2. REVIEW OF LITERATURE

2.1. Cereal storage proteins

Seeds are the richest source of plant proteins, and contain 10-50% protein, most of them are storage proteins. They have no enzymic activity and simply provide a source of nitrogen and carbon skeletons for the developing seedlings. These storage proteins are deficient in some of the essential amino acids. Since seeds provide an important source of protein for human and livestock nutrition, much research has been devoted to increase the content of essential amino acids and improve the nutritional quality of seed proteins.

The fundamental understanding of the chemistry of storage proteins starts from the work of Osborne and Mendel. They classified the proteins into four groups on the basis of their solubility differences. These four classes are the albumins- extracted in water, globulins- extracted in salt solutions, prolamins- extracted in aqueous alcohol and glutelins- extracted by alkaline or acidic solvents (Osborne and Mendel, 1914). The composition of these fractions in different cereals is shown in Table 1.

Table 1. Protein content and solubility fractions of cereal grains

<table>
<thead>
<tr>
<th>Cereal grain</th>
<th>Protein (%)</th>
<th>Albumins (%)</th>
<th>Globulins (%)</th>
<th>Prolamins (%)</th>
<th>Glutelins (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wheat</td>
<td>10-15</td>
<td>3-5</td>
<td>6-10</td>
<td>40-50</td>
<td>30-40</td>
</tr>
<tr>
<td>Rice</td>
<td>8-10</td>
<td>Trace</td>
<td>2-8</td>
<td>1-5</td>
<td>85-90</td>
</tr>
<tr>
<td>Maize</td>
<td>7-13</td>
<td>Trace</td>
<td>5-6</td>
<td>50-55</td>
<td>30-45</td>
</tr>
<tr>
<td>Sorghum</td>
<td>9-13</td>
<td>Trace</td>
<td>Trace</td>
<td>60</td>
<td>considerable</td>
</tr>
<tr>
<td>Rye</td>
<td>9-14</td>
<td>5-10</td>
<td>5-10</td>
<td>30-50</td>
<td>30-50</td>
</tr>
<tr>
<td>Barley</td>
<td>10-16</td>
<td>3-4</td>
<td>10-20</td>
<td>35-45</td>
<td>35-45</td>
</tr>
<tr>
<td>Oats</td>
<td>8-14</td>
<td>1</td>
<td>80</td>
<td>10-15</td>
<td>5</td>
</tr>
</tbody>
</table>

Source: Konzak (1977)
As seen from Table 1 the storage proteins, prolamin of cereals account for about half of the total protein in the mature seeds of all cereals except oats and the rice, where glutelins and globulins perform the function of storage protein, respectively (Larkins, 1981). In the original Osborne's classification, no reducing agent was used in the aqueous alcoholic solvent. Many workers have subsequently noted that additional protein could be extracted with aqueous alcohol if a reducing agent was added to it. Landry and Meureaux (1970) developed such a method to fractionate maize protein which was then extended to sorghum (Jambunathan and Mertz, 1973), foxtail millet (Monteiro et al., 1982), kodo millet (Sudharshana et al., 1988) and barnyard millet (Monteiro et al., 1988).

Cereal prolamins are characterized by a higher content of proline and glutamine and a lower level of charged amino acids, especially lysine. The prolamins of most cereals are complex mixtures of polypeptides that occur in protein bodies. The difficulties of purification of a single polypeptide from a heterogeneous mixture and the lack of a defined biological (e.g., enzymic) activity made the understanding of the three-dimensional structure of prolamins difficult in earlier times (Williams and Watson, 1938; Entrikin, 1941). Now, details of prolamin structure are emerging from structures prediction, spectroscopic and computer modelling studies. Extensive work has been carried out on the prolamins of wheat, barley, rye, maize and sorghum.

2.2. Prolamins of wheat, barley and rye

Wheat, barley and rye are closely related members of the tribe Triticeae and contain homologous group of proteins (Shewry et al., 1984). The prolamins of these cereals are called gliadins, hordeins and secalins, respectively. Shewry et al. (1986) classified these prolamins into three groups on
the basis of their amino acid composition and sequences. The high-molecular weight (HMW), S-poor and S-rich prolamins.

