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REVIEW ARTICLE

Legume Seed Storage Proteins–A Review

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ABSTRACT
Malnutrition seems to be alarming in every country in the world. Legumes are the reservoir of seed storage proteins which serves as the cheapest source of dietary proteins. These seed-specific proteins have the potential to partially replace non-vegetable proteins in human diet. Insufficient intake of proteins leads to serious health problems e.g., cancer, blood pressure, hypertension, diabetes, obesity and immune deficiency. Besides, seed proteins retain insecticidal and antimicrobial properties. These reserve biomolecules undergo proteolysis to release energy, carbon skeleton, nitrogen and sulphur required for germination and seedling growth. There exists a wide interspecific and intraspecific variation in quantity and quality of seed storage proteins among legumes. The author reviewed the present status of genetic variation in legume seed proteins in addition to their biosynthesis, transport, hydrolysis, electrophoretic separation, inheritance pattern and molecular basis of expression. This may be useful for planning for genetic improvement of seed storage proteins in cultivated legume species.

Key words: *Malnutrition, legumes, seed storage proteins, biosynthesis, genetic basis, genetic variation*

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INTRODUCTION

Legumes are second to the cereals in providing foods in most of the countries. The total world value for leguminous crops is approximately more than two billion US dollars per annum. These food crops are rich in seed protein which serves as the major and cheapest source of dietary protein of half of the world population [1,2] and it can be as high as 40% in legumes. Because of the low glycaemic index (GI) and the high content of undigestible fibres, legume seeds can prevent type II diabetes. Legume seed proteins have the potential to partially replace meat and dairy products in the human diet. This will help to meet the increasing worldwide demand for proteins. This is a major concern of our policy makers and researchers to ensure nutritional security of the country which houses one-fourth of undernourished living in the world. In India, the availability of pulse grains is 31.6g/capita/day against a normal requirement of 80gm/capita/day and a minimum of 50gm/capita/day [3]. Legume seed proteins protect human health due to their reduced calorific content, reduced or no effect on blood glucose levels (glycemic index) and improved heart health [4]. In contrast to cereals, these are deficient in S-containing amino acids (methionine, cysteine and cystine) and tryptophan but rich in lysine [5]. Seed proteins maintain structural integrity against desiccation in matured seed and undergo hydrolysis at germination to provide free amino acids, ammonia and carbon skeletons to the developing seedlings. Besides, these not only serve as source of constructive and energetic compounds, but they also have bio-active roles by themselves and/or act as precursors of biologically active molecules. Seed proteins are often involved in plasma lipid and glucose homeostasis, inhibition of hydrolytic enzymes, blood pressure control and immuno-modulation. In this pursuit, the author explore quantitative and qualitative variation of different sources of seed proteins in addition to their cellular localization, hydrolysis, inheritance pattern, molecular basis of expression, electrophoretic separation and insecticidal and/or antimicrobial properties in legumes.

DEPOSITION OF SEED STORAGE PROTEINS

Legumes are important source of protein for human diet. The majority of the food proteins are present in seeds in from of storage proteins. These are characterized by their ability to store stable reserve amino acids and provide carbon, nitrogen and sulphur to support seed germination and seedling growth. Each of the storage proteins in the matured seed as well as at different stages after germination can be identified by Mass spectrometry (MS) or 2D-PAGE [6].

Storage proteins of legumes are deficient in sulfur amino acids and those of cereals are poor in lysine and tryptophan. Storage proteins contain an N-terminal signal peptide which depicts their transportation to endoplasmic reticulum (ER). Storage proteins are located in the endosperm in cereal seeds and in the cotyledons of most legume seeds. In legumes, seed proteins are specifically transported via the golgi apparatus and stored in protein storage vacuoles (PSVs) of cotyledonary cells of mature seeds [7,8], whereas cereal prolamins are mainly localized in cisternal space of endoplasmic reticulum [9]. The PSVs are gradually filled up with storage proteins and subdivide to form tens of thousands protein bodies (PBs). In the young parenchymatous cotyledonary cells, the PSVs are large vacuoles which fragments to form protein bodies (around 1 μm diameter) and subsequently disappear with the advancement seedling development.

