PAPER PREPARED FOR THE TECHNICAL ADVISORY COMMITTEE

1. Need and scope for intensive basic research for the further improvement of pulse crops:

The knowledge gained by concentrated work in the last few years which has been summarised in a recent publication of IARI makes it clear that the yield of pulses can be increased substantially from their present low levels. Even these yields, however, are unsatisfactory compared to the yield levels of the present-day high yielding varieties of cereals. Also, the realisation of these potential yields in pulses may have the paradoxical effect of lowering the competitiveness of pulses. For with greater production, pulses may loose the advantage of higher prices they command at present vis-a-vis the cereals. But, from the point of view of ensuring adequate nutrition of the more vulnerable sections of the community, it is essential that prices should be low enough for even the poorest to be able to include pulses, in adequate quantities, in their diet. It is essential, therefore, that the yield levels in pulses should be raised to that obtained with cereals so that pulses can compete in their own right with the high yielding varieties of cereals.

The reasons for the apparently lower yield-ceiling in pulses are not clear. It has been suggested that pulses have an inherently lower yield-potential than cereals. This may be a reflection of the evolutionary history of the legumes. However, that may be the need for concentrated, basic investigations to understand the causes for the apparently lower yield-ceiling cannot be doubted. Since these problems are likely to be common for all the pulse crops there would appear to be great advantage in concentrating such basic work at one centre. Some of the major aspects on which such basic information is needed are:

2. Factors affecting the photosynthetic efficiency:

In USA, Canada and Australia, extensive studies on photosynthesis in relation to crop production have been initiated in sorghum, maize and soyabean. These studies clearly indicate that high photosynthesis plants are characterised by the enzyme PEP carboxylase, low CO_2 compensation point, high cyclic/non-cyclic ratio, special bundle sheath, high efficiency of translocation in sorghum and maize (Hatch and Slack, 1969). Further it appears that high photosynthesis character may always not be associated with PEP carboxylase (McNaughton and Fullem, 1970). In fact, it has come to be realised that such a large number of characters, which by themselves are dependent upon several enzymes, may not be genetically linked. In some genotypes, by coincidence or, moreprobably, by selection, most of these characters have come together. One can conceive that the most optimum combinations of all these characters have not yet been incorporated into any one genotype. Therefore, attempts are now being made in several laboratories to establish relationship of these characters at the level of various taxa. Scientists at the Carnegie Institute in USA have been able to obtain two species of Mimulus which grow under two distinct environments of high altitude and dry conditions. Photo-synthesis rates and enzyme composition of these species show characteristics of low and high photosynthesis groups. Interspecific crosses have been successful and the genetics of this character is being studied. Similarly, two species of Atriplex representing two distinct photosynthesis groups have been isolated and successfully crossed. The F_1 shows no dominance but in F_2, genotypes with varying level of PEP carboxylase were observed (Carnegie Institute Report, 1970). With the exception of these two instances, no serious attempt has been made to understand the genetics of photosynthesis mechanism in higher plants. Even in this case, emphasis is on carbon dioxide fixation aspects. This is by and large true of most laboratories where photosynthesis work in relation to crop production is in progress. Studies of this nature in respect of grain legumes are very essential.

However, the utilisation of solar energy is in the production of ATP. Once produced, ATP need not only be used in the fixation of carbon dioxide; instead, it can be utilised for other energy requiring processes such as synthesis of protein, fats etc. Therefore, variability in photosynthesis in dark reaction needs extensive investigation. It is all the more important, because several enzymes are involved in this process. One can expect, therefore, genetic variation in this very important trait of plants. At the species level variation has been shown by Chen, Brown and Black (1969). Recently Sinha and Khanna (1971) studied photophosphorylation during growth and development in wheat species and varieties. The existence of variation was obvious. Such studies have to be extended to pulse crops.

Another aspect of photosynthetic efficiency is the capacity to effectively utilise high levels of solar energy. Some crops like maize and Sorghum have the ability to use light intensity of even 8,000 f.c. and above. Other crops like soyabean reach the saturation limit at a light intensity of about 2500-3000 f.c. Little information is available about the behaviour of pulse crops but the available data on the performance of the same set of genotypes in summer and in the rainy season suggest that the ceiling of light utilisation of the presently available varieties of pulses may be as low as in soyabean, if not lower.

