

Biochemical Approaches in Crop Plant Evaluation

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It is essential to evaluate and characterise the collected germplasm of various crops for its proper exploitation in conventional plant breeding programmes. Various methods of evaluation, based on chemical, biological and molecular analysis, provide tools for cataloguing of genetic diversity. In this article, information available on crop plant evaluation based on nutritional quality, proteins, enzymes, isoenzymes and other chemical constituents has been summarised.

Considerable improvements in procedures and techniques for biochemical analysis of plants in recent times have resulted in the formulation of well defined strategies for evaluation of crop plants. The biochemical evaluation is primarily based on chemical analysis or study of enzyme polymorphism. Phylogenetic relationship has been shown in many crop species based on such studies. Using newer techniques such as hybridisation with suitable probes, chromosome mapping and location of genes has been achieved. Powerful tools of DNA sequencing have made it possible to do genomic analysis, both in respect of gene diversity and gene regulation. DNA restriction fragment length polymorphism (RFLP) provides an additional versatile tool for generating useful genetic markers. Different biochemical approaches used for evaluating crop plants are : (i) Evaluation based on nutritional composition *vis-a-vis* processing and utilisation, (ii) Assessment of nutritional quality, (iii) Characterisation of biochemical constituents (proteins, carbohydrates, lipids, enzymes, isoenzymes, etc., (iv) Varietal identification and genetic purity, (v) Phylogenetic studies, and (vi) Use of RFLP.

EVALUATION OF CROP PLANTS

Available information on the evaluation of some of the important species of crop plants using these approaches, is given below.

Cereals

The methodology has been developed for large-scale evaluation of germplasm in different cereal species for chemical constituents namely, proteins, carbohydrates, lipids, vitamins, fibre and anti-nutritional factors (Paech and Tracey, 1964; AOAC, 1970). Considerable variability was observed in cereal grains in the quality of proteins (Lodha et al., 1976; Eggum, 1977; Hulse et al., 1980). Rice has the highest nutritive value among cereals (Chavan and

Duggal, 1978), but aleurone layers rich in protein and vitamins are lost during milling. The induced mutants of *japonica* and *indica* rice showed differential protein distribution in endosperm (Vilawan and Siddiq, 1973). All other cereal grains are extremely deficient in lysine and/or tryptophan (Indira and Naik, 1971; Eggum, 1977; Sikka and Johri, 1979). However, in maize, barley and sorghum, high lysine genotypes have been identified or induced (Eggum, 1977; Balaravi et al., 1976; Lodha et al., 1976). All these high lysine mutants have substantially improved protein quality as a result of decrease in alcohol soluble proteins, the prolamines.

Screening for protein content has led to identification of high protein wheat, Pusa 5-3 (16%). In maize, Opaque-2, Floury-2 and Opaque-7 have higher lysine content. The varieties RISO-1508, Notch-1, Notch-2 and Hyproly of barley and Opaque P-721, IS-11758 and IS-11167 of sorghum have high lysine content. In wheat and rice, no high lysine strains are available in the world germplasm collection analysed so far. However, Nephad wheat has slightly higher lysine than other genotypes.

It is well documented that when barley, rice, maize and sorghum are fertilised with increasing amounts of nitrogen, grain protein increases. However, this increase is largely contributed by prolamines, thus resulting in poor protein quality (Eggum, 1977). For rice and oats, this is not true since prolamines accounts for a very small fraction. Tannins are also present in a number of plant materials including sorghum and barley which reduce digestibility (Jambunathan and Mertz, 1973; Eggum, 1977). Chemical analysis has shown variability for tannin content in sorghum varieties.

The quality after processing is also an important aspect of grain evaluation. However, the role of different constituents in determining technological and rheological properties has not been studied in great depth. Albumins and globulins are responsible for part of the differences in baking characteristics (Pence et al., 1954). Gliadin and glutelin together constitute gluten in wheat. The presence of strong gluten is a major factor in reducing the disintegration of paste while being cooked and thereafter, producing a cooked product that is of the firmness or consistency desired by the consumer (du Cros et al., 1982) and is a major aim of the current durum wheat (*Triticum turgidum* var. *durum*) improvement programme.

