Chalmers, W.S. (1972) The conservation of wild cocoa populations: the plant breeders most urgent work. Proc. IV Internat. Cocoa Res. Conf., Trinidad 1972, 114-118.

Hallauer, A.R. (1985) Compendium of recurrent selection methods and their application. Critical Reviews in Plant Science, Vol 3. 1-33.

End, M. (1991) International Cocoa Germplasm Database, version 2.1.

Kennedy, A.J., Lockwood, G., Mossu, G., Simmonds, N.W. and Tan, G.Y. (1987) Cocoa breeding: past, present and future. Cocoa Growers' Bull., **38**, 5-22.

Marshall, D.R. and Brown, A.H.D. (1975) Optimal sampling strategies in genetic conservation. In D.H.Frankel and J.G.Hawkes (eds), Crop genetic resources for today and tomorrow, 53-80. Cambridge University Press, Cambridge.

Montserrin, B.G., de Verteuil, L.L. and Freeman, W.E. (1957) A note on cocoa hybridisation in Trinidad with reference to clonal selection and hybrid seed. Caribbean Comm. Public Exch. Serv., No. 33.

Posnette, A.F. (1943) Cocoa selection in the Gold Coast. Trop. Agric., Trinidad, 22, 184-187.

Pound, F.J. (1938) Cacao and Witches-broom disease, Port of Spain.

Pursglove, J.W. (1968) Cocoa. In Tropical Crops. Dicotyledons 571-598. Longman, Essex.

Toxopeus, H. (1969) Cacao: *Theobroma cacao* L. In Outlines of Perennial Crop Breeding in the Tropics. (eds. Ferwerda and Wit). Veenman, Wageningen, 79-109.

Williams, J.T. (1984) A decade of crop genetic resources research. In Crop Genetic Resources, Conservation and Evolution. (eds Holden and Williams) Allen and Unwin. London.

RP 985

ISOZYMES AND THE DESCRIPTION OF COCOA GERMPLASM IN TRINIDAD

G.Sirju-Charran *, E.Johnson ** and J.M.Warren **

INTRODUCTION

The International Cocoa Genebank, Trinidad (ICG,T) maintained by the Cocoa Research Unit (CRU) of The University of the West Indies, St. Augustine is the most genetically variable cocoa germplasm collection in the world. Limited land space and the high cost of maintaining a collection of this size requires that there is no unnecessary duplication of the material.

A rapid and reliable method for the identification of accessions was required at the CRU to supplement the use of morphological descriptors. To this end, isozyme analyses by starch gel electrophoresis were initiated using peroxidase. The results obtained demonstrated that additional enzyme systems are needed to discriminate between accessions. The main objective of the current work is to discover enzyme systems suitable for characterization of the germplasm. Suitable enzyme systems are those which show variable isozyme banding patterns between accessions and which are stable over normal environmental conditions. Constitutive enzyme systems in flush leaves are being investigated. Ultimately it is intended that each accession will be described by a series of isozyme banding patterns which may be analogous to a fingerprint.

MORPHOLOGICAL DESCRIPTORS

The present method of describing accessions at the CRU is based on the measurement of leaf, flower, fruit and seed characteristics using the agreed IBPGR methodology (Anon, 1981). Although measurement of these morphological descriptors may be useful, this procedure is very time consuming and labour intensive. Furthermore these phenotypic descriptors may well be influenced by environmental variables. Flower, fruit and seed characteristics can only be recorded for sexually mature plants. Pod and seed data may need to be recorded anyway because they are of economic interest. There are thus a number of drawbacks to this methodology.

Isozyme analysis could be an effective tool for identification of accessions since analyses can be performed on tissue extracts from a plant at any stage of development. When the technique is perfected a large number of individuals can be rapidly evaluated. Isozyme descriptors are probably less likely to be influenced by the environment than are morphological characteristics.

* Dept. Plant Science, University of West Indies, St Augustine, Trinidad, West Indies

** Cocoa Research Unit, University of West Indies, St Augustine, Trinidad, West Indies

CLASSIFICATION AND ISOZYME ANALYSIS

Isozyme analyses by various electrophoretic techniques have been widely used to identify crop plant cultivars of barley and oats (Fedak, 1974; Almgard and Norman, 1970), sorghum (Shechter and De Wet, 1975), pear (Santamour and Denuth, 1980), grape (Walters et al., 1989), and cocoa accessions (Atkinson et al., 1986; Yidana et al., 1987; Lanaud, 1986).

