JPP JOURNAL OF PROTEINS AND PROTEOMICS
5(2), May-August 2014, pp. 109-119

Research Article

CHANGES IN RICE SEED PROTEINS OVER DEVELOPMENTAL AND GERMINATION STAGES

Arvinder Singh and N. K. Matta

Department of Botany, Kurukshetra University, Kurukshetra 136119, India

Abstract: Changes occurring in protein fractions and their constituent polypeptides were studied at different developmental and germination stages in seeds of rice line 'Pusa-HH'. During development, seed protein content kept increasing upto 10 days after flowering (7.5%) after which it dropped down to 5.9% at maturity. Proportion of glutelins, the dominant fraction throughout seed development, followed a decrease in its proportion from 78% at 4 days after flowering to 58% at maturity. Proportion of prolamins increased steadily from 7% to 16%; those of albumins from 9.1% to 10.8% and of globulins from 5.6% to 15.1%. On SDS-PAGE most of the polypeptides as seen in the total protein extracts of mature seeds were also present at 4 days after flowering stage. Bands of molecular weight 60 kDa, 58 kDa, 52 kDa, 51 kDa and 49 kDa yielded polypeptides of molecular weight 57 kDa, 53 kDa, 40.5 kDa, 38 kDa, 36 kDa, 29 kDa, 25 kDa, 21.5 kDa and 20 kDa on reduction with 2-mercaptoethanol. Densitometric gel scanning showed an increase in relative concentration of all polypeptides except those of molecular weight 32 kDa and 21.5 kDa which decreased by 69% and 60%, respectively.

Protein content during germination revealed a decrease from 5.9% in the ungerminated seed to 1.7% at 6th day of germination. Whereas proportion of glutelins exhibited a decrease (57.3% to 35.2%), a subsequent increase was noticed in the relative proportion for albumins, globulins and prolamins. Under reducing conditions, glutelin polypeptides of molecular weight 88 kDa, 57 kDa, 53 kDa, 36-40.5 kDa, 32 kDa, 29 kDa and 16 kDa degraded first. Polypeptides of molecular weight 32 kDa, 29 kDa and 16 kDa completely degraded by 3rd day, those of 88 kDa, 57 kDa and 53 kDa by the 4th day and those of molecular weight 36-40.5 kDa by 5th day of germination. Degradation products appearing midway as new bands of molecular weight 50 kDa and 35 kDa disappeared by 4th and 5th day leaving only a few bands of molecular weight 20 kDa, 18 kDa and 13 kDa on the 6th day.

Keywords: Rice; germination; development; seed storage proteins; gel electrophoresis.

Introduction

Seed evolve as a propagule for ensured supply of nutrients required by the embryo for its growth into a seedling. For this, reserve foods in the form of carbohydrates, lipids and proteins are synthesized and accumulated during seed development. Of these different seed reserves, proteins serve as a source of nitrogen and sulphur for the growing seedling. These proteins which are synthesized at one stage and are utilized at another have been, thus, rightly called as seed storage proteins. On the basis of their solubility, plant proteins have been classified into four groups. The albumins are soluble in water and globulins in salts; those soluble in dilute alkali or dilute acids called glutelins and the fourth type named prolamins are soluble in aqueous alcohol (Osborne, 1924). Whereas globulins are the major storage proteins in seeds of leguminous plants, prolamins and glutelins are mainly present in cereals (Juliano, 1972). On the other hand, glutelins represent the dominating protein fraction in case of rice, and glutelins together with

Corresponding Author: **Arvinder Singh**
E-mail: arvinder_kuk@redifmail.com

Received: May 20, 2014
Accepted: July 30, 2014
Published: August 31, 2014

prolamins contribute about 75% of total protein in pearl millet (Chandna and Matta, 1990).

