NEW APPROACHES TO BREEDING FOR IMPROVED PLANT PROTEIN

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FOREWORD

The Panel on New Approaches to Breeding for Plant Protein Improvement, held at Röstånga, Sweden, from 17 to 21 June 1968, was organized jointly by the International Atomic Energy Agency and the Food and Agriculture Organization of the United Nations. There is a pressing need for new and better sources of protein to feed the expanding world population, and interest in the problem is intense.

Various solutions to the problem of inadequate levels of protein and/or essential amino acid in the diet of the poor man in less developed countries have been proposed. These rely generally on supplementing the diet directly by fish or seed protein or by synthetic amino acids; such approaches, however, are complicated because they involve major capital investment, controlled food processing and distribution, and frequently change in dietary habits.

Another solution does exist, potentially far more feasible from an economic and social point of view: that of developing crop varieties having improved protein, by which is meant either higher protein content or higher content of certain essential amino acids (such as lysine), or both. These improvements may be achieved through plant breeding, since it has recently become clear that gene mutations can markedly raise the levels of the limiting amino acids in seed protein, as was first shown with maize. Providing such improved crop varieties for local growers has the advantage of (a) long-term return after initial investment for improvement, in contrast to the continuing costs of diet supplements, and (b) adaptability to present local cropping and eating habits, unlike new high protein food products and dietary additives. It is now essential, therefore, that these unique advantages of genetic improvement in cereal grains and legumes, which are dietary staples throughout the world, should be recognized and exploited and that special emphasis should be given to inducing such mutations.

The FAO and the IAEA, through the Joint FAO/IAEA Division of Atomic Energy in Food and Agriculture, have embarked on a program designed to increase the world protein supply by this method. This is particularly appropriate, since nuclear techniques can be used to unique advantage in solving problems associated with increase of protein quantity and quality by plant breeding. Some of these problems and the contribution of nuclear techniques to their solution may be cited:

(1) Selection in crop plants for high protein and/or high content of a specific amino acid has not been practised in the past and as a result existing variation within these characters may not be very wide. Induction of mutations, as by treatment with ionizing radiation, offers the possibility of expanding this natural variation. Such mutation plant breeding also has the potential of adding such a desirable character as high protein without loss of other desirable characteristics in a variety, as might occur through hybridization.

(2) The plant breeder has been handicapped in offering the quick results needed to close the 'protein gap'. The lack of rapid, relatively inexpensive screening techniques for protein and for specific essential amino acids such as lysine is the single most important factor. Until true mass screening for amino acids and protein becomes available to the plant breeder, identification and selection of plant materials for improved protein lines is a slow, expensive procedure. In several chemical, microbiological and physical methods the use of radioisotopes - either added or induced - permits considerable increase in rates of analysis, with resulting lower costs.

(3) Chemical assay for amino acid content in a seed protein is not a complete indication of the nutritional value of that food source. After mass screening for protein quality, limited screening for nutritional value is necessary. Here methods are needed which are more rapid and less expensive and give results that are valid for human beings, in order to assess the nutritional availability to the consumer of the seed protein and its constituents. Here use of isotopically labelled compounds and model systems may quickly approximate slow animal feeding tests.

The co-ordinated program, as established by the IAEA in October 1968, has three major phases. These are:

(1) Development, evaluation, and standardization of methods for the rapid mass screening of plant material, with emphasis on nuclear techniques. Present quantitative assay methods - chemical, enzymatic and microbiological - may be greatly speeded up by using radioisotopically tagged compounds which permit isotope dilution procedures and/or very rapid and simple assays based on scintillation counting techniques. In addition, physical assays based on neutron activation of protein nitrogen have been developed.

(2) Development, evaluation, and standardization of methods to determine nutritional availability of the essential amino acids in seeds, with emphasis on nuclear techniques. Present animal feeding tests, which are expensive and time-consuming, can be supplemented by relatively simple absorption tests based on radioisotopically tagged compounds in model systems. Further investigation into these simple indicative tests is needed, with correlation to the more elaborate feeding tests.

(3) Development of crops with improved protein through plant breeding. Here the use of ionizing radiation for induction of mutants is a recognized tool of value to the plant breeder. It should be pointed out that this phase may proceed with present screening techniques, but that better screening methodology, as it is developed, will be applied to plant breeding programs in progress.

