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SEED PROTEIN ELECTROPHORESIS STUDIES IN CUCURBITS-A REVIEW

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ABSTRACT

Seed protein electrophoresis is increasingly being utilized as an additional approach for species identification and as a useful tool for tracing back the evolution of various groups of plants. This paper summarizes the main features of the seed protein profile-stability, uniformity and additive nature. In addition, the significance of this approach for resolving specific taxonomic and evolutionary problems in cucurbitaceous family has been pointed out.

Varietal characterization based on morphological data is becoming difficult because these morphological traits are highly influenced by environment. Morphologies reflect not only genetic constitution of cultivars, but also interaction of the genotype with the environment. Due to the GE effects, it is inappropriate to discriminate ambiguity among similar morphological expressions. Descriptions based on morphologies are fundamentally flawed in their ability to provide reliable information for calculation of genetic distance or validation of pedigrees. Therefore, *in vitro* identification/selection of cultivars/genotypes for various qualitative and quantitative traits is of great significance as it helps in obtaining desirable result and hence proved a boon to the researchers as well as farmers. Therefore, a simple technique is required for identification of different varieties. Electrophoresis of seed or seedling extracts followed by appropriate protein or activity stains has been suggested as a possible method for distinguishing cultivars (Larsen and Benson, 1970; Wilkinson and Beard, 1972). These techniques are all based on the concept that each cultivar is distinct and relatively homogeneous at the genetic level. Thus by screening enough loci one should be able to uniquely define each cultivar. Soluble proteins of seeds are the physiologically active constituents, which constitute bulk of enzymes involved in plant metabolism and are responsible for the nutritional and technological property of plant (Johari *et al.,* 1989). Soluble proteins being primary gene products provide a valuable tool of making genetic system and hence, different methods of electrophoresis are used in chemo taxonomical studies of plant species (Ahl *et al.,* 1982; Agrawal, 1985 and Wilkinson *et al.,* 1985). This technique is least influenced by environment and is used as "Fingerprint" to identify genotypes (Smith and Smith, 1992).

Electrophoresis is basically a process of forced diffusion within an electric field. Protein molecules of the sample are moved through a medium that is gel, paper, or cellulose by applying an electrical gradient (Pierce and Brewbaker, 1973). The protein molecules are separated on the basis of their molecular weight or electrical charge. During electrophoresis the lighter molecules move faster and travel more distance in the gel medium and vice-versa. Therefore, the protein molecules with low molecular weight will be stacked at the bottom of the gel. By using markers of known molecular weight, it is therefore possible to estimate the molecular weight of the proteins (poly peptide chains).

Markert (1968) proposed the term "isozyme" for multiple molecular forms of an enzyme, showing a common catalytic activity, derived from a tissue of a single organism. Different molecular forms or isozyme of an enzyme can be separated by various biochemical methods, but the electrophoresis is perhaps the most versatile and easily applied to problems in the plant sciences. The use of

isozyme phenotype for cultivars identification was proposed by Brewbaker (1966) and the progress in this field was reviewed by Nielsen (1985).

Proteins and DNA can be used to provide varietal profile. They are in popular usage because the variation for these markers is distinct and this variation is understandable in genetic terms. These characters are in routine, usage and are widely accepted as source of reliable data in evolution, taxonomy and genetics (Smith and Smith, 1992). The proteins can represent primary gene products and seeds are considered physiologically stable and easy to handle (Landizinsky and Hymowitz, 1979; Sarkar and Bose, 1984). Seed proteins have the advantage of being scorable from inviable organs or tissues and the electrophoretic protocol for bulk protein assays is generally simpler than that for isozymes (Cooke, 1984; Gepts, 1990).

Stability is one of the main features of seed protein profile. For this reason, it has been suggested as an additional tool for species identification besides other traditional biosystematics approaches. Furthermore, the composition of seed protein is highly stable and is affected only slightly by environmental conditions or seasonal fluctuations (Gray *et al.*, 1973). Seed proteins are mainly storage proteins and are not likely to be changed in dry mature seed. Thus, the mature seeds of different age still possess the same profile (Robinson and Megarrity, 1975). In addition, intrinsic changes in the plant such as chromosomal rearrangements or even doubling of chromosome numbers have no, or very small, effects on the seed protein profile (Nakai, 1977).