2.2.1. HMW prolamins

HMW prolamins are extracted in the presence of reducing agent, and represent less than 5% of the total prolamins in barley and rye and about 7.9% in wheat (Field et al., 1983; Miflin et al., 1983).

HMW prolamins consist of 3-5 polypeptides with MW ranging from 95-145 kD (Shewry and Miflin, 1985). These are further classified into two groups on the basis of MW values and sequences: x-type (83-88 kD) and y type (67-77 kD). Genes for the different HMW subunits have been isolated and the amino acid sequences of the encoded protein deduced (Shewry and Tatham, 1990).

The HMW prolamins of barley and rye are called D-hordeins and HMW secalins, respectively. These proteins are rich in glycine, glutamine and proline (Field et al., 1982; Kries et al., 1984). Significant sequence homology is noted between HMW secalins, D-hordeins and HMW glutenins (Shewry et al., 1988). CD spectroscopy of HMW secalin fraction gave spectra which are identical to those of the HMW subunits of glutenin indicating a similar conformation (Tatham et al., 1990 a,b)

2.2.2. Sulfur-poor prolamins

The S-poor prolamins comprise the omega-gliadins of wheat, C-hordeins of barley and the omega-secalins of rye and account for about 10-20% of the total prolamin fractions from these species (Shewry and Miflin, 1985).
2.2.2.1. **C - Hordeins of barley**

Electrophoretic separation of C-hordein from different barley cultivars showed a number of proteins ranging in MW from 54-60 kD (Shewry et al., 1981). Amino acid sequencing show unique N-terminal and C-terminal domains of 12 and 6 residues respectively flanking a repetitive domain of over 400 residues. This repetitive domain consists mainly of octapeptides (Pro- Gln- Gln- Pro- Phe- Pro- Gln- Gln) and at least three related pentapeptides (Pro- Gln- Gln- Pro- Tyr) close to the N-terminus (Forde et al., 1985). Pernollet and Mosse (1983) and Tatham et al. (1989) predicted the presence of three β-turns in the pentapeptide region adjacent to the N-terminus and suggested that these could form a pseudohelical structure.

2.2.2.2. **S-poor prolams of wheat and rye**

The S-poor prolams of wheat (omega-gliadins) and rye (omega-secalins) have MW of 44-78 kD and 48-53 kD respectively as determined by SDS-PAGE (Kasarda et al., 1983). The N-terminal amino acid sequences of omega-gliadins and omega-secalins are homologous to C-hordeins, with short non-repetitive domains followed by several repeated peptides. Structure predictions show the presence of β-turns in positions similar to those predicted in C-hordein (Tatham and Shewry, 1985).

2.2.3. **S- rich prolams**

The S-rich prolams are quantitatively the major group of prolams in wheat, barley and rye and the most structurally diverse. They include monomeric protein with intra-chain disulfide bonds, and polymers linked by inter-chain disulfide bonds (Shewry and Miflin, 1985 ; Tatham et al., 1990 c). Mostly they consist of about 250-300 residues, with apparent MW values by SDS-PAGE of about 36-44 kD. One group of secalins have higher MW
values, about 75 kD by SDS-PAGE (Shewry et al., 1984). There is considerable diversity in their sequences, but they can be classified into three types, the τ-type (present in barley, wheat and rye), the α-type (present only in wheat) and polymeric type (present in wheat and barley).

The τ-type prolamins may correspond most closely to the ancestral type of S-rich prolamin, as they are present in all members of the Triticeae and distantly related meadow grasses (Shewry et al., 1986). The amino acid sequence data of τ-type gliadins show a unique N-terminal domain of 12 residues followed by a series of repeats of Pro- Gln- Gln- Pro- Phe- Pro- Gln. The proline poor non-repetitive domains contain all the cysteine residues (Shewry and Tatham, 1990).

The α-type prolamins are monomeric and present only in wheat and in closely related species of Triticum and Aegilops (Autran et al., 1979). Although they have a similar structural organisation to the τ-gliadins, the repetitive domain is less conserved, consisting of two interspersed repeats of (Pro- Gln- Pro- Gln- Pro- Phe- Pro) and (Pro- Gln- Gln- Pro- Tyr). In addition, the C-terminal domain contains two regions which are rich in glutamine called the poly-Gln region (Shewry and Tatham, 1990).