A theme underlined by the Panel was that the problem of plant protein improvement requires the contributions of several scientific disciplines. Co-ordination of the efforts of geneticists and plant breeders, analytical chemists and biochemists, physicists and nutritionalists is called for. The Agency's co-ordinated program can facilitate such co-operation by (1) providing a mechanism for the rapid and direct exchange of ideas and results, (2) providing a means of critical analysis of current and projected research projects before large amounts of time and money have been expended, and (3) concentrating efforts to identify the significant problems and to design and co-ordinate experiments aimed at their solution.

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I. THE NEED FOR PROTEIN

THE WORLD'S PROTEIN SITUATION AND CROP IMPROVEMENT

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Abstract

THE WORLD'S PROTEIN SITUATION AND CROP IMPROVEMENT. Although calculations with statistical data available show quite clearly that on the basis of average protein requirements and supplies no protein gap exists, clinical indications in the health standards of populations and household surveys show that such a gap does in fact exist, both between rich and poor countries and also within countries. Income and protein supply are closely related and those who are also deficient in calorie intake, the poorest, not only get insufficient protein in their daily diet but probably also use it for energy conversion. A luxurious consumption of protein, particularly of animal protein, is confronted with an insufficient protein intake from pure cereal diets lacking in essential amino acids. Immediate and most efficient help to close this protein gap can be expected from increasing the protein content in cereals and, at the same time, improving the biological value of this protein by raising its level of the amino acid lysine. This would mean enough and better food for those in real need, without changing food habits, at a low price and without additional cost for transportation, propaganda, distribution, etc.

I feel honoured to have been asked to present an introductory paper to this Panel of outstanding experts. Before trying to speak briefly on the world's protein situation, I should like to ask your indulgence since I am not a nutrition specialist nor an expert in economy, and as a simple agronomist I may even wrongly interpret what I have grasped from all the publications in this field. Furthermore, my personal views may not always reflect the official opinion of FAO, which I would like to add, may well be modified as the Director-General, Mr. A.H. Boerma, has chosen as one of the four main priorities of FAO's activities how to solve what is regarded as a worldwide problem, namely how to fill the protein gap.

What is the size of this protein gap? This was naturally my first question. But neither FAO's 1964 edition of the SOFA Report (The State of Food and Agriculture), which deals in Chapter III with protein needs and prospects, nor the ECOSOC Report for 1967 (Report of the United Nation's Economic and Social Council) "Increasing the Production and Use of Edible Protein" gives any information on this aspect. The ECOSOC Report indicates: "The Advisory Committee has not tried to calculate the size of the protein gap. Various methods have been devised in attempting to do so. Although there may be wide differences in the resulting figures, they all indicate that such a gap exists, and the experts are unanimous in emphasizing that the gap is undeniable and increasing." The FAO Report states in its chapter on protein: "It is not easy, however, to assess the nature and extent of the world's protein problem, quite apart from proposing how it can be overcome."

The only concrete information I was able to find was in the May 1967 number of the International Division Service of the US Department of Agriculture, which reads: "... by 1970 the world deficit of all protein will be 10 million tons, about 12% of total world needs. Half of this shortage will be animal protein."

Being short of time and unable to confirm this figure from other publications, I have tried to make a very rough estimate of my own. For this calculation I used the FAO/WHO Report of the Joint Study Group 1965, 'Protein Requirements", which gives detailed data of protein requirements by age and stage of development in grams of reference protein per kilogram of body weight per day.

This reference protein had been introduced because the expert group felt that, since all of the amino acids are inter-related in protein synthesis and turnover in the body and in other metabolic processes and since there must be a sufficient supply of all of them to support these processes, the pattern of amino acids in a protein is a more important determinant of its nutritional quality than its content of essential amino acids, which had been stressed previously. Reference protein may be defined as one with an amino acid pattern that will allow complete utilization of the absorbed products for the synthesis and maintenance of body protein, which is their principle physiological function, and will support the other metabolic processes adequately. For working purposes the reference protein was considered to have an NPU (net protein utilization) value of 100.

Assuming a normal age distribution of world population, the world's needs will come with 35 g reference protein per day per capita for 3.4×10^9 to 120 000 tons per day, or about 44 million tons per year. Corrected for protein with an NPU of 65 the quality of a diversified daily diet and a 10% retail allowance, the requirement will be around 77 million tons of protein.

To get the supply figures I used statistical data provided by Dr. W. Schulte of FAO's Statistics Division on current levels of diet based on regional estimation of major food crops. The present supply amounts, thereafter, to about 83 million tons. The world's total production of protein can be regarded as 30-35% higher, exceeding 100 million tons.

