1.087	154
2	73	13.00	1.70	3.5	2	955	1.085	211

Cross #	Clone #	Total prot.	"av."methionine	Chip color	Rest p.	Fresh wt.	Spec. grav.	Total d.m.
2	78	14.01	1.50	1.5	1	1505	1.079	313
2	81	10.34	1.52	1.5	1	785	1.090	182
2	83	8.43	1.22	7.5	3	1360	1.084	298
2	85	13.17	1.42	9.5	1	1560	1.072	301
2	86	14.08	1.60	3.5	3	825	1.078	170
2	87	13.45	1.40	7.5	2	695	1.077	142
2	89	11.44	1.30	3.5	1	870	1.067	159
2	90	10.48	1.52	7.5	1	770	1.069	144
2	92	8.62	1.13	5.5	3	710	1.084	155
2	93	8.40	1.25	5.5	3	605	1.080	127
2	95	12.85	1.27	1.5	2	1095	1.079	228
2	97	15.00	1.40	3.5	1	935	1.075	187
2	98	9.42	1.45	9.5	1	775	1.054	120
2	104	13.25	1.50	5.5	2	500	1.075	100
2	105	17.31	1.40	1.5	2	630	1.068	116
2	109	13.77	1.20	3.5	1	1765	1.073	345
2	110	13.23	1.32	3.5	2	930	1.075	186
2	111	11.47	1.20	7.5	3	1340	1.072	259
2	112	12.97	1.40	3.5	2	585	1.073	114
2	113	12.25	1.35	1.5	2	415	1.078	86
3	1	11.85	1.75	2.0	2	1065	1.115	304
3	2	8.96	1.55	1.5	3	2330	1.079	485
3	3	13.51	1.70	2.5	5	1080	1.085	239
3	4	11.61	1.13	2.5	3	505	1.086	113
3	5	11.39	1.45	1.5	5	2180	1.082	468
3	6	16.59	1.65	3.5	4	505	1.074	100
3	7	9.33	1.20	4.5	4	785	1.083	170
3	8	11.94	1.77	4.5	3	410	1.093	98
3	9	11.54	1.75	6.5	4	685	1.079	143
3	10	9.23	1.60	6.0	5	810	1.087	183
3	11	9.16	1.45	1.5	3	950	1.092	224
3	14	9.78	1.75	5.5	1	1450	1.078	299
3	15	11.08	1.55	3.5	4	1180	1.083	256
3	18	14.38	1.80	2.5	3	1030	1.084	226
3	19	10.11	1.85	5.5	5	1065	1.081	226
3	21	13.67	1.50	9.5	1	650	1.092	154
3	22	11.59	1.92	7.5	2	1300	1.079	271
3	23	10.87	1.25	3.5	2	1475	1.073	288
3	24	10.77	1.85	3.5	4	520	1.094	125
3	25	13.88	1.72	5.5	3	695	1.094	167
3	26	10.55	1.65	1.5	2	1390	1.086	310
3	27	11.23	1.65	1.5	3	1660	1.085	367
3	28	10.99	1.65	9.5	3	1180	1.088	269
3	30	12.42	1.55	3.5	3	1450	1.082	311
3	31	13.13	1.65	1.5	1	870	1.087	196
3	32	9.72	1.42	3.5	3	1000	1.092	236
3	34	11.71	1.97	1.5	2	1250	1.087	282
3	36	10.96	1.77	3.5	4	475	1.092	112