The aggregated prolamins include low-molecular weight (LMW) subunits of wheat glutenin and β-hordeins of barley. The LMW subunits of wheat have short non-repetitive N-terminal sequence, which may contain a single cysteine residue, followed by a proline rich repetitive domain of interspersed repeats of two heptapeptides: Pro- Gln- Gln- Pro- Phe- Ser and Gln- Gln- Gln- Pro- Val- Leu (Shewry and Tatham, 1990).
The B-hordeins are characterized by their high degree of polymorphism. Two-dimensional (IEF/SDS-PAGE) analysis of B-hordein fractions from eight cultivars show a total of 47 major polypeptides present in any one variety (Faulks et al., 1981). The polypeptides are further classified into three groups on the basis of their cyanogen bromide cleavage patterns.

Forde et al. (1981) sequenced two cDNA clones related to B1 and B3 hordeins. The amino acid sequences were about 80% homologous and appeared to consist of two structural domains. The region close to C-terminus was proline-poor with no clear repeated sequences. In contrast the second domain towards the N-terminus was proline-rich and had a series of octapeptide repeats based on the sequence Pro- Gln- Gln- Pro- X -Pro- Gln- Gln- which is similar to the octapeptide present in C-hordein (Pro- Gln- Gln- Pro- Phe- Pro- Gln- Gln- Gln) suggesting a common origin for atleast some parts of B- and C-hordein polypeptides.

2.3. Prolamins of Maize

Extensive work was carried out on prolamins (Zein) of maize. Zeins were extracted from maize endosperm using 70% ethyl alcohol or 55% isopropyl alcohol containing reducing agents. Until the late 1960’s zein was primarily defined as the protein extractable with 60-70% alcohol from com meal. Subsequently many investigators have shown that additional protein can be extracted with alcohol if a reducing agent was added to it. This fraction was referred to as glutelin-I, alcohol soluble reduced glutelin (Paulis and Wall, 1971), zein-2 (Sodek and Wilson, 1971), zein like (Misra et al., 1975), reduced soluble protein (Wilson, 1987) and τ-zein (Esen, 1987).

N-terminal sequencing of zeins for 33 residues, indicates significant homology among the zein subunits. Zein polypeptides from normal
com hybrids have very similar amino-terminal sequences and only relatively minor differences in sequences are noted between zeins from near-isogenic normal and opaque-2 hybrids (Bietz et al., 1979).

After studying the solubility properties of zeins in different concentrations of alcohol containing reducing agents, Esen developed a method of fractionating the different zeins by exploiting their extractability differences (Esen, 1986). Based on the findings he proposed a nomenclature for the zeins, which has been accepted widely (Esen, 1987). According to his classification the three zein fractions are referred to as α- , β- and τ- zeins.

2.3.1. α- Zein

The α-zeins (MW 24, 21 and 10 kD) which constitute 75-85% of the total zeins are soluble in 50-95% isopropyl alcohol but insoluble in 30% isopropyl alcohol /30 mM sodium acetate. The α-zeins are encoded by a large multigene family (Shotwell and Larkins, 1989). α-zeins are made as preproteins with an NH₂-terminal signal peptide of 20 or 21 amino acids (Marks and Larkins, 1982). Complete amino acid sequences derived from cloned cDNAs have shown that the 19 and 22 kD α- zeins consist of about 210-220 and 240-245 residues respectively. Unique domains of about 36-37 and 10 residues are present by a repetitive domain consisting of blocks of about 20 residues (Rubestein and Geraghty, 1986). All the α-zeins have high contents of glutamine (25%), leucine (20%) alanine (15%) and proline (11%) and none has been identified that contained lysine (Esen, 1987).