Rice is one of the important crops which stands next to wheat and maize with respect to its annual grain production and provides food for about 60% of the world's population (Khush, 1997). Though with a lower protein content varying from 5.9 to 11.1% (Villareal and Juliano, 1978), rice protein is known to have better nutritional quality in comparison to the other cereals because of its higher lysine content of 3.5 to 4%. Whereas glutelins constitute about 80%, prolamins represent only 5% of the total rice proteins (Juliano, 1972). However, using different extraction protocols, Krishnan and White (1995) reported a lower proportion of 53% for glutelins and a higher one for prolamins as 35%. Whereas prolamins are deposited in the spherical protein bodies called PB-I located in the centre of endosperm, glutelins are stored in the irregular protein bodies called PB-II present in the peripheral regions (Tanaka *et al.*, 1980). Glutelins are known to be represented by the polypeptide-pair of mol. wt. 57 kDa which on reduction breaks into a large acidic and a small basic subunit with molecular weights 37-39 kDa and 22-23 kDa respectively (Yamagata *et al.*, 1982). In respect of their molecular weights, rice glutelins are thus considered similar to the legumin-like proteins constituting 11S globulins of other crops. The biosynthesis of glutelin as a precursor from a single gene followed by its processing into a disulphide-bonded polypeptide pair is also reported to be similar to that of the 11S proteins of pea (Croy *et al.*, 1980) and soybean (Katsube *et al.*, 1999). The two proteins also show homology with respect to the amino acids sequence (Zhao *et al.*, 1983) and the nucleotide sequence of their genes (Takaiwa *et al.*, 1987). However, rice glutelin polypeptide pairs form multimolecular aggregates through disulphide linkages unlike the highly organized hexameric structure of legumin-like proteins. Prolamins, the alcohol-soluble proteins, are known to be constituted by polypeptides of mol. wt. 17 kDa, 15 kDa, 14 kDa and 13 kDa (Singh, 2006).

Accumulation of different polypeptides in rice grains is known to begin early during seed development and reaches its maximum between 8 d.a.f. and 10 d.a.f. (Yamagata *et al.*, 1982; Luthe, 1983). The two major proteins i.e. glutelins and prolamins are reported to follow different temporal accumulation patterns during the course of seed formation. Whereas glutelin polypeptides (mol. wt. 37 - 39 kDa and 22 - 23 kDa) and globulin polypeptide (26 kDa) are synthesized early and are known to appear by 5 d.a.f. increased intensity of prolamin polypeptides of mol. wt.10-16 kDa on gels could be seen at 10 d.a.f (Yamagata *et al.*, 1982). With synthesis and accumulation of prolamins exceeding that of glutelins in later stages of seed development (Li and Okita, 1993), rice proteins follow different temporal accumulation patterns.

During germination, seed proteins are hydrolyzed to provide small peptides and amino acids which may remain in the storage tissue or be translocated to the growing seedlings. A number of reports are available with respect to activities of different proteolytic enzymes in cereals during seed germination (Shutov and Vaintraub, 1987; Mitsuhashi and Oaks, 1994; Kato and Minamikawa, 1996). According to these reports, a series of peptidases are responsible for degradation of seed storage proteins. Very few studies have also been carried out for patterns of protein mobilization in germinating seeds of cereals like maize, barley and rice (Horikoshi and Morita, 1981; Torrent *et al.*, 1989; Kumar and Matta, 2011). In maize, degradation of major storage proteins, glutelins and zeins is completed between 3 to 8 days of germination. In rice, it has been shown that the acidic subunits of glutelin were the first to undergo proteolysis leaving behind the basic subunits intact (Yano *et al.*, 2001). However, detailed studies on the changing pattern of rice proteins during their accumulation in the developing seeds and their degradation in the germinating seeds have not been taken up earlier. Therefore, the present work aimed at concerted study on changes in different grain protein characteristics occurring in the developing and germinating rice seeds.

Materials and Methods

Materials : Plants of rice line 'Pusa-HH' were field grown at the Botanical gardens of Kurukshetra University and seeds of different

developmental stages were harvested at an interval of 2 days from 4 days after flowering (d.a.f.) to 12 d.a.f. and at maturity. For seed germination studies, surface sterilized seeds were placed on moist filter paper in covered glass dishes at $30^0C \pm 2$ in the dark. Seeds were collected at different germination stages as follows:

I. When the seed was swollen with radicle just emerging from the micropyle, after one day of germination.

II. When the radicle had grown to a length of approx. 15mm i.e. after 2 days of germination.

III. After 3 days, when the radicle grew to about 25mm and plumule to about 10mm.

IV. When the plumule had grown to about 20mm length i.e. after 4 days of germination.

V. After 5 days of germination, when the plumule was about 40mm.

VI. After 6 days, when the plumule had further grown longer to more than 50mm height.

The seeds collected at different developmental and germination stages were dried under vacuum and stored in the deep freeze for various analyses.

Preparation of total seed protein extracts - Total seed protein extracts were prepared as described by Matta (1981) with minor modifications. 40 mg of the seed meal was suspended in 400µl of 0.2M Tris-HCl buffer (pH 6.8) containing 2% sodium dodecyl sulphate (SDS) in eppendorf tubes. The suspension was heated in a water bath at 80°C for about 40-45 min with frequent vortex mixing. The suspension was centrifuged at 2,000*g* using a microfuge and the extract used for electrophoretic studies.

