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Cultivars identification based on biochemical markers

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ABSTRACT

For many years, cultivars with narrow morphological deviations were evaluated using traditional field plot techniques. This technique is tedious and time consuming. Furthermore, the morphological characters do not guarantee the correct determination, because they are unstable and influenced by environmental conditions,. The advent of biochemical markers makes more exact identification possible. In addition, the biochemical techniques are rapid, accurate and dependable. The biochemical cultivar identification is useful to ensure the genetic purity of plant cultivars and their parental lines in commercial seed production of hybrids, aid for identification and characterization of diversity in crop cultivars and their wild varieties, analyze phylogenetic relationship of the accessions, asses the geographical origin of germplasm, describe a new cultivar, and speed up distinctness, uniformity, stability (DUS) test for candidate cultivar. It is also useful for the registration of new varieties, and plant variety rights applications. In this review, I made a survey for biochemical cultivar identification in the plant families: *Poaceae, Leguminosae, Solanaceae, Compositea, Euphorbiaceae, Citraceae, Elaeagnaceae, Palmaea, Brassicaceae, Fagaceae, and Apiaceae* in terms of the reproducibility of the techniques and their wide applications.

KEYWORDS

SDS-PAGE;
Isozyme analysis;
Genetic diversity;
Poaceae;
Leguminosae;
Solanaceae;
Compositea.

INTRODUCTION

For many years, cultivars with narrow morphological deviations were evaluated with reference to yield and quality[1-3], using traditional field plot techniques. However, the identification with this technique is tedious and time consuming. Furthermore, the morphological characters may be unstable and influenced by environmental conditions[4-7]. Therefore, cultivars did not guarantee the correct determination. Because of that, biochemical and molecular markers were used for cultivars identification to achieve more exact identification[8-12]. Electrophoretic analysis of proteins and isoenzyme offers an efficient and cost effective method towards cultivar identification and varietal purity tests in seeds lots[13-16]. The analysis of the seed proteins and isozymes by polyacrylamide gel electrophoresis (SDS-PAGE and A-PAGE) is the rapid, accurate and dependable technique. The technique is useful to ensure the genetic purity of plant cultivars and their parental lines in commercial seed production of hybrids. SDS-PAGE and A-PAGE are most economical simple and extensively used biochemical techniques for analysis of cultivar identification. As seed storage proteins are largely indepen-

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dent of environmental fluctuation, their profiling using SDS-PAGE technology is particularly considered as a consistent tool for economic characterization of plant cultivars[17-20]. The electrophoretic protein profiles and their high stability and independence of the ecological conditions were used as cultivar markers[21,22].

Analysis of seed storage proteins provide aid for identification and characterization of diversity in crop cultivars and their wild varities and phylogenetic relationship of the accessions[23,24]. Polymorphism in seed storage proteins has been associated with geographical origin of germplasm[25,26]. Cultivar identification is useful for describing a new cultivar, testing genotype purity and speeding up-distinctness uniformity stability (DUS) test for candidate cultivar[27,28]. It is also useful for the registration of new varieties, pedigree analysis studied and plant variety rights applications.

A-PAGE and SDS-PAGE analysis have been used widely for cultivar identification in many plant families. In this review, we will focus on cultivar identification using biochemical techniques in different plant families.

Family poaceae

The *Poaceae is* one of the largest angiosperm families with approximately10 000 species[29]. Wheat (*Triticum aestivum* L.) seed-storage proteins represent an important source of food and energy. In the U.S., kernels within wheat classes traditionally have had uniform phenotypic characteristics. Those features denoted the wheat class, thereby predicting its end-use and quality[29]. Changes in breeding and marketing have made this system obsolete. Absolute numbers of varieties as well as the proportion of those utilizing wide crosses have increased variation within classes, and multiple biotypes are more common. Because of the number of wheat varieties that may possibly be grown, using kernel characteristics can no longer be much successful in predicting wheat class. Researchers therefore are increasingly using biochemical methods of PAGE and HPLC to "fingerprint" gliadin or glutenin proteins, which may identify varieties and indicate quality. These methods may be used to help identify and select genotypes and varieties with good quality characteristics, to analyze mixtures and varieties having kernels with heterogeneous biotypes, and to ensure varietal purity. Plant breeders can also use fingerprinting for plant varietal protection and hybrid analysis[30-33].

A comparative study of total grain protein was carried out by SDS-PAGE to characterize the differences between and within two species of wheat *(Triticum aestivum* and *Triticum durum*) represented by 12 cultivars[34]. The densitometric profile data of the polypeptides showed an obvious variation in the number and position of bands from one cultivar to another. Cladistic analysis showed that the four cultivars belonging to species *T. aestivum* separated earlier in evolutionary history, and can be recognized from the rest of the cultivars which belong to species *T. durum*.

