PROCEEDINGS OF THE NUTRITION SOCIETY

The Three Hundred and Sixty-second Scientific Meeting was held in the Medical and Biological Sciences Building, University of Southampton, on 16/17 July 1981

SYMPOSIUM ON
'BREEDING LEGUMES FOR NUTRITIONAL VALUE'

The composition and nutritional value of legumes in relationship to crop improvement by breeding

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The aim of this paper is not to review the composition of legume seeds over-all but to concentrate on their nutritionally important components in relationship to crop improvement by plant breeding. Legume seeds are primarily important for their supply of protein in diets in many parts of the world. They are also important sources of energy and contain both oil and carbohydrate. In developed countries grain legumes may be primarily used as animal feed or as a source of oil. More recently they have been utilized to a small extent for the production of protein concentrates, e.g. textured plant protein for human consumption. Grain legume seeds contain little retinol, riboflavin and ascorbic acid, but have reasonable quantities of thiamin and nicotinic acid as well as the nutritionally important minerals, calcium and iron. It is the relative proportions of these constituents, especially protein, and the composition of individual constituents which largely determine the nutritional quality of the seed, e.g. a large content of both protein and oil is desirable, although these are usually negatively correlated. However, legume seeds contain a variety of anti-metabolites (Liener, 1969). Some, such as phytate, interfere with mineral availability and are involved in undesirable processes such as those leading to hard cook; some, such as tannins, may affect digestion processes and others, such as saponins, lead to physiological disturbances. Still others are heat labile, such as lectins, which interfere with the protective barrier of the intestinal membranes or enzyme inhibitors which disrupt the digestive process and may lead to other undesirable physiological reactions. Components, such as protein, which are used as nutrients, occur in relatively large amounts as compared to anti-metabolites. Throughout the agricultural period, man has consciously or unconsciously selected for improved nutritional quality with regard to these different components.

The nutritional importance of a seed constituent must be related to the purpose for which the seed meal is being used, i.e. animal feed, human food, etc. and also to the other feed components or to the diet. Thus a deficiency in the sulphur amino

acid content of legume seeds, in a diet which has sufficient of these amino acids supplied from other sources, is relatively unimportant.

Generally speaking, legumes are used as high-protein crops that play a secondary role to cereal or root crops which are higher yielding and supply much of the energy requirement in a diet. Legumes have the advantage of fixing nitrogen and so often lead to soil improvement, and in developed countries they play an important role as a break crop. Soya bean and peanuts are often grown as a cash crop for oil or, in the former case, used as animal feed and it is important to distinguish whether their ultimate role is direct human consumption or animal feed. Many legumes, of course, are important forage crops but this aspect was not to be dealt with in this paper.

Before embarking on a breeding programme for crop improvement, the plant breeder wishes to know (1) the importance of a character for improvement; (2) does sufficient variation for this character exist in the breeding materials available to him; (3) are there suitable screening methods?—these must be fast, simple, inexpensive and sensitive; (4) lastly, it is a help if something is known about the inheritance of the character.

In considering grain legumes as a source of food for humans, I focus on their high protein content. The primary objective in that respect is to obtain the maximum stable protein yield per area of ground. For some geographic areas there is also a need to improve the sulphur amino acid composition of the legume protein since cysteine and methionine are the first limiting amino acids nutritionally, i.e. areas where a large part of the protein of the diet is obtained from a legume, the diet over all, may be first limiting in sulphur amino acids especially for young children, nursing mothers, the sick etc.

Legumes often contain anti-metabolites and even toxic substances. This problem, except in specific instances, is of less significance because, either the varieties commonly consumed have been selected to contain relatively small amounts of these substances, or, the food is cooked or processed so as to eliminate or inactivate them (this doesn't apply if used as an animal feed). Furthermore, there is an increasing body of evidence that some of these substances play a protective role (Gatehouse *et al.* 1979), e.g. in insect or fungal resistance, and if this is the case their removal could cause problems in the field.

Sufficient variation exists for the main components in legume seeds since oil concentrations vary from 1 to more than 40%, carbohydrate from 2–3 to 60% and protein from about 15 to 50% of the dry weight. Manipulation of the proportion of these components, however, may not be accomplished easily since the different combinations of the major components are each associated with a complex biochemical machinery about which we know very little, e.g. what is the biological regulation whereby soya beans are oil seeds while broad beans mainly store starch. With respect to individual classes of constituents, relatively little variation in the sulphur amino acid content of different legume varieties has so far been reported, but considerable variation exists in the content of anti-metabolites. For example, there are *Phaseolus* varieties which contain no lectin, whereas others have

relatively large amounts and a similar situation exists for most of the other anti-metabolites.

Protein yield

Protein yield per area of ground can be increased either by increasing over-all yield whilst at the same time keeping the percentage protein content of the grain approximately constant, or secondly, by increasing the percentage of protein in the grain, keeping over-all yield approximately constant.

It is generally conceded that legume yields are well below optimum levels, especially in comparison with cereals, due to (1) lack of breeding effort so far (soya beans are the exception); (2) lack of basic understanding of the underlying physiological constraints; (3) low pest and disease resistance of many semi-tropical and tropical legumin varieties in cultivation.

The best strategy therefore for improving protein supplies lies in stabilizing and increasing over-all grain yields. Breeders such as Jain (1971) have suggested that this is best accomplished by the development of a new legume ideotype. Chapman *et al.* (1979) and Gates (1981) have also suggested that flower and pod abortion could be reduced by the breeding of improved floral architectures.