Additiveness is another typical feature of seed protein profile. When proteins of two electrophoretic variants are mixed, the uncommon bands will persist in the gel. The common bands will merge but no new band will be formed. One can detect the parents of a specific hybrid by comparing its protein profile with a profile obtained by protein mixture of the suspected parents. Allopolyploids are permanent interspecific hybrids perpetuating themselves following a doubling of their chromosome number. Information accumulated thus far indicates that the seed protein profile of synthetic allopolyploids represents an exact summation of the number of bands of their diploid parents (Hall and Johnson 1963; Shephard 1968; Murray *et al.*, 1970; Chen and Bushuk 1970; Houts and Hillebrand 1976). This apparently does not hold in high ploidy levels where many genomes are combined together.

Although uniformity and uniqueness of the seed protein profile are typical of many groups of plants, variation in the number of bands and their position in the profile has been reported, especially where a great number of accessions were examined. Unfortunately, except for a few genera, extensive screening of germplasm to uncover variability in the seed protein profile is woefully lacking. Differences between accessions of the same taxon in darkness and thickness of various bands are the most commonly reported types of variation, suggesting that the formation of many of the bands in the seed protein profile are under control of quantitative gene systems. Caution should be taken in interpreting darkness and thickness of bands as quantitative gene systems. This kind of variation may be due to differential extraction or solubility of seed protein from different accessions. In addition, a third possibility exists that the thickness and darkness of bands may be due to the lack of separation on the gels of several proteins having similar migration rates. In any case, no attempt has been made to estimate the number of genes causing quantitative variation in seed protein bands (Ladizinsky and Hymowits, 1979).

Usage of seed protein electrophoresis

Isozyme/ protein markers have been developed in several vegetable crops for characterization, varietal identification, sex determination, assessment of genetic diversity and distinguishing zygote from nucellar seedlings (Dhall and Cheema, 2001). The common uses of the markers have been described as follows:

Assessment of species relationships by similarity of protein profiles

Measurement of the degree of similarity of the profile of conspecific categories by the methods described is legitimate from the biological point of view since most of the variation observed probably can be attributed to a small number of genes that cause the different migration rates of various protein bands. By applying the same techniques for comparing categories above the species level, one might face a situation in which bands with the same migration rate in profiles of two species do not necessarily represent similar proteins (Mies and Hymowitz 1973).

Identification of hybrids and their parents by the protein profile

When proteins of two electrophoretic variants are mixed, the uncommon bands will persist in the gel. The common bands will merge but no new bands will be formed. Furthermore, the individuality of the uncommon bands will be expressed in the F_1 hybrids of the two variants. In other words, one can detect the parents of a specific hybrid by comparing its protein profile with a profile obtained by a protein mixture of the suspected parents. Thesee techniques have been used for cultivar identification in cucumber (Meglic and Staub, 1996), bittergourd (Tewari, 1997), melons (Sujatha *et al*. 1991 a) and several other vegetable crops (Wills *et al*. 1977).

Seed protein electrophoresis and the origin of cultivated plants

The high stability of the seed protein profile and its additive nature make seed protein electrophoresis is a powerful tool in elucidating the origin and the evolution of cultivated plants. A cultivated plant and its immediate wild progenitor still form a common gene pool (Harlan and de Wet 1971) and can be considered from the genetic point of view, as members of the same species. Therefore, despite conspicuous morphological differences between them they will share, more or less, the same protein profile. Indeed similarity between the seed protein profile of wild species and their cultivated counterparts has been reported.

Plant variety protection, registration, certification and patents

A precise description of newly bred cultivars is necessary to distinguish it from other cultivars of the same kind in order to protect the right of plant breeders and producers (Arus, 1983; Bailey, 1983). Traditionally morphological field surveys were conducted to detect the seed contamination and establish cultivars identity. These methods need large field areas or green house space, are labour intensive and require an expert to distinguish between morphologically similar cultivars of a crop. Biochemical methods would be of great value for cultivar identification. Protein electrophoresis or isozyme analysis is not only quicker and less labour intensive but also more reliable since the expressions of isozyme loci are codominant and are not altered by environmental factors (Henn *et al.,* 1992; Smith and Smith, 1992). In order for cultivars to be nationally or internationally registered and for breeders to be granted plant breeders' rights of protection, varieties must successfully pass inspection for the criteria of distinctness, uniformity, and stability (DUS)(Bailey, 1983). Certification is a test of the trueness to declare varietal type. Application of these tests can help promote the breeding of novel genotypes. A detailed discussion on the utility of biochemical data in providing descriptors for the granting of plant breeders' rights has been given by Bailey (1983).