For more accurate identification of breeding material, high molecular weight glutenin subunits (HMW-GS) have so far been used for wheat cultivar identification. However, HMW-GS can often not distinguish between cultivars for its overestimation of molecular mass and incorrect identification of HMW-GS due to its low resolution[35]. Therefore, Labuschagne and Maartens[36] compared HMW-GS, low molecular weight glutenin subunits (LMW-GS) for wheat cultivar identification. Five commercial wheat cultivars were analyzed using the above two methods. The HMW-GS failed to distinguish between the five cultivars. LMW-GS' could clearly distinguish between the cultivars, but banding pattern interpretation was more complicated than for HMW-GS. They concluded that HMW-GS should be used for initial cultivar identification, as it is simple and reliable. Where they fail to distinguish between cultivars, the LMW-GS should be used for final identification.

Separation of proteins according to their solubility by Osborne[37] is widely used for the characterization of seed proteins and their subsequent SDS PAGE can be suitable tool for variety identification[8]. Although Osborne protein fractionation is known and has been often used for almost one century, many studies proclaim, that bounders among protein fractions are not sharp and method is influenced by temperature, time of extraction and intensity of shaking. It was found that HMW glutenin zones are passing to the pattern of gliadins and also mutual overlapping between LMW glutenins and electrophoretic pattern of alfa, beta and gamma gliadins in conditions of the SDS-PAGE[38]. Whence it follows, that a considerable part of next protein fractions (globulins, gliadins and glutenin) were included in this albumin fraction. Changes in protein pattern can be influenced by external factors e.g. cultivation of spelt cultivars in conditions of low and high sul-

phur level in the soil or using of reducing detergents e.g. dithiotreitol as extraction buffer[39].

Low-molecular-weight glutenin subunit (LMW-GS) composition in common wheat is one of the critical determinants of gluten properties. However, the nomenclature of *Glu-3* encoding LMW-GSs has not been consistent among laboratories, due to the complexity of the LMW-GSs and the distinct separation methods used by different researchers. It is very important to unify the nomenclature systems in current use, to facilitate the sharing of information about the effects of individual LMW-GS on gluten properties. Therefore, Ikeda *et al*.[40] analyzed 103 cultivars (having various *Glu-A3*, *Glu-B3* and *Glu-D3* alleles from Argentina, China, France, Japan and Mexico) using 1D SDS-PAGE and 2D analyses. They found differences in nomenclature particularly for *Glu-A3* and *Glu-B3*, including new *Glu-3* alleles among laboratories. They proposed a new list of standard cultivars representing *Glu-3* alleles.

A-PAGE and SDS-PAGE have been applied to characterize 38 durum wheat varieties and 12 bread wheat varieties. Number of bands resulted by applying the first method (A-PAGE) was 13 polymorphic bands, while 12 HMW- GS (high molecular wheat glutenin subunits) have been resulted by applying SDSPAGE. Data were combined together (25 polymorphic bands) to calculate the genetic similarities between studied individuals using Jaccard's coefficient followed by setting up the cluster analysis using Unweighted-Pair Group average Method with Arithmetic mean (UPGMA) method[41]. The value of genetic diversity was 56% between durum wheat varieties, while the same value was increased to reach 100% in the bread wheat varieties. Dendogram for all 50 genotypes showed a separation of bread wheat varieties in a sub cluster which is distinct from the sub cluster containing the bread wheat varieties.

Genetic variation was studied on HMW-Gs in twelve common wheat varieties using SDS-PAGE[42]. Each variety contained a range of two to five subunits and 14 different glutenin subunit patterns were observed in hexaploid wheats. Based on SDS-PAGE data, the average of similarity coefficient was 0.525. This indicated high variation between varieties.

Wheat grains of thirteen varieties were collected from different ecological regions of Iran. The variability of seed storage-proteins was analyzed by SDS-PAGE[43]. Based on the data of high molecular weight (HMW) and low molecular weight (LMW) gluten subunit bands, cluster analysis was performed and Jaccard,,s similarity index (JSI) was calculated. It was concluded that SDS-PAGE analysis of wheat endosperm protein is useful for evaluation of genetic variability and cultivars identification that help in wheat breeding program.

The biochemical characteristics and phylogenetic relationships of ten populations of *Triticum turgidum* ssp. Dicoecum Schrank in Italy were assessed[44]. Analysis of the Glu-1A locus, which resulted active in all populations considered revealed four different mobility profiles, Four combination of HMWG subunits encoded by Gl-1B were identified while A-PAGE analysis of gliadin fractions disclosed from one to five genotype. This analysis indicatess that the *T. turgidum* ssp. Dicoccum Schrank considered are less suited for the preparation of pasta and bread. Varities more suitable for these commodities could be obtained by selection or through crosses with appropriate varities of durum wheat.