The presence of a specific gliadin (band 45) is correlated with gluten strength in durum cultivars and an allelic band (gliadin 42) can be correlated with weak gluten and poor cooking quality (du Cros et al., 1982). In UK many of the wheat milling industries do electrophoretic analysis of incoming grain lots for quality control. This makes it possible to distinguish between supplies of feed wheat and those suitable for bread or biscuit manufacture.

A more critical biochemical approach involves the characterisation of protein in stored seed *vis-a-vis* cultivar identification for their genetic purity which is the most important parameter of seed quality. These studies fall in the area of polymorphism which exists between genotypes and have, therefore, been widely used as

markers for cultivar identification (Wringley et al., 1982) and in determination of species and genome relationship (Konarev et al., 1979). Gel electrophoresis of storage proteins/isoenzymes has been successfully used in identification of cultivars in wheat, rice, maize, sorghum, oats and rye (Almgard and Clapham, 1975; Suh et al., 1977; Shewry et al., 1978; Park and Stegemann, 1979; McDonald, 1980; Smith, 1986). In sorghum, isoenzyme polymorphism has successfully been used to characterise different cytoplasm and maintainer lines (Tripathi et al., 1983). In other crops also, studies on storage protein, polypeptide pattern and isoenzyme polymorphism have helped in understanding the basis of dwarfness in crop plants, disease resistance and ability of plants to withstand stress (Hayward and McAdam, 1977; Mali and Mehta, 1977; Shewry et al., 1979). Enzyme polymorphism in crosses with distinct genomic background, have helped in studying gene dominance and nucleocytoplasmic interactions. Even heterosis has been associated with enzyme polymorphism, specially with the enzymes of mitochondria and their ability to complement on crossing (Srivastava, 1972).

Homology between bands of different species, based on similarity in migration, provides criteria of genetic affinity from which evolutionary relationship can be inferred (Hall et al., 1966). Johnson et al. (1967) obtained evidence about the origin of genomes of polyploid wheats using gel electrophoresis. The study also confirmed the place of domestication of cultivated tetraploid wheat and for identifying C and D genomes of *Ae. cylindrica* (CC, DD).

Studies of Baum (1978) indicated a close relationship of *Haynaldia* and *Eremopyrum* to *Secale* (ryes), the three genera forming a discrete and isolated group. *H. villosa* is of particular interest as a potential donor of useful genes to bread wheat (Della Gatta et al., 1983) through electrophoretic and chemical characterisation of major seed storage proteins. Variation within and between the three sub-species of *Oryza sativa* i.e. *indica*, *japonica* and *javanica* has been studied using similar techniques (Siddiq et al., 1972).

Sequence analysis of prolamines is of considerable interest. Protein mixtures from oats, rice, normal and high lysine sorghum, two varieties of pearl millet, two strains of teosente and gamma grass were isolated and subjected to NH₂-terminal amino acid sequence determination (Bietz, 1982). This type of approach has demonstrated that prolamine sequence analysis can successfully predict evolutionary relationship.

DNA restriction fragment length polymorphism (RFLPs) is an additional versatile tool for generating useful genetic markers. Botstein et al. (1980) suggested the concept of using RFLPs as genetic markers in mapping of chromosomes. Only limited data are available on the occurrence of RFLPs in plants. The frequency of occurrence of RFLPs in barley and wheat cultivars, using a well characterised α -amylase cDNA probe, has been investigated (Muthukrishnan et al., 1983). The widespread occurrence of enzyme polymorphism and RFLPs in plants has prompted the researchers to generate an RFLP map of agronomically important wheat genome. A detailed RFLP map of wheat genome is likely

to have many useful applications of linkage relationship between agronomic traits and specific RFLPs. This will allow monitoring the inheritance of these traits in breeding studies merely by scoring for linked RFLP.*