Assessment of genetic purity

The purelines, hybrids, inbred and other type can be successfully identified from a varietal mixture (Smith and Smith, 1992).

Marker assisted selection

Progress has been made in mapping and tagging of many agriculturally important genes with protein/ isozyme markers, which forms the foundation for marker-assisted selection. The tagged trait can be used directly for breeding purposes. Similarly availability of tightly linked genetic markers for resistance genes will help in identifying plants carrying these genes without subjecting them to the pathogen or insects attack in early generation. Several loci of linked with morphological markers have been identified in watermelon (Howkins and Dane, 2001) and melons (Baudracco-arnas and Pitrat, 1996) Genes responsible for resistance to *Fusarium* wilt has been tagged and on the basis of this it has become easier for itrogression of resistant genes into susceptible lines in marker assisted selection (Zheng and Wolf, 2000)

Measurement of genetic diversity

An important component for efficient and effective management of plant genetic resources as well as their utilization is characterization of germplasm. Such a characterization is essential not only for the identification of various species but also to determine genetic relatedness among them. The information generated could be used successfully in breeding programme wherever possible. Genetic distance in terms of similarity of dissimilarity between two genotypes can be easily measured on the basis of protein profiles (Chen *et al*., 1990; Vaughan and Denford, 1968). This technique has been found effective in determining genetic diversity in melons (Baudracco-arnas and Pitrat, 1996), watermelon (Lee *et al*. 1996) and several other vegetable crops (Hoey *et al*. 1996).

Seed protein electrophoresis is done either by isozyme method or poly acrylamide gel electrophoresis (PAGE)/ sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). Seed protein (Gepts, 1990) and isozyme variants (Wendel and Weeden, 1989) that migrate at different rates under electrophoresis have been the most widely employed molecular genetic markers during the last quarter centaury. Isozymes are generally fractioned by starch gel electrophoresis (Kephart, 1990) in studies of genetic diversity and divergence, whereas, seed proteins are generally analysed via polyacrylamide gels (Cooke, 1984). When conducted according to proper laboratory procedures, protein electrophoretic migration rates are generally highly heritable, and ample polymorphism is available for may germplasm management purposes (Simpson and Withers, 1986).

Isozyme studies in cucurbits

To date, isozymes have been the genetic markers most frequently applied to plant germplasm management (Simpson and Withers, 1986). They are generally (But not always)) governed by single Mendalian genes with codominant alleles and, after the appropriate genetic analysis, are interpretable by locus/ allele models (Weeden and Wendel, 1989). They can be assayed from a wide variety of organs and tissues, and analytical procedures are not exceptionally complicated (Murphy *et al.*, 1990; Wendel and Weeden, 1989). In the cucurbitaceous family isozyme studies have been carried out to study intra and interspecific relationships of the cultivated species as well as to classify the wild species and evaluate relationship among the wild and cultivated taxas (Tanksley and Orton, 1983). Wall (1969) examined natural populations of *Cucurbita foetidissima* from several localities in the southwest USA for electrophoretic variants of the two enzymes. Three phenotypes were identified for leucine aminopeptidase activity, which were believed to be controlled by two codominant alleles. A rare alcohol dehydrogenase

allele was identified in the heterozygous condition in a population from Taxas.

Ford and Simon (1972) reported that cucumber plant introductions from all over the world and well-known US varieties had an identical peroxidase-banding pattern. The PX_1, peroxidase, present in most of the *Cucumis* species, was absent but a cluster of three bands at the PX_2 locus, identical in electrophoretic mobility to the PX_{2B} cluster of *Cucumis melo* was present. Price *et a., (*2003) reported the molecular and biochemical characterization in lupin (*Lupins alba* L.) by observing peroxidase enzyme pattern.

Esquinas-Alcazar (1977) examined 125 *Cucumis melo* populations for variability in six enzyme systems. Crude extracts of roots hypocotyls of 4-6 weeks old seedlings were subjected to starch gel electrophoresis and stained for peroxidase activity. They observed variation in isozyme patterns.

Staub *et al.* (1983) studied 47 enzymes and general protein in the fruit, seed and cotyledons of *Cucumis sativus* with respect to resolution for possible genetic analysis. In subsequent studies 19 enzymes in green cotyledons of 69 plants belonging *Cucumis sativus* and 8 other species were found to be polymorphic.

