Disulphide Linkages Occur in Many Polypeptides of Rice Protein Fractions: a Two-dimensional Gel Electrophoretic Study

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Abstract: Most of the work on rice storage proteins focused on the major fraction – glutelin. In contrast to previous reports of the occurrence of single legumin-like polypeptide pair in glutelin, two-dimensional gel electrophoresis revealed many glutelin bands, with some having and some lacking disulphide-linkages. Five legumin-like polypeptide pairs exhibited wide heterogeneity over a range of molecular weight ($M_r$) 25 to 60 kDa, each having a large subunit ($M_r$ ranged from 18 to 40.5 kDa) disulphide-bonded to a small subunit ($M_r$ ranged from 16.5 to 18.0 kDa). A band of 49 kDa was homodimeric with two subunits of 29 kDa each; a polypeptide of 51 kDa which altered position to ones corresponding to 53 kDa and 57 kDa on 2-D gels contained intrapolypeptide linkages. Polypeptides of 65 kDa and 60 kDa occurred as aggregates of 110 kDa. The number of polypeptides in other seed protein fractions albumins, globulins and prolamins, varied from four to ten. One of the albumin bands had intrapolypeptide disulphide linkages (20 kDa) and the globulins contained two such bands (13.5 and 20 kDa). Thus, the present study provides a description of the polypeptide composition of different rice protein fractions that is finely resolved with respect to the occurrence of disulphide linkages.

Key words: seed storage protein; glutelin; prolamin; globulin; *Oryza sativa*

Rice (*Oryza sativa* L.) is, next to maize, the second largest major food crop with an annual production of 645 million tonnes at the global level. For the poor and malnourished populations of Asian countries, it provides a highly accessible source of dietary energy and proteins. Although its seed protein content (5.9%–11.1%) is relatively low compared to other cereals, rice is known for its better nutritional quality on account of its higher lysine content (Juliano, 1990). Plant proteins have been classified into four types on the basis of their differential solubility: water soluble albumins; salt soluble globulins; alcohol soluble prolamins; and dilute alkali/acid soluble glutelins. Unlike leguminous seeds, which have globulins as the most abundant storage proteins, cereal seeds are rich in glutelin and prolamin fractions, with the exception of oats, which have globulins as the dominating fraction. The imbalance in supply of essential amino acids in human nutrition through these two major groups of food crops is due to the relatively low contents of sulphur-containing amino acids in the globulins of legumes and lysine in the prolamins of cereals. Studies on the characterization of seed storage proteins, therefore, are parts of an important strategy towards the improvement of the nutritional quality of different crops.

In rice, glutelins are reported to be the major storage protein fraction, accounting for 80% of the total seed protein; prolamins are present in a very low proportion of approximately 5% (Villareal and Juliano, 1978). Using different extraction conditions, however, Krishnan and White (1995) reported a relatively lower proportion of glutelins (53%) and a higher proportion of prolamins (30%). Glutelins are rich in the essential amino acids lysine and tryptophan, whereas prolamins are comparatively lacking in these (Hibino et al, 1989; Juliano, 1990).