SDS-polyacrylamide gel electrophoresis - SDS-polyacrylamide gel electrophoresis was carried out on 14% gels following the method of Laemmli (1970). For gel electrophoresis under reducing conditions, 2% 2-mercaptoethanol was added to the seed protein extract and the samples were heated in an oven at 90°C for 10 min before loading these onto the gels. The gel was run at 17 mA and after the tracking dye moved down into the separation gel, current was increased to 25 mA. The gel was stained with Coomassie brilliant blue (0.05%) dissolved in a solvent containing methanol, acetic acid and distilled water in the ratio 50:7:43 (v/v) and destained in the same solvent mixture but lacking the dye.

Densitometric scanning of gels - Relative concentration of polypeptides constituting various protein samples separated by SDS-PAGE was determined by densitometric scanning of the gel. Stained gels were photographed and saved images analysed by 'TotalLab' software from Nonlinear Dynamics Ltd. (downloaded from www.nonlinear.com) for relative concentration of different polypeptides.

Seed protein fractions - Four seed protein fractions were separated by using the methods employed by Luthe (1983) and Schaeffer & Sharpe (1990) with slight modifications. The water soluble albumins were extracted in 10mM Tris-HCl buffer (pH 7.5) and for the sequential extraction of other protein fractions, different solvents buffered with Tris-HCl (pH 7.5) used were 0.5 M NaCl for globulins, 55% n-propanol for prolamins and 0.5% SDS for glutelins.

Protein estimation - For estimation of seed protein content, semi-micro Kjeldahl method was followed. Protein concentration in different protein fractions was determined using the method given by Bradford (1976).

Results and Discussion

A. Investigations on developing seeds

Seed protein content and polypeptide patterns - In the developing seeds, protein content showed an increasing trend in the initial stages followed by a decrease towards maturity (Figure 1). Estimated as 4% at 4 d.a.f. stage, it increased steadily to 7.5% at 10 d.a.f. and then was found to decrease down to 5.9% as the seed approached maturity.

When electrophoresed on SDS-gels, a number of polypeptides could be resolved in the total seed protein extracts from seeds of different developmental stages. All bands as observed in the mature seeds under non-reducing conditions i.e. of molecular weight 110 kDa, 88 kDa, 78 kDa, 60 kDa, 58 kDa, 52 kDa, 51 kDa, 49 kDa, 43 kDa,

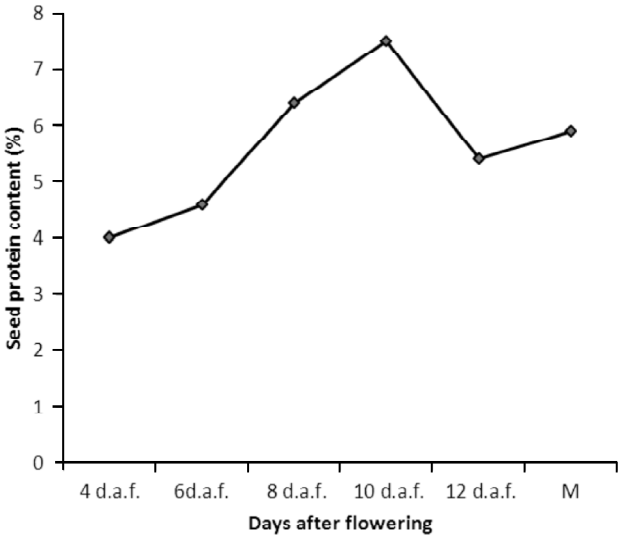

Figure 1: **Seed protein content at different developmental stages in rice line 'Pusa HH'; 4 d.a.f., 6 d.a.f., 8 d.a.f., 10 d.a.f., 12 d.a.f and M represent development stages**

38 kDa, 32 kDa, 27 kDa, 20 kDa, 16 kDa, and 13 kDa (Figure 2) could be seen at the early stage of 4 d.a.f., those of mol. wt. 110 kDa, 60 kDa, 58 kDa, 52 kDa and 20 kDa being the darkly staining ones. In the initial stages (Figure 2), intensity of various bands like those of mol. wt. 88 kDa, 78 kDa, 60 kDa, 58 kDa, 52 kDa, 51 kDa, 49 kDa, 38 kDa, 20 kDa, 16 kDa, and 13 kDa was low and it kept on increasing later with progress in seed