HYDROLYSIS OF SEED STORAGE PROTEINS

A major metabolic event in the germinating seed is the hydrolysis of the seed protein reserves to provide nutrients for the growing seedlings necessary before photosynthesis is established. Following germination the storage proteins are initially hydrolysed by endopeptidases particularly cysteine peptidases(CPs) producing oligo-peptides within the vacuoles and then the oligo-peptides are transported to cytosols by (CPs)where they finally degraded by exopeptidases (serine carboxypeptidases, aminopeptidases etc. -non-specific cleavage of peptde bonds) to form free amino acids which serves as precursors for the synthesis of new proteins and other nitrogenous compounds in the seedling [10]. The CPs fall into two major families, the papain like CPs and legumain-like CPs. The most active forms of the above two categories are proteinase A and proteinase B respectively. The former exhibits non-specific cleavage of peptide bonds while the Legumain like CPs cleave specific peptide bonds. The Legumain- like CPs acts its proteolytic activity only after the seed storage protein being subjected to limited hydrolysis by papain-like CPs.

SEED PROTEIN CONTENT AND INHERITANCE PATTERN

Storage proteins account for 70–80% of the total quantity of reduced nitrogen in mature grains. Mungbean contains globulin (70%), albumin (15-20%) and traces of prolamin [11]. There exists a wide variation in protein content that ranged from 18.42 to 29.96%.in munbean[12]. Varieties e.g., cv.Jyoti, Kodala local A, Jagatsinghpur local A, Sudhasarangi local B and Anandapur local-A have been reported to be protein rich (>26%). Yohe et al. [13] evaluated 313 accessions of mungbean and the range of protein content was 19.1 - 28.3% with a mean of 24%. Bhadra et al. [14] found high protein cultivars to be small seeded and low yielder. Akram and Arshad[15] estimated the range of crude protein to be 25.2-38% in mungbean. Working with 37 land races in Odisha, Naik [16] and Naik et al.[17] found a wide range of variation in protein content which varied from 17.2 - 29.9%. Pigeon pea seed is made up of 85% cotyledon, 14% seed coat, and about 1% embryo and it contains 23.8% protein but, harbours high proportion of anti-nutritional factors, e.g, stachyose and verbascose. Besides, seed protein content ranges from 20-30% in *Phaseolus vulgaris* L[18], 13.7-30.7% in *Pisum sativum* L.[19], 22.4-27.9% in *Vigna unguiculata* [20] , 22-38% in *Vicia faba* L. [21] and up to 38–40% in soybean and lupin [22].

Singh and Singh[23] and Malhotra and Singh[24] reported that protein content in mungbean seeds was moderately heritable. Both additive and non-additive gene actions were important in the inheritance of protein content but additive gene action played the predominant role [23]. Tiwari and Ramanujam [25] found additive gene effect on total protein yield. Malhotra and Singh [24] faced difficulty in simultaneous improvement of yield and protein content in seed. Chandra *et al*. [26] opined protein percentage in mungbean seed as a polygenic trait coupled with some degree of maternal influence. Tomooka *et al*. [27] illustrated that variation in seed protein content present in mungbean could be used for differentiating genotypes. In mungbean, two F_2 populations were shown to have monogenic segregation of polypeptide bands, presence being dominant over absence [16]. Further he could establish linkage between some polypeptide bands.

INSECTICIDAL AND ANTIMICROBIAL ACTIVITIES OF SEED PROTEINS

Legume seed extracts also serve as source of proteinase inhibitors, trypsin inhibitors and lectins [28]. Insecticidal properties of proteinase inhibitor from pigeon pea [29] and trypsin inhibitor from adzuki bean [30] have been demonstrated. Horse gram (*Dolichos biflorus*) seeds are rich in lectins (30kD) that reduces fecundity of insects while, alfalfa (*Medicago sativa* L.) seeds are reported to contain putative plant antibacterial peptides [31].