Glutelins are the most extensively studied protein fraction in rice and are known to be represented by a polypeptide pair of molecular weight ($M_r$) 57 kDa consisting of disulphide-bonded polypeptides of 34–39 kDa and 21–23 kDa (Yamagata et al, 1982). Sequencing studies of glutelin polypeptides by Zhao et al (1983) and cDNA by Takaia et al (1987) indicated considerable homology between rice glutelin and pea legumin. Like pea legumin, rice glutelin is also synthesized as a precursor which is later processed and cleaved into two disulfide-bonded subunits (Yamagata
et al, 1982). Despite its similarity to the salt-soluble pea legumin in terms of subunit composition, biosynthesis of polypeptide pairs, and amino acid sequence, rice glutelin differs from pea legumin by being insoluble in salt solutions. Takaiwa et al (1987) related this insolubility to the less hydrophilic sequences observed in the COOH-terminal region of the acidic subunits of rice glutelins. Also, unlike polypeptides of the highly organized 11S legumin-like protein, rice glutelin polypeptide pairs do not constitute a hexameric structure, but instead form multimolecular aggregates through disulphide linkages (Tanaka et al, 2004). A number of other polypeptides have also been reported in the glutelin fraction under reducing conditions. Juliano and Boulter (1976) observed polypeptides with molecular weights of 38, 25 and 16 kDa, whereas Zhao et al (1983) reported those of 36, 22 and 14 kDa in the glutelin fraction. Analyses of the polypeptide composition of other rice protein fractions, albumins, globulins and prolamins, have also been carried out by many researchers (Wen and Luthe, 1985; Krishnan and Okita, 1986; Steenson and Sathe, 1995). When considering the occurrence of polypeptides with the same molecular weight, these reports have generally postulated that the presence of one or other polypeptide in a particular fraction is the consequence of contamination by certain other protein fractions. Thus, a number of bands in positions corresponding to molecular weight lower or higher than those of the major glutelin bands, as described above, have been simply explained as contamination by prolamin (14 kDa) (Wen and Luthe, 1985; Krishnan and Okita, 1986) or albumin/globulin (in excess of 58 kDa) (Snow and Brooks, 1989) polypeptides. Moreover, Zhao et al (1983) described the polypeptide with the Mr of 14 kDa as being similar to those present in glutelins of other cereals. In the studies on rice mutants by Kumamaru et al (1998) and Takemoto et al (2002), bands other than those of 57 kDa, 40 kDa and 20 kDa as seen in the glutelin fraction were left unexplained. Thus, the polypeptide composition of the different rice protein fractions is not properly resolved or understood. We have therefore, in the present study, made efforts to separate four seed protein fractions using standard protocols based on solubility differences and compared these in terms of their polypeptide composition.

**MATERIALS AND METHODS**

**Materials**

Seeds of rice line ‘Taroari Basmati’ were obtained from the Division of Genetics, Indian Agricultural Research Institute, New Delhi, India.

**Total seed protein extract**

For preparation of total seed protein extracts, 40 mg of defatted seed meal suspended in 400 μL of 0.2 mol/L Tris-HCl (pH 6.8) containing 2% sodium dodecyl sulphate (SDS) was heated in a water bath at 80°C for 40–45 min with frequent vortex mixing, followed by centrifugation at 2000×g using a microfuge, as described by Chandna and Matta (1990).

**Fractionation**

Separation of four protein fractions was based on methods employed by Luthe (1983), and by Schaeffer and Sharpe (1990), with slight modifications. All aqueous extraction solvents were buffered with 10 mmol/L Tris-HCl (pH 7.5). After extraction of albumins in water, the residue was used for separation of globulins with 0.5 mol/L NaCl, followed by 55% n-propanol for prolamins and 0.5% SDS for the glutelin fraction. Each extraction was repeated twice and the supernatants were pooled.

**SDS-polyacrylamide gel electrophoresis**

SDS-polyacrylamide gel electrophoresis was carried out on 14% gels following the formulation of Laemmli (1970). For non-reducing electrophoretic analysis of protein fractions, 100 μL of 0.2 mol/L Tris-HCl buffer (pH 6.8) containing 2% SDS and 10% glycerol was mixed with 100 μL of a given protein fraction. For gel electrophoresis of seed protein extracts and protein fractions under reducing conditions, 2% 2-mercaptoethanol was included. The samples were heated in an oven at 100°C for 10 min before loaded onto the gel.

**Two-dimensional gel electrophoresis**

Two-dimensional gel electrophoresis of total
seed protein extracts and extracted protein fractions was carried out following the method as described by Singh and Matta (2008). The 1.5 mm thick gel strip with polypeptides separated under non-reducing conditions (1D, -2ME) was equilibrated for 2 h with gentle shaking in 0.2 mol/L Tris-HCl buffer (pH 6.8) containing 2% SDS and 2% 2-mercaptoethanol, and loaded onto another gel of 2 mm thickness for electrophoresis in the second dimension (2D, +2ME).