Cross #	Clone #	Total prot.	"av."methionine	Chip color	Rest p.	Fresh wt.	Spec. grav.	Total d.m.
3	37	9.99	1.25	5.5	2	840	1.084	184
3	38	12.46	1.80	1.5	3	740	1.088	168
3	39	9.63	1.45	5.5	3	410	1.079	85
3	40	9.15	1.05	5.5	2	700	1.069	131
3	41	12.08	1.70	3.5	3	920	1.089	211
3	42	12.22	1.62	9.5	4	1220	1.080	257
3	43	9.04	1.55	7.5	4	965	1.084	211
3	45	9.38	1.35	5.5	3	1325	1.077	270
3	46	11.63	1.50	5.5	3	810	1.080	170
3	47	7.53	1.50	1.5	4	325	1.102	84
3	48	10.01	1.75	2.0	3	790	1.090	183
3	50	9.29	1.37	3.5	3	1005	1.080	211
4	1	13.82	1.65	3.5	3	1495	1.087	337
4	141	10.79	1.47	7.5	5	1230	1.079	256
4	142	12.31	1.05	5.5	2	235	1.093	56
4	145	10.31	1.15	3.5	3	1470	1.077	300
4	147	11.62	1.35	5.5	3	545	1.069	102
4	148	12.58	1.50	5.5	3	1030	1.084	226
4	149	13.45	1.75	3.5	4	440	1.073	86
4	154	11.81	1.55	7.5	3	1120	1.082	240
4	156	11.53	1.45	5.5	3	645	1.084	141
4	159	11.14	1.35	5.5	4	650	1.083	141
4	160	12.57	1.32	3.5	3	380	1.086	85
4	161	11.11	1.10	5.5	2	575	1.075	115
4	162	12.45	1.50	7.5	3	815	1.079	170
4	163	11.98	1.45	5.5	2	540	1.080	114
4	165	12.34	1.60	1.5	3	2340	1.083	507
4	166	11.36	1.45	5.5	2	930	1.081	198
4	168	12.11	1.47	5.5	2	430	1.075	86
4	170	11.84	1.17	9.5	1	635	1.067	116
4	180	13.80	1.55	3.5	2	570	1.096	140
4	181	7.50	1.45	9.5	4	830	1.071	158
4	189	10.37	1.17	3.5	2	2020	1.086	451
4	191	11.76	1.50	7.5	3	2330	1.071	445
4	194	14.20	1.42	5.5	5	690	1.087	156
4	197	15.17	1.82	1.5	5	1130	1.076	228
4	198	11.83	1.45	3.5	3	1615	1.080	340
4	297	11.77	1.55	1.5	4	590	1.092	139
4	314	12.46	1.45	5.5	3	910	1.077	186
4	331	11.48	1.40	5.5	2	825	1.078	170
4	333	13.44	1.60	5.5	1	795	1.082	171
4	341	12.26	1.35	1.5	3	780	1.076	157
4	345	11.01	1.45	5.5	2	785	1.083	170
4	349	10.61	1.45	3.5	3	960	1.091	225
4	357	8.04	1.25	9.5	4	910	1.070	172
4	365	11.25	1.55	3.5	5	770	1.077	157
4	367	12.02	1.60	5.5	3	970	1.078	200
4	370	12.45	1.57	9.5	2	740	1.065	132

