GENETIC MARKER TECHNIQUES IN THE FAMILY CUCURBITACEAE

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SUMMARY

The Cucurbitaceae is a moderately large plant family of about 130 genera and 900 species. Approximately 30 of these species are used as cultivated plants. Some progress has been made in understanding the taxonomy and phylogeny of cucurbits through the use of alpha taxonomy. Biochemical and molecular marker techniques are increasingly being utilised as a useful tool in elucidating taxonomic relationships, tracing phylogenetic patterns and for genetic linkage analysis for crop improvement. A literature review was undertaken to assess the extent of utilisation of various marker techniques in the Family Cucurbitaceae.

INTRODUCTION

The Cucurbitaceae is a predominantly tropical plant family of about 130 extant genera and 900 species, the overwhelming majority of which are distributed in three main areas: Africa and Madagascar, Central and South America and Southeast Asia (Jeffrey, 1980). In tropical Africa, cucurbit distribution is especially characteristic of the drier regions. For example, Namibia, which includes both the Namib and Kalahari Deserts, has been identified as an important centre of cucurbit diversity (Maggs & Guarino, 1995).

Several species in the Cucurbitaceae are of economic importance including the musk or sweet melon (*Cucumis melo* L.); cucumber (*Cucumis sativus* L.); watermelon (*Citrullus lanatus* (Thunb.) Matsumura & Nakai) and summer or winter squash (*Cucurbita pepo* L., *C. mixta* Pangalo, *C. moschata* Poir., *C. maxima* Duch. and *C. ficifolia* Bouche). A number of other species are produced in relatively small quantities for local consumption or are gathered from the wild, but they are nevertheless a vital component of subsistence diets. The Cucurbitaceae are an important source of vegetables, fruits, edible seeds and seed oil, domestic utensils, medicines, water, animal fodder and fuel. In spite of this potential, fewer than 0,5% of the species are being commercially exploited and the family is relatively poorly known.

The long and intimate association of man with cucurbits is typified by several species which are known only in cultivation. Evolution of these crop species has been unravelled through remains from archaeological deposits and present-day distribution patterns. The domestication of cucurbits is a success story reflected in the current world-wide cultivation of major cucurbit crops. Initial breeding and improvement activities focused on the elimination of bitterness, sex expression, the increase of the facility of F1 hybrid production and disease resistance. It was, in fact, in watermelon that the transfer of disease resistance from an inedible land-race to horticulturally acceptable cultivars was first demonstrated (Whitaker, 1979). This pioneering research in the first decade of this century had far-reaching implications not only for watermelons, but for plant breeding in general. With innovative biotechnological methods now available, a new scope of possibilities has been opened up for improvement and development in cucurbit crops.

The family as a whole is characterised by trailing, vine-like stems and tendrils. Except for some variation in flower size and colour among the species, the general morphology is very similar. This has contributed to confusion in the taxonomy and the classification of the family has been fraught with ambiguity and abounding synonomy. A major cause of this chaotic state was the indiscriminate use of characters which, when used in conjunction with the sum total of features of the plants concerned are useful for delimitation, but when used alone are liable to lead to misplacing of taxa.

Following an extensive literature survey undertaken in order to determine the extent of utilisation of marker techniques within the Family Cucurbitaceae, it became clear that markers have been employed for two main purposes:

* basic research in plant taxonomy; noteably, delimitation of taxa, inter- and intra-specific relationships including evolution and phylogeny.

* applied research in crop breeding; for especially determining marker linkages to important agronomic traits, construction of linkage maps and for testing genetic purity of hybrid seed.

Utilisation of the various marker techniques within the Family Cucurbitaceae will be discussed in the light of these two objectives.

MORPHOLOGICAL MARKERS

The study and descriptions of the variation of organisms and the investigations of the causes and consequences of this variation has relied traditionally on morphological information. Although this science of taxonomy has declined from a pre-eminent position last century, it remains both the most basic and ultimate of the biological sciences.

Early taxonomic treatments of the Family Cucurbitaceae were essentially based on easily detected morphological variation. Greatly aided by developments in anatomy and palynology, taxonomic understanding of the family has advanced considerably in recent years.