The several previous investigations on the isozymes of *Cucurbita pepo* have primarily dealt with peroxidases (Dvorak and Cernohorska, 1967; Denna and Alexander, 1975), esterases (Wall, 1969; Puchalski and Robinson, 1978). Polymorphism was observed in all these isozyme systems, indicating that *Cucurbita pepo* possesses a signigficant level of inherent allozyme variation.the results of these studies have been summarized by Dane (1983). More recently, two studies have sampled a greater range of isozyme systems and produced valuable genetic information on several loci (Pitrat, 1982). In *Cucurbita pepo*, considerable variation between different cultivars were found especially for the aspartate aminotransferase and malate dehydrogenase isozyme systems (Ignart and Weeden, 1984). Each of the 5 fruit types represented in the 21 cultivars tested were distinguished by specific allozymes or combination of allozymes. Cultivars within a fruit type gave very similar allozyme phenotypes and often could not be distinguished on the basis of 6 assays used. Despite the out crossing nature of the species allozyme polymorphism within most cultivars was low.

Engalychev *et al.* (1987) reported 42 forms representing various sub-species and botanical varieties of *Cucumis melo*, both cultivated and wild with other wild *Cucumis* species. They observed heterogeneity for peroxidase isoenzymes with specta containing 8-11 fractions of different mobility. Differences were observed between species, within species, within sub-species and in many cases between cultivars. Engalychev and Zhemchuzhnikova (1988) found marked heterogeneity for electrophoretic mobility of the protein fractions between varieties of different ecological groups in cultivated, semi-cultivated and wild forms of melon. Chen *et al.* (1990) analysed four inbreds, belonging to vars. *reticulatus* and Maukwa and their progenies for isozyme variation in seed and cotyledon extracts. Of the 10 enzyme systems studied, only peroxidase (PRX) and shikimate dehydrogenase (SkDH) were polymorphic. The PRX and SkDH phenotypes of the 4 lines and their hybrids indicated that SkDH was controlled by 2 alleles at a single locus, each allele encoding a different mobility band.

Similarly, isoenzyme variation was found in 13 species of *Cucumis* from India, South Africa and Holland for the enzyme systems peroxidase (PRX), glutamate oxaloacetate transaminase (GOT) and glutamate dehydrogenase (GDH) using PAGE (Sujatha *et*

al., 1991a). The different species possessed their own individual zymograms. There was little similarity among the species for the PRX and GDH zymograms, but significant similarity was observed for GOT, where all species possessed a genus specific isozyme of GOT 4. No species of South African origin appeared to have contributed to the evolution of *Cucumis melo*. Again Sujatha *et al.* (1991b) studied 32 muskmelon varieties of 3 types (Indian dessert, Indian non-dessert and semi-wild and exotic dessert types) by PAGE for isozyme variation in 4 enzyme zymograms esterase (EST), peroxidase (PRX), glutamate oxaloacetate transaminase (GOT) and glutamate dehydrogenase (GDH). Four major isozyme groups were detected in PRX, EST and GOT and allotted to 4 loci. GDH had 2 groups only. Three out of the 14 loci scored were dimorphic with two alleles at each locus, but the remaining 11 loci were monomorphic. While both standard and variant alleles at the PRX_2 locus were well distributed amongst the collection, variant alleles detected on GOT_2 and EST_2 were confined to Indian non-dessert and semi-wild cultivars Phut and Kachri, respectively. Accession 566, catalogued as *Cucumis melo* var. *callosus*, was unique in possessing all the variant alleles identified.

Sujatha and Seshadri (1991) studied isozyme variation in 32 melon cultivars for 4 enzyme systems esterase (EST), peroxidase (PRX), glutamate oxaloacetate transaminase (GOT) and glutamate dehydrogenase (GDH). Of 14 loci scored, 11 were monomorphic and 3 (PRX_2, GDT_2 and EST_1) were polymorphic. Genetic studies proved the monogenic nature of inheritance of the alleles at these 3 polymorphic loci. Parent cultivars were homozygous at the PRX_2 locus where the standard allele PRX_{2a} was completely dominant over the variant allele PRX_{2b}. Conversely, the 2 alleles at GOT_2 and EST_1 showed codominant reaction.