Cross #	Clone #	Total prot.	"av."methionine	Chip color	Rest p.	Fresh wt.	Spec. grav.	Total d.m.
4	383	11.50	1.65	5.5	3	520	1.083	113
4	387	12.47	1.20	5.5	2	1000	1.081	213
4	390	13.86	1.20	3.5	3	1290	1.079	269
4	394	9.52	1.35	5.5	3	1180	1.078	243
5	1	13.49	1.75	3.5	3	1490	1.072	288
5	2	12.44	1.74	1.5	3	1675	1.067	305
5	3	13.13	1.46	5.5	2	945	1.080	199
5	5	13.62	1.67	9.5	3	1050	1.065	187
5	6	12.94	1.85	9.5	2	910	1.064	160
5	7	14.28	1.80	2.0	3	1150	1.070	217
5	8	15.78	1.70	3.5	2	1430	1.067	261
5	9	13.60	1.67	3.5	3	400	1.081	85
5	10	16.43	1.31	7.5	2	550	1.068	101
5	11	12.85	1.40	7.5	3	1355	1.067	247
5	12	12.36	1.88	1.5	2	900	1.071	172
5	13	12.95	1.46	5.5	2	930	1.075	186
5	14	10.53	1.97	9.5	4	1170	1.068	216
5	15	14.67	1.60	1.5	3	1015	1.074	200
5	16	8.58	1.49	9.5	2	1315	1.078	271
5	17	13.16	1.32	5.5	2	1010	1.074	199
5	18	13.86	1.63	1.5	4	850	1.076	171
5	19	11.34	1.75	5.5	2	1575	1.071	301
5	20	12.65	1.29	1.5	3	1655	1.071	316
5	21	11.16	1.67	9.5	2	2340	1.068	432
5	22	16.49	1.79	5.5	3	785	1.054	121
5	23	12.23	0.97	5.5	4	535	1.081	114
5	24	15.24	1.58	7.5	5	1050	1.060	176
5	25	10.63	1.41	9.5	3	770	1.069	144
5	26	11.59	1.35	1.5	4	385	1.085	85
5	27	10.90	1.44	1.5	4	520	1.083	113
5	28	9.77	1.22	7.5	5	1680	1.077	343
5	29	13.95	1.67	1.5	3	990	1.076	200
5	30	13.86	1.52	1.5	4	1475	1.073	288
5	31	11.85	1.90	7.5	3	1170	1.068	216
5	32	15.16	1.76	1.5	4	545	1.069	102
5	33	15.23	1.77	5.5	4	910	1.071	174
5	34	13.68	1.50	5.5	1	990	1.076	200
5	35	12.89	1.54	7.5	3	1500	1.071	286
5	36	12.74	1.64	1.5	1	845	1.076	170
5	37	12.78	1.71	9.5	5	1230	1.074	243
5	38	8.84	1.54	7.5	5	2195	1.071	419
5	39	12.26	1.71	7.5	3	1010	1.080	212
5	40	12.05	1.45	7.5	2	1255	1.073	245
6	1	11.05	1.42	9.5	3	1000	1.064	176
6	2	9.23	1.25	9.5	5	1320	1.065	235
6	3	11.69	1.35	3.5	5	1460	1.070	276
6	4	11.97	1.30	7.5	2	1145	1.070	216
6	5	14.59	1.25	5.5	3	1000	1.075	200