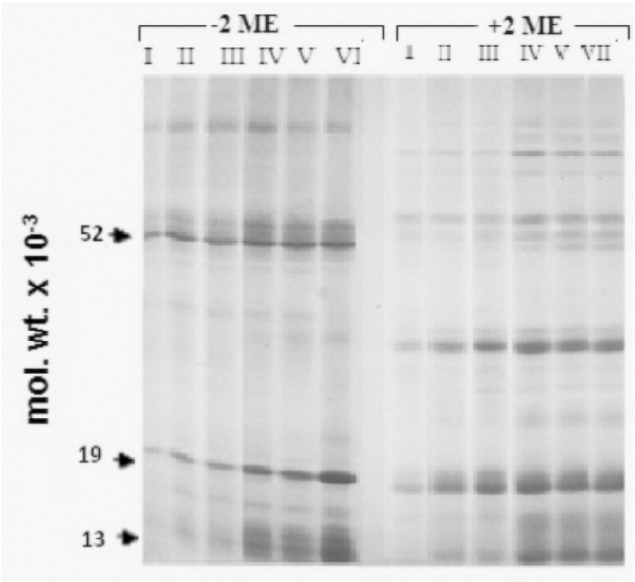

Figure 2: **SDS-polyacrylamide gel electrophoresis of seed protein extracts of seeds at different developmental stages of rice line Pusa - HH.**
I = seed protein extract of developmental stage 4 d.a.f ; II= 6 d.a.f; III =8 d.a.f; IV= 10 d.a.f; V=12 d.a.f; VII= mature. -2ME and +2ME stands for without and with 2-mercaptoethanol

development. On the other hand, bands of mol. wt. 110 kDa and 43 kDa exhibited a gradual decrease in their intensity. In addition to the changes in the concentration of polypeptides, a new band of molecular weight 14 kDa could also be detected at10 d.a.f.; this band kept increasing in intensity till maturity. With progress in the seed development, these bands were observed to be increasingly synthesized and looking darkest at 10 and 12 d.a.f. In their detailed studies on polypeptide composition of different protein fractions, Singh and Matta (2011) have reported that bands of molecular weight 110 kDa, 88 kDa, 78 kDa, 60 kDa, 58 kDa, 52 kDa, 49 kDa, 38 kDa, 20 kDa represent the glutelin fraction and those of molecular weight 16 kDa and 13 kDa represent the prolamins. On the other hand, bands of molecular weight 51 kDa, 43 kDa, and 32 kDa were reported as globulin polypeptides and those of molecular weight 27 kDa and 14 kDa as albumin polypeptides.

Under reducing conditions, bands representing polypeptides of molecular weight 88 kDa, 78 kDa, 65 kDa, 60 kDa, 57 kDa , 53 kDa, 40.5 kDa, 38 kDa, 36 kDa, 32 kDa, 29 kDa, 27 kDa, 25 kDa, 21.5 kDa, 20 kDa ,16 kDa, and 13 kDa could be observed at 4 d.a.f.

A comparison of polypeptides under different electrophoretic conditions revealed that the bands of mol.wt. 60 kDa, 58 kDa, 52 kDa, 51 kDa and 49 kDa present under non-reducing conditions disappeared under reducing conditions whereunder new bands of molecular weight 57 kDa, 53 kDa, 40.5 kDa, 38 kDa, 36 kDa, 29 kDa, 25 kDa, 21.5 and 20 kDa could be seen. In this way, polypeptides lacking and those containing disulphide linkages could be observed at the very early stage of 4 d.a.f. Whereas band of molecular weight 60 kDa, 58 kDa, 52 kDa and 49 kDa are known to represent glutelin subunit pairs, those of molecular weight 40.5 kDa, 38 kDa, 36 kDa represent the large acidic and those with molecular weight 25 kDa , 21.5 kDa, and 20 kDa small basic subunits of glutelin in the presence of 2-mercaptoethanol (Singh and Matta, 2011). In their characterization studies of rice glutelin polypeptides, Wen and Luthe (1985) had earlier reported a number of large subunits of mol. wt. 28.5 to 30.8 kDa and small subunits of molecular weight 20.6 to 21.6 kDa.