Grain legumes

Chemical evaluation in respect of legumes (arhar, gram, peas, urad, cowpea, mung bean, french bean, lentil and horse gram) and some potential minor legumes (rice bean, winged bean and adjuki bean) is essentially similar to cereals. However, unlike cereals, storage proteins in pulses are less numerous but more complex. The major problem with some of the grain legumes is the presence of anti-nutritional and toxic factors including enzyme inhibitors. Nutritional evaluation, therefore, has primarily taken into account the presence of all such factors like trypsin, chymotrypsin and α -amylase inhibitors, phytolectinins, cyanogenic glycosides, lathyragens, saponins, estrogens, allergens, phytic acid and flatulence causing oligosaccharides (Liener, 1982; Salunkhe, 1982). The storage proteins, vicilin and legumin, are deficient in sulphur amino acid methionine (Boulter et al., 1973). However, consumption of cereals, with legumes complements this deficiency.

Fortunately, most of these anti-nutritional factors reported in legumes are destroyed on cooking. However, in case of *Lathyrus sativus*, the presence of neurotoxin, BOAA causes paralysis. Thus, the screening has centred around identifying genotypes low in BOAA content (Misra et al., 1979). The chemical nature of carbohydrates and proteins in the grains influence the digestibility of legumes. The rate of α -amylolysis in green gram is faster than in others and, therefore, the rate of digestibility is better in this pulse. Non-reducing oligosaccharides present in legumes have been reported to be involved in flatulence. The low digestibility of the native soybean proteins has been demonstrated partially due to the compact structure of the native storage proteins and partially to enzyme inhibitors and hemoglutenins (Liener, 1976).

Oilseeds

Evaluation of oilseeds primarily consists of determination of the oil contents and fatty acid profile. On the basis of this data, one can predict the quality of oil present in the oil seeds and its probable energy potential (Singh, 1975). The oil from different crop plants falls into two groups—edible and non-edible. For dietary fats, nutritional studies are also performed in order to judge their suitability for human consumption.

The commonly used edible seed oils usually contain 90% non-polar fraction (free storage lipids) while some species also contain, in addition, 3–4% polar fraction (phospholipids) of bound lipids. A survey of literature would reveal that gas-liquid chromatography (GLC), high pressure liquid chromatography (HPLC), column chromatography, UV and IR spectroscopy have widely been used for

characterisation of seed lipids of various oilseed crop species and identification of cultivars (Poneleit and Davis, 1972; Singh, 1975). Analysis of various oilseed crops has shown not only genetic variability but also changing pattern of fatty acid composition due to environment (Sen et al., 1976). It is, therefore, imperative that while evaluating crop plants, one should look for stability in oil quantity and quality both from the point of view of genetic and environmental effects.

Tuber crops

One of the main tuber crops is potato which is a good source of starch. In addition, it also contains protein, vitamins and minerals and is considered a staple food throughout the world. Biochemical studies for varietal identification and genetic purity have been carried out in potato also. Polyacrylamide gel electrophoresis patterns have been used to screen potato germplasm in Germany (Stegemann and Loescheke, 1962). Due to the complicated genetics of the family, the taxonomic importance of protein pattern studies for cultivar identification is greater than inheritance studies (Stegemann, 1984).

Trees/fruit crops

In contrast to the extensive literature on cereal and legume seed protein, relatively few studies have been conducted towards characterisation of protein in forest trees. Disc electrophoresis and isoelectric focussing have been used for studying the enzymatic variation in *Pinus radiata* (Langridge et al., 1981). However, these studies need to be extended to other trees which may help in identifying different genotypes.

In conclusion, it may be said that a number of biochemical approaches are now available which may assist not only in phylogenetic studies but also in germplasm evaluation and identification. Biological evaluation of proteins can further help in protein characterisation. Electrophoresis and iso-electric focussing techniques, in which separation of proteins and enzymes based on differences in their molecular weight and charges of the constituent polypeptides, offer an opportunity of analysing not only genetic variability among crop plants but also offers scope for studying phylogenetic relationship and specific nucleocytoplasmic interactions.

Keeping in view the objectives and suitability of analytical procedures, either singly or in combination, several alternative techniques are available for evaluating crop plant species.

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