The genetic diversity of the seed storage proteins (Glutenin and Gliadin) was assessed among 24 wheat genotypes ((14) durum wheats and (10) bread wheats) using A-PAGE and SDS-PAGE[45]. In durm wheats, Jorjet, kechek, sham9 and kahlahadba recognized by gamma 45 and subunits (17+18) that had positive effect on the dough. In bread wheats, Abozec, Sham10, Doma32058, Doma32457, Doma4, Bohoth8 have good technology characteristics due to that have subunits (5+10). This date are useful in breeding programs to improve quality by selecting of the best genotypes.

Spelt wheat

The polymorphism level in three spelt wheat cultivars (Hercule, Altgold and Rouquin), three new–breeders' spelt lines (H92.27, H92.28 and M92.20 (originated from hybridization between spelt and common wheat) and reference common wheat cultivar was evaluated using the electrophoretic profiles of seed albumins and globulins, gliadins, glutenins, glutenins[46]. Entire evaluation of all four-marker systems showed differences between common wheat cultivar and spelt cultivars and spelt breeders' lines. Also significant differences between old spelt cultivars (Hercule, Altgold and Rouquin) and new spelt breeders' lines were found. The seed storage proteins (gliadins and glutenins) were

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the most used marker system for wheat and spelt identification. Albumins and globulins were used only marginally. The esterase isozymes were only important for the spelt cultivars identification[47]. The seed storage proteins are associated with agriculturally significant traits and they are used in a legal protection of cultivars[8,48,49]. Canadian authors found the basic differentiating regions of spelt gliadins, in which the heaviest group of omega gliadins (100–113 kDa) and higher number of alpha gliadin bands are absent in comparison with common wheat[38]. German authors constructed ideal spelt gliadin pattern, where the heaviest group of omega gliadins was also absent, in comparison with common wheat, and additionally on the basis of A-PAGE analyses they described a new D-zone of bands, located between omega and gamma gliadins. That D-zone was not found in common wheat[48].

Buckwheat

Four buckwheat varieties 'Pyra', 'Spacinska I', 'Kara-Dag' and 'Jana' registered in the Czech Republic were evaluated by discontinuous SDS-PAGE electrophoresis[50]. In the four registered buckwheat varieties 36 significant band positions were described. The highest polymorphism of buckwheat proteins was represented by bands with a molecular weight of 30-60 kDa. No single specific bands for the tested varieties were detected. The designated bands showed a similar frequency and coincidence in all varieties. In spite of this fact, varietal differences in frequency and coincidence of some designated bands were found among the tested varieties. Significant differences in protein band frequencies and their coincidences could serve as a supplemental identification criterion, especially in such cases when the electrophoretical analyses of bulked samples are not sufficient.

Electrophoretical study of protein fractions (albumins + globulins, prolamins and sum of glutelins) in SDS-PAGE conditions and their quantification were tested in eight varieties of common buckwheat and in two tartary buckwheat accessions[51]. The electrophoretical visualization showed as a main fraction albumins + globulins with high inter- and intra-varietal polymorphism in tested varieties. Tartary buckwheat accessions showed completely uniform electrophoretical spectra. A low appearance of prolamins was confirmed in all tested varieties (inclusive tartary buckwheat accessions). Spectrum of soluble glutelins in common buckwheat was characterized by lower frequency and intensity of protein bands. The high polymorphism of buckwheat storage protein provides good presumption for its utilization as genetic markers. The inter- and intra-varietal polymorphism of buckwheat storage protein was confirmed by several authors[52,53].

Barley

SDS-PAGE was used to analyze the hordein polypeptide patterns of Brazilian barley varieties (*Hordeum vulgare* L.) and of two native species of *Hordeum* from southern Brazil (*H. euclaston* Steud. and *H. stenostachys* Godr.)[54]. Twelve of the 14 varieties examined showed intra-varietal polymorphism. Phenograms using each seed as an operational taxonomic unit (OTU) showed that the seeds from most varieties did not form distinct clusters. Seeds from different plants of the native species varied considerably. The molecular weights of the hordein polypeptides of the two native species were quite different from those of *H. vulgare*. There was a greater similarity between the native species than with *H. vulgare*, although *H. stenostachys* was slightly closer to the cultivated species than *H. euclaston*

Biochemical investigation of seven Egyptian barley cultivars were performed using SDS-PAGE of both water soluble and water-non soluble seed storage proteins under reducing conditions[55]. Twenty-four protein bands were obtained from the SDS-PAGE of the water soluble seed storage protein fraction, three out of them were common while the other 21 were polymorphic. The protein profile of the buffer soluble seed storage protein fraction showed 28 protein bands, four out of them were common and the other 24 were polymorphic. UPGMA denderogram depicated wide genetic diversity among cultivars.