Densitometric scanning studies - Relative concentration of polypeptides and their densitometirc scanning profile at different developmental stages as analyzed under reducing conditions is given in Table I and Figure 3. As is vivid, relative concentration of polypeptides of mol.wt. 88 kDa, 78 kDa, 65 kDa, 60 kDa, 57 kDa, 53 kDa, 40.5 kDa, 38 kDa, 36 kDa, 29 kDa, 25 kDa, 20 kDa, 16 kDa and 13 kDa registered an increase in accumulation, the maximum being for that of mol. wt. 36 kDa which increased from 5.45% at 4 d.a.f. to 11.26% in the mature seeds. The rate of accumulation was also high for polypeptides of molecular weight 78 kDa, 65 kDa, 60 kDa, 40.5 kDa, 38 kDa, 36 kDa, 29 kDa, 25 kDa, 19 kDa, 16 kDa and 13 kDa. On the other hand, relative concentration of polypeptides of molecular weight 32 kDa and 21.5 kDa decreased during seed development from 4 d.a.f. to maturity from 5.04% to 1.57% and 4.12% to 1.66%, respectively.

Table 1
Relative concentration of polypeptides in developing seeds of rice line 'Pusa-HH'

Polypeptide Mol. wt. (kDa)	*Relative concentration (%) at different developmental stages*					
	4 d.a.f.	*6 d.a.f.*	*8 d.a.f.*	*10 d.a.f.*	*12 d.a.f.*	*Mature*
88	6.28	6.57	6.59	6.66	7.19	7.29
78	6.16	6.76	7.55	7.32	8.41	8.33
65	8.37	8.42	9.05	9.84	10.40	10.36
60	0.45	1.85	1.59	1.71	2.65	2.47
57	2.07	2.62	3.22	3.07	3.89	3.92
53	– – – -	1.65	1.81	2.05	2.15	2.95
40.5	4.52	4.58	4.83	6.09	7.44	7.39
38	2.80	2.36	3.42	4.96	5.07	6.24
36	5.45	10.88	13.28	11.89	11.39	11.26
32	5.04	4.59	4.85	3.54	2.60	1.57
29	1.90	3.36	3.67	4.47	4.45	5.37
27	0.84	1.91	2.94	3.55	4.04	4.97
25	2.19	1.43	3.20	5.26	5.21	5.34
21.5	4.12	3.73	3.73	2.49	1.44	1.66
20	12.15	14.92	15.55	15.59	16.58	16.49
16	6.77	7.64	9.97	9.28	9.50	9.94
13	11.17	12.28	10.06	13.25	13.60	13.41

Proportion of four protein fractions - Table 2 shows the proportion of four protein fractions in the seeds of different developmental stages i.e., 4, 6, 8, 10, 12 d.a.f. and at maturity. In the mature seed, glutelins represented the major protein fraction (58%) whereas the proportion of remaining three fractions i.e. albumins, globulins and prolamins was in the range of approximately 11%, 15% & 16%. At the early stages i.e. at 4 d.a.f., glutelins represented a relatively higher (78.3%) proportion as compared to the lower proportion of albumins (9.1%), globulins (5.6%) and prolamins (7.0%). As the seed development progressed from 4 d.a.f. to maturity, albumins did not exhibit much of variation in their proportion at different stages whereas globulins exhibited a low proportion of 5.6% at 4 d.a.f., increased upto approximately 15% at 8 d.a.f. and remained more or less the same in the mature seeds. On the other hand, prolamins registered a constant increase in their proportion from 7.0% at 4 d.a.f. to 16.1% at maturity and the proportion of glutelins showed a decrease from 78% to 58%.

In the present study, accumulation of different polypeptides during seed development has been studied. Protein synthesis and accumulation kept on increasing as the seed development proceeded. Rice line under present investigation showed a high protein accumulation at very early stages i.e upto10 d.a.f., after that it showed a sudden reduction. It is likely that the activity of proteinases leads to reduction in protein content. These proteolytic enzymes are known to be functional at some stages during seed development (Harris and Chrispeels, 1975). In present investigation, the four seed protein fractions were seen to follow different synthesis rates and accumulation patterns during seed development, and thus showed that regulation was based on time specific expression of their genes. In rice, studies by Li and Okita (1993) showed increased accumulation of prolamins as compared to glutelins at later stages of seed development and this was explained to be due to overall abundance of prolamin mRNA transcripts. During early stages, the mRNA and protein synthesis are known to be regulated at transcriptional level. The degree of translatability and stability of a specific mRNA also plays an important role in protein synthesis. In this way, when transcriptional level of various genes shut down, the overall expression is determined by the post-transcriptional controls. Thus, the regulation