2.3.2. β- Zein

The β-zein are polypeptides of 14 and 16 kD and these proteins show less charge heterogeneity than the α-zeins on IEF analysis (Marks et al., 1985 ; Pedersen et al., 1986). The encoded proteins consist of 160 residues,
and rich in methionine (18 residues) and cysteine (7 residues). B-zeins do not show any sequence homology to the α-zeins, and do not contain repeated sequences. However, the amino acid sequence data show that the methionine residues are clustered, particularly between positions 121 and 132. The β-zein gene has a short region of nucleotide sequence homology to the cysteine-rich domain of the 27 kD τ-zeins, as well as the cysteine-containing β-hordein of barley. This peptide also shares several amino acids in common with the cysteine-rich τ/omega - gliadins (Pedersen et al., 1986). The β-zein has less glutamine (16%), leucine (10%) and proline (9%) than the α-zeins, but contains significantly more of the sulphur amino acids methionine (11%) and cysteine (4%) (Esen et al., 1985; Pedersen et al., 1986).

2.3.3. τ-Zein

The τ-zein of 27 kD, also referred to as the reduced-soluble-protein (RSP), has the unique property of being soluble in saline solutions in addition to its solubility in aqueous alcohol. The N-terminal amino acid sequence data of the 27 kD zein indicate that the polypeptide is characterized by an initial 11-residue sequence followed by the repeating hexapeptide sequence Pro- Pro- Pro- Val- His- Leu, which is repeated six times starting at position 12. The N-terminal sequence of this zein is found to be extremely hydrophobic with proline accounting for 43% of the first 58 residues (Esen, 1981; Esen et al., 1982; Vitale et al., 1982).

The primary structure of the mature ASG was determined from the sequence of one near full length cDNA clone. The encoded protein consists of 204 residues. In the N-terminal, one half of the sequence contains eight identical tandem repeats of the hexapeptide Pro- Pro- Pro- Val- His- Leu and two octapeptide Gln- Pro- His- Pro- Cys- Pro- Cys- Gln repeats. There are four
successive proline residues flanking the stretch of eight tandem repeats at both ends (Wang and Esen, 1986). The \( \tau \)-zein, has two regions of homology to the \( \beta \)-zein, there are matches of 10 residues each between amino acids 34 to 49 of the \( \beta \)-zein and 112 to 125 of the \( \tau \)-zein, and between amino acids 61 to 73 of the \( \beta \)-zein and 130 to 141 of the \( \tau \)-zein. These regions of homology, probably account for the small degree of cross-reactivity between the \( \beta \)-zein and \( \tau \)-zein antisera. (Esen et al., 1985).

2.3.4. Delta - Zein

Kirihara et al. (1988) reported the sequence of a gene encoding a 10 kD zein, which is not related to any other prolamin. This protein which is also extracted under reducing conditions is called delta-zein. The protein consisted of 129 residues and do not contain any repeated sequences but has a central domain containing 17 of the 29 methionine residues, mostly as doublets separated by 2 or 3 other residues.

2.4. Prolamins of oat

Oat is the cereal, most closely related to the Triticeae, being placed in a separate tribe (the Aveneae). The prolamins of oat (avenins) account for about 10% of the total grain protein (Peterson and smith, 1976). Like other cereal prolamin, avenins are also found to be located inside the protein bodies (Pernollet et al., 1982). On SDS-PAGE, avenins separated into 3-4 bands ranging in MW from 20-34 kD. The amino acid composition of avenins is characterized by high levels of glutamine-glutamic acid, leucine and proline (Kim et al., 1979b). N-terminal amino acid sequencing of avenins shows no homology with prolamins of wheat, barley, maize, sorghum and pearl millet (Bietz, 1982).
2.5. Prolamins of Rice

Rice prolamins account for about 5% of the total grain proteins, since the major rice storage proteins are glutelins (Mandac and Juliano, 1978; Padhye and Salunke, 1979). They are classified into three groups: 10 kD, 13 kD and 16 kD (Mandac and Juliano, 1978).

The 10 kD rice prolamins consist of about 110 residues, and contain low contents of glutamine and proline. The 13 kD prolamins consist of about 140 residues and show 70% homology with the 16 kD prolamin. There are no repeated sequences and the available sequences showed a high degree of heterogeneity (Kim and Okita, 1988a; Okita et al., 1989; Shyur et al., 1992). The prolamin fraction of rice is poorly characterised in terms of secondary and tertiary structures. Horikoshi et al. (1991) reported that the N-terminal sequence of the major prolamin was found to be PGx- Phe- Asp- Val- Pleu- Gly- Gly- Ser- Tyr- Arg. The protein consisted of one polypeptide of 131 residues and had a MW of 14930. The protein was rich in glutamine (22%), leucine (13%) and alanine (7%). The proline content of rice prolamin was lower than in other cereals. No methionine and lysine residues were found in the major rice prolamin (Kim and Okita, 1988a,b).