A related question is the occurrence of photorespiration in pulses. Obviously such photorespiration would have a negative effect on the 'realised' photosynthesis. Photorespiration has been shown to be present in soyabean and field bean and it is, presumably, important also in the pulses. It is, possible, however, that genotypes may exist which do not exhibit such photorespiration.

3. Factors affecting the harvest efficiency:

The importance of a favourable harvest index, in terms of the partitioning of the photosynthate to the economically useful part of the plant, has come to be well recognised.

In the pulses, little selection would appear to have been exercised in respect of maximizing the harvest index by selection of suitable plant type. This question will need to be studied in depth to find out the plant type which, keeping in view the several factors involved would be optimum. While results of such studies on the cereals can form the starting point, they cannot be transposed uncritically to pulses. For instance, one of the important considerations is determining the optimum plant type in cereals has been the presence of erect or nearly erect leaves, which allows of better penetration of the sunlight in close stands. However, in some clovers it has been suggested that photosynthetic efficiency and not the leaf angle is important. There is, therefore, a great need for basic research on this aspect in pulse crops.

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Although high potentiality of photosynthesis is a pre-requisite for obtaining higher yields, this obviously cannot be the only factor responsible for high economic yield. So far, the storage capacity of a genotype usually called the sink-capacity has been determined in terms of yield components, such as grain number and grain weight etc. While the significance of and the simplicity with which these characters can be studied should not be underestimated, the fact remains that these characters are very much influenced by photosynthetic ability of different plant parts and translocation rates of photosyntheates of the plant. Therefore, scientists are turning to the physiology of developing grains and studying the activity of enzymes associated with the synthesis of starch, proteins and fats. In maize, it has been shown by Shannon (1969) that in the presence of sugary gene, very poor activity of starch synthesising enzymes exists. Tsai and Nelson (1968) have demonstrated in maize, the essentiality of certain phosphorylases if starch is to be accumulated. In peas, Turner (1969) observed that changes in starch content closely followed activity of starch synthetase. The activity of these enzymes seems to be regulated by inorganic phosphate, potassium, ADP and PGA. Obviously, then phosphate and potassium availability could have a profound effect on these enzymes and in turn on starch synthesis and grain development. A simple method to assess the storage capacity of a genotype could be by studying the incorporation of a precursor into the reserve product. Using sucrose-C^{14} uptake and its incorporation into starch, it has been observed that varietal differences exist in tall, medium and dwarf wheats (Sinha 1971). Information on these aspects in pulse crops is almost completely lacking and is urgently needed. Finally, on the basis of these studies, it appears that the future plant type in crop plants is not to be based on morphological architecture alone, but also on the metabolic capacity.

The important aspects of such investigations on variability in photosynthetic efficiency in relation to yield are:

a) Field conditions:

i) Differences in the retention of carbon dioxide in plant canopies of different pulse crops and genotypes.

ii) Photosynthesis rates under field conditions and in relation to canopy structure. The canopy structure can be changed by using different genotypes and by manipulating nutrition and populations.

iii) Photorespiration rates under field conditions.

iv) Respiration of plant canopies in relation to canopy structure, CO_2 concentrations, temperature and humidity.

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b) Laboratory:

i) Variation in photophosphorylation both cyclic and noncyclic at the species and varietal level in relation to developmental stages.

ii) Response of photophosphorylation components to light intensities.

iii) Variation in PEP carboxylase and RUDP carboxylase at species and varietal level.

iv) Response of carboxylases to light.

v) Genetics of photophosphorylation and carboxylation.