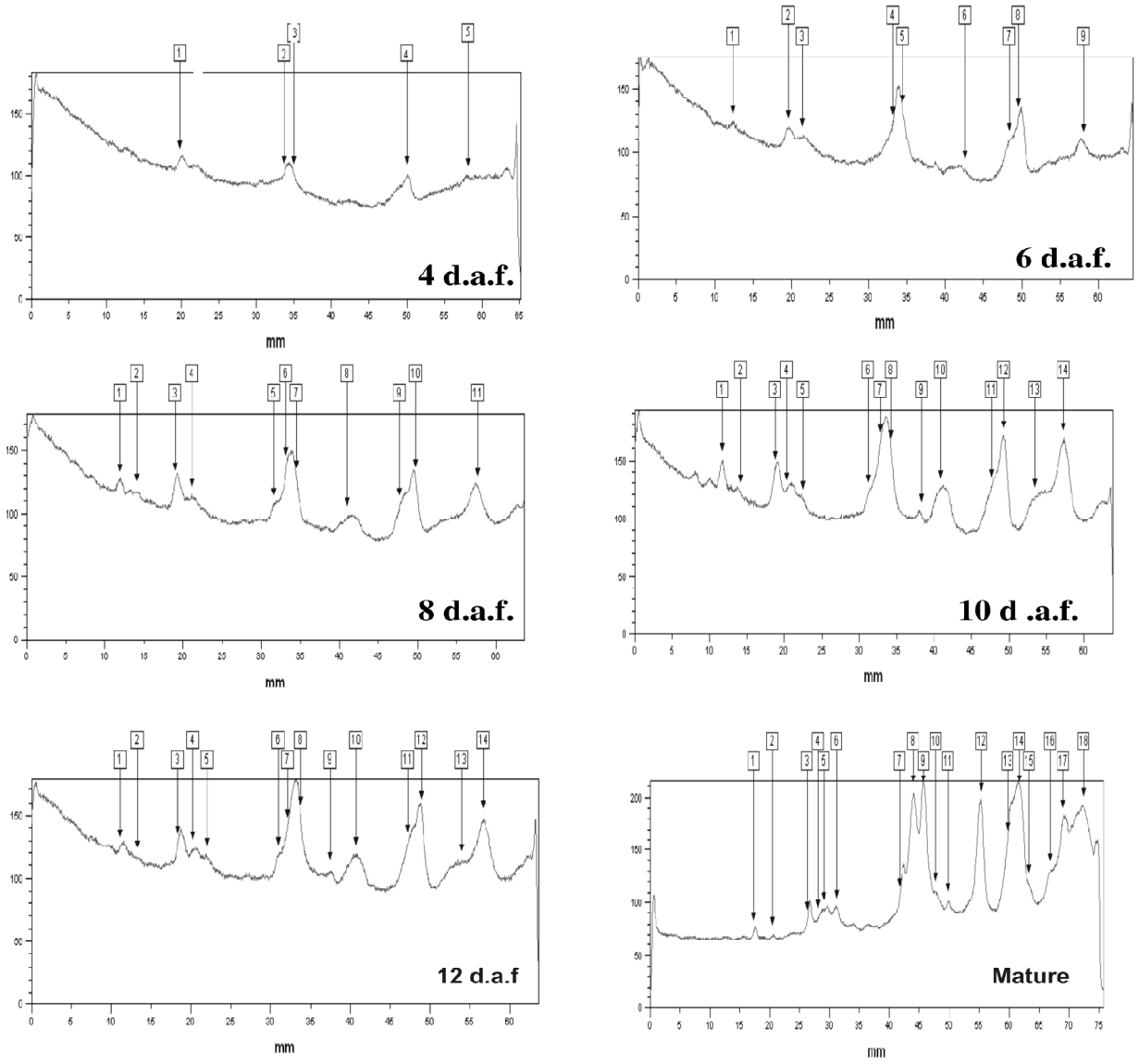

Figure 3: **Densitometric scanning profiles of different polypeptides at different developmental stages of rice line Pusa - HH**

Table 2
Proportion of four protein fractions at different seed developmental stages in rice line 'Pusa-HH'

Seed developmental stage	*Proportion of seed protein fractions (g/100g)*			
	Albumins	*Globulins*	*Glutelins*	*Prolamins*
4 d.a.f.	9.1	5.6	78.3	7.0
6 d.a.f.	10.8	8.0	72.7	8.5
8 d.a.f.	12.5	15.0	61.0	11.5
10 d.a.f.	12.8	15.7	59.1	12.4
12 d.a.f.	12.1	15.9	58.2	13.8
Mature	10.8	15.1	58.0	16.1

of storage proteins synthesis is operative at post-transcriptional level also. The present work did not involve studies at the mRNA level. However, based on studies on mRNA synthesis and stability undertaken recently by other workers viz. Kim *et al.* (1993), Zhu *et al.* (2003) and Xu *et al.* (2012) and based on current data on proportion of four fractions and their polypeptides , it may be stated that factors for molecular regulation of storage protein genes are functional differently for the four protein fractions, these being operational upto 10 d.a.f. in case of albumins and globulins and till quite late in case of prolamins during seed development under normal conditions.