Hordeins extracted from dry grains and green malt of six two-rowed winter barley varieties (Angora, Sladoran, Rodnik, Rex, Martin and Barun) separated by SDS-PAGE and isoelectric focusing were used varietal discrimination[56]. In all separation experiments better resolution of proteins was achieved with dry grain extracts, than with malt extracts. Angora, Sladoran and Martin variety could be distinguished from other varieties by differences in hordein patterns obtained by gradient gel SDS-PAGE (8-18% T), and Angora, Sladoran, Martin and Rodnik by isoelectric focusing in pH gradient 5.5-8.5.

Genetic variation and relationships among thirty four barley cultivars (*H. vulgare* L.) improved in Turkey were assessed by hordeins[57]. Totally, 15 different hordein patterns were observed among 34 cultivars and twelve of these were cultivar specific. Genetic similarity (*GS*) calculated on hordein ranged from 0.52 to 1.00. Cluster analyses based on hordein data showed that most of the cultivars are closely related in genetical point of view. The dendrogram of hordeins couldn't precisely separate the barley cultivars. Correspondence analysis by using two marker systems showed that hordein data were not able to perform to distinguish barley cultivars. Some polymorphic and repeatable RAPDs markers should be equipped with morphologic markers in order to identify candidate cultivars and registered cultivars before and after registration procedure.

Heisel et al.[58] identified fifty-five United States barley cultivars by SDS-PAGE patterns of the hordein proteins. The analyzed cultivars yielded 34 different hordein patterns. Twenty-four cultivars gave unique patterns, whereas each of the others fell into one of 10 groups, each of which comprised from two to seven cultivars.

Oryza

One hundred and fifteen varieties (including cultivars and lines) with different ecotypes in japonica rice (*Oryza sativa* L.) were analyzed for endosperm storage proteins by SDS-PAGE to estimate their genetic diversity for the purpose of genetic improving and variety identification[59]. Nineteen types of profile were identified according to 1) presence/absence of 65 kDa bands, 2) staining intensity of 70, 60, 57, 37-39, 22-23, 13 and 10 kDa bands, 3) migration velocity of 35 kDa (α-4) and 4) band number at 57 kDa location. UPGMA dendrogram based on the cluster analysis of genetic similarity of the protein bands showed a small genetic variation among the tested materials, with the similarity coefficients varying between 0.75 and 1.00. However, studied at the similarity coefficient level of 0.894, three distinct groups were identified from the cluster analysis of the rice varieties. The first group included eight varieties with high amylose content, the second group contained fifteen varieties with high protein content, and the third group had the remaining ninety-two varieties, which accounted for 80% of the total materials. Clear relationship between ecotypes distinguished by maturity and groups revealed by cluster analysis was not found in this study. Only the group of high amylose linked with medium-maturity medium japonica ecotype. The bands of 70 kDa and 65 kDa can be used as protein markers to identify F1 seed purity of japonica hybrid rice Liuyanyou 422.

Eighteen traditional and improved basmati rice (*Oryza sativa* L.) varities were studied for morphological descriptors, total soluble proteins and isozymes as biochemical markers or determining distinctive features[60]. SDS PAGE for total soluble proteins and isozymes analysis revealed moderate and moderate to high degree of polymorphism respectively. UPGMA analysis of combined isozyme data of different enzymes could discriminate all varities except Hansraj from KLS 24. It can be conclude that in situations where the morpho-physiological DUS descriptors are not able to establish distinctiveness of a variety then biochemical and molecular markers may be used as additional or complement descriptors for resolving distinctiveness of basmati rice varities.

Zea mays

The albumins and globulins of the inbred and hybrid corn (Zea mays) were electrophrotically analyzed on improved lactate – PGE[61]. Each inbred or hyrid had its own unique band pattern distinguishable from the others regarding as its fingerprinting. The band pattern of all kernel was basically similar to that of all embryo, except that of the endosperm showing less bands with weaker staining intensity. The band number of the hybrid was exactly equivalent to the number of the common bands and the specific bands of the two parents. This procedure could be used in corn cultivar identification and as a test for genetic purity.

Sorghum

Sorghum (*Sorghum bicolor (L.)Moench)* is an important cereal crop, a significant dietary food for one-third of world population and principle source of energy, protein, vitamins and minerals. Conventionally Grow out Test is used to assay the purity of sorghum hybrid seed lots on a representative sample of the seeds. Grow out Test entails a lot of cost in terms of locked-up capitals and problems of storage. Kovimandon and Khon[62] replaced the Grow out Test with biochemical (protein) assays. In a biochemical assay, A-line (cytoplasmic male sterile), B-line (maintainer), R-line (restorer) and H-line (hybrid) have been screened by

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means of protein markers for polymorphisms. In addition to this, eight open pollinated (pure line) varieties have also been screened by means of protein polymorphisms. Electrophoretic protein profiles could be efficiently used for distinguishing varieties, hybrids and its parents and could be used as substitute of Grow out Test. Biochemical test can also be used very efficiently, in investigating phylogenetic relationships among six Sorghm genotypes[63]. A total of 56 polypeptide bands were scored of which 30 were polymorphic and 26 were monomorphic. The similarity coefficients among six genotypes ranged from 0.500 to 0.900, indicating a moderate genetic relationship.