4. Adaptability to stress environments:

Pulses are predominantly grown in conditions of moisture stress with limited rainfall. One of the limitations in increasing their production is their intense local adaptation within each region. The recent success in wheat and rice have been mainly due to the wide adaptation of the new varieties. This involves several mechanisms like changes in plant type, photo and thermal insensivity and utilisation of nutrients in the early stage of growth, and better harvest index due to efficient mechanism for transfer of photosynthesis from leaf to grains. Some of the primitive cultivars are found to possess several adaptative gene blocks, which if incorporated into these high yielding varieties can extend the degree and range of their adaptation. They will also provide the basis for improved disease resistance and nutritional quality, normally found in the primitive cultivars. During the past 5 years, studies on the biology of adaption in wheat and barley (Finlay and Wilkinson, 1963) have yielded information that there is considerable genetic variation among the collections for their response to changes in environment. They developed simple statistical techniques to identify such genotypes and to demonstrate that a crossing programme between such types improves both the yields and range of adaptation.

One of the mechanisms used in the case of millets in India has been the utilisation of heterosis using photo-insensitive male steriles of sorghum and pearl millet. Similar studies as those carried out in wheat and barley as mentioned above have to be done in these crops particularly for stability of yield under a wide range of moisture stress. This will help to identify genotypes with diverse mechanisms of adaptation to such environment, which can be brought together into one genotype.

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The paucity of information on the biochemical basis of adaptability has slowed down the improvement programme for this character. Indications are available that certain sulphur compounds may be involved in coping with environmental stress. It is necessary to undertake detailed programmes of physiological investigations, by following the pattern of distribution of sulphur containing amino acids. This will be done by examining different genetypes of varying adaptability for these constituents. These studies will be conducted both in the field and under controlled environment. Based on this information further studies on specific chemical constituents will be undertaken.

a) Field conditions:
- i) Partitioning of dry matter.
- ii) Translocation of photosynthates from leaves to seeds.
- iii) Seed development.
- iv) Effect of growth regulators on the partitioning of dry matter.
- v) Anatomical studies on the translocation channels.

b) Laboratory:
- i) Genetical differences in the translocation of labelled compounds under controlled conditions.
- ii) Studies on seed development and capacity to synthesize proteins and carbohydrates.
- iii) Study of starch synthesis in relation to seed development.
- iv) Effect of temperature on the development of these enzymes.

5. <u>The indeterminate flowering habit in pulses</u>:

Flowering in pulse crops is of the indeterminate type. The processes of vegetative growth on the one hand, and flowering and seed-development on the other go on simultaneously. This is in contrast to the position in the cereals where there appears to be a sharp demarcation between vegetative growth and reproductive development. This difference can have a bearing on several aspects.

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The importance of photosynthetic activity at the time of grain development has been clearly brought out in cereals. The position with respect to legumes is not so clear. Apparently, screening for photosynthesis and its efficiency at different developmental stages would need to be done. The extended growth and flowering periods would mean that photosynthates would be partitioned not only to the developing fruits and seeds but also to the newer vegetative growth and newer inflorescences.

The results obtained with cereals may not be wholly applicable for pulses and it is essential that investigations on this aspect should be undertaken.

A second aspect which needs investigation is the high rate of flowering shedding in pulses. Recent studies have emphasised that one of the objectives in pulse breeding should be to breed for greater seed production per unit of vegetative growth and one of the methods of obtaining this would be to have a greater number of pods/unit of vegetative growth. Prevention of the shedding of flowers and young fruits could make a substantial contribution to this. Basic investigation on the existence of genotypic variation in respect of this character are needed. An understanding of the reasons for such flower and fruits sheeding - whether it is due to limitations of nutrients, hormonal imbalance, light or temperature dependence etc. are also urgently needed.

The indeterminate flowering habit of pulses also results in the fruits maturing in successive flushes. Especially where the mature fruit has a tendency to split open and expose the seeds, this may necessitate several pickings, a laborious, time and labour consuming operation. One solution to this problem would be to breed for synchrony of fruit maturity. The genotypic variability in respect of this character and its genetic control would, however, need to be better understood before this problem can be tackled effectively.