Wild urdbean is immune to several important bruchid species. A novel 40-kDa peptide in urdbean and 33kD arcelin in wild *Phaseolus vulgaris* seed are reported to cause lethality to bruchids [32]. The vicilin protein fraction (7S globulins) isolated from resistant cowpea seeds inhibits the midgut digestive enzymes of bruchids (*C. maculatus*) [33]. There exists a significant positive correlations of seed resistance to bruchids with trypsin inhibitor in cowpea. The highest trypsin inhibitor activities has been reported in cowpea genotype GC82-7 whereas highest proteinase inhibitor and α-amylase inhibitor activities are inherent to cv. TV 7. *Cajanus albicans* and *Vigna bourneae* show bruchid resistance due to higher levels of trypsin/chymotrypsin inhibiton. In human system, intake of α-amylase inhibitor in legume seeds (as food component) has a role in body weight control and diabetes management. Similarly, lectins in various sources of legume seeds has role in immuno-modulation and anti-cancer properties while, Conglutin-γ in lupin seeds has Hypoglycamic property.

ELECTROPHORESIS OF SEED PROTEINS

Electrophoresis is the migration of charged molecules in solution in response to an electric field. Their rate of migration depend on the strength of the electric field as well as the net charge, size and shape of the molecules; and also on the ionic strength, viscosity and temperature of the medium in which the molecules are moving. SDS is an anionic detergent which denatures proteins by wraping around the polypeptide backbone and it binds to proteins fairly in a mass ratio of 1.4:1 which confers a negative charge to the polypeptide in proportion to its length i.e., the denatured polypeptides become "rods" of negative charge cloud with equal charge or charge densities per unit length. It is usually necessary to reduce disulphide bridges in proteins before they adopt the random-coil configuration necessary for separation by size. As an analytical tool, sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) is a simple, rapid and highly sensitive technique. It is used to study the properties of a single charged species and as a separation technique of polypeptides of different molecular weights. This technique offers two distinct advantages. Polypeptides migrate according to molecular weight on SDS gels so that molecular weight of polypeptides can be easily estimated. At the same time, SDS-PAGE has become a potent technique for resolving mixtures of many insoluble proteins, especially membrane proteins which become solubilized by SDS.

TYPES OF SEED PROTEINS

Total seed protein consists of several kinds of protein fractions which differ in sedimentation constant (S), molecular weight (kd), amino acid composition and solubility properties. From extraction point of view, seed proteins are classified into four protein classes on the basis of their solubility [34]. These are (1) Albumins- soluble in water, (2) Globulins- insoluble in water, but soluble in salt solutions, (3) Prolamins- insoluble in water and salt solutions, but soluble in alcohol /water mixtures (4) Glutelins- insoluble in dilute acids or alkali solutions. In legumes, seed proteins comprised mainly globulins (70%) and albumins (15-20%) as reported by Aykroyed *et al*.[11]. Besides, several other proteins, such as, arcelin, lectin and prolamin are also found in lower proportions. In contrast, major storage proteins of cereals are prolamins and glutelins with exception in oat where globulin is the major class of storage protein. The prolamin fraction of *Hordeum vulgare* is termed as hordein and that of *Zea mays* is named as zien. A new chimeric seed storage protein "Zeolin" is composed of phaseolin (from P. vulgaris) and 89 amino acids of gamma-zein(from zea mays) and it tends to accumulate to very high amounts in leaves of transgenic tobacco [35]. It is insoluble unless its disulfide bonds are reduced and forms endoplasmic reticulum-located protein bodies. Because the storage proteins of cereals and legumes nutritionally complement each other, zeolin can be used as a starting point to produce nutritionally balanced and highly stable storage proteins. Albumins and globulins are the major seed protein fractions in leumes which are detailed below.

Globulins: Globulin is the major seed storage protein fraction in pulses [36] which constitutes about 65% of total seed proteins [37]. The globulins consist of two major families of proteins e.g., 11S legumin and 7S vicilin which differ in size and sedimentation values but are structurally related and have similar evolutionary roots. In leguminous plants, the 7s and 11s globulins appear to be in the same protein bodies with no spatial separation. The mature 11S globulins are typically composed of six subunits of

approximately 60kd each in size and these are deficient in cysteine and methionine. Post translational processing cleaves each subunit into acidic and basic polypeptides linked by a single disulphide bond. In contrast, the mature 7S globulins consist of three subunits ranging between 40 and 80kd in size. These globulins (7S) lack cysteine residues and are also poor in other sulphur amino acids [38]. The 7S globulin α′ chain in soybean seed protein brings about up-regulation of LDL-receptors, plasma cholesterol and triglyceride reduction in human system.