Traditional taxonomic studies have resulted not only in the description and delimitation of species and production of keys for the Cucurbitaceae, but have proved of value in the identification of new sources of genetic variation for the improvement of existing crops.

Morphological variation as markers are useful in both breeding programmes and genetic studies. Despite their economic importance, few genes have been recorded for most cucurbit crops although the situation is gradually improving e.g. Zink (1986) determined that yellow corolla mutant in muskmelon was controlled by a single recessive gene. Zink (1977) was also the first to describe a genetic linkage in muskmelon between short internode and yellow virescent leaf colour and to calculate the genetic distance between the two genes.

Linkage selection has improved breeding strategies in recent years. Morphological markers which are relatively easy to recognize can simplify the recovery of genes of interest linked to them which are more difficult to score. Cordate leaf shape in *Cucumis sativus*, is a recessive morphological feature linked to disease resistance and other traits (Vakalounakis, 1992); while umbrella leaf in the same crop species is associated with sensitivity to low humidity (den Njis, A.P.M. & de Ponti, O.M.B., 1983). Vakalounakis (1992) was able to arrange 11 disease resistance and morphological loci in four distinct linkage groups following backcrossing and analysis of segregation in two lines of cucumber.

To supplement existing information on the independence between different characters and the description of only four linkages, Pitrat (1991) initiated a systematic study to identify genetic linkages between various markers in muskmelon. Attention was focused on disease resistance genes and several morphological characters (both vegetative and floral). Following analysis of crossing and segregation data, the existance of the previously described linkage groups was confirmed and an additional four groups were recognized. These groups appear to be independent. Five markers tested seem to be independent from any of the eight linkage groups. These results are a first step towards a genetic map of *Cucumis melo*.

A list of the genes of the watermelon was initially published in 1976 (Robinson *et al.*, 1976) and recently updated (Henderson, 1995). The initial few genes identified for watermelon based on morphological characters and disease resistance have swollen in number to total 88 genes, supplemented through isozyme marker analysis. Seven linkage groups with 24 loci have subsequently been constructed. Unfortunately, the general properties of morphological markers are far from ideal. This is reflected in cucurbit breeding in general, where it appears that the linkage relations of most of the simply inherited morphological traits have been largely overlooked (Fanourakis & Simon, 1987).

Cucurbit crop species, like *Citrullus lanatus*, present difficult taxonomic and phylogenetic questions because wild forms may be morphologically so similar to the cultigen, that they are not readily distinguishable. It is clear that morphological variation alone is insufficient to clarify the relationship and additional sources of analysis will have to be employed in order to discern patterns of evolution among the watermelon and its wild relatives.

MICROMOLECULAR MARKERS

This type of marker comprises a series of biological compounds which are products of the secondary metabolism of plants. These compounds are characterised by a relatively low molecular weight and are chemically very heterogenous. Chromotographic techniques are employed for their identification and analysis.

The Family Cucurbitaceae is characterised by the presence of cucurbitacins and other triterpenoids. The occurrence of these bitter-tasting compounds in the vegetative parts and fruits has been shown to be of taxonomic significance. Following extraction of the compounds and subsequent identification by paper chromatography, a definite correlation between chemical composition and taxonomic delimitation of the family was identified (Rehm *et al.*, 1957). For example, cucurbitacin B is characteristic of the genera *Coccinia, Cucumis, Lagenaria* and *Trochomeria*, whereas cucurbitacin E is the main bitter compound in *Citrullus* with the exception of *C. naudinianus*. Following taxonomic revision, the latter species was later reclassified under the genus *Acanthosicyos*. This example illustrates that secondary metabolites can be a useful tool in improving plant classification.

There is, however, no reason to suppose that chemical markers are more important than structural data. Occasionally conflicting evidence is also presented. For example, chromatographic flavonoid patterns of leaf extracts from 21 *Cucumis* species indicated species-specific patterns (Dane *et al.*, 1980). However, the subgeneric groupings from this study did not conform with groupings of Deakin *et al.* (1971) which were based on sexual affinities and morphology.