2.6. Prolamins of Sorghum

The total protein in the whole seed of sorghum ranges from 9.8 to 14.3% and in that the prolamin fraction ranges from 40.6 to 60.3% (Jambunathan and Mertz, 1973; Jambunathan et al., 1975; Paulis and Wall, 1979). Taylor et al. (1984b) optimized the protein fractionation procedure for sorghum. Sorghum prolamins (Kafirins) are classified into two group on the basis of their extractability in aqueous alcohols α-kafirin and β-kafirin (Sastry and Virupaksha, 1969). Haikerwal and Mathieson (1971) separated
kafirins into two fractions on sephadex G-100, one excluded fraction of above 100 kD and a smaller sized fraction ranging from 4-30 kD. The amino acid composition of kafirins is characterized by low levels of lysine, but contains more valine, alanine, isoleucine and glutamine (Taylor et al., 1984 a). Krishnan et al. (1989) separated kafirins on SDS-PAGE and assigned molecular weights to the different polypeptides. The major bands were of 27 and 25 kD, minor proteins of 18 and 12 kD were also observed.

Evens et al. (1987) had earlier observed that the \( \tau \)-kafirin was extractable in water containing a reducing agent and termed this protein as reduced soluble protein (RSP). N-terminal amino acid sequence of sorghum RSP shows that ten of the first 11 residues are found to be identical with the corresponding sequence of maize RSP. The sorghum homologue also contains the repeating hexapeptide Pro- Pro- Pro- Val- His- Leu found in maize RSP. The nucleotide sequence of a cDNA clone encoding \( \tau \)-kafirin encodes a protein of 212 amino acids with a single peptide of 19 amino acids. The mature protein contains four repeats of the hexapeptide Pro- Pro- Pro- Val- His- Leu (de Barros et al., 1991). Genomic cDNA clones have been made for \( \alpha \)-kafirin which showed extensive homology with \( \alpha \)-zein (de Rose et al., 1989). Conformational studies show that sorghum prolamins contain a mixture of \( \alpha \)-helix and aperiodic structure with no \( \beta \)-sheet (Wu et al., 1971).

2.7. Work carried out on minor millets

Though extensive work has been reported on the cereal proteins, work on minor millets is scandy. Indira and Naik (1971) first studied the nutritional composition and protein quality of the minor millets. According to them protein in the grains was resistant to extraction by conventional solvents. The prolamin fraction was deficient in lysine and tryptophan as that of sorghum
and maize. They have suggested that the digestibility can be enhanced by heating or autoclaving. Later, Monteiro et al. (1982) studied the nutritional composition, fractionation and amino acid composition of Italian millet/foxtail millet. The same authors in 1988 and 1992 reported their work on kodo millet and barnyard millet (Monteiro et al., 1988, Sudharshana et al., 1988 and Suman et al., 1992). According to them, Italian millet found to contain higher amount of prolamin whereas barnyard millet and kodo millet contain higher amount of glutelin. Naren and Virupaksha (1990) have isolated α- and β- setarins from Italian millet and found that both are low in molecular weight and rich in sulphur amino acids. Mosse et al. (1989) studied the relationships between amino acid composition and nitrogen content of foxtail millet varieties and reported that the composition of amino acids are independent of grain protein content. Geervani and Eggum (1989) reported that the amino acid composition of minor millets was comparable to other cereals. The high values for energy and protein digestibility showed that starch as well as protein are highly digestible. There are a few reports about the puffing quality, milling characteristics and rheological properties of proso millet (Lorenz and Dilsaver, 1980 a,b ; Lewis et al., 1992).

Like other cereals, germination found to increase the lysine and tryptophan content of proso millet (Parvathy and Sadasivam, 1994). Germination also increases the α- and β- amylase activities gradually which reached the maximum level in the 5th and 7th day respectively in some of the minor millets (Parvathy and Sadasivam, 1982). Differences in the enzyme activities of β-amylase, protease, cellulase and hemicellululase are appeared to be influenced by the location and soil condition, where the millets are grown (Skovron and Lorenz, 1979).
2.8. Interrelationships between cereal prolamins

The commonalities and divergences in structure of prolamins from different cereals may be brought out by studies of their amino acid composition, primary sequence and by the use of immunochemical methods (Shewry and Tatham, 1990).