Walters *et al.* (1991) evaluated 8 species representing 6 genera (*Benincasa, Bryonia, Citrullus, Ecballium, Lagenaria* and *Luffa*) using starch gel electrophoresis. Comparison of single and multiple condition electrophoresis indicated that undetected comigration generally increased with increasing genetic differentiation of taxa. All the taxa were allozymically connected, either directly or via other taxa in the study.

Isshiki *et al.* (1992) examined Indian wild cucumber (*Cucumis sativus* var. *hardwickii*) and 81 accessions of cucumber (*Cucumis sativus*) for isozyme variation of six enzymes. They concluded that the Indian wild cucumber is a distant relative of the cultivated cucumber, but that the relationships between ecotype differentiation among cucumber cultivars and isozyme phenotype were not valid.

Isozyme variation in seven African species within *Cucumis,* subgenus *melo*, subgroup Myriocarpus (4x *C. aculeatus, C. anguria* var. *anguria*, *C. dipsaceus, C. ficifolius, C. myriocarpus* subspecies *leptodermis*, *C. prophetarum,* and 2x and 4x *C. zeyheri*) and 2x *C. sativus* of subgenus *Cucumis* using horizontal starch gel protein electrophoresis to characterize interspecific and intraspecific variation (Staub *et al.*, 1992). Cluster and classification and regression tree analysis of allelic frequencies among 14 polymorphic loci indicated that *C. myriocarpus* subspecies *leptodermis, C. prophetarum* subspecies *dissectus, C. ficifolius, C. anguria* var. *anguria* and 2x and 4 x *C. zeyheri* had biochemical affinities and could be distinguished from *C. aculeatus* and *C. dipsaceus*, which were similar. *Cucumis sativus* was dissimilar from all other spices studied.

Berg and Gabillard (1994) examined the genetic variability of melon seed protein. Electrophoretic analysis of 74 geographically diverse accessions, consisting of 19 morphologically distinct groups, revealed 270 reproducible seed protein bands of which 70 were

variable. Genetic evaluation led to the conclusion that at least 20 loci govern the variation observed. Phylogenetic trees constructed using protein and morphological data were compared. Lines derived from Cantaloupe F_1 hybrids by single plot descent, after selfing and diplohaploidization were also studied revealing 265 reproducible protein bands of which 72 were variable on a presence/absence basis. Vaz *et al.,* (2004) worked on different cultivars of *Lupins alba* and discriminated and isolated varietal differences in the variety by seed protein electrophoresis.

Lebeda and Dolezal (1995) screened 11 cucumber cultivars and accessions with various levels of field resistance to *P. cubensis* for peroxidase isoenzyme polymorphisms. Considerable variation, characterized by the occurrence of 7 different zymograms, was detected in the studied set. It is thought that these zymograms could be used for the discrimination of specific cucumber genotypes with high levels of field resistance to *P. cubensis*. Staub *et al.* (1996) developed a series of *Cucumis sativus* populations containing alternative alleles for 20 enzyme coding loci. Allelic variation was originally noted in commercially available processing cucumber lines and in germplasm collections. Crosses were made among these elite lines and F_4 lines developed and used to determine the inheritance of isoenzyme banding patterns. These lines were crossed to European line derived from intermating 3 glasshouse lines from Numhems Zaden BV, De Ruiter Zonen BV, and Nickerson Zwaan BV and Poinsette 76 (US fresh market type) and GY 14 (US processing type). Further crossing resulted in the production of 6 European glasshouse lines (W6743), 11 US processing types (W6744) and eight US fresh market types (W 6745). The genetic stock should prove useful in genetic marker research, genetic drift detection and plant breeding research.

Isozymes analysis of eight enzymes was done in *Cucumis hystrix* Chakr. and two cultivated *Cucumis* species (*C. sativus* L. and *C. melo* L.) electrophoretically to investigate the biosystematics of these three species (Chen *et al.,* 1997). Cluster analysis using data from six enzymes indicated that considerable genetic distance existed between both *C. hystrix* and melon and between *C. hystrix* and cucumber. *C. hystrix* might be a key species for studying the evolution and taxonomy of genus *Cucumis*.