Poa

P. pratensis is a valuable plant which, under temperate climatic conditions can be used for livestock feeding (hay harvesting and grazing), lawn planting, and soil improvement. Total seed storage proteins were analyzed by SDS-PAGE in genotypes belonging to three *Poa* species, *P. trivialis* and *P.angustifolia*) collected from Turkey[64]. The dendrogram based on protein data with UPGMA clustering method indicated a low intra-specific genetic diversity while *P. pratensis* and *P. trivialis* genotypes stated closer relation as compared to *P.angustifolia* genotypes. The low intra-specific genetic diversity in the three species confirmed the previous work of Tamkoc and Arslan[64] who found a low genetic diversity among 11 Turkish Kentucky bluegrass genotypes. It can be inferred that seed storage protein profiles could be useful markers in the studies of genetic diversity and genetic relationships of *Poa* species, and can be used to determine the correct starting material for plant breeding.

Avena

Oat genotypes exhibit valuable and reproducible polymorphism in the avenin pattern. Avenin pattern is important to identify oat genotypes and to use in breeding programs. Dumlupinar *et al*.[65] used SDS-PAGE technique to identify 96 Turkish oat landraces (*Avena sativa* L.) and three commercial cultivars (Checota, Faikbey and Seydisehir) based on oat avenin protein band numbers and molecular weights. Results showed a high polymorphism in avenin protein band numbers and molecular weights of genotypes.

***Festuca pratensis* Huds**

Fifty two accessions of *Festuca pratensis* Huds. were analyzed using SDS-PAGE of seed proteins to detect genetic variation among ecotype populations[66]. 18 of which had been collected in Switzerland and 32 in Bulgaria. The two cultivars Preval and Cosmolit were used as standards. Cluster analyses and correspondence analyses/scatter plot were used as statistic approaches for determining genetic diversity among individual ecotypes and groups of ecotypes. Electrophoretic spectra of proteins show clear differences among local accessions in relation to their origin. In Swiss ecotypes 32 protein fragments were determined whereas in Bulgarian local populations their number was 68. Each of the two eco-groups possesses fragments that appear in all accessions of the group. The number of monomorphic bands within Bulgarian local ecotypes is four whereas their number in Swiss ecotypes is 12. Four monomorphic bands appearing in all proteinograms no difference of eco-groups was identified with Rm values of: 0.43, 0.55, 0.58, 0.82. A higher level of protein band polymorphism was proven in Bulgarian ecotypes in comparison with Swiss ecotypes. Thirty seven polymorphic bands occurred exclusively in the Bulgarian local ecotypes and had a frequency of 0.03 or higher whereas within Swiss ecotypes was detected one unique protein fragment. SDS-PAGE "fingerprinting" is suggested as a fast and easy approach to differentiate *F. pratensis* ecotypes by their origin as well for detection of foreign germplasm for inclusion in breeding programs

Family leguminosae

Seed protein profiles of 47 accessions belonging to eleven species and four tribes of grain legumes were analyzed by SDS-PAGE[67-73]. All eleven species were clearly recognizable from their protein banding patterns, but only *Phaseolus vulgaris* expressed high intraspecific variations, followed by *Lathyrus sativus*. Variation among accessions of other species was very limited. Cluster analysis, after quantifying the protein bands, using UPGMA procedure, showed phylogenetic relationships which were in a good concordance with species classification based on morphological characters. Accessions of tribe Vicieae formed one cluster *(Vicia faba, Lens culinaris, Pisum sativum, Lathyrus sativus* and *Vicia ervilia)* having nearly equal amounts of three categories of polypeptide: high, moderate and low molecular weight. The second cluster was a small tribe of Cicereae *(Cicer arietinum* accessions) having moderate and low molecular weight polypeptides. Accessions of Phaseoleae tribe formed the third cluster

(Phaseolus vulgaris, Vigna unguiculata and *Vigna radiata),* having predominantly high molecular weight polypeptides. Finally, the more distinct tribe, Aeschynomeneae *(Arachis hypogaea* accessions), formed a separate cluster exhibiting a special banding pattern. A unique discrepancy was observed about *Glycine max,* which belongs to Phaseoleae but was clustered with Cicereae. To ascertain the extent of genetic variation and its geographical distribution, Asghar *et al*.[74] analyzed twenty-nine accessions of *Cicer arientimum* (Chickpea) germplasm for total seed protein profile using SDS-PAGE. A considerable variation in protein banding pattern was observed which was localized to various geographical regions. The albumin and globulins components of the seed proteins of nine Egyptian cultivars of *Vicia faba* were analyzed by SDS-PAGE to measure genetic variation[75-79]. The electrophoregram showed identity profile for each cultivars supporting the validity of electrophoresis of seed protein components in cultivar identification. Principal component analysis and cluster analysis indicated higher role for seed albumins over seed globulins in genetic variation within *Vicia faba* in Egypt

