Interfaces between Agriculture, Nutrition, and Food Science. Workshop. 10-12.11.81

The Improvement of nutritional quality by genetic means

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Plant breeding supplies the methodology for improving crop plants. It has been likened to the process of building, although in fact it is more like replacing the coloured marble pieces in an existing mosaic by pieces of another colour, ultimately changing the pattern substantially.

In general, the plant characters can only be changed one at a time: so an order of priority is required. The second requirement is that the character under improvement should be readily identifiable by the breeder. Crop improvement is a "numbers game", in which the plant with the desired combination of characteristics may be quite rare. The breeder may have to sort through a large number of plants in order to identify the very few possessing the character which he needs. If such a character is evident, a careful look at each plant will suffice to choose those desired. However, few quality characters can be seen, and they must therefore be identified by other means.

Sometimes, one is lucky, and a close linkage or high correlation with an evident character is found: for example, kernal weight or kernal volume may be highly correlated with oil content. The breeder picks out the plants with larger grains, which greatly reduces the numbers he must handle. The oil-content of these is then found by analysis, and the plants having the highest values are selected, and grown out in the next season in head-rows, preferably replicated.

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An important concept of Nutrition is that of balance. The various nutrients are required by the body in balanced quantities. The obvious way of achieving this is thoough a balanced diet. However, the poorer sections of the human race are often unable to produce or to afford to purchase a balanced diet, so we have to do the best we can with the foodstuffs available to them, improving them if possible.

A sample from the whole row is taken after harvest, and analysed. There is more grain to work with, and a better analysis can usually be made of the average oil-content in the row. Growing the head-row in two replications, both of which would be analysed, helps to indicate the influence of soil conditions on oil content, and permits a better judgement on whether the high oil values selected are probably due to genetic rather than environmental effects.

Often with nutritional characters, differences are not visible and there are no good correlations with evident plant characters. Then the whole screening process requires an analytical technique, with a methodology which can handle small quantities of grain (grain is available only from single plants, and most is required for seed purposes) in large numbers. Generally, nutritional factors are difficult for the breeder to work with, both because an analytical procedure is needed, and because rapid analytical techniques for small quantities of grain are seldom available. Modern technology is steadily removing the latter difficulty.

Priorities

The breeder's two priorities are yield and evident quality. Both are complex: yield involves a series of positive genetically controlled factors which enhance grain production, which need to be accompanied by a series of resistances to the yield reducers, such as pests, diseases, drought, as well as a general tolerance of difficult soil and weather conditions.

Evident quality includes grain type, hardness, colour, size, texture and flavour. All are likely to be controlled by several to many small genes each, and so requiring selection in large populations. Even evident quality characters are themselves quite difficult to handle. Consumer panels for tasting and cooking evaluations are needed. Further, any breeding for nutritional improvement must not result in an adverse effect on the yield, or on the evident quality, unless it brings with it an obvious advantage to the farmer, a trade-off. He is not going to accept lower yields unless a premium price is paid for the nutritionally superior type: nor will he want to change the evident food quality if he uses it in his own home. He would only grow it for sale.

Clearly, breeding for nutritional quality is fraught with difficulties. The plant breeder must work with a (chemical) analytical technology: the new cultivar developed must be at least as good in yield and at least as acceptable in grain appearance and food preparation as existing commercial types, or else the improved food value must be sufficient to justify premium payments: the "improved" cultivar has got to be economically slightly more rewarding to the grower than are existing types. This approach to nutritional improvement has therefore not been widely used for nutritional defects which can be easily corrected by additions to the diet.

Generally, Plant breeding has been important in three areas: (i) evident characters associated with nutritional improvement (ii) inhibitors and toxic factors (iii) Protein quality and quantity.

1) Evident characters. In the tropical crops, colour is often important. Sometimes it may be associated with tannin-like substances which help to confer some level of disease or pest resistance. Black beans are an illustration of this. Most people prefer the white ones, once farming standards no longer require the benefits associated with the colour ~~confers~~.

Sorghum provides a good example of evident nutritional quality. Sorghum grains which have a persistent sub-coat, the testa, are high in polyphenols. Often the grains are brown in colour and as the intensity of this pigment increases the polyphenol content also increases. Not only are the polyphenols bitter in taste, they also have adverse nutritional effects. Jambunathan & Mertz (1973) showed in rat-feeding trials that a low polyphenol sorghum gave an average gain per rat of 34.8 g. in 28 days, whereas a high polyphenol grain showed an actual average weight loss of 0.6 g. per rat over the same period. Oswalt (1973) demonstrated that sorghums with dark-coloured testas had a lower IVDMD than those lacking the dark testa. The mechanism of this nutritional inhibition is well known to my audience, as indeed it is to the African farmers who use these sorghums, and who have devised various methods, often involving soaking or germinating in wood-ash or lime, for ~~of~~ reducing the tannin-content of the grain ~~in~~ during food preparation.