Sodium dodecyl sulphate poly acrylamide gel electrophoresis (SDS-PAGE) in cucurbits

Almost all analytical electrophoresis is carried out in poly acrylamide gels under condition that ensure dissociation of the proteins into their individual poly peptide subunits and that minimize aggregation. Most commonly, the strongly anionic detergent SDS (sodium dodecyl sulphate) is used in combination with a reducing agent and heat to dissociate the proteins before they are loaded on the gel. The denatured polypeptide binds SDS and become negatively charged. All components of the system contain 0.1 % SDS (Laemmli, 1970). The SDS-PAGE method is found very much effective in identification of different genera, species, cultivar etc. (Smith and Smith, 1992; Chen *et al.*, 1997).

Pasha and Sen (1991) examined salt-soluble proteins of seeds of several taxa of cucurbitaceae by SDS-PAGE. Three to five major protein bands were detected in most of the 22 species studied, with some variations. Most genera had distinctly different protein patterns; however, *Gymnopetalum* was very similar to *Trichosanthes* (tribe Trichosanthinae) and there were some similarities between *Benincasa, Coccinia* and *Citrullus* (tribe Benincaseae). Mukia appeared to be distinct from *Cucumis* although both of them belong to the same sub-tribe. *Sechium* differs from most of the cucurbits. Within any genus, different taxa showed almost identical protein patterns as in

Momordica (two varieties), *Luffa* (three species), *Cucumis* (two species), *Cucurbita* (two species) and *Trichosanthes* (four species). The dendrograms obtained from cluster analysis of similarity matrices of proteins revealed both similarities and dissimilarities with current concepts of taxonomic relationships of the taxa examined.

Singh (1996) carried out electrophoresis in 10 varieties of bottlegourd, 3 of bittergourd, 2 of pumpkin and 2 of ridgegourd. The bottlegourd cultivar gave rise to a seed protein profile of 10 bands. In terms of presence/absence of bands, these varieties fell into three distinct groups. The bittergourd cultivars gave 7 bands and formed two distinct groups. The pumpkin cultivars also exhibited a distinct seed protein profile with a total of eight bands and were grouped into two categories according to presence/absence of bands. The two ridgegourd cultivars displayed similar banding pattern of 6 bands.

Tewari (1997) subjected seed proteins of bittergourd cultivars (PBIG-1 and PBIG-2) to polyacrylamide disc gel electrophoresis. Based on electrophoresis (PAGE) of water soluble protein, parental lines PBIG-1 and PBIG-2 were easily distinguishable as PBIG-1 had 7 bands and PBIG-2 had only 5 bands. Jackson *et al*., (1992) isolated cytochrome C peroxidase and the isomers of peroxidase enzymes by polyacrylamide gel electophoresis.

In 53 lines of muskmelon a total of 13 bands distributed in 4 zones i.e. A, B, C and D. Zone A had 3 bands, zone B had 2 bands, zone C had 7 bands and zone D included one band. There was similarity of band among the genotypes in zone B, C and D. However, zone A showed differences in banding pattern and the 53 germplasm lines could be grouped in 5 dissimilar protein profile groups and thus, it was possible to distinguish certain germplasm lines on the basis of protein profiles (Yadav *et al.*, 1998). Similarly, Chaudhary and Ram (2000) characterized 65 germplams lines of muskmelon (*Cucumis melo*) using SDS-PAGE. A total of 15 seed protein bands could be resolved which were distributed into four distinct zones (A, B, C and D). There was no difference in banding pattern in C and D zones. The differential bands were located mainly in A zone. However, a single specific genotype possessed 2 extra bands B3 and B4 in B region. The 65 genotypes were classified into 7 different groups based on protein profiles. Three morphologically indistinguishable genotypes (oblong fruited) were identifiable on the basis of their seed protein profiles. However, it was also observed that genotypes having contrast morphological traits (round vs oblong fruit) possessed the similar protein profile in some cases.

In cucumber storage protein of seed of 19 germplasm lines could be resolved into a total of 17 bands distributed into 3 zones i.e. A, B and C zones. A zone comprised of 6 bands, zone B had 7 bands and zone C included 4 bands. The 19 germplasm lines could be classified into 8 different groups based on protein profiles. Thus, it was possible to distinguish certain germplasm lines on the basis of protein profiles (Singh and Ram, 2001)

CONCLUSION

Thus, for the foreseeable future, biochemical descriptors, and in particular DNA based descriptors, will be valuable tests for providing information on genetic identity and similarity to the plant breeding and agricultural community at large. Many new techniques are in their infancy of development. It is expected that future advances in this technology will allow these techniques to become even more discriminatory with more widespread application, and they will become both easier and less expensive to use.

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