RESULTS

Electrophoresis of total seed protein extracts of rice line ‘Taroari Basmati’ revealed a number of bands with molecular weights of 110, 88, 78, 60, 58, 57, 53, 52, 51, 49, 40, 38, 37, 33, 31, 27, 25, 23.5, 23, 21, 20, 19, 17, 16.5, 16, 15, 14.5, 14, 13.5, 13 and 12.5 kDa under non-reducing conditions (Fig. 1, 1D, -2ME). In the presence of 2-mercaptoethanol (Fig. 1, 2D, +2ME), bands of 110, 60, 58, 52, 51, 49 and 25 kDa disappeared whereas new bands with different molecular weights were seen at positions of 65, 60, 57, 40.5, 38.5, 38, 36, 29, 25, 21, 20, 18 and 16.5 kDa. The relationships of individual bands as seen under non-reducing conditions with those resulting from their reduction could be elucidated through the use of two-dimensional gel electrophoresis (Fig. 1). Bands with the molecular weights of 88, 78, 40, 38, 37, 33, 31, 27, 25, 23.5, 23, 21, 19, 17, 16.5, 16, 14.5, 13.5, 13 and 12.5 kDa as seen in the first dimension, appeared as spots along the diagonal in the second dimension. Polypeptides moving off the diagonal were seen to resolve at different positions. Whereas certain spots rose to higher molecular weight positions, others resolved at lower molecular weight positions below the diagonal. For clarity, bands moving to different positions off the diagonal resulting on their reduction could be discerned through the use of two-dimensional gel electrophoresis (Fig. 1). Bands with the molecular weights of 60, 58, 52 and 25 kDa as seen in the first dimension moved below the diagonal as two spots each at different molecular weight positions in the second dimension. Thus, on the basis of two-dimensional gel electrophoresis (Fig. 1), following relationships of bands seen in the first dimension (1D, -2ME) with their respective spots appearing below the diagonal in the second dimension (2D, +2ME) could be discerned:

Band (1D, -2ME)   Pair of spots (2D, +2ME)
60* kDa = (38*+21*) kDa
58* kDa = (40.5*+25*) kDa
52** kDa = (38.5*+20*) kDa, (36*+20*) kDa
25* kDa = (18*+16.5*) kDa

The band of 49* kDa moved off the diagonal, resolving as a single spot of 29+ kDa in the second dimension. The band of 110* kDa seen in the first dimension resolved in the second dimension into two spots of 60* kDa and 65* kDa. Certain other bands seen under non-reducing conditions in the first dimension (51*, 20** and 13.5** kDa) converted to spots with greater apparent molecular weight, in positions higher up the diagonal in the second dimension. Whereas the band of 51* kDa resolved to positions of 53* and 57* kDa, that of 20** kDa converted to spots at the positions of 21.5** and 27** kDa, and the band of 13.5** kDa moved as a spot to the higher molecular weight position of 16** kDa in the second dimension.

To assign various bands, as seen in the total seed protein extracts, to their respective solubility fractions, albumins, globulins, prolamins and glutelins were
separated by electrophoresis on SDS-gels (Fig. 2; Table 1). A few lightly staining bands with molecular weights of 23.5, 20, 16.5, 14 and 13 kDa, could be seen in the albumin fraction under non-reducing conditions; a new band of 26 kDa was observed under reducing conditions. Using 2-D gel electrophoresis, the band of 26* kDa observed under reducing conditions was shown to result from the polypeptide of 20* kDa present under non-reducing conditions (Fig. 3-A). The globulin fraction consisted of a large number of polypeptides, with those of molecular weights of 57, 53, 40 and 37 kDa as low intensity bands and those of molecular weights of 27, 23, 20, 16, 14.5, 13.5 and 12.5 kDa as dark bands (Fig. 2). The band of 20 kDa moved to the higher positions of 21.5 and 27 kDa, and that of 13.5** kDa to the position of 16** kDa in the second dimension (Fig. 3-B). The remaining globulin polypeptides were found to resolve as spots along the diagonal. The prolamins were represented by fewer polypeptides, such as those with molecular weights of 21, 17, 15, 14 and 13 kDa under reducing conditions (Fig. 2), the band of 21 kDa disappeared and the band of 17 kDa exhibited enhanced intensity. The glutelin fraction under non-reducing conditions was represented by bands of 110, 88, 78, 60, 58, 52, 51, 49, 38, 25, 20, 16.5, 14.5 and 13.5 kDa (Fig. 2). As observed for total seed protein extract on 2-D gel, the bands of 60, 58, 52 and 25 kDa in the glutelin fraction also moved down as two spots each to molecular weight positions as seen for those in the total seed protein extracts (Fig. 3-C); the band of 49+ kDa moved down as single spot of 29+ kDa. In addition to these bands, the band of 51* kDa shifted to