Forty-one wild types and 41 cultivars of common bean (*Phaseolus vulgaris*) from Meso- and South America were screened for variability of phaseolin seed protein using SDS/PAGE and two-dimensional isoelectric focusing SDS/PAGE[80]. Wild accessions from the Andean region showed phaseolin types which had not been previously identified in wild material from that region. Other wild accessions from Argentina exhibited novel phaseolin patterns ('Jujuy') type, and one accession from northern Peru exhibited a novel phaseolin type ('lnca ') type. The 'H" and "C' phaseolins, previously identified only in cultivars, were observed in several wild accessions,from Argentina. Among cultivars, two minor variants of the "S" phaseolin type were identified. The 'Sb" ('S Brazil') was characteristic of a limited number of cultivars from Brazil whereas the 'Sd" ('S Durango 222') predominated in cultivars of the Mexican central highlands. The distribution of the previously described "B" phaseolin appeared to be larger than formerly known as it extended not only in Colombia but also in Central America. It is possible to correlate the "Sb', 'Sd', and 'B" phaseolin types with certain agronomic traits.

Abrus precatorius L. belongs family Fabaceae (Leguminosae), is commonly known as Rosary pea. De Britto *et al*.[81] investigated the genetic variation among seven *Abrus precatorius* L. varieties using SDS-PAGE analysis. Variations as well as similarities were observed in protein profile. Overall 56% of similarity between varieties was observed. Dendrogram showed two major clusters. Varieties white and red were grouped in one major cluster and the remaining varieties were grouped with another major cluster.

The genetic diversity within 60 Ghanaian cowpea (*(Vigna unguicuulata* (L.) Walp) germplasm was examined by SDS-PAGE techniques[82-84]. This study was carried out for the effective utilization of germplasm collections (largely local landraces) for crop improvement that has been hampered by the existence of duplicates and genetically redundant accessions which are not noticeable by morphological markers. Similarity index and simple matching coefficient indicated a high degree of homogeneity in banding patterns. The seed protein data could be used, together with other data, for the elimination of duplicate accessions and for the setting up of a core collection, to reduce maintenance cost and ensure efficiency in the use of the germplasm.

Seed storage protein profiles of three germinated horse gram (*Macrotyloma sar-garhwalensis* Gaur and Dangwal) varieties were analyzed by SDSPAGE[85]. Genetic diversity of germinated horse gram was evaluated by constructing the dendrogram for high molecular weight (HMW) and low molecular weight (LMW) gluten subunit bands. In conclusion, SDS-PAGE of germinated seed storage proteins can be economically used to assess genetic variation and relation in germplasm. The specific bands of germinated seed storage protein profiles may be used as markers for identification of the mutants/genotypes.

The genetic variation of seed protein was assayed by SDS-PAGE for 19 genotypes of soybean (*Glycine max*)[86-88]. 60% of the separated bands were polymorphic, exhibiting good genetic diversity between genotypes. SDS-PAGE data explains %78.018 of the total variation on the first five axes. UPGMA dendrogram depicted seven groups. The SDS-PAGE analysis.

Family solanaceae

Tomato (*Lycopersicon esculentum* L.) is a member of the family Solanaceae and significant vegetable crop of special economic importance in the horticul-

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tural industry worldwide[89-90] Although the genus *Lycopersicon* includes a few species, its taxonomy is still questionable and phylogeny has not been completely established[91]. Tomato (*Lycopersicon esculentum* Miller) is being grown universally. The existing germplasm from Azad Jammu & Kashmir, Pakistan was characterized morpho-genetically during the year 2002-2003 at Rawalakot, to determine the variability among the cultivars and to identify some selectable markers based on total seed protein profiles. The purpose of the investigation was to develop new varieties based on genetical variability among the cultivars and to document the germplasm for future record. The total seed proteins comparisons based on SDS-PAGE profile did not indicate variability among the major bands, however some miner bands were found to be variable in terms of distance traveled in the gel[92].

Biochemical and molecular characterization of eight tomato varieties were carried out based on seed storage proteins electrophoresis[93]. The electrophoretic pattern of water soluble protein produced 4 monomorphic bands, 6 polymorphic band and 3 unique bands. The pattern of non soluble protein produced 9 bands, one band is unique and considered a positive specific band of tomaten cartago variety and the others are polymorphic bands. 37 bands out of the total number were polymorphic and 19 were unique. It could be concluded from the dendrogram based on SDS-Protein that electrophoretic analysis is important for genetic analysis and indicate a considerable amount of genetic diversity between the different studied varieties of *Lycopersicon esculentum* L.