B. Investigations on Germinating Seeds

As is evident from Figure 4, the seed protein content exhibited a decrease with progress in germination. The seed protein content as compared to 5.9% protein content in the ungerminated seeds, it was reduced to 1.7% by the 6th day of seed germination. The maximum decrease was noticed on the 3rd day of germination when the plumule had also emerged and radicle had grown to good length.

SDS-polyacrylamide gel electrophoresis of total protein extracts from seeds at different germinating stages (Figure 5) showed that the concentration of different polypeptides decreased constantly as the germination progressed from hydration to emergence of the radicle and then through growth of the seedling. As seen under non-reducing conditions, bands of molecular weight 110 kDa, 88 kDa, 60 kDa, 58 kDa, 52 kDa, 51 kDa, 49 kDa, 43 kDa, 38 kDa, 32 kDa, 20 kDa, 18 kDa, 16 kDa and 13 kDa observed in the ungerminated seeds decreased in intensity and almost disappeared leaving very faint bands of molecular weight 52 kDa, 43 kDa, 32 kDa, and 13 kDa at 6th day of germination. Polypeptides of molecular weight 110 kDa, 60 kDa, 58 kDa, 52 kDa, 51 kDa, 49 kDa, 38 kDa and 20 kDa were the first to be degraded as the decrease in their intensity could be noticed at 2nd and 3rd days; by the 4th day of seed germination, these bands except that of molecular weight 20 kDa, became very faint and by the 6th day of germination, all these polypeptides had completely disappeared. On the other hand, intensity of certain bands like those of molecular weight 32 kDa and 13 kDa increased up to the 4th day of germination after which it kept declining.

When analyzed in the presence of 2-mercaptoethanol, the polypeptides of molecular weight 88 kDa, 57 kDa, 53 kDa, 36-40.5 kDa, 32 kDa, 29 kDa and 16 kDa were revealed to degrade first. Whereas polypeptides of molecular weight 32 kDa, 29 kDa and 16 kDa degraded by 3rd, those of molecular weight 88 kDa, 60 kDa, 57 kDa, 53 kDa and 36-40.5 kDa disappeared by 4th and 5th day of germination. However, on 1st and 2nd day of germination, the intensity of bands of molecular weight 65 kDa, 21.5 kDa, 20 kDa, 18 kDa, 16 kDa and 13 kDa increased but later on

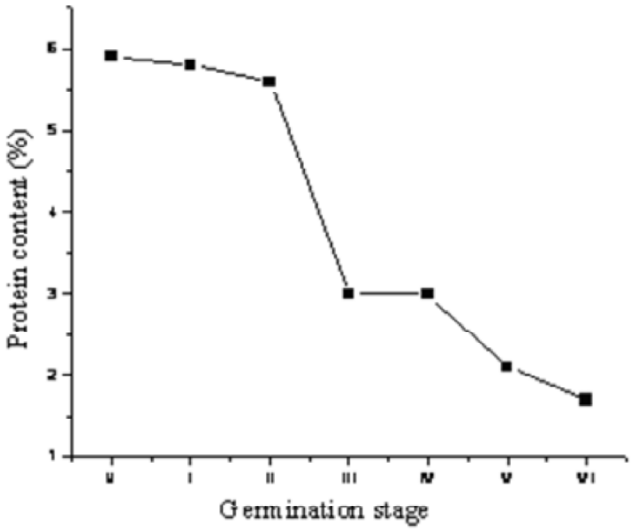

Figure 4: **Seed protein content at different germination stages in rice line 'Pusa HH'; U - Ungerminated seeds; I, II, III, IV, V, VI represent germination stages**