The plant-breeding task of selecting for white grain types is simple, and is also well known to African farmers. However, the birds eat the white types, so the people have the choice where the birds

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are bad of no sorghum grain, or a bitter grain which the birds will not eat and which needs processing before the people can eat it themselves. The processes of beer-making greatly reduce tannin contents, and African beers are often important in the diet.

I used to work in the Lake region of Tanzania. The small weaver birds, quelea, nest in Central Tanzania, and their numbers reach many millions when the young fly. All the sorghum cultivars grown on the east side of the Shinyanga district, adjoining the Wembere steppe where the birds nest, have dark grains with high polyphenol content. Those in Western Shinyanga district, away from the usual flight path and feeding grounds of the quelea, have sweet, corneous, white, high quality grains. These are parts of the same district, most of the people living there are Wasukuma, there is free interchange between the East and the West. Somebody has got to get rid of the birds before the plant breeders can usefully get rid of the polyphenols.

There is one place where plant breeders can help: some of the high-tannin sorghums are very bitter and unpalatable to birds when the grain is in the milk stage, but the tannin level is much reduced by the time the grain has ripened. Provided that the birds are not too numerous at harvest time, this grain reduces the food processing problems. The cultivar "Serena" released from Serere in Uganda and quite widely grown in the brown-grain areas of East Africa, is a normal high tannin sorghum. A line from the 1965 cross 5D x 135 at Serere has now completed its field testing. Although only equal in performance to Serena for grain yield, the grain has this character of the tannin content falling markedly, as judged by taste, during the ripening of the seed. It is now being multiplied and released from Serere, for Uganda, & also in Kenya. It will be interesting to see to what extent it replaces "Serena".

Quinua (Chenopodium quinoa) is an important grain crop in South America, especially in the Altiplano of Bolivia and Peru. The situation there is exactly parallel to that of sorghum. The grains often contain saponins, and a breeding programme was started to get rid of those. However, it was apparent to me when I visited our project there eighteen months ago that the saponins are in the grains in the areas where birds are bad, and there are good quality grains without saponins grown in other areas. (The saponins are removed by soaking bagged grain in the rivers.)

Another example of grain colour is of course yellow maize, in which the carotene can be a useful source of vitamin A. Not everyone likes a yellow maize, and other sources of vitamin A can easily be found in a mixed diet. The carotene levels known in sorghum at present do not warrant a serious breeding effort to include carotene in the breeding programme.

Another class of nutritional problems, the toxic substances, are very important. The haemolytic anaemia "favism" from eating Vicia faba beans is mentioned in every text-book. Another important one in the sub-tropics and tropics is lathyrin. Lathyrus, the grass-pea, is a most useful crop plant. It grows in rough conditions better than any other pulse I know. The farmer can just broadcast it into his rice field among the stubble immediately after the rice harvest, no land preparation, no weeding. It comes up, grows, and gives a yield under almost any conditions. It can be grazed or cut, and still regenerate to give a yield of pulse. I do not know of any legume which can do the job for the poor farmer which Lathyrus can do, even half as well. It is true that the lathyrin can be destroyed by soaking and boiling the grain: but when the pressure is on the food supply, some of it gets ground up for use in bread, and then the neurotoxin begins to have its horrible effect. It is essential to get rid of the lathyrin content by breeding, and much progress has already been made here in India in achieving this, the cultivar PUSA 24 being a low lathyrin line (Laxman Singh, 1975). In Bangladesh, a pulse project supported by IDRC has taken up work on Lathyrus. An excellent range of germplasm has already been collected, including some unusually productive types, and a research worker has been here in Hyderabad for training in the techniques of lathyrin assessment. I hope Dr. Kaul will find time to say a word about this.

Again, in the Altiplano of S. America, lupins are a beautiful source of oil - the fields in flower are a wonderful sight - but the presence of the alkaloid lupinin requires long periods of treatment in water to prepare it as a cooking oil, and work on breeding low-lupinin lines is in hand.

Cyanogenic glycosides in sorghum furnish an example of the improvement of nutritional quality by breeding. Dhurrin is not found in ungerminated sorghum seeds, but sprouted sorghum liberates it after two days of germination, and it may be present in substantial quantities in young shoots one week after emergence from the soil. Young side

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branches, young tillers, droughted and freshly ratooned plants are a dangerous source of poisoning to cattle. Much progress has been made in the USA in breeding for forage sorghums and Sudan grasses of very low dhurrin content. Rancher was regarded as particularly good (Franzke 1945) but Piper is today the most widely used of the low HCN cultivars (Hein, 1957).

The main target for nutritional improvement by breeding has been the protein quantity and quality in cereals. This was given an impetus by the concern over protein deficiency in the Developing World, before it was recognised that the basic cause of protein deficiency in most situations is food deficiency, people are not getting enough food to eat.