Cucurbitacins, like other low-molecular weight markers, are under genetic control. Investigation as to the genetics of bitterness in four genera of Cucurbitaceae, revealed that a single dominant gene is responsible for this trait in each case (Robinson *et al.*, 1976). As cucurbitacins are reportedly toxic (Steyn, 1950) and are responsible for imparting an undesirable and bitter taste to cultivated cucurbits, the frequency and level of expression in individuals or populations is important to plant breeders.

MACROMOLECULAR MARKERS

PROTEIN MARKERS

Storage Proteins

Storage proteins are synthesized in plant organs of reproduction, propagation and dispersal where they are recognized in discrete deposits. Because of their abundance in foodstuffs, storage proteins were amongst the first to be studied (Shewry, 1995). Storage proteins of two types have been identified in the seeds of the Family Cucurbitaceae *viz.* 11S globulin (Hara-Nishimura *et al.*, 1985) and 2S albumins (Hara-Nishimura *et al.*, 1993). As these secondary plant products are found ubiquitously throughout the family they are of very little taxonomic value other than to distinguish the group as a whole from other plant families. However, seed storage protein profiles, obtained by electrophoresis, can be used for resolving taxonomic and evolutionary problems within the family. Furthermore, Dunnhill & Fowden (1965) recorded that the composition of seed protein in Cucurbitaceae is affected only slightly by environmental conditions or seasonal fluctuations.

Pichl (1978) found the overall variation to be small in his characterisation studies of seed storage albumins in

Cucurbita maxima. This epitomises the main feature of seed protein profiles i.e. conspicuously species specific and highly stable. This is particularly true for cultivated plants. In a similar study, Navot & Zamir (1987) found that within the genus *Citrullus*, only two of the six major bands in the profile were variable and each of the 3 species had a unique pattern for storage proteins.

In comparison with the genetic information about other economically important crops, the watermelon has been somewhat neglected. However, linkage maps for *Citrullus* have been constucted using seed protein markers in conjunction with isozymes (Navot & Zamir, 1986). In this study, nineteen segregating electrophoretic markers (two seed protein and seventeen isozyme loci) were considered and four linkage groups were identified using an interspecific cross between *C. lanatus* and *C. colosynthis*.

No research appears to have been conducted on the tuber storage proteins of Cucurbitaceae which could differ significantly from seed storage proteins. Underground storage organs are found in several African taxa as an adaptation to extreme, arid conditions.

Sozymes

Isozyme phenotypes of some enzymes are recognized to be useful as genetic markers, as most isozymes are stably controlled by codominant alleles and are not influenced by the environment. Isozyme markers are compared by monitoring their migration in gels during electrophoresis and isoelectric focusing; they are detected by enzyme-specific stains. Within the family under review, isozyme studies have been carried out mainly in the genera of economic importance like *Cucumis* and *Cucurbita*, not only to study intra- and interspecific relationships of the cultivated species, but also to classify the wild species and evaluate relationships among the wild and cultivated taxa (Dane, 1983).

Esquinas-Alcazar (as reported in Dane, 1983) examined 125 populations of *Cucumis melo* and six enzyme systems encompassing 11 isozyme loci. For five of the isozymes there was no variation and the other six were dimorphic. He concluded that little or no allelic variation was found at most of the loci in all of the populations studied. Perl-Treves *et al.* (1985) examined 5 varieties of *C. melo* and one wild variety with 29 nuclear-coded enzymes. They found 22 of the isozymes to be monomorphic. Of the other seven isozymes, six were dimorphic and one was trimorphic. Thirty accessions of *C. melo* from Iran were examined by Staub *et al.* using nine isozymes. There was no variation for five of the isozymes, two were dimorphic and two were trimorphic. These studies indicate that there is little isozymic variation in *C. melo* germplasm.

Using 18 enzymes, relationships among cross-compatible wild diploid species of *Cucumis* were classified biochemically (Staub *et al.*, 1987); however, relationships within species were not well defined. This illustrates that the technique is not without shortcomings and that, perhaps intraspecific relationships may be better elucidated through analysis of DNA markers.