2.8.1. Amino acid composition

The amino acid composition of the prolamins from different cereals reveals that they all belong to one class with little or low levels of lysine, tyrosine and tryptophan (Shewry and Tatham, 1990; Horikoshi et al., 1991). The prolamins differ within themselves in their content of glutamic acid, proline, cysteine and methionine. The sulphur amino acid content has been used for the classification of the different prolamins as S-poor or S-rich. The cysteine content in the S-poor prolamins would help further in the distinction between those that aggregate and those do not. α-zein and rice prolamin belong to S-poor category while β-zein, τ-zein, τ-gliadin and LMW glutelin to S-rich category.

2.8.2. Primary sequence comparison

The proline-rich repetitive sequences of the S-rich prolamins are related, to varying extents, to the repeat motifs present in the S-poor prolamins. The conclusion is that they have a common evolutionary origin. In contrast, the repeats of the HMW subunits are not related to repeats in the S-rich or S-poor prolamins.

Although prolamins are thought to occur only in the grasses, recent studies have shown sequence similarities between different prolamins and between group of prolamins and other seed proteins. The non-repetitive C-terminal domains of the S-rich prolamins contain three conserved domains.
They vary in length from about 20 to 35 residues, and are called A, B and C; they are flanked and separated by more variable regions, which are homologous within, but not always between the different families of S-rich prolamins. Regions A, B and C also related to each other, indicating that they may have arisen from a triplication of a single domain of about 30 residues (Kries et al., 1985).

Sequences related to A, B and C are also present in the HMW prolamins, regions A, B in the N-terminal domain and region C in the C-terminal domain. The S-poor prolamins do not contain regions A, B and C but the repeat motif is clearly related to the S-rich prolamins, implying an evolutionary relationship. On the basis of these studies it was proposed that S-rich, S-poor and HMW prolamins evolved from a single ancestral domain.

A comparison of the conserved sequences of the Triticeae with other prolamins shows that regions related to A, B and C are present in the avenins, zeins (β and α-zeins) and rice (10 kD), although other parts of the prolamin sequences show little or no homology. Regions corresponding to A, B and C are also present in two groups of non-prolamin seed proteins. The first is a group of serine proteases and α-amylases that are present in the seeds of many cereals. The proteins consisted of about 120 to 130 residues and regions A, B and C account for most of the protein sequence. The second group is the 2S storage albumins present in the seeds of many dicotyledenous plants, notably oilseed: rape, sunflower and Brazil nut. This protein consists essentially of regions A, B and C, but most have been modified by post-translational peptide cleavage (Shewry, 1993).
2.8.3. Immunochemical studies

Antibodies have been useful probes for the comparison of sequence homologies between different prolams. Polyclonal antisera have been raised to α-gliadin by several groups. Tobler et al. (1982) prepared rabbit antibodies to α-gliadins and showed that little reaction occurred to B- and other gliadins. Skerritt and Underwood (1986) and Skerritt et al. (1991) have prepared several monoclonal antibodies that bound α-rather than β-gliadins while omega-gliadins are more immunologically distinct. A number of monoclonal and polyclonal antibodies have been identified that bound α-, β- and τ- gliadins but not omega-gliadins (Skerritt and Underwood 1986; Freedman et al., 1988). Brett et al. (1993) have recently obtained a specific antibody binding to chromosome 1D-encoded LMW-gliadin subunit (GS) and found correlations with baking performance. HMW-GS have been the group of prolams most unequivocally related to variation in dough properties (Payne, 1987). Monoclonal antibodies developed specifically for HMW-GS has got high degrees of homology between individual subunits.

Antibodies raised against α-, β- and τ-zeins were found to cross-react with their corresponding kafirin polypeptides (Watterson et al., 1990; Shull et al., 1991) while antibodies raised against C2 coxins cross-reacted with C1 coxins, while C3 coxins antibodies reacted only with C3 antigen. It was found that C1, C4 and C5 antisera cross-react strongly with α-zeins, while C3 antisera cross-reacted with τ-zein (Leite et al., 1990; Ottoboni et al., 1990).