6. <u>Factors affecting the symbiotic relationship between legumes and rhizobia:</u>

A factor unique to the legumes is the symbiotic relationship existing between them and certain bacteria which are capable of fixing atmospheric nitrogen which the legumes can utilize. It is this property of adding nitrogen to the soil which makes legumes an invaluable component of agriculture in such less-developed countries as India. However, optimal efficiency in this process is highly dependent upon the specific requirements of the microorganisms being adequately met. There appears to be considerable specificity in the host-rhizobium relationship.

Such specificity may exist not only at the generic level but may extend to the species and even the varietal level. The host genotype may also play an important role as has been shown concurently in soyabean. Among the pulses, such work has not been extensive as yet but there are indications of specific differences in *Phaseolus spp.* in the type and region of nodule formation which appears to be under genetic control. Again, it has been found that mutants obtained by irradiation differ from the control in their infectability and symbiotic relationship with given strains of the bacterium. Varietal differences have also been shown to exist in factors toxic to the bacterium located in tgea seed coat of some pulses.

Another complicating factor is the fact that there need not be a close relationship between efficiency as nitrogen fixers and the competitive ability of bacterial strains or their resistance to bacteriophage. In countries like India where pulses have been grown from time immemorial the possibility is there that highly competitive bacteria specific to these crops may exist in the soil. Such bacteria may then suppress the improved strains used to inoculate the seed.

These and other complicating factors such as the adverse effect of nitrogen application on rhizobial infection has led to the line of thinking that it might be preferable to do away with this symbiotic relationship and feed the legume plant directly with high doses of nitrogen as is done with cereals. The availability of mutants (natural or induced) lacking the capacity to form nodules (even when the appropriate strain of the bacterium is present) in soyabean and some other legumes such as pea, has made this a feasible proposition.

Attractive as this may look, these are certain difficulties in accepting this proposition fully. First, it is not clear whether application of high doses of nitrogen would at all lead to higher yields of grain; studies on the application of relatively high doses of nitrogen (more than sufficient to compensate the loss of rhizobial N) to pulse crops have shown that this results in excessive vegetative growth but not in commensurate increases in grain yield. Again, at least under the condition under which pulses are cultivated in general, it may be difficult to apply very high doses of fertilisers. Secondly, and a more important consideration in developing countries like India, where pulses have a crucial role to play in nutrition, it appears doubtful if the required quantities of fertilizers would be available. The wisdom of converting pulses (which have been estimated to add each year probably more than the amount of N added in the form of fertilisers in the whole of India) from suppliers of Nitrogen to the soil to consumers appears debatable.

Little is known about many important factors governing the host-bacterium relationship and such fundamental studies are badly needed.

7. Studies on the biosynthesis of storage proteins:

There is now considerable information on the biosynthesis and regulation of certain amino acids in microorganisms. The plants which are a major source of proteins and essential amino acids for human populations have been poorly studied.with regard to the biosynthesis of amino acids and proteins. In bacteria, several mechanisms are known to regulate biosynthesis of threonine, isoleucine, lysine and methionine, which have aspartate as an initial precursor and are essential for our nutrition (Gest and Datta 1964 and Datta 1969). Apparently, lack of suitable material has been partially responsible for this poor knowledge. In bacteria one can resort to the analysis of mutants on supplemented media to determine biosynthetic pathways. Complex organisation in higher plants did not offer any such advantage until only recently when isolated cells were cultured and made to differentiate under controlled conditions. The first paper on the regulation of lysine biosynthesis in plants appeared only recently (Bryan et al.,1970).

From the point of view of fundamental and applied research, it should be essential to know the biosynthesis of amino acids in germinating seeds, vegetative and reproductive organs including developing grains. This information is necessary in the growing and differentiating calli of different crops. Studies with plants grown under controlled conditions would have a direct bearing on the various aspects of protein synthesis and quality, while in vitro studies will be important to establish biosynthetic pathways of amino acids.

8. Conclusion:

The foregoing account of the results obtained in India on the possibilities for pulse improvement and of the need for an intensive attack on some of the basic problems relating to breaking the ceilings to high yields in these crops would suggest that an International research effort involving an inter-disciplinary cluster of highly talented scientists would yield a good pay off. Such a research effort could become the single most significant effort in the battle against protein malnutrition.

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