Isozyme studies in *Citrullus* revealed that very little variation occurs in cultivated watermelon but significant divergence from wild forms was found (Navot *et al.*, 1990). This uniformity in cultivated varieties is probably due to strong human selection for rare mutants like lack of bitterness and red colour.

The phylogeny of *Citrullus* and two related genera was suggested following electrophoretic analysis of 26 enzyme coding genes (Navot & Zamir, 1987). Measurement of genetic distances between species and races investigated, places *Acanthosicyos* and *Praecitrullus* as two distinct outgroups to *Citrullus*. *Citrullus* is also subdivided into two: one branch representing *C. colosynthis* and the second, the southern African species, *C. ecirrhosus* and *C. lanatus*. Allozymic variability is also concordant with the variation of other morphological traits which are used to define the taxonomic groups of the genus *Citrullus*.

Linkage maps have been constructed for *Citrullus* (Navot & Zamir, 1986) using isozyme markers. Further isozyme studies utilising data from four different crosses involving three *Citrullus* species, reveal that genetic distances between markers and their linear order in maps were homogenous and therefore suggest that genome organisation within this genus is conserved (Navot *et al.*, 1990). However, as only a finite number of isozymes can be examined to find sufficient variation for establishing linkage maps, additional markers need to be identified and utilised.

The practical application of isozymes was illustrated by Isshiki *et al.* (1991), who investigated the usefulness of these markers in melon breeding. *Kekiri* is a cultivar of *Cucumis melo* which displays certain agronomically useful traits. Glutamate oxaloacetate transaminase (GOT) isozymes in this and other cultivars were analysed by electrophoresis. Two phenotypes of GOT were distinguished, triple-banded only observed in *kekiri* and single-banded common in all cultivars of *C. melo* investigated. As the cotyledons displayed the same phenotypes with those of mature leaves in GOT, it implies that this marker can be employed very early in the growth stage for melon breeding. This genetic marker could be applied in the testing of purity of hybrids in commercial seed production.

DNA MARKERS

Restriction Fragments Length Polymorphism (RFLP)

Restriction fragment length polymorphisms (RFLPs) are differences in hybridisation patterns among DNAs revealed after digestion with a restriction endonuclease and subsequent probing with a randomly selected, genomic or cDNA cloned sequence. Changes in the electrophoretic mobility indicate differences in the length of the restriction fragment due to insertion or deletion. Changes in the number of fragments indicate the loss or gain of restriction sites for such an enzyme caused by base substitution.

This approach has been used in the Family Cucurbitaceae to produce more comparable data. Good results have been obtained with various defined repeated sequences as probes (Torres Ruiz & Hemleben, 1991; Zentgraf *et al.*, 1992), ribosomal RNA (Torres *et al.*, 1989; Torres Ruiz & Hemleben, 1991) and chloroplast DNA (Perl-Treves & Galun, 1985). Data from work such as this can have value at all taxonomic levels. One frequently used technique is to determine the length of restriction fragments in different populations or species.

Individual genotypes of a species can be characterised using the RFLP technique with applications in cultivar separation. Neuhausen (1992) assessed the degree of RFLP in *Cucumis melo* and subsequently determined relationships among cultivated varieties. The level of variability detected in melon was suprizingly low although a higher level of variability was detected when comparing *C. melo* with *C. sativus* (cucumber). This indicates that within *C. melo*, the differences among accessions are due to infrequent base substitutions, thus confirming earlier deductions of Shattuck-Eidens *et al.* (1990), whereas between the two species, differences are mainly due to genome rearrangements such as insertions and deletions. It is interesting to note that this low variability with RFLPs mirrors the findings of isozyme profile analysis undertaken on the melon (Dane, 1983).