From this very rough calculation it appears that from a statistical point of view no protein gap exists, and in fact the SOFA report which is based on much more detailed information, e.g. food balance sheets for 43 countries, comes to the following conclusion: "It appears that the available supplies exceed average requirements on a per capita basis in all of the countries for which there are data except Ceylon, although these requirements appear to be only just covered in a number of other countries including Colombia, Pakistan, Peru and the Philippines" - and later - "It is probable that even on a world basis the total available supplies of protein are enough to satisfy the calculated requirements of the whole population."

This statistical game with average per capita demands and supplies is in fact misleading! It is comparable to the famous example of a man living in average comfortable temperature conditions with one leg on a stove and the other in an ice box.

The protein gap exists, despite the average figures showing a balance - Kvashiokor, reduced growth rates, poor general physical and mental development are significant medical symptoms of inadequate protein intake to be found in many developing countries. But it is a gap between advanced and developing countries and between rich and poor people inside the countries, particularly marked in developing (low calorie) countries where the protein supply of the lowest income class is restricted to cereals only, often coupled with an insufficient calorie intake where protein is 'misused' as energy source. This insufficient protein intake by the poorest has been estimated by Dr. Sukhatme (Director, FAO Statistics Division) in "The World's Food Supply" (1964) to affect one quarter to one third of India's population.

The report of the President's Science Advisory Committee, Vol. III, "The World Food Problem", gives a number of examples from household surveys in Indian villages, e.g. in Vellore, Madras State, the lowest income group with less than $12.5 per year had 1260 calories per day per capita (65% of all Indian average) and 32 g protein per day per capita including 0.3 g animal protein; the highest income class, $50-75 per year, reached only 89% of the calorie requirements, but 93% of the protein requirements including 7.6 g of animal protein. Mr. A. Alschschule, in his paper "The Case for Sophistication" says: "25 million tons of protein come from animal sources. But these are available primarily to 500 million people out of the total world population." The relationship between per capita income and daily intake of total protein and animal protein all over the world shows that total protein intakes form a growth curve rising sharply up to a per capita income of $600-800 per year, but after that it flattens out, whilst intake of animal protein on the other hand shows a linear relationship with income over the whole range of incomes up to $2000.

This close relationship of income level and protein supply per person leads on the one hand to the gap between "luxurious and insufficient consumption" and, on the other hand, shows that with present trends any improvement through raising living standards will need many years. There is no hope of a redistribution, the gap will become even wider in the future. In the economically more developed countries there has been an increase of about 6% in protein intake since before the war, whereas in developing countries, where greater supplies of protein are most needed, it is estimated to have declined by about the same percentage. With the higher population growth rate in the developing regions this gap will soon reach a critical point. It is against this background that the Economic and Social Council of the United Nations made its recommendations to scientific and research workers, to governments and the United Nations agencies to increase the production and use of edible protein. And that is also our main reason for being here. I think that the task before us is challenging. Nevin S. Swerimshaw from Massachusetts, in his well-known report to the Protein Advisory Committee in October, 1966, says in his introduction: "Protein-rich foods are especially scarce and costly, and protein malnutrition is a major health problem. There is no prospect within the near future that the developing countries will be able to apply the scientific and technical practices of modern agriculture rapidly enough to solve their food problem. For this reason a solution to the world protein problem from conventional protein sources alone cannot be expected. Maximum effort to improve conventional agriculture in developing countries must be supplemented by the production and use of new and unconventional sources of protein of the type described in this report."

Is he right in this statement that there is no prospect in the near future of solving the problem by conventional means applying modern agricultural production methods?

No doubt new unconventional sources of protein have to be developed and have to contribute to solve the problem in a future where the increasing competition for available agricultural land between animal and man will become harder because of the increasing population density. Nowadays the meat consumption in many industrialized countries - by far exceeding the natural requirements - can only be met by direct imports of animal products or by importation of plant material from developing regions for fodder mixtures. The Federal Republic of Germany, for example, imported in 1965/66 660 000 tons of animal products and 7.2 million tons of fodder concentrates to meet its consumption demand of 3.9 million tons of meat.

Despite the promise of science in the development of unconventional sources of protein it will take some time before research and industry are able to put unconventional protein-rich food products on the market and until the consumer acceptance of these new foods is assured.