Cross #	Clone #	Total prot.	"av."me-thionine	Chip color	Rest p.	Fresh wt.	Spec. grav.	Total d.m.
6	6	9.00	1.09	7.5	3	915	1.070	173
6	7	14.56	1.43	1.5	4	590	1.073	115
6	8	18.53	1.25	3.5	3	1000	1.063	174
6	9	14.54	1.52	7.5	3	1165	1.064	205
6	10	12.39	1.15	7.5	5	1525	1.070	288
6	11	12.36	1.35	3.5	3	1320	1.069	246
6	12	13.97	1.35	7.5	3	2300	1.070	434
6	13	12.65	1.65	9.5	2	735	1.073	144
6	14	15.84	1.45	3.5	4	1400	1.065	249
6	15	12.90	1.25	7.5	3	830	1.071	158
6	16	11.29	1.30	7.5	2	2070	1.062	355
6	17	13.02	1.13	5.5	2	1340	1.068	247
6	18	15.92	1.40	3.5	3	920	1.070	174
6	19	11.86	1.36	7.5	1	2230	1.064	392
6	20	11.67	1.37	7.5	3	2120	1.071	405
6	21	16.79	1.40	7.5	3	560	1.067	102
6	22	8.57	1.36	9.5	3	1715	1.069	320
6	23	13.63	1.26	1.5	2	2010	1.069	375
6	24	14.72	1.28	5.5	3	1240	1.074	245
6	25	11.53	0.99	9.5	4	1145	1.070	216
6	26	12.11	1.25	9.5	3	560	1.077	114
6	27	11.56	1.49	5.5	4	850	1.069	159
6	28	16.31	1.09	7.5	3	535	1.059	88
6	29	11.71	1.29	9.5	3	1520	1.067	277
6	30	11.47	1.41	7.5	4	1970	1.071	376
6	31	11.01	1.35	7.5	5	780	1.076	157
6	32	12.77	1.42	7.5	5	1065	1.081	226
6	33	14.82	1.27	7.5	3	945	1.074	187
6	34	15.86	1.19	9.5	5	360	1.075	72
6	35	14.52	1.37	5.5	4	850	1.076	171
6	36	12.68	1.13	7.5	3	595	1.072	115
6	37	13.52	1.35	9.5	2	1145	1.065	204
6	38	12.98	1.50	3.5	3	940	1.080	198
6	39	12.34	1.47	7.5	5	990	1.070	187
6	40	11.32	1.37	3.5	3	735	1.089	169
7	1	11.26	1.67	7.5	3	780	1.076	157
7	2	12.17	1.36	7.5	3	810	1.052	121
7	3	13.40	1.63	5.5	5	1425	1.059	235
7	4	12.86	1.79	9.5	4	1020	1.062	175
7	5	11.56	1.29	7.5	3	1460	1.070	276
7	6	9.99	1.74	7.5	3	990	1.070	187
7	7	12.11	1.39	9.5	5	1800	1.071	344
7	8	11.06	1.65	9.5	4	1500	1.060	251
7	9	13.98	1.73	7.5	2	1095	1.063	190
7	10	10.52	1.51	3.5	3	1365	1.071	261
7	11	12.07	1.47	1.5	4	990	1.082	213
7	12	13.71	1.51	7.5	4	740	1.057	119
7	13	13.21	1.73	7.5	3	1010	1.068	186

Cross #	Clone #	Total prot.	"av."methionine	Chip color	Rest p.	Fresh wt.	Spec. grav.	Total d.m.
7	14	12.55	1.87	5.5	3	750	1.041	95
7	15	15.61	1.77	1.5	2	535	1.059	88
7	16	16.72	1.60	9.5	3	1435	1.067	262
7	17	16.31	1.68	5.5	4	1010	1.063	175
7	18	12.53	1.42	7.5	5	1890	1.068	349
7	19	14.56	1.69	7.5	3	915	1.064	161
7	20	11.93	1.64	9.5	3	660	1.056	105
7	21	12.31	1.61	5.5	4	1315	1.065	234
7	22	13.11	1.61	7.5	4	1435	1.067	262
7	23	13.26	1.40	1.5	2	670	1.081	142
7	24	11.54	1.58	7.5	2	1435	1.071	274
7	25	12.72	1.70	7.5	2	1040	1.061	176
7	26	13.76	1.59	3.5	3	710	1.076	143
7	27	11.57	1.53	7.5	5	1120	1.067	204
7	28	10.68	1.54	7.5	4	870	1.067	159
7	29	16.20	1.60	1.5	2	975	1.066	176
7	30	11.86	1.79	1.5	3	480	1.079	100
7	31	13.37	1.52	5.5	2	940	1.080	198
7	32	12.01	1.45	9.5	5	810	1.066	146
7	33	13.62	1.60	1.5	3	1065	1.070	201
7	34	17.59	1.60	5.5	1	2340	1.066	422
7	35	10.75	1.06	5.5	3	820	1.079	171
7	36	11.69	1.32	9.5	5	745	1.064	131
7	37	11.81	1.50	8.0	5	1315	1.065	234
7	38	10.23	1.70	1.5	6	1355	1.080	285
7	39	12.10	1.24	9.5	3	1080	1.048	153
7	40	12.27	1.55	1.5	2	1255	1.077	256
8	1	10.81	1.65	5.5	3	1970	1.071	376
8	2	12.05	1.46	7.5	4	890	1.066	160
8	3	12.14	1.34	5.5	3	1405	1.073	274
8	4	16.60	1.47	5.5	2	1340	1.059	221
8	5	13.78	1.28	3.5	3	1095	1.068	202
8	6	11.76	1.65	5.5	2	1900	1.064	334
8	7	11.02	1.84	8.0	3	615	1.069	115
8	8	12.63	1.72	4.5	5	1535	1.077	313
8	9	13.56	1.20	4.5	2	2010	1.066	362
8	10	13.41	1.27	2.5	3	900	1.078	185
8	11	11.81	1.51	4.5	5	1375	1.062	236
8	12	14.46	1.41	5.5	3	810	1.073	158
8	13	11.49	1.24	8.5	3	1000	1.064	176
8	14	12.52	1.36	6.5	6	1230	1.060	206
8	15	11.98	1.24	4.5	3	1455	1.062	250
8	16	11.29	1.52	5.0	3	1050	1.071	200
8	17	10.18	1.22	8.5	6	1640	1.055	257
8	18	12.96	1.11	9.5	2	1365	1.054	211
8	19	9.65	1.31	6.5	4	720	1.083	156
8	20	13.86	1.49	7.0	5	610	1.061	103