![Fig. 2. SDS-polyacrylamide gel electrophoresis of seed protein fractions of rice line 'Taroari basmati'.](image)

- a, Prolamins; b, Glutelins; c, Globulins; d, Albumins.
- `-2ME' stands for absence of 2-mercaptoethanol; `+2ME' stands for presence of 2-mercaptoethanol.

<table>
<thead>
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<th>Table 1. Polypeptide composition of four seed protein fractions of rice under reducing and non-reducing conditions.</th>
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<tr>
<td>Albumin polypeptide</td>
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<tr>
<td>-2ME</td>
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<tr>
<td>23.5</td>
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<tr>
<td>20*</td>
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<td>16.5</td>
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<td>14</td>
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<td>13</td>
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<td>20**</td>
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<td>16</td>
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<tr>
<td>14.5</td>
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<td>13.5**</td>
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<td>12.5</td>
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- ●, ◆, ◊, ▲, +, **, ■ and ● denote the relationship of polypeptides in two columns of a given protein fraction representing non-reducing (-2ME) and reducing conditions (+2ME); details in the text.
the higher molecular weight positions of 53* and 57* kDa.

**DISCUSSION**

Two-dimensional gel electrophoresis, in which polypeptides are separated according to their apparent molecular weight under non-reducing conditions in the first dimension, followed by reducing conditions in the second, is particularly valuable for analyzing the occurrence of disulphide linkages. In the present study, it also provided highly useful information about the relationships between bands of different rice proteins seen under non-reducing conditions and their respective polypeptides under reducing conditions. A large number of bands such as those with molecular weights of 88, 78, 40, 38, 37, 33, 31, 27, 25, 23.5, 23, 21, 19, 17, 16.5, 16, 14.5, 13.5, 13 and 12.5 kDa resolved as spots at the same molecular weight position along the diagonal on 2-D gels. In this way, these polypeptides which remained unchanged in molecular weight under reducing and non-reducing conditions did not show the occurrence of disulphide linkages. Meanwhile, spots occupying positions above and below the diagonal resulted from the reduction of different types of disulphide linkages, whereas intrapoly peptide disulphide linkages were observed in certain cases, and interpoly peptide linkages forming homodimeric/heterodimeric pairs were seen in others.

Thus, through the use of two-dimensional gel electrophoresis, rice proteins could be shown to have a number of heterodimeric polypeptide pairs with the following composition:

<table>
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<tr>
<th>Polypeptide pairs</th>
<th>Disulphide-bonded subunits</th>
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<tr>
<td>60* kDa</td>
<td>(38*+21*) kDa</td>
</tr>
<tr>
<td>58* kDa</td>
<td>(40.5*+25*) kDa</td>
</tr>
<tr>
<td>52** kDa</td>
<td>(38.5*+20*) kDa, (36**+20**) kDa</td>
</tr>
<tr>
<td>25* kDa</td>
<td>(18*+16.5*) kDa</td>
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The band of 49° kDa resolving as a single spot of 29° kDa in the second dimension could be explained as a homodimer having interpoly peptide disulphide linkages. Similarly, the band of 110° kDa resolving into two spots of 60° and 65° kDa in the second dimension suggested that it represented the aggregate of polypeptides of 60° and 65° kDa. The bands of 51°, 20° and 13.5° kDa, which were different in shifting to higher positions of 53° and 57° kDa, 21.5° and 27° kDa, and 16° kDa, respectively, thus, showing the presence of intrapoly peptide linkages.