Meanwhile crops and livestocks will continue to be the major sources of protein for human consumption and it seems worthwhile to discuss in this Panel what we can do. Can we increase the protein content of grains by 1 or 2% or more, basing on a variability in protein content of barley from 8-19%? Can we raise the protein percentage of roots and tubers and other starchy crops? Can we improve the quality of the cereal protein by changing the amino acid pattern increasing the lysine content? Can we raise the tryptophane content in corn, that of threonine in rice and wheat? And how fast can we do all this? Let us remember that cereals, the staple food of the poorest, supply half the total of 80 million tons of protein available each year - an increase in the protein content of cereals from say 10 to 11% would already mean 5 million tons of protein more per year - a raise in the NPU from 60 to 70 would mean a gain of another 7-8 million tons.

Together with the recently begun agricultural revolution through the introduction of high-yielding cereal varieties in such countries as India, Pakistan and the Philippines, it could mean not only sufficient protein and calories for the poorest - the most needy in the world living on cereal diets - it would also mean raising health standards, vitality and the activity of millions of people to take their future in their own hands.

II. BREEDING FOR PROTEIN IMPROVEMENT

ACTIVITIES OF THE BREEDING WORK AT SVALÖF TO PRODUCE MORE AND BETTER PROTEIN

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Abstract

ACTIVITIES OF THE BREEDING WORK AT SVALÖF TO PRODUCE MORE AND BETTER PROTEIN. Swedish agricultural produce is characterized by a surplus of starch and a deficit in protein and therefore a great effort is being made to increase protein production. The growing interest in protein problems is also a consequence of the global need for more and better protein. Swedish agricultural research in the field of protein studies is therefore a result of both the national and the international situations. The Swedish Seed Association, Svalöf, has undertaken various measures in recent years to study the formation of protein in agricultural crops and to breed varieties with a higher protein content and better protein quality from the nutritional point of view. A survey is given of the actual protein problems that either have been or will be studied in the work at Svalöf.

INTRODUCTION

The vegetable output of Swedish agriculture is characterized by a surplus of starch and a deficit in protein. Therefore a great effort is being made at the present time to increase protein production. The growing interest in protein problems is a consequence of the global situation where there is a very great need for more and better protein. Current Swedish agricultural research in the field of protein studies is therefore a result of both the national and the international situations.

An increase in protein production and improvement in the protein quality is to a great extent a question of breeding. The Swedish Seed Association, Svalöf, has undertaken various measures in recent years to study the formation of protein in agricultural crops and to breed varieties with a higher protein content and better protein quality from the nutritional point of view.

The breeder is now much better prepared for this work. Modern biochemical research has given him excellent new tools to refashion our agricultural crops and make them still more useful as basic materials for food and feed. Current oil-crop breeding in Sweden and Canada gives a good example of this. Modern biochemical screening methods have made it possible to change the fatty acid composition in the rape seed to advantage.

For the agricultural crops grown in the Skåne province of Sweden we can calculate the following protein production per hectare:

Clover-grass mixture	800-900 kg
Oil crops (winter rape)	750-800 kg
Pulses	750 kg
Potato	600 kg
Cereals	400-500 kg

But one must also consider the protein quality when judging the value of this protein production. The figures may serve as a background to the following survey of actual protein studies at the Swedish Seed Association.

RESEARCH PROGRAMS

Problems of protein production, quantity and quality in cereal grains

Investigators: A. Hagberg and R. Olered

Studies on the protein in the grain kernel have been in progress for some time at the Chemical Department of the Swedish Seed Association, particularly from a technological point of view as a quality problem for bread grain. Baking tests have been the conventional way of testing protein quality in bread grain. These studies now have been combined with intensive research work on the enzyme systems, particularly the enzymes alpha- and beta-amylase, which break down starch. During recent years nutritional value has received topical interest and work has been carried out to improve the protein content and composition in all cereals. The nutritional value is an extremely important aspect in both bread and fodder grain. Extensive research has been carried out using feeding experiments with laboratory animals. This research work has led to a more detailed definition of the concept of nutritional value in fodder grain and resulted in the inventory work now going on in barley and oats in order to find the available biological variation in protein content for both quantity and quality within these cereals. A large variation has already been shown, ranging from about 8% protein units up to between 18 and 20% units in different barley varieties grown under the same conditions. Thus, it is possible to make a considerable increase in the protein production in our cereals. This is all the more important because cereal crops at present dominate Swedish agriculture. An increase in the protein content of 2-3% units in fodder grain should decrease the need for supplementary protein to a very great extent and thus limit protein imports.