Vicilin (7S) is the major storage globulin in mungbean [39]. It is rich in acidic amino acids and deficient in sulphur amino acids (cystine, cysteine and methionine). Amino acid composition of mungbean vicilin resemble to that of *vicia faba* [40]. However, considerable variation is noticed in terms of number and size of vicilin subunits among *Vicia faba* and *Cicer arietinum.* At least four clearly definable bands comprising four subunits with molecular weights of 63.5, 50, 29.5 and 24kd were reported following SDS-PAGE of vicilin seed protein of mungbean [41]. In contrast, Thakare *et al.* [42] observed four polypeptide bands at 58, 54, 36 and 27 kd of mungbean vicilin in the developing cotyledon of a variety, ML 5. Legumin (11S), is another storage globulin that constitutes lower proportion in seed [41]. Other proteins include albumin, lectin and trypsin inhibitors [43].

Albumins: Albumin fraction of seed protein constitutes about 10-15% of total seed proteins. The major albumin family is 2S albumin which consists of several groups of unrelated proteins. Notable among these are trypsin inhibitor and phytolectin [44]. 2S albumins are processed as large and small subunits. Structurally, albumins contain relatively more number of sulphur-amino acids than globulins. Most 2S-albumins contain conserved cysteine residues which are bound by intra-chain disulphide bonds within large subunits and inter-chain disulphide bonds between different subunits. The Brazil nut albumin (BNA) and sunflower seed albumin (SSA) are rich in methionine. Genes for these proteins could be introduced into sulphur- poor legumes [38].

VARIATION IN SEED PROTEIN PROFILES

Protein electrophoresis is a powerful tool for population genetics [45]. As storage proteins are not substantially affected by environmental fluctuations, their profiling using SDS-PAGE technique is considered to be a reliable tool for economic characterization of germplasm [46, 47]. Variation on the basis of protein peptides has been reported by Rao *et al*. [48] and Jha and Ohri [49]. Ferguson and Grabe [50] and Murphy *et al.*[51] indicated the potential of electrophoresis techniques for determining the extent of genetic variation in crop germplasm.

a) Inter-specific variation

Seed protein profile is species-specific. Pollard *et al*. [52] reported electrophoresis (SDS-PAGE) as one of the most effective method for identification of grain legume species and varieties. Different banding pattern of seed proteins has been documented among grain legumes e.g. French bean [53], *Vicia faba* [54]. Teparybean [55], Groundnut [56], chickpea [57], Soybean [58], *Lathyrus* [59] and Yearbean [60]. Cooke [61] and Hussain *et al.* [62] advocated studies on electrophoretic patterns of seed protein to verify varietal identity of different species. Gallez and Gothileb [63] used gel electrophoresis techniques to trace the taxonomic difference in plants. Valizadeh [64] studied seed storage protein profiles of 47 accessions belonging to eleven species including mungbean, *Lathyrus*, cowpea, soybean, *Vicia faba*, pea, *Lens culinaris*, chickpea, groundnut *Phaseolus vulgaris* etc and four tribes of grain legumes. All eleven species were clearly recognizable from their protein profiles, but only *P. vulgaris* expressed high intra-specific variations followed by *L. sativus*. Thakare *et al*. [42] used SDS-PAGE to examine the banding pattern of vicilin. The closely related species e.g. mungbean and urdbean and also their wild putative progenitor Vigna sublobata are altogether different in polypeptide banding pattern. The accessions of *V. sublobata* resemble mungbean and urdbean with varying degree of similarity in electrophoretic banding pattern. The results supported the theory that *Vigna radiata* and *Vigna mungo* might have been evolved from two distinct forms of *Vigna sublobata* namely Var. Sublobata and Sylvestris respectively. Ghafoor *et al*. [65] revealed specific bands of seed proteins to identify vigna radiata and *Vigna mungo*. Tripathy *et al.*[66] reported that Mayurbhanj local (urdbean) and TCR 213 (a wild accession of *V. sublobata*) had absence of a globulin band with molecular weight 27.5 kd and 30.2 kd respectively, but such bands are present in all accessions of mungbean leading to serve as molecular marker(s) for species identification. Chandel *et al*. [67] stressed on comparative morphological studies as well as protein finger printing pattern to trace evolutionary relationship of mungbean and urdbean with their wild ancestors. Eggi and Potokina [68] suggested that SDS-PAGE of seed storage protein could be used to determine the breeding systems of legume species. Specific bands of seed proteins can be used to identify vigna radiata and *Vigna mungo*. Rao *et al*. [48] observed species -specific seed storage polypeptide bands in 10 *Vigna* species analysed through SDS-PAGE.