One attempt to develop a cereal with a higher protein content which may prove to be successful is triticale, from the cross between wheat and rye. There was much fantasy in the early days, when remarkable protein contents and qualities were quoted. Little attention was being paid to the fact that low grain yields are usually associated with higher protein contents, while the amino-acid profile of grains with a poorly developed endosperm is often of good quality. However, now that productive types are coming into the field, having plump grains, it does look as though protein content may be some one or two percent more than in wheat, with protein quality at least as good. (Hulse and Laing, 1974). Triticale outyields wheat on acid soils in the tropics and sub-tropics in the highlands of East Africa and in South America.

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The prospects of protein quality and quantity improvement in wheat itself are promising. There is a good range of variation in both protein content and lysine content. On a dry weight basis, in a large sample, mean lysine values increased from 0.33 percent to 0.63 percent as the grain protein increased from 10 percent to 20 percent. There was a strong positive relationship between protein content and lysine (Mattern et al 1975).

Rice grain protein has a good amino-acid profile, with a high glutelin fraction. Milled grain usually contains some seven percent of protein. Semi-dwarf breeding lines have been developed at IRRI which give yields comparable to those of IR-8, but with two percent more protein. The higher protein rice is nutritionally superior because of a higher level of all essential amino-acids, including lysine, in the milled rice (Juliano & Beachell, 1975). Among the best known quality breeding programmes is that using the Opaque-2 gene in maize. A considerable amount of work has led thus far to the conclusion that opaque-2 maize derivatives have grains that are too soft to keep well in many areas, while yields are around ten percent below those of the normal type. The former problem has been partly solved by the development of types with vitreous kernals, while the latter defect probably cannot be solved with the opaque-2 gene, as the kernals of the high lysine types are suffering from arrested development. Normal maize has around 2.5-2.8 g. lysine per 100g protein, while the opaque-2 types may have around 4.0 g. It is hard to increase total protein

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content usefully above 10 percent, as the additional protein consists mainly of zein, of low nutritive value. We may conclude that farmers would do better to grow normal endosperm types, the richer farmer purchasing supplementary food rich in lysine, the poorer farmer sowing a couple of climbing bean seeds at the foot of each maize plant, as he has done from time immemorial.

Sorghum is a crop greatly in need of improved protein quality. Hulse et al (1980) state "Recognizing the inferior nutritional quality of normal sorghum protein, the inhibition of protein by polyphenols, together with nitrogen losses that occur during domestic and small industrial processing, and probably, in storage, it is recommended that research be continued to stabilize a higher than average lysine in combination with an average (c 10% N x 5.7) protein content". One cannot make bricks without straw: we have not yet found the straw.

Sorghum is a cereal in which the increased protein resulting from better nutrition of the plant is largely prolamine, of low nutritive value. Protein levels are low under conditions in which nitrogen is limiting at grain filling. Riley (1980) did a thorough study of the protein situation in sorghum, of progress made in trying to use the two high lysine genes from Ethiopia, and the mutant in P-721 from Purdue. There were few grounds for encouragement. Plump seeds were unobtainable with the Ethiopian source in crosses: lysine content transferred better from P-721, but yields were poor. Some Figures from Riley's table 1.1 are shown below:-

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Nitrogen applied	Line	Protein percent (N x 6.25)	Lysine (Percent of protein)
20 kg N/ha	CSH - 1	4.95	3.02
	P-721	9.87	2.44
	Q.50662[a]	9.30	2.60
170 kg N/ha	CSH - 1	13.18	1.71
	P-721	15.81	2.62
	Q.50662[a]	12.49	2.48

(a) Q.50662 was a "bulgy" line derived from the Ethiopian line IS 11758

The big change in lysine content with protein content of CSH-1 will be noted: the bulgy line Q 50662 had lysine levels and a response to N. very similar to those of P-721. Many analyses and much effort went in to this work, and there was little at the end of it showing any practical promise.

The next approach to this problem should consist of making up a population from lines with good yields drawn from diverse sources which have higher than average lysine contents and around 10 percent protein when grown on land of moderate fertility. Mass selection should then be practised for ten generations, using the Udy analytical method, and the progress measured.

Conclusions and Abstract

Factors associated with nutritional inhibitors (such as polyphenols in sorghum) or nutritional assets (such as carotene in maize) and which can be seen by inspection (i.e., are evident) can be dealt with effectively by plant breeding.

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Inhibitors and toxic factors which can only be identified by analyses or tests (i.e. cryptic factors) require laborious procedures, but levels can often be much improved. The breeding approach should always be tried. Examples are lathyrin in grass pea or lupinin in lupins.

Improvements in cereal protein quantity and quality also require laborious procedures. In cereals such as rice and wheat, in which increased protein levels are also associated with improved lysine levels, worthwhile progress can be made by plant breeding.

Cereals such as maize and sorghum in which increased protein levels are associated with a higher prolamine content of low quality have not been responsive to plant breeding, and do not warrant expensive programmes. A low level recurrent-selection-in-populations approach ought to be tried.

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