At the species level, results of this technique have also been of value in phylogenetic studies. Different species within the genus *Cucumis* were characterised with molecular markers by Zentgraf *et al.* (1992). Various highly repetitive satellite DNA and middle repetitive ribosomal RNA genes were used as hybridisation probes with mixed results. RFLP analyses and hybridisation with rDNA probes confirmed the division of the genus *Cucumis* into an African and an Asian subgenus. Using different rDNA spacer probes, *C. anguria* and *C. melo* of the subgenus *Melo* can clearly be distinguished from each other by their spacer organisation resulting in RFLPs. This confirmes the taxonomic division of the subgenus *Melo* into the "anguria group" and the "melo group". Satellite DNA is taxon specific and can be a useful tool for the identification of species. Satellite hybridisation also curiously revealed that *C. melo* of the African group was more closely related to the Asian species in having similar organisation of the rDNA spacer, than to *C. anguria*, which also belongs to the African subgenus.

This generic subdivision was already demonstrated in earlier research conducted on the plastome of *Cucumis* (Perl-Treves & Galun, 1985). Restriction patterns based on digestion of chloroplast DNA (chlDNA) with nine endonucleases and hybridisation to heterologous chlDNA probes were obtained in some twenty wild species and cultivated varieties of *Cucumis*. By comparing restriction sites, which is a direct and accurate way to measure distances between species, a phylogenetic tree was constructed by parsinomy analysis for the *Cucumis* plastome. Most systematists using chlDNA for phylogenetic analysis have adopted this approach (Bremer, 1991). A definite African group was identified, which is distant from the melon (*C. melo*), the cucumber (Asian Group) and a few morphologically distinct African species.

The results of this study were compared with isozyme phylogeny for the same taxon in a subsequent publication (Perl-Treves *et al.*, 1985) and found to have great overall similarity. Although cpDNA is useful for phylogenetic analysis due to relatively slow sequence evolution, its application is limited. The taxonomic level at which cpDNA sequences are optimally informative is the differentiation of genera. At lower taxonomic levels, especially for studies on population genetics and speciation, cpDNA usually provides little informative variation (Bachmann, 1992).

Cultivars of *Cucurbita pepo* and other *Cucurbita* species have also been characterised by RFLP analysis using different fragments of the ribosomal intergenic spacer (IGS) of *C. pepo* as hybridisation probes (Torres & Hemleben, 1991). In contrast to the highly conserved rRNA coding regions, the spacers which separate them, especially the IGS, are more divergent thereby generating RFLPs in the rDNA of the Family Cucurbitaceae (Torres *et al.*, 1989). Several cultivars of *C. pepo* could be distinguished by a diagnostic rDNA restriction pattern, whereas other cultivars showed an identical RFLP pattern which suggests a closer relationship. Cultivars differing in RFLP patterns with the probes and enzymes used in this study also show clear differences in certain characters like fruit morphology. However, cultivars exhibiting identical patterns are not necessarily completely identical in phenotype.

Within the genus *Cucurbita*, two species (*C. moschata* and *C. maxima*) exhibited strong cross-hybridisation with the *C. pepo* spacer probes as reflected in their RFLP patterns. As the DNA of two *Cucumis* species did not cross-hybridise significantly with *Cucurbita pepo* spacer probes, these probes can therefore be used to differentiate between the genus *Cucurbita* and other Cucurbitaceae genera.

Data revealed in these studies of *Cucurbita* show marked differences to findings of similar work undertaken with the genus *Cucumis*. In contrast to *Cucurbita*, restriction patterns for *Cucumis melo* are highly uniform (Neuhausen, 1992). Satellite probes isolated from *Cucumis melo* hybridise exclusively with DNA from *C. melo* and do not cross-hybridise with other *Cucumis* species.

Molecular genetic maps are commonly constructed by analysing the segregation of RFLPs. However, this technique has yet to be used extensively for the creation of linkage maps in Cucurbitaceae which would allow for indirect selection strategies for crop improvement programmes to be developed. However, it must be borne in mind that the usefulness of RFLP markers is largely dependent on the degree of polymorphism and therefore its application in cucurbit crops with low variability like *Cucumis melo* may be limited. The situation, however, in other cucurbit species may be entirely different as the apparant level of variation detected as RFLPs within a species differs greatly from one species to another (Helentjaris *et al.*, 1985).