Previous studies by different researchers have reported the presence of either only one type of glutelin subunit pair with the molecular weight of 57 kDa (Yamagata et al, 1982; Krishnan and Okita, 1986), or two subunit pairs with molecular weights of 50 and 62 kDa (Robert et al, 1985). In the present electrophoretic study, however, greater numbers of polypeptide pairs have been observed in the rice glutelin fraction, with those of 60°, 58°, 52° and 25° kDa representing heterodimers and that of 49° kDa as homodimer. In various other studies involving mutants, polyploids and different wild species of rice, such polypeptide pairs were consistently observed by us (2006). In the electropheretograms of glutelin fractions studied by previous researchers, a number of bands at molecular

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**Fig. 3. Two-dimensional gel electrophoresis of albumins (A), globulins (B) and glutelins (C) of rice line ‘Taroari basmati’.**

1D (-2ME), SDS-polyacrylamide gel electrophoresis under non-reducing conditions in the first dimension; 2D (+2ME), SDS-polyacrylamide gel electrophoresis under reducing conditions in the second dimension.
weight positions lower and higher than those of the major bands described above have been simply explained as contamination by albumin/globulin (in excess of 58 kDa) and prolamin (14 kDa) polypeptides (Wen and Luthe, 1985; Krishnan and Okita, 1986; Snow and Brooks, 1989). Meanwhile, Zhao et al (1983) described the polypeptides of 14 kDa as being similar to those present in glutelins of other cereals.

In the present study, polypeptides in the molecular weight range of 13 to 21 kDa in a particular fraction were also similar to those of the polypeptides observed in certain other fractions. These have been described by previous researchers as contamination, without giving specific evidence. In our studies, however, we have categorically shown that polypeptides which are common to any two given fractions exhibited different characteristics. Polypeptides at the position of 20 kDa are seen in albumin, globulin and glutelin fractions. As observed from their behavior on 2-D gels, these polypeptides cannot be considered to be cross-contamination, whereas the albumin polypeptide of 20* kDa changes to the 26* kDa position in the second dimension, the globulin polypeptide moves to the 21.5* and 27* kDa positions. Thus, this 20 kDa polypeptide in the albumin and globulin fractions contains intrapolyptide disulphide linkages. The 20 kDa glutelin polypeptide seen under non-reducing conditions, however, remains unchanged on a 2-D gel and thus does not include disulphide linkages. The 20 kDa glutelin polypeptide is also seen in the presence of 2-mercaptoethanol as the reduced product of the 52* kDa band. The 16.5 kDa polypeptides seen in the albumin and glutelin fractions do not exhibit any change in molecular weight positions under non-reducing and reducing conditions. The polypeptide present in the albumin fraction, extracted at the first step of the extraction sequence, is lighter in intensity, whereas that present in the glutelin fraction, extracted at the last step, is relatively darker and thus these are unlikely to represent cross-contamination. In addition to bands with the molecular weights of 57, 40 and 20 kDa, their studies on rice mutants showed the occurrence of a number of other bands in the glutelin fraction, but these were left unexplained. Luthe (1983), and Schaeffer and Sharpe (1990) used Tris-HCl containing SDS-urea after extracting albumins, globulins and prolamins. Kumamaru et al (1988) tried the extraction of glutelins in 1% lactic acid buffered with Tris-HCl (pH 7.5) after albumins and globulins, and also at the last step of sequential extraction, after albumins, globulins and prolamins. In addition to bands with the molecular weights of 57, 40 and 20 kDa, their studies on rice mutants showed the occurrence of a number of other bands in the glutelin fraction, but these were left unexplained. Luthe (1983), and Schaeffer and Sharpe (1990) used Tris-HCl containing SDS to extract glutelins after separation of albumins, globulins and prolamins. Whereas all the above workers have used 2-mercaptoethanol for extraction of glutelins, our studies involving the extraction of glutelins with and without 2-mercaptoethanol showed no difference in the composition of low molecular weight polypeptides. After different extraction trials, we preferred to follow the method given by
Luthe (1983), and by Schaeffer and Sharpe (1990) extracting proteins in order as albumins, globulins, prolamins and glutelins. Also, the polypeptide composition described by earlier workers was based on gel electrophoresis only under reducing conditions and thus the occurrence of disulphide linkages in different polypeptides could not be studied by these workers. Through the use of one- and two-dimensional gel electrophoresis, our study has provided a clearer picture of the polypeptides present in different fractions.