Attempts to increase the percentage protein content may be more difficult to bring about. Penning de Vries *et al.* (1974) have pointed out the probable thermodynamic yield penalty involved and Sinclair & de Wit (1975) have suggested that the drain of materials needed to sustain the high protein content may have disruptive effects in terms of leaf and nodule senescence with consequent yield lowering. Although one can question some of the assumptions which inevitably have had to be made in developing this analysis, the second law of thermodynamics tells us that a high protein content is expensive in terms of materials and energy. In practice, however, breeders have reported either none or a weak negative correlation between protein yield and over-all yield in legumes (e.g. see Griffiths & Lawes, 1978; Bond, 1977), indicating that both could be improved simultaneously. Furthermore, improving over-all yield would allow greater leeway for subsequent improvement of protein content, i.e. the two problems are inter-connected. Increasing and stabilizing over-all yield is still, in my opinion, the first priority and for its achievement will require new understanding of the underlying basic developmental physiology of flower and pod set.

Improving protein composition

A negative correlation has been found between grain protein content and the percentage of the limiting sulphur amino acids, methionine and cysteine, per unit of protein, in *Vicia faba* (Hanelt *et al.* 1978). This result can be safely generalized to other legumes. Energy calculations based on the approach of Penning de Vries *et al.* (1974) have also been applied to legume seed protein composition improvement (Boulter & Gatehouse, 1978) and these show that the yield penalty would be relatively mild in this case.

Is there sufficient available variation to increase the sulphur amino acid content

of legume proteins? This question could be answered either by conducting a survey (at present there is insufficient information available), or be answered theoretically. Most of the sulphur amino acids in the legume seeds are in protein and the levels of free sulphur amino acids are small. However, since legume seeds lose very little sulphur amino acids in normal cooking procedures, approaches aimed at increasing the free sulphur amino acid content would appear a possibility; selection in tissue culture using sulphur amino acid analogues to isolate mutants in which the biosynthetic, sulphur amino acid pathway enzymes were not subject to feedback inhibition, followed by re-generation of whole plants which retained these characteristics is one possibility. Alternatively, one could attempt to increase the sulphur amino acid composition of the seed protein itself. This strategy depends upon (1) the presence of high sulphur amino acid containing proteins in legume seeds and (2) whether lines could be developed in which these proteins were produced in large amounts.

Thus, many of the albumin proteins have relatively high sulphur amino acid contents, but most so-called house-keeping enzymes may not be capable of being 'de-regulated' so as to be produced in large quantities. Strategies which involve increasing this class of proteins as a whole, however, might be important, e.g. increasing the relative proportion of embryo axis to endosperm since the proportion of albumin protein to total protein is higher in the axis. The dominant position of the seed storage proteins in the protein profile has led Boulter (1977) to suggest that this might be a possible route for legume improvement. Thus different storage proteins differ in their sulphur amino acid content (Gillespie & Blagrove, 1974) and a single storage protein can provide the bulk of a sulphur amino acid in a seed, e.g. legumin in *Vicia faba* supplies 60% of the seed methionine (Boulter, unpublished results). Legumin type proteins contain a higher percentage of sulphur amino acids than the vicilin type 7S proteins and since both types normally occur in different legumes, some improvement could be made in legumes with a relatively low legumin:vicilin value by changing this in favour of legumin. However, calculations indicate that although this would give some improvement, it would not raise sulphur amino acid levels to the FAO standard (Boulter, 1980). Another approach along these lines has been advocated by Palacco (1977). Urease, which can occur in very high levels in some legumes and is generally distributed in others in small amounts, contains relatively more sulphur amino acids than the average composition of the seed protein (Bailey & Boulter, 1971). Palacco has suggested several ways whereby over-producers of urease could be selected in legume cell cultures. In some cases this approach may not be satisfactory since increased sulphur amino acid content due to increase in a particular protein will only be nutritionally acceptable if the sulphur amino acids of that protein are freely available in the human digestive tract. Thus the trypsin inhibitor of many legumes is a high sulphur amino acid containing protein which in the case of the *Phaseolus vulgaris* enzyme is not readily digested in the rat gut (Bradbeer and Boulter, unpublished results), but see the results of Abbey *et al.* (1979) for *Vicia faba* proteinase inhibitors.

Screening

Suitable methods for screening for protein content are dye binding coupled with automated N determination or by using IR reflectance. Fast screening methods also exist for the sulphur amino acids, e.g. IR reflectance. In screening for sulphur amino acids both methionine and cysteine must be taken into account since the latter can spare the former. Total sulphur analysis, with prior removal of aqueous alcohol soluble sulphur components is often satisfactory (Evans & Boulter, 1974). This must be followed by a secondary protein screen, to ensure, for example, that the high sulphur content is not due to an increased trypsin inhibitor content.

Investigations in this laboratory using *Pisum, Vicia* and *Phaseolus vulgaris* meals have shown that the major storage proteins from these legumes are highly digestible in rat assays. Tannins had a very small deleterious effect with the legume seed varieties which we have investigated over the years (Phillips *et al.* 1981). Biological value (BV) and net protein utilization (NPU) in legumes are limited principally by their sulphur amino acid contents.

With regard to the several other anti-metabolites present in different legumes I do not intend to refer to these in detail as usually the cooking and processing procedures minimize their interference, although I realize that they may assume great importance in some diets, e.g. lathyrism in India (Misra *et al.* 1980).

In closing, however, I would like to refer to the phenomenon of hard cook. Storage at high temperature and high relative humidity decreases the cooking rate of black beans (*Phaseolus vulgaris*). This phenomenon is due to leakage of Ca and Mg from the cell, caused by membrane deterioration and phytin breakdown, forming cation bridges within the pectin of the middle lamella, decreasing its solubility and therefore the rate of cotyledon cell separation (Jones and Boulter, unpublished results).

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Printed in Great Britain