Florina[94] analyzed the protein profiles of tomato seeds by SDS-PAGE method using 2 variants of extraction solution in order to obtain a wider viewing of tomato seed protein subunits. Analysis of protein patterns of 12 different samples showed no significant qualitative differences between genotypes. Four varieties)Pontica, Carisma, Siriana and Coralina) showed a higher number of bands. Both extraction methods have led to the development of several bands located between 3 kDa and 142 kDa in most genotypes. Small quantitative differences were observed in the level of 90 kDa and 41 kDa using the first version of solution extraction compared to the second one. Anyway, a high level of homogeneity was detected with both methods used.

Hasan *et al*.[95] distinguish fifty-four accessions of cultivated and wild groups of eggplant *(Solanum melongena* L.) and its nearest wild species by SDS-PAGE of soluble seed proteins. The results of electrophoretic banding patterns show that there was a great deal of variation within and between groups of eggplant in terms of numbers, sizes, positions, staining intensities and presence or absence of protein bands in the profile, This variation can be used for characterization and identification of eggplant cultivars. Elongated fruit eggplant cultivars exhibited nine common group-specific bands which distinguish them from the other groups. Meanwhile, a great variability in seed protein profiles was detected in the round fruit type and primitive cultivars, weedy and wild groups of eggplant including the truly wild *S. incanum*, which indicated that these groups consist of diverse genotypes.

Soluble protein pattern of seeds of ten chilli varieties as obtained on SDS Polyacrylamide Gel Electrophoresis (SDS-PAGE) and Isoelectric Focusing (IEF) were used to identify these varieties[96]. Qualitative as well as quantitative differences in the bands were obtained. Isozymes study was also done at two different stages i.e. at 7 and 14 days after germination (DAG) of chilli seedlings. IEF was found to be more effective for cultivar identification as compared to SDS-PAGE. Isozymes study at 7 DAG of seedling proved better for identification of different chilli cultivars as compared to 14 DAG. In conclusion, electrophoretic technique can be utilized efficiently for varietal identification in Chilli.

Family compositea

The identification of sunflower hybrids KBSH-1, TCSH-1, PKVSH-27, APSH-11 and DSH-1, their parental lines and the varieties, Morden, Surya, AKSF-9, CO-2, CO-3, CO-4, GAUSF-15, NDSH-15 and SS-56 was possible from the genotype specific intensity of dark, light and medium bands as well as their relative position in the seed protein and isozymes profiles analyzed by SDS-PAGE[97]. Their characterizations were difficult through the total number of bands and as several of them were common in more than one genotype.

Family euphorbiaceae

Jatropha curcas has recently come into prominence as source of bio-diesel. This species has naturalized stands spread across China and due to varied climatic

conditions is likely to have a high degree of variability. Biochemical and electrophoretic analysis in *J. curcas* was studied in seven seed sources collected from Central Himalayan zone[98]. Considerable differences in the quantity of individual polypeptides as displayed by the density of bands, was found among the seed sources. Among all the seed source Kalachauna and Saknidhar had highest similarity of 100% whereas Gandhari had the lowest ranging from 42 to 57%. Cluster analysis based on protein bands identified Saknidhar, Kalachauna, Naithana and Basnal in one group, Basnal and Dhruvpur in another group and Gandhari as a distinct seed source. Five esterase isoenzymes and 3 each of acid phosphatase and peroxidase were recorded in the seed sources of which more variation between seed sources were in esterase than in rest two enzymes. Kalachauna and Dhruvpur had 100% similarity sharing all the isoenzymes present whereas Sakanidhar and Naithana also had 100% similarity but had 1 isoenzymes of esterase lacking. As compared to esterase and peroxidase the isoenzyme pattern of acid phosphatase was quite similar in all the seed sources of *J. curcas* studied here.

Family citraceae

Genetic differences of 18 cultivars and/or ecotypes were detected by isozymes[99]. Fruits and endosperms of loquat cultivars were taken early in the morning and analyzed in the same day using PAGE for alcohol dehydrogenase (ADH), catechol oxidase (CO), glutamate oxaloacetate transaminase (GOT), acide phosphatase (HP), and peroxidase (PER). All enzyme systems were also analyzed in the leaves but ADH was not detected in this plant tissue. As a result of this experiment, for ADH, all cultivars had the same banding patterns in fruits. Some biotypes within the same cultivars such as Yuvarlak Çukurgöbek and Tanaka had different banding patterns of endosperm. In CO enzyme system extracted from leaves, fruits and endosperms had different banding patterns. All cultivars had the same banding of GOT in each of the plant tissue. But band patterns changed among tissues. In HP, three patterns were observed in the endosperm. On the other hand, leaves and fruits of loquat cultivars had only one isozyme pattern with five bands. There were one banding pattern with two bands for PER in loquat fruits, while three distinct banding patterns were observed in endosperms and leaves. Akko 1 and Akko 13 can be easily distinguished with PER isozymes of leaves, whereas Tanaka and Yuvarlak Çukurgöbek had the same banding patterns for PER in the each of three plant tissues. Isozymic differences between cultivars suggested that isozymes may provide useful markers for cultivars identification in loquat.