b) Inter-varietal variation

Mungbean : Sahai and Rana [69] reported almost similar protein profiles (SI 93.3%) of two varieties (S-9 and Pusa Baisakhi) of mungbean(*Vigna radiata* L. Wilczek). They did not assign the polypeptide bands to any particular protein type. Tripathy *et al*. [66] using a set of mungbean genotypes reported that two mungbean varieties (C No.3 and C No.36) having similar banding pattern differed in thickness of bands. Kole *et al.* [70] identified five distinct zones in the polypeptide banding pattern of storage seed protein. Tomooka *et al.* [27] carried out electrophoretic assessment of 581 local strains of mungbean collected from different regions of Asia. They observed 8 protein types on the basis of combination of four albumin and two globulin bands. The frequency of each protein type strain showed a clear geographical cline. Naik [16] studied crude proteins of mungbean, extracted with 0.5 M NaCl through SDS-PAGE. He observed nine densely stained polypeptide bands at molecular weight 62.4, 61, 58.2, 55.6, 48.4, 35.1, 30.2, 27.5, 19.5 kd. Mohanty *et al*.[71] could differentiate 24 test genotypes into 14 protein types based on SDS-PAGE of albumin and globulin seed storage protein fractions. Ten genotypes were observed to have different unique protein types which facilitated varietal identification. Ghallab *et al.* [72] correlated superiority of two mungbean genotypes (L 2520 and L 1720) in seed yield with absence of two bands at around 94.6kd and presence of a 12.1kD polypeptide band under drought stress condition. Similarly, a low molecular weight polypeptide marker approximately at 12.8kD revealed for drought tolerance in mungbean [73].

Urdbean: Seed storage protein profiling has been standardized in urdbean [74]. Tripathy *et al.* [75] revealed contrasting genotype-specific polypeptide bands in 14 genotypes out of 20 test enotypes based on total seed storage protein profiling. Thakare et al. [42] observed two out of 86 accessions of urdbean (*Vigna mungo*) to differ from the rest in vicilin protein banding pattern following SDS-PAGE. One such accession, U 196 had an extra band at 50 kd in addition to the four major polypeptide bands of molecular weights 69, 59, 56 and 55 kd. However, Sahai and Rana [69] obtained completely similar (SI 100%) banding patterns in two varieties (Mashin-48 and Pusa-1). Ghafoor *et al*. [74] observed low inter-accession diversity and no clear differentiation among a set of 111 genotypes of urdbean on the basis of SDS-PAGE of seed proteins. This was attributed to either exchange of similar germplasm lines between neighbouring regions or the germplasm lines may represent as descendant/progenies of the same ancestors. Genotypes with similar banding patterns are suggested to be studied for detailed agronomic and biochemical analysis including 2-D electrophoresis and DNA finger printing for better management of the gene bank [76, 77]. Clustering of advanced breeding lines along with an approved variety in one group revealed that only a portion of the genetic diversity has been exploited for improvement of black gram.

Pigeonpea : Seed storage protein profiling of 30 pigeon pea genotypes including two popular land races of Odisha revealed 21 polypeptide bands ranging from 11.0 to 135.4kD using silver staining technique [78]. Pigeon pea was shown to have two characteristic dense and broad yellow bands approximately at 39.0 and 52.5kD; and one dense black band at 16.4kD using silver staining technique. They revealed characteristic genotype-specific polypeptide banding pattern in 15 test genotypes. Maruteru, Manak, Gajapati local, ICPL 2376 and ICPL 88039 had distinct unique protein types as these revealed high degrees of dissimilarity with rest of the genotypes.