The major hexameric 11S globulin subfraction in the pea family consists of heterodimeric subunit pairs. Initially, a single type of subunit pair of 60 kDa (Wright and Boulter, 1974) was reported and proposed to form the hexamer. Later studies in *Vicia faba*, however, revealed the 11S protein to be highly heterogeneous (Matta et al, 1981), with as many as 10 different types of subunit pairs, in the molecular weight range of 37–79 kDa, associating randomly to yield different hexameric forms; in addition, nine pairs with the molecular weights of 42–89 kDa, were reported in *Lathyrus sativus* (Sood et al, 1995). The five heterodimeric subunit pairs observed in the present study of rice glutelin also exhibit wider heterogeneity in molecular weight of subunit pairs and their respective subunits. Rice glutelin exhibits appreciable similarities to legumin-like proteins in respect of a number of characteristics, including its occurrence as polypeptide pairs of large and small subunits, their molecular weights (Wen and Luthe, 1985), biosynthesis (Yamagata et al, 1982) and amino acid sequences (Zhao et al, 1983). In the case of legumin, wide heterogeneity has been reported with respect to the large acidic subunits, which have the molecular weight in the ranges of 23–53 kDa, each having a large subunit (ranging over 15–39 kDa) and a small subunit (ranging over 10–26 kDa), were reported. Thus, rice glutelins show more similarity to *C. melo* globulins, in having wider heterogeneity in the molecular weight of their small basic subunits.

On the basis of X-ray crystallographic studies of the 11S protein in *Cucurbita pepo* (Blagrove and Lilley, 1980), it has been suggested that acidic subunits are present on the surface, and the basic subunits towards the inside, of the hexameric protein molecules. The occurrence of acidic subunits towards the surface of protein molecules may make them more prone to the influence of cellular microenvironment and may have acted as one of the possible causes of variation in the sizes of acidic subunits in legume proteins. Various α-subunits of rice glutelin have been grouped into two classes, glutelin A and glutelin B, with α₁, α₃ and α₄ being classified as A type, and α₁, α₅ and α₆ as B type (Hibino et al, 1989). As hypothesized by Tanaka et al (2004), some of these A and B type glutelin subunits polymerize into larger macro-molecular complexes/higher order structures whereas others remain as low molecular weight species. Unlike 11S globulins, rice glutelin subunit pairs are not organized to internalize the small basic subunits and this may be one of the causes of heterogeneity observed in the molecular weight of glutelin small subunits. In view of the occurrence of different glutelin subunits observed in the present study and those reported by Tanaka et al (1987), it will be highly relevant to carry out immunological studies and MALDI-TOF-MS for further useful information on these glutelin polypeptides.

Duplications are generally considered as an important source of variation, which play an important role during evolution. Variation in the sizes of acidic subunits in 11S proteins has been attributed to the presence of several repeated sequences rich in Gly and Glu as in cruciferin (Simon et al, 1985) or Asp and Glu as in legumin (Lycett et al, 1984) and glycinin (Negero et al, 1985). It would be of interest to purify different acidic and basic subunits of rice glutelin followed by sequence analysis to determine if their heterogeneity can also be attributed to such repeated sequences.
The heterogeneity of acidic and basic subunits of rice glutelin points towards the occurrence of multiple glutelin genes. Through the studies on cDNA clones, Takaiwa et al (1987) reported two types of glutelin genes, type I and type II, which code for the rice glutelins having acidic and basic subunits with slight differences in their molecular weights. Later, more sequences of a new subfamily of glutelin genes were also described by the same group (Takaiwa et al., 1991). The recently sequenced rice genome (Goff et al., 2002; Sasaki et al., 2002; Yu et al., 2002) also indicates the occurrence of additional glutelin genes. Consequent to reports of heterogeneity occurring in the legumin fraction (Matta et al., 1981), studies were undertaken on different pea legumin types in terms of their cDNA clones, precursor polypeptides, and gene organization (Domoney et al., 1984; Bown et al., 1985). More than ten legumin genes, mapping to at least three genetic loci, were estimated to occur in the haploid pea genome (Domoney et al., 1986; Gatehouse et al., 1988). Thus, in view of the occurrence of different glutelin polypeptides reported in the present paper, studies may be planned for elucidation of their biosynthesis, processing, gene families and their organization along the lines followed for pea legumins mentioned above.

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REFERENCES