Family elaeagnaceae

Elaeagnus umbellata (Thunb.), commonly known as autumn olive, belongs to the family Elaeagnaceae and is native to the Himalayan regions of Pakistan, China, and India and is also found in Korea and Japan. The seeds of 8 ecotypes from Azad Jammu and Kashmir, Pakistan, were analyzed for comparisons of their relationship and evolution based on SDS-PAGE of total seed proteins[100]. The results indicated that each autumn olive population can be distinguished by their own specific protein bands with reference to a molecular weight marker included in the gel. The dendrogram based on computer package analysis indicated that populations having the same base of origin fall under 2 simultaneous groups. The distribution of the populations was variable irrespective of their physical location as the seeds of the plant may have been dispersed by birds from distant places. SDS-PAGE thus provided valuable information for the identification of populations and could be utilized for population and varietals discrimination as well as seed quality test in true to type seed producing plants.

Family palmaea

Date palm (*Phoenix dactylifra* L.) is one of the most important fruit crops in the Middle East. Little knowledge is currently available about the molecular characterization of date palm cultivars. Determination of genetic variability and variety identification in date palm are two major importance in breeding programs, characterization of germplasm, and conservation purposes. Four varities in Saudi Arabia (Med300l b1, Sugay1 b1, Khalas b1 and Sukkarib1) were analyzed by SDS-PAGE and other molecular markers (RAPD and ISSR)[101]. Protein markers showed inter-varietal polymorphism. Cluster analysis by UPGMA grouped the four varities into two clusters. Cluster A included Sukkarib1 and cluster B consisted of 3 other varities. Sugay b1 and Khalas b1 were the two most closely related varities among the four cultivars with the highest similarity value (0.85). The average similarity among

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the four cultivars was a bit more than 55%. The varities had narrow inter-varietal genetic diversity. The result of the analysis can be used for the selection of possible parents to generate mapping populations

Family brassicaceae

Seed protein profile and esterase isoenzme were studied in ten accessions of lemongrass [*Cymbopogon flexuosus*] collected from northeastern India belonging to two major chemotypes – citral and geraniol rich essential oil[102]. Four esterase isozyme bands were observed with Rm values in the range of 0.394 to 0.798. Among them one with Rm value 0.747 was unique as it was consistently present in all the accessions irrespective of citral or geraniol rich chemotype. For seed protein, SDS-PAGE analysis of seven accessions (RLJ-TC-1, RLJ-TC-4, RLJ-TC-5, RLJ-TC-8, RLJ-TC-9, RLJ-TC-10 and OD-19) revealed a total of 13 bands among the accessions ranging in the size from 21.5 to 92.0 kd. Six citral rich accessions exhibits very similar seed protein profile with 10 to 11 protein band each. However, the geraniol rich chemo-type RLJ-TC-8 exhibit different profile with only six high molecular proteins. Four seed proteins with molecular weight 92.0, 86.0, 80.4 and 61.6 kd were consistently found in all the chemotypes irrespective of citral or geraniol rich and can be considered as marker for the species. Although esterase isozymes exhibited low polymorphism, yet close similarity of isozyme and seed protein profile can be considered as evidence of genetic homogeneity among the accession of lemongrass.

Family fagaceae

Total seed proteins of four *Quercus* species extracted from bulked seed samples were analyzed by modified SDS-PAGE in order to obtain additional taxonomically useful descriptors[103]. A total of 7 alternative protein bands with different mobility rates were identified within a molecular weight range of 24 kDa to 36 kDa. *Quercus robur* L. and *Q. petraea/Matt.fLeibl.* showed equal electrophoregrams. *Q. pubescens* Willd. can be discriminated from *Q. robur* L. and Q. petraea/ Matt./Leibl. by its two additional bands. *Q. rubra* L. showed a significantly different electrophoregram with completely novel protein bands).

Family apiaceae

Coriander (*Coriandrum sativum* L) is an annual herb (2n=22), which belongs to the family Apiaceae and generally grown in winter season as main crop in India. Singh et al.[104] evaluated SDS-PAGE as a tool for characterizing coriander varieties. The SDS-PAGE electrophoresis of total soluble seed proteins revealed a total of 7 bands with Rm value ranging from 0.347 to 0.926. A polymorphism of 71.4% was recorded among 20 varieties of coriander. The maximum dissimilarity value was shown by Sindhu. Varieties like Sadhana, Sindhu, RCr-436 and RCr-684 were observed different from the rest of the coriander varieties whereas, RCr-436 and RCr-684 could not be differentiated from each other.

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