Cowpea: Cowpea (*Vigna unguiculata*) is a close relative of mungbean. A comprehensive study of the polymorphism in banding pattern of "Vicilin like" Vignin (or G1 fraction) of cowpea involving 81 cultivars and 55 wild accessions through SDS-PAGE revealed 17 unique banding pattern [79]. Out of this, 13 unique protein profiles were observed in one accession each. Wild accessions within the species exhibited more variation in banding pattern than cultivated varieties. However, it was not possible to ascertain the geographical distribution, centre of diversity, or the progenitor of cowpea due to tremendous variation in wild taxa. In contrast, Gomathinayagam and Ramaswamy [80] obtained 9 definable bands showing uniform banding pattern in two varieties of cowpea by SDS-PAGE of seed protein. Prasad *et al.*[81] were able to associate the major variation in the protein bands of molecular weight about 24kd in relation to genetic variation in six varieties of cowpea for insect resistance.

French bean: Seed protein profiles of french bean have been widely investigated by many workers [82, 83, 84]. Brown *et al*.[84] while screening 107 cultivars reported that the majority of the lines contain T- (25%) or S- (69%) phaseolin types (Globulin-1) but polymorphism for C-phaseolin pattern was up to the extent of 6%. Lioli and Bollini [85], however, observed four phaseolin variants in five cultivars. Land races and wild accessions have been shown to have much variation in phaseolin banding pattern than cultivated varieties [82]. In screening 23 wild Mexican accessions, six new phaseolin types (M1- M6) were observed. In addition, Gepts et al. [86] reported another two new phaseolin types, A and H.

Screening of different sets of germplasm accessions by some workers [87, 88] has been reported to have genetic variation for lectins and the genotypes were classified into four groups A, B, C and D. They observed 11 different lectin variants and the absence of lectin based on two dimensional iso-electric

focusing and SDS-PAGE. However, wide range of polymorphism in arcelin protein is the characteristic feature of wild accessions of french bean [89]. Four definable variants were recognized and these were named as arcelin 1, 2, 3 and 4.

Chickpea: Ahmad and Slinkard [90] carried out separation of seed storage proteins of a set of cultivated varieties of chickpea (*Cicer arietinum* L.) and eight wild related species; and compared their polypeptide banding pattern by SDS-PAGE. Kharkwal [91] conducted, a study for intra-specific relationship using electrophoretic analysis of seed proteins of four chickpea varieties. The results indicated that the desi and kabuli types were of same species. Additionally, no evidence was obtained to indicate that kabuli types should be *Cicer kabulicum.* All four varieties were highly homologous. The numbers of bands observed were nine in each. Singh *et al.* [92], identified 2-3 major bands and a variable no. of minor bands in nine chickpea varieties analysed by non-denaturing PAGE. Afzal *et al*. [93] could establish genetic relationship in the genus, Cicer L. They characterized the total seed storage protein electrophoregrams of the test genotypes into different protein types.

Iqbal *et a*l.[47] Identified 12 SDS-PAGE markers, six of which were polymorphic among 57 genotypes. They reported no association between disease reaction to Ascochyta blight and SDS-PAGE markers, and the cluster analysis revealed mixed grouping of susceptible and tolerant genotypes. SDS-PAGE of seed storage protein seems to be less effective in resolving intra-specific genetic diversity in cultivated chickpea for disease reaction and therefore, wild Cicer spp. are suggested to be included.

Electrophoresis of seed storage proteins was found to be a potent technique for genotyping and genetic diversity in chickpea [94]. They used 50mM phosphate buffer (pH 7.8) for extraction of total storage protein and the electrophoretic data of SDS-PAGE of eight Kabuli (white seeded) genotypes revealed three clusters. Identified protein markers i.e., KSSP-100, KSSP-93, and KSSP 64 could be used for identification of an exotic genotype ILC 195 and two mutants e.g., CM 98/99 and CM 2000 respectively. These genotypes were reported to be highly divergent from other test entries. A combination of above identified markers was also found to be potent enough to discriminate CM 315/99 and Noor 91 from rest of the test genotypes. However, three genotypes e.g., Pb 1, CM 94/99 and PKV Kabuli 2 exhibited 100% homology and grouped in one cluster. Use of 2-D electrophoresis of proteins and DNA finger printing have been suggested to discriminate genotypes with similar protein profiling.