This approach has some potential for practical application in the commercial seed sector as reported by Matsuura *et al.* (1994). In cucumber, F1 hybrid cultivars are preferred because of combined superior characteristics. Instead of costly and risky field observation testing which is the current screening method, the authors examined the possibility of using RFLP markers for this purpose. Two parental inbred lines and the F1 hybrid were used in the experiment. After screening 196 genomic and 49 cDNA clones, four groups were distinguished - three being monomorphic while the fourth showed RFLPs. It was from this fourth group that a probe was selected, pure for both parental lines, for further testing. Comparison of patterns following digestion and hybridisation with the selected probe of the parental lines and the F1, illustrate that inheritance of RFLP markers is codominant and as such, a useful tool for purity testing in hybrid seed production. Although the authors advocate this approach as being "available and convenient" for practical use, it is questionable as to whether this method is superior to that of isozyme markers in which codominant allelic expression is interpreted from electrophoretic banding patterns. However, data from the study of Matsuura *et al.* (1994) provides the base to isolate useful RFLP probes and gives a good indication as to the minimum amount of DNA required for the analysis and the stage of the seedlings for maximum DNA isolation in the cucumber.

Random Amplified Polymorphyc DNA (RAPD)

The basis of Random Amplified Polymorphic DNA (RAPD) methodology is the Polymerase Chain Reaction (PCR) which is an *in vitro* method for producing large amounts of a specific DNA fragment. Using primers, short sequences of DNA are amplified by means of a thermoresistant DNA polymerase and repeated cycles of denaturing, annealing and polymerisation. Differences in electrophoretic bands are due to changes in primer annealing sites and as such can be used as genetic markers.

The most important aspect of this polymorphism is that it can be mapped as a standard genetic marker (Williams *et al.*, 1990). Since RAPD markers segregate in a Mendelian fashion, they can be used to develop genetic linkage maps or perhaps saturate existing RFLP linkage maps. The use of RAPDs has already allowed detection of linked markers to major genes controlling agronomically desirable traits like disease resistance in certain crops e.g. tomato (Newbury & Ford-Lloyd, 1993).

This method is new and not yet fully tested, hence a paucity in literature available regarding this approach and its application in genetic linkage mapping for the Family Cucurbitaceae.

However, RAPDs have proven useful for DNA fingerprinting to facilitate the identification of cucurbit crop varieties (Jeon *et al.*, 1994) and to determine parentage within breeding material of watermelon (Hashizume *et al.*, 1993).

Six *Cucurbita pepo* and nine *C. moschata* cultivars were tested for the presence of RAPD markers that could distinguish them (Jeon *et al.*, 1994). Fifty 10-mer primers were used of which six produced 64 useful RAPD markers. Eight (11%) of these markers were common to both species, whereas forty (62,5%) were species specific. The remainder were polymorphic in either or both species. Although this polymorphism within each crop was extensive, *C. pepo* and *C. moschata* could be separated by several species-specific bands. Conversely, several bands were monomorphic across all cultivars tested indicating some interspecific relationship. The F2 population of a hybrid between *C. pepo* and *C. moschata* was tested using the same primers. Bands specific to either one of the parent species segregated in the expected 3:1 Mendelian ratio for the presence:absence of bands.

The extensive polymorphism present in these *Cucurbita* cultivars, coupled with the speed and simplicity of the technique, suggests the use of RAPD as a promising new and efficient method for molecular characterisation and taxonomic delimitation in other members of the Family Cucurbitaceae.

The application of various markers in the purity testing of hybrid seed has been discussed. It is perhaps in this regard that RAPD analysis will prove to be most effective. Hashizume *et al.* (1993) tested two inbred lines of watermelon and the hybrid which is used extensively in Japan for commercial seed production. 59 oligonucleotides were screened as primers with nine out of 286 bands observed to be polymorphic between the parents, suggesting that only 3% of the genome differs. One primer was selected and generated a product specific to the F1 and male parent, thus enabling discrimination between the hybrid and the female parent.