Cross #	Clone #	Total prot.	"av."me-thionine	Chip color	Rest p.	Fresh wt.	Spec. grav.	Total d.m.
8	21	13.12	1.29	5.5	5	1325	1.064	233
8	22	11.79	1.78	4.5	4	2195	1.066	395
8	23	13.83	1.37	5.5	3	1840	1.064	324
8	24	12.85	1.20	5.5	2	1810	1.071	346
8	25	11.14	1.35	5.5	3	1850	1.066	333
8	26	12.45	1.40	5.5	4	1500	1.064	264
8	27	13.57	1.35	5.0	0	895	1.065	159
8	28	11.77	1.46	3.0	2	1340	1.076	270
8	29	10.64	1.31	5.5	4	1240	1.069	231
8	30	12.13	1.27	5.5	4	715	1.075	143
8	31	9.68	1.44	5.5	5	600	1.081	128
8	32	11.77	1.35	5.5	3	820	1.072	158
8	33	10.13	1.61	5.5	5	2010	1.066	362
8	34	10.54	1.24	8.0	3	2340	1.059	386
8	35	7.77	1.50	5.5	3	1165	1.074	230
8	36	8.04	1.47	7.5	5	1165	1.069	217
8	37	13.22	1.43	4.5	4	1105	1.068	204
8	38	15.16	1.33	6.5	3	1845	1.060	309
8	39	11.69	1.71	6.5	3	1505	1.067	274
8	40	12.36	1.47	3.5	4	2240	1.074	442

B. DATA OF THE PARENTAL CULTIVARS

Cultivar #	Hill #	Sample of hill	Total prot.	"av." methionine	Specific gravity
320- 6	1	A	9.00	1.08	
320- 6	1	B	8.79	1.03	1.118
320- 6	2	A	9.67	1.09	
320- 6	2	B	10.41	1.10	1.124
320- 6	3	A	13.61	1.04	
320- 6	3	B	13.15	0.95	1.103
320- 6	6	A	10.35	1.02	
320- 6	6	B	10.00	1.13	1.099
320- 6	7	A	9.90	1.00	
320- 6	7	B	9.96	1.00	1.112
320- 6	8	A	9.94	1.05	
320- 6	8	B	9.41	0.98	1.117
320- 6	9	A	13.03	1.19	
320- 6	9	B	13.15	1.07	1.090
321-38	2	A	15.01	1.07	
321-38	2	B	14.56	1.07	1.071
321-38	3	A	13.55	1.08	
321-38	3	B	13.42	1.10	1.074
321-38	4	A	17.31	1.08	
321-38	4	B	17.47	1.05	1.068
321-38	5	A	15.52	1.08	
321-38	5	B	15.55	1,17	1.074
321-38	6	A	15.81	1.10	
321-38	6	B	17.89	1.09	1.071
321-38	7	A	16.64	1.12	
321-38	7	B	15.63	1.17	1.080
321-38	8	A	13.98	1.13	
321-38	8	B	14.46	1.14	1.094
321-70	1	A	12.17	1.22	
321-70	1	B	13.25	1.21	1.089
321-70	2	A	16.78	1.11	
321-70	2	B	13.74	1.10	1.094
321-70	3	A	12.56	1.14	
321-70	3	B	14.17	1.20	1.096
321-70	4	A	13.75	1.08	
321-70	4	B	16.86	1.14	1.111
321-70	5	A	17.98	1.28	
321-70	5	B	18.86	1.31	1.082