The variation in occurrence of toxic phenols in rye

Investigator: A. Hagberg

Rye has a favourable amino acid composition but a bad reputation as an inferior fodder plant. This is particularly valid for malted ryes not approved for milling purposes. The occurrence of phenol compounds in rye is the cause of the negative effect in the fodder stuffs, as has been shown by Wieringa in the Netherlands. Corresponding substances also occur in wheat but in lower concentrations and are therefore less harmful. When fed with only malted rye the experimental animals did not grow at all and some died. This was because of the toxins contained. Research has now been started in the genetic variation in occurrence of the phenols in question in different rye populations and to find material with a low phenol content or no tendency to contain these toxins.

Leaf protein as a supplement in bread baking

Investigator: O. Hall

The protein laboratory at the Swedish Seed Association is running a program of studies on the technological effects of the enrichment of wheat and rye flour with protein concentrates of vegetable origin (leaf protein concentrate, protein precipitations from mill waste, rape meal, pea meal, field bean meal etc.). The program has been set up with a view to studying the ancillary problems in the developing countries as well as considering Swedish domestic agriculture.

The nutritio-physiological effect of protein supplements in different baking procedures

Investigators: L. Munck et al.

This work has the aim of studying different protein supplements and how they continue to exist in different types of bread from a nutritio-physiological point of view. The present research work started three years ago. Doughs and bread have been analysed for amino acid and carbohydrate composition and for denaturation effects on proteins. Feeding trials with mice were also done. Sugar has proved to have a very bad effect on the nutritional value of the protein. Damage to the protein can be reduced to a minimum if the method of baking thin cakes for short periods is used.

Production of protein concentrate from dicotyledonous plant seed

Investigators: L. Munck et al.

The work, carried out in co-operation with the Biochemical Institute of the University of Lund, includes the following main topics:

(1) Studies of three leguminous crops Vicia faba, Soya hispida and Cicer arietinum. Comprehensive studies of the seed development in Vicia faba, its chemical composition and toxic substances;

(2) Storage of seed from these three crops under varying conditions;

(3) Extraction of the protein, to include study of different factors for optimum extraction. Appraisal of the nutritional value of the protein.

Pulses: protein content and quality

Investigator: S. Bingefors

In the work with pulses at the Swedish Seed Association the questions associated with protein have received considerable interest for some time. The first task was to investigate variation in protein content in the available material and thereby to discover possible differences between varieties. A survey was published in the Journal of the Swedish Seed Association in 1958 showing the results of the analyses over a long period of years. It was demonstrated that vetches had a significantly higher content of crude protein than peas and field beans. Field beans had a higher protein content than peas, but the difference was not significant. By their greater productivity the field beans, however, gave the highest crude protein yield per unit area; the peas gave also higher crude protein yield per unit than vetches. The variety variations were proportionately small.

Even if there are possibilities of raising the protein content in pulses - especially in peas - any variation in protein composition is of still greater importance. The protein in pulses does not contain very much of the essential amino acid methionine. The Division of Pulse Crops of the Swedish Seed Association has rather extensive material covering a great number of varieties and amino acid investigations are planned both for these varieties and for more topical breeding material. Some of the material has been analysed in co-operation with Professor W. Schuphan at the Bundesanstalt für Qualitätsforschung pflanzlicher Erzeugnisse of the Federal Republic of Germany. Important differences in methionine content in some pea varieties have been found in the preliminary analyses carried out so far. This fact makes it attractive to continue the investigations, possibly with a view to starting a plant breeding program to improve protein quality.

Toxic compounds in oil seed meal (including vegetative parts of Brassica plants)

Investigator: E. Josefsson

Preliminary work with glucosinolates in oil seed meal were started at the Swedish Seed Association by L.-Å. Appelqvist in 1961. Since 1 January 1964 E. Josefsson has been working on these problems in close co-operation with the Division of Oil Plants at the Seed Association and also in co-operation with Svenska Extraktionsföreningen, Karlshamn.

The main purpose of the investigations is to obtain varieties with low glucosinolate content through plant breeding. To reach this goal rapid and sensitive methods of analysis and plant material containing genetic variation in the content of the substances in question are required. One method adapted for Brassica plants and Crambe and another suitable for white mustard are the results of the work. Some studies have also been carried out to judge the influence of environment on the glucosinolate content. This content has been determined in a large number of varieties and single plants. Interesting and promising results have been obtained, particularly during the last year. Among other things we have noted that a Polish spring rape variety contains only 5-10% of the average glucosinolate content in rape and that this low content is genetically based.

Besides investigations of seed material, analyses of the glucosinolate content of the vegetative parts of kale, rape and turnip rape were started four years ago, since marrow stem kale and fodder rape give high protein yields and may be possible sources of protein for human consumption. Qualitative determinations of glucosinolates have been done in both the seed and vegetative plant parts in some material.