The homologies between prolams of wheat, rye, barley, and oats have also been established. The results of these investigations are presented in Table 2.
### Table 2. Immunological cross-reactivity between some cereal prolams

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Barley</th>
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<th>Rye</th>
<th>Oats</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B-</td>
<td>C-</td>
<td>r-</td>
<td>α-</td>
<td></td>
</tr>
<tr>
<td>C-Hordein</td>
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<td>+</td>
<td>+</td>
<td>+</td>
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</tr>
<tr>
<td>r- Gliadin</td>
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<td></td>
<td>+</td>
<td>-</td>
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<tr>
<td>B-Hordein</td>
<td>+</td>
<td></td>
<td>-</td>
<td>-</td>
<td>Zawistowski and Howes (1990)</td>
</tr>
<tr>
<td>Avenin</td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Zawistowski and Howes (1990)</td>
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</table>

**Wheat Mabs**

<table>
<thead>
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<th>Wheat</th>
<th>Rye</th>
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<td>+</td>
<td>-</td>
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<tr>
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<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Skerritt and Lew (1990)</td>
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</tbody>
</table>
Most of the antibodies distinguish between S-poor and S-rich prolamins but many of these do cross-react between them as well. There have been fewer antibodies generated to prolamins from cereals, other than wheat, barley, rye and oat. Most of the recent immunochemistry on maize grain storage proteins has been performed by Esen and Co-workers. α-zeins have been studied extensively and appear to have little immunochemical homology to prolamins from wheat, rye or barley (Esen et al., 1987; 1989). Esen (1988) also studied the structural homologies between different zeins. Polyclonal antisera to α-, β- and τ- kafirins do not cross-react with kafirins other than the immunogen, although varying degrees of cross-reaction with prolamins from millets, rice, maize and wheat are noticed (Mazhar et al., 1993). Naren and Virupaksha (1990) have studied the synthesis of α-setarin using immunochemical techniques.

2.8.4. Peptide mapping of proteins

Peptide maps on proteins are generally run for two purposes, either to see if the molecules from different sources are structurally related or to identify changes in structure of an individual molecule which has arisen as a consequence of normal or aberrant metabolism viz., comparison of protein with the same function isolated from different tissues or species, comparison of products of translation produced \textit{in vitro} with equivalent molecules isolated from cells and comparison of subunits of multimeric proteins.

The principle of peptide mapping is to fragment proteins into several pieces by peptide bond cleavage and then to compare the physical behaviour of these polypeptides or peptide mixtures. The fragments may be generated by proteolytic enzymes or by chemical treatments. The fragments are separated by three ways.
Bietz et al. (1970) compared the peptide maps of peptic digests of whole gliadin and its α-, β-, τ-1 and τ-3 - components using cation and anion-exchange chromatography. Peptide maps from α- β- and τ- gliadin showed many similarities both within each variety and between corresponding components. The two τ-gliadin chromatograms are nearly identical. Peptides from gliadin and glutenin are also compared (Bietz and Rothfus, 1970). Similarities between peptide maps from cation and anion-exchange chromatography of digests of gliadin, glutenin and their derivatives suggested that most gluten contain segments of polypeptide sequence that are similar or identical. Gliadin peptides eluted from cation-exchange resins at pH 1.8 are especially rich in glutamic acid and proline but are deficient in lysine, arginine, cysteine and methionine. Corresponding peptides from glutenin are also rich in glutamic acid and proline. But peptide size is the most striking difference between glutenin and gliadin.

Size exclusion and RP-HPLC methods have been used by Bietz (1983; 1984; 1985) to examine cereal storage proteins. But until 1985 HPLC has not been applied to study the peptide mapping from cereal proteins. Jones and Lookhart (1985) used HPLC for the first time for separating peptides for sequencing from wheat storage protein. RP-HPLC separation is more advantageous because of speed, sensitivity and easy recovery of the peptides.
Nowadays HPLC is rapidly becoming the method of choice for studying, separating and purifying proteins and peptides (Horikoshi et al., 1991; Lew et al., 1992).