As shown in this study by Hashizume *et al.* (1993), lines which are genetically close are often selected for commercial hybrid seed production in cucurbit crops. In these cases, the morphological determination of genetic purity of the hybrid is rather difficult. Isozyme analysis does not detect polymorphism in watermelon (Navot & Zamir, 1987) and the use of RFLPs is very labour-intensive when attempting to detect a difference between closely related parents (Matsuura *et al.*, 1994). RAPD assay could therefore be an effective tool for the detection of genetic differences in parental lines even if they are closely related.

DISCUSSION

Although steps have been taken in employing molecular marker techniques to understand the evolution of a few major cucurbit crop species, it is evident that more marker studies coupled with morphological, genetic and biochemical research need to be undertaken to establish phylogenetic relationships among taxa in the Cucurbitaceae. This potential for further research is clearly demonstrated by the lack of studies undertaken on lesser-known taxa like *Momordica* and *Acanthosicyos* which are both locally important food crops in their areas of distribution.

Given the innovative techniques which are now available to plant systematists, future taxonomic treatments within the Family Cucurbitaceae would clearly benefit enormously from these data in monographic considerations. Problems with *a priori* character weighting which is typically associated with morphological comparisons in making systematic and phylogenetic inferences, will be eliminated with biochemical and molecular marker techniques.

Molecular markers may be employed to verify taxonomic delimitations, trace phylogenetic histories and clarify discrepancies in this notoriously problematic family. The use of DNA variation by plant systematists has increased considerably during recent years and phylogenetic studies utilising especially cpDNA appear consistently in taxonomic treatments. Doyle (1991) illustrates both the utility and the pitfalls of DNA data in plant systematics in his discussion of molecular studies of the genus *Glycine*. The author cautions plant systematists against the weighting of molecular characters, particularly relative to non-molecular data.

In fact, the importance of morphological markers must not be overlooked. Basic phenotypical comparisons remain essential preliminaries to all programmes of rational resource utilisation, such as conservation, crop introduction and crop improvement. For example, in breeding for disease resistance, knowledge of the linkage of a resistance gene with a morphological marker and use of this feature in the screening procedure might permit easier and quicker selection of host resistant genotypes. Furthermore, as most taxa within the Cucurbitaceae are diploid species with low chromosome number (n = 7, 11, 12), gene maps may be uncomplicated and easy to develop using simply inherited morphological traits.

A wide range of variability in vegetative and fruit characters is available in the Cucurbitaceae which is as of yet underexploited by plant breeders. Barriers between the cultivated species and their wild relatives in the Family Cucurbitaceae are generally high and crosses between them commonly fail to produce fertile hybrids. A practical aim of marker analysis would be to utilise the knowledge on

relatedness between cultivated and wild species, in order to enable the increase of genetic resources of cultivated cucurbit species by introgression of wild genes through rapidly developing biotechnological approaches. In future, much crop production in Africa will need to come from land now considered marginal or unsuitable. New cultivars of domesticated melon or pumpkin crops with adaptations to specific environments may be desirable. Breeders may also be concerned with domesticating or developing cultivars in species which are now considered wild, like *Acanthosicyos horridus* (!nara) and *A. naudinianus* (gemsbok-komkommer).

Much emphasis is currently being placed on the need to be able to carry out large-scale screening of genetic resources held in genebanks (Newbury & Ford-Lloyd, 1993). To assist in the maintenance of the large Cucurbitaceae germplasm collection held at the National Plant Genetic Resources Centre in Namibia, a marker technique like RAPD would allow for screening of duplicate samples and identification of diverse or representative samples. This would allow for informed planning for further germplasm collecting. Passport and characterisation data of morphological traits will be further enhanced by any additional genomic information gleaned through molecular marker assay.

It is clear that the above techniques differ in strengths and weaknesses and that for precision in the characterisation and estimation of genetic variability in populations as well as taxonomic delimitations and estimation of phylogeny, the techniques must be regarded as complementary. These techniques can all be applied to answer a range of questions regarding the degree of variation within the Family Cucurbitaceae.

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