At the Cocoa Research Unit, isozyme analysis using starch gel electrophoresis began with the study of the peroxidase system in leaf and bark tissue of cocoa (Yidana et al., 1987). Leaf and bark samples of the accession being studied were macerated to obtain tissue extracts. These crude extracts were absorbed onto filter paper wicks which were then placed in a starch gel. A direct electric current was applied to the gel for a specific period of time. This resulted in separation of the peroxidase isozymes according to their charge and mass. These isozymes were subsequently detected by placing slices of the gel into an appropriate staining solution. After a few hours, zones of isozyme activity appeared in the gel. The number of bands and the distance travelled was measured for each accession. These data were used to group accessions according to their banding patterns.

The number of peroxidase isoforms were greater in bark tissue than in leaf tissue of cocoa. Based on the banding patterns obtained in bark, six definitive bands were identified (Yidana et al., 1987). Using the observed variation of these definitive bands between accessions, three main reproducible patterns were obtained called Types ICS 1, IMC 67 and M 8 after the first accession in which they were identified.

Work on peroxidase in bark tissue of cocoa has continued at CRU for some two years with the analysis of about five hundred accessions. This yielded one additional reproducible isoperoxidase banding pattern. Some accessions were also observed to show seasonal variation implying peroxidase may be influenced by the environment. This observation, and the greater potential for detecting biochemically unmodified gene products in developing tissues, led to the decision to concentrate on flush leaf tissue rather than bark.

Currently the analyses of acid phosphatase (ACP), isocitrate dehydrogenase (IDH), leucine aminopeptidase (LAP), malate dehydrogenase (MDH), phosphoglucoisomerase (PGI), and shikimate dehydrogenase (SKDH) in flush leaf tissue are in progress on a routine. So far twelve reproducible banding patterns were obtained for MDH and eight for ACP. Using these two enzyme systems ninety-six combinations are mathematically, though not necessarily genetically, possible. Discrimination between most accessions within a particular peroxidase Type was achieved. A few of the ACP x MDH combinations however, contained more than one cocoa accession. Obviously, ninety-six groups cannot possibly characterize the two thousand plus accessions held in the genebank. Therefore additional enzyme systems are required.

Work on other enzyme systems is promising. PGI and IDH appear to be polymorphic and research is continuing. SKDH and LAP exhibit very little variation between cocoa accessions and so do not satisfy the criteria. Work is continuing on other constitutive enzyme systems in flush leaf tissue to find polymorphic systems which can be used to further characterize cocoa germplasm. Ideally only a few systems will be needed to characterize each of the two thousand plus accessions.

FUTURE

Another approach being developed at the CRU is to make use of enzyme systems which can be analysed on a minimum number of buffer resolution systems. In this way maximum benefits may be derived from the use of starch gel electrophoresis as a gel can yield several slices, each of which can be stained for a different enzyme system. This will speed up the work and decrease the cost of analysis of accessions.

In addition to identification of germplasm, the data obtained from electrophoretic isozyme analyses can perhaps be used for the assessment of the genetic variation in the populations present in ICG,T. This can be estimated by determination of allele frequencies from the isozyme data. Enzymatic markers have also been used to detect major genes contributing to quantitative traits. This has been done in tomato (Tanksley et al., 1982), and may be of value in the planning of cocoa breeding programs.

It has also been suggested that economically important, quantitative polygenic traits, such as yield (Tanksley et al., 1982; Frei et al., 1986) and disease resistance (Stavely and Hanson, 1967) may also be associated with isozyme markers. This is possible if the enzyme marker is linked to a major gene influencing these traits and so research is also in progress at the CRU to detect major genes contributing to traits of economic importance in cocoa.

Isozyme analyses should assist in documentation of ICG,T, whose objective is to conserve wild cocoa genotypes as a living genebank. The cost of maintaining such a collection can be greatly reduced if incoming material can be screened on arrival to evaluate its genetic importance. This should decrease duplication, redundancy and ensure conservation of genotypes rather than phenotypes.

REFERENCES

Almgard, G. and Norman, T. (1970) Biochemical techniques as an aid to distinguish some cultivars of barley and oats. Agri. Hortique Genetica, 28, 117-123.

Anon. (1981) Genetic Resources of cocoa. IBPGR Working Group on genetic resources of cocoa. International Board for Plant Genetic Resources. Rome.

Atkinson, M.D., Withers, A.L., and Simpson, M.A.J. (1986) Characterisation of cocoa germplasm using isoenzyme markers. I. A preliminary survey of diversity using starch gel electrophoresis and standardization of the procedure. Euphytica, 35, 741-750.

Fedak, G. (1974) Allozymes as aids to Canadian barley cultivar identification Euphytica, 23, 166-173.