2.9. Synthesis and deposition of cereal storage proteins

Prolamins are synthesized on rough endoplasmic reticulum (RER) and subsequently deposited into protein bodies (Shull et al., 1990). Investigations on developing wheat endosperm by electron microscopy have also implicated the RER in the synthesis and transport of wheat prolamsins (Campbell et al., 1981). Immunocyto-chemical studies confirmed that gliadins are transported via the golgi apparatus and are deposited in the protein bodies (Kim et al., 1988; Stenram et al., 1991). Changes in the protein fractions of developing normal and opaque-2 maize endosperm were studied by Murphy and Dalby (1971). Misra and Mertz (1975) also studied the developmental change in the endosperm protein of high-lysine mutants of corn. Protein fraction distribution from 14 days after pollination through harvest was followed by Ortega et al. (1991).

Electron microscopy of developing endosperm have shown the presence of three types of protein bodies in rice endosperm, the large spherical, small spherical and crystalline protein bodies (Harris and Juliano 1977; Bechtel and Pomeranz, 1978). Quantification of prolamins and glutelins of rice during seed development was studied by immunoblot analysis (Li and Okita, 1993). The storage proteins of rice exhibited different temporal accumulation patterns during seed development and comprised a much larger proportion of the total storage protein fraction than previously recognized.

Developmental differences in the rate of deposition of three kafirins were observed by Mazhar and Chandrasekar (1993). The hard
endosperm kernels deposit more \( \beta \)-kafirins than the soft endosperm kernels during all stages of development. The extent of cross linking of \( \beta \)-and \( \tau \)-kafirins is also greater in the hard-endosperm kernels which indicates that grain hardness may be a function of kafirin composition.

2.10. Changes in biochemical constituents during germination

During germination and early seedling development, cellular activity in the aleurone is directed rapidly to the synthesis and secretion of hydrolytic enzymes that catalyze the depolymerization of storage macromolecules in the cells of the starchy endosperm. Hydrolysis of storage proteins is initiated by the action of proteinases that cleave the internal peptide bonds without causing extensive modification. Proteolytic enzymes (proteases) involved in the mobilization of proteins are present in both ungerminated and germinating seeds. The activity of protease increases from the 3rd day of germination in maize endosperm up to 8th day and then decreases (Harvey and Oaks, 1974a; Fujimaki et al., 1977; Moureaux, 1979). Reducing agents enhance the activity of protease. The maximum degradation of zeins and glutelins occur between 3-8 days of germination coincided with the appearance of acid protease. Electrophoretic analysis of germinating maize and sorghum indicates that the major zein and kafirin bands gradually disappear without changing into any other bands indicating that they degrade to produce very low molecular weight peptides and amino acids (Fujimaki et al., 1977; Taylor, 1983).

\( \alpha \)- and \( \beta \)-amylases and other glucanases mediate starch hydrolysis during germination which in turn increase the total sugars. \( \alpha \)- and \( \beta \)-amyloses are most important for they can be used in domestic and commercial brewing. Extensive reports are available on the activity of these enzymes in intact and
germinated cereals (Macgregor et al., 1984; 1988; Warner et al., 1991; Ratnavathy and Balaravi, 1991). But limited reports are available on the activity of amylases in millets. Changes in the activity of $\alpha$- and $\beta$- amylases in millets during germination were reported by Parvathy and Sadasivam (1982).

SDS-PAGE, immunoblotting and immunocytochemical analysis of germinating maize with anti-zein antibodies indicate that $\tau$-zeins are degraded first, followed by the $\beta$-zeins, while the $\alpha$-zeins are the most resistant and are detected up to 10 days after germination (Torrent et al., 1989; de Barros and Larkins, 1990; Mohammed and Esen, 1990). Immunoblotting, ELISA and radio-immunoassays have also been used to study hydrolysis of barley protein during germination (Skerritt, 1988b; Skerritt and Henry, 1988). Mazhar and Chandrasekar (1993) used ELISA to follow the kafirin degradation during germination.

Literature survey revealed that extensive work has been carried out on the seed storage proteins of major cereals like, wheat, rice, maize, sorghum, barley and oats. However very limited work has been reported on the minor millets. Hence attempts were made to study the prolamin storage proteins of minor millets to certain extent.