Cultivar #	Hill #	Sample of hill	Total prot.	"av." methionine	Specific gravity
321-70	6	A	12.90	1.10	
321-70	6	B	12.29	1.10	1.100
321-70	7	A	14.27	1.08	
321-70	7	B	16.18	1.07	1.104
709	1	A	13.43	1.38	
709	1	B	14.10	1.50	1.078
709	2	A	13.92	1.51	
709	2	B	15.46	1.38	1.079
709	3	A	14.00	1.54	
709	3	B	16.07	1.43	1.073
709	4	A	15.62	1.52	
709	4	B	15.80	1.55	1.073
709	5	A	15.19	1.40	
709	5	B	14.24	1.38	1.076
709	6	A	15.78	1.44	
709	6	B	15.70	1.40	1.076
709	7	A	15.10	1.43	
709	7	B	16.02	1.50	1.077
706-34	1	A	18.08	1.22	
706-34	1	B	15.09	1.27	1.068
706-34	2	A	12.74	1.17	
706-34	2	B	15.98	1.15	1.067
706-34	5	A	15.32	1.17	
706-34	5	B	14.76	1.16	1.062
706-34	7	A	12.33	1.05	
706-34	7	B	12.54	1.05	1.059
706-34	8	A	9.84	1.05	
706-34	8	B	12.07	1.05	1.060
706-34	9	A	17.70	1.25	
706-34	9	B	13.10	1.17	1.072
706-34	10	A	14.38	1.15	
706-34	10	B	18.42	1.23	1.064
735- 1	1	A	10.22	1.04	
735- 1	1	B	10.48	1.04	1.079
735- 1	2	A	10.58	1.14	
735- 1	2	B	10.59	1.12	1.082
735- 1	3	A	12.04	1.22	
735- 1	3	B	10.46	1.25	1.078
735- 1	4	A	10.41	1.07	
735- 1	4	B	9.10	1.08	1.065
735- 1	5	A	9.80	1.23	
735- 1	5	B	10.73	1.27	1.066
735- 1	6	A	11.08	1.13	
735- 1	6	B	10.29	1.09	1.067

Cultivar #	Hill #	Sample of hill	Total prot.	"av." methionine	Specific gravity
735- 1	7	A	11.67	1.04	
735- 1	7	B	11.17	1.00	1.071
711- 8	1	A	12.04	1.45	
711- 8	1	B	16.76	1.44	1.068
711- 8	2	A	15.95	1.52	
711- 8	2	B	15.49	1.49	1.065
711- 8	3	A	15.01	1.57	
711- 8	3	B	14.54	1.63	1.067
711- 8	4	A	17.42	1.49	
711- 8	4	B	15.97	1.44	1.070
711- 8	5	A	18.47	1.44	
711- 8	5	B	15.67	1.50	1.065
711- 8	6	A	15.21	1.54	
711- 8	6	B	14.88	1.45	1.069
711- 8	7	A	17.91	1.43	
711- 8	7	B	18.31	1.39	1.067
321-65[+]			9.00	1.45	1.111

[+] mean values from the 1969 crop of the same field