Fieldpea: Electrophoresis of cotyledon storage proteins could be employed for varietal identification in field pea [95]. Proteins isolated from storage protein vacuoles in the cotyledon of 18 h water imbibed seeds contained stored protein reserves in addition to the hydrophobic integral tonoplast membrane protein TP 25(25kd) [96]. Jose et al. [97] observed wide array of seed storage protein variation in 20 land races collected from Nilgiris district of Tamil Nadu, India.

Faba bean: Fava bean(*Vicia faba* L.) accumulates large amounts of proteins during seed development [98]. Globulin are the major seed proteins in faba bean [99]. Globulins comprise about 69% to 78% of the total seed proteins and are generally rich in aspartic and glutamic acids, leucine, and arginine [100]. Vicilin and legumin are the major globulins in fava bean. Vicilin is formed in the developing seed before legumin [101], but legumin is more abundant than vicilin in seed. Albumins are biologically active water-soluble proteins, with a higher amount of methionine and cysteine compared to globulins [100], involved in cotyledonary cell metabolism.

Pea : Ghafoor and Arshad [102] carried out SDS-PAGE of seed storage proteins of pea (*Pisum sativum* L.) and observed 25 subunits among these 20 were polymorphic. The protein banding data were investigated in relation to agronomic traits evaluated for two years that indicated influence of polymorphic bands on quantitative traits. Particular clusters based on seed storage protein finger printing were better for specific desirable agronomic traits that were suggested to utilize in crop improvement programme. Ullah *et al.* [103] characterized 34 pea accessions based on SDS-PAGE of seed proteins especially glutenin sub-units. Variations were observed in the density of some common bands as well as presence or absence of the minor bands. The phyllogenetic analysis revealed that genetic diversity is independent or origin or source of the genotypes. Cluster analysis showed separate groups for many genotypes having same origin.

Adzuki bean: The seed storage proteins of 434 strains of Adzuki bean (Vigna angularis) were analysed by SDS-PAGE. Fifteen protein types were identified basing on the combination of polymorphic bands. The geographical distribution of total protein types differed among regions from where the strains originated [104]. Yamaguchi and Kosuge [105], Mori *et al.* [106] and Takehisa *et al.* [104] have revealed variation in seed protein polypeptide banding pattern among Adzuki bean (*Vigna angularis* L.).

Lentil: Seed protein of 14 cultivars of lentil (Lens culinaris) was studied by Yuzbasioglu et al. [107] using sodium dodecyl sulphate polyacrylamide gel electrophoresis. Similarity coefficients(SM) were converted into distances (D) using formulae D= 1-SM. Seed protein distances ranged from 0.00- 0.80. The dendrogram based on distance matrix indicated two distinct clusters that included three and eleven

varieties respectively. Sultana *et al.* [108], however, analysed 144 lentil genotypes collected from different parts of Pakistan using SDS-PAGE and obtained 13 polypeptide bands. The cluster analysis did not prove any correlation between ecological diversity with genetic diversity in lentil. This could be due to the fact that a small sample of accessions from a particular region might not reflect the actual diversity within that region.

MOLECULAR BASIS OF STORAGE PROTEIN GENE EXPRESSION

Storage protein genes are often members of some gene families. Members of each family usually show minor variation in gene sequences resulting in major differences in amino acid content within the family of storage proteins. For example, the rice glutelin B sub-family has about five members sharing 80-88% identified at the nucleotide level. Each member of the gene family may differ in expression as well as regulation level. Recently, a number of genes for seed storage proteins have been identified. In majority of the cases, the regulatory mechanism is concerned with conserved sequence (e.g., *legumin box, vicilin box*) in the promoter region for seed-specific expression of the genes. An attempt to use duplication of legumin box(CATGCATG) in the seed-specific promoter may be useful in improving the nutritional quality of seed proteins. Besides, the transcriptional factor (PvAif) mediated over expression of genes for seed storage proteins (phaseolin and phytohaemaglutinin) have been achieved in french bean [109]. Methionine- rich seed protein has been made possible by modifying genes for more methionine codons by site directed mutagenesis [44]. Besides, this can be achieved by constructing a chimeric gene by fusion of a seed specific promoter to a gene for methionine rich protein and then to introduce the chimeric gene to a cultivated species of legume plant [109]. Mutagenic treatments are known to play role in modification of genes for legume seed storage proteins. Gamma ray (T 2-1) and EMS induced (OUM 7,OUM 11-5, OUM 52-3 and OUM 75-1) mutants of mungbean differed in polypeptide banding pattern as compared to their parent (Dhauli) indicating the mutation of genes in multigene families for seed storage protein expression [66].

GENETIC DIVERSITY IN RELATION TO SEED PROTEINS

A set of genotypes of *Vigna mungo* and *V. radiata* resembling to *V. mungo* for seed characters were analysed by SDS-PAGE. Four clusters of genotypes were observed. Based on SDS-PAGE, specific bands were suggested to be used for identifying *Vigna radiata* from mixed germplasm with *Vigna mungo* [65]. The viny wild forms of munbean (TCR 213, TCR 243 and TCR 192) clearly revealed distinctly different protein profiles from all land races based on albumin and globulin seed protein fraction and these form three initial divergent single clusters [110]. Asghar *et al*.[111] evaluated 29 cultivars of chickpea for genetic diversity based on seed storage proteins following SDS-PAGE. 18 bands were observed in total and all the accessions were classified into five clusters on the basis of similarity in banding pattern. The varieties included in one cluster were compared with those included in other clusters for presence/absence of specific bands as a basis of genetic diversity. For instance, cluster I included nine local wild accessions which lack minor band numbers 1 to 9. Similarly, cluster 3 included two accessions both of which revealed absence of band number 15. Roy [112] observed similarity coefficient values ranging from 68-100% with high average coefficient value of 90% and consequently obtained very restricted level of polymorphism among some Indian cultivars and wild accessions of mungbean.

Gallab *et al*. [72] carried out cluster analysis of 10 mungbean genotypes through electrophoretic banding pattern of soluble seed protein fractions (albumins and globulins) which revealed three different genetic clusters. The similarly value of 0.90 was observed between L 3430 and L 2920 indicating that two genotypes are closely related in their protein polypeptide patterns. Other genotypes showed variable genetic diversity in relation to similarity values among them. Further, they enunciated superiority of varieties L 2520 and L 1720 in seed yield could be due to the absence of band at molecular weight 94.65 kd and presence of band at 12.1 kd under drought condition. Tripathy *et al*. [113, 114] analysed albumin and globulin protein fractions through SDS-PAGE of 41 test genotypes including 34 improved varieties, five local land races, one wild accession of mungbean and one local variety of urdbean. Clustering pattern of albumin was different from globulin. Albumin seed protein analysis revealed subtle genetic difference among genotypes, whereas, the genotypes in globulin polypeptide banding pattern seemed to be less diversed at even highest phenon level (SI = 1.00) indicating higher genome homology for globulin protein expression. Cluster analysis based on albumin electrophoresis revealed six clusters. Pant M-5 and RCM 15 constituted separate clusters owing to their typical characteristic polypeptide banding pattern. A few genotypes namely, OGG 57, OUM 7, COGG 912 and ML 613 were also clubbed in separate clusters at lower phenon level indicating their genetic divergence from rest of the genotypes.

Tripathy *et al.*[75] revealed contrasting genotype-specific seed protein profiling in 14 urdbean genotypes out of 20 test enotypes.. Clustering pattern revealed distinctly divergent group formed by cv. TPU 95-1 and TPU 4. In a set of pigeonpea ermplasm lines, Maruteru, Manak, Gajapati local, ICPL 2376 and ICPL 88039 have been identified as genetically distant from rest of the genotypes based on total seed protein profiling [78]. Crossing of elite divergent genotype would open a possibility for improvement in protein quality and quantity per se in cultivated legume species.

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