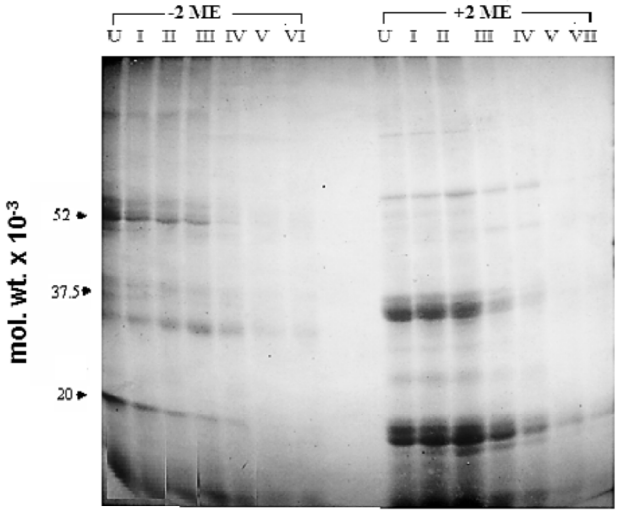

Figure 5: **SDS-polyacrylamide gel electrophoresis of seed protein extracts of seeds at different germination stages of rice line ' Pusa - HH' . U - Ungerminated seeds; I, II, III, IV, V, VI represent germination stages.**

most of these disappeared by 5th and 6th day of germination when bands of molecular weight 20 kDa, 18 kDa and 13 kDa could still be seen as faint bands. Some new bands of molecular weight 50 kDa and 35 kDa were observed to appear but were degraded completely by 4th and 5th day of germination.

Proportion of four protein fractions - Changes in the relative amount of four protein fractions during seed germination can be seen in Table 3. As the germination progressed, prolamins and

Table 3
Proportion of four protein fractions at different germination stages in seeds of rice line 'Pusa-HH'

Seed germination stage	*Proportion of seed protein fractions (g/100g)*			
	Albumins	*Globulins*	*Glutelins*	*Prolamins*
U	10.5	15.7	57.3	16.5
I	11.4	16.4	52.3	19.9
II	12.8	15.2	50.1	21.9
III	13.5	14.8	46.1	25.6
IV	14.0	17.1	42.9	26.0
V	14.7	19.3	39.7	26.3
VI	15.2	22.6	35.2	27.0

albumins followed an increase and glutelins exhibited a decrease in their proportion. The salt soluble globulins also exhibited an increased proportion at the end of seed germination. In this way, glutelins showed the maximum degradation and decreased in their proportion from 57.3% in the ungerminated seed to 35.2% at 6th day of germination. Whereas proportion of albumins and prolamins increased from 10.5% and 16.5%, respectively, in ungerminated seeds to 15.2% and 27.0% respectively at 6th day, that of globulins which was 15.7% in ungerminated seeds, increased to 22.6% by day 6th of germination. It is likely that the amino acid composition of degradation products of large subunits of glutelin subunit-pairs was such that these were extracted alongwith prolamins in aqueous alcohol without 2-mercaptoethanol, thus resulting in an increase in the proportion of prolamins as observed presently. On the other hand, proportion of globulins decreasing upto 3rd day of germination and showing an increase thereafter followed the same trend as described earlier (IRRI, Annual report, 1968).

Densitometric scanning studies - Table 4 and Figure 6 respectively show the relative concentration and densitometric scanning profile of polypeptides at different seed germination stages as analyzed under reducing conditions. As the germination stage advances, most of the polypeptides exhibited a decrease in their relative concentration except those of molecular weight 20 kDa, 18 kDa and 13 kDa which revealed an increase from 13.16% to 26.30%, 7.27% to 22.51%

Table 4
Relative concentration of polypeptides in germinating seeds of rice line 'Pusa-HH'.

Polypeptide Mol. wt. (kDa)	*Relative concentration (%) at different seed germination stages*						
	U	*I*	*II*	*III*	*IV*	*V*	*VI*
88	1.98	1.94	1.79	1.23	-----	-----	------
65	2.02	2.19	2.56	2.19	1.98	-----	------
60	1.97	1.75	1.17	0.76	-----	------	-----
57	1.65	1.48	1.43	1.01	-----	------	-------
53	1.34	1.24	1.02	0.87	-----	------	------
50	------	-----	1.44	3.04	3.08	------	------
40.5	5.26	3.87	3.52	3.50	2.08	------	------
38	9.61	7.52	7.58	6.05	3.02	------	-----
36	9.44	10.38	8.27	2.68	2.04	------	------
35	------	-----	4.84	1.81	-----	------	------
32	2.92	2.40	1.01	-----	-----	------	------
29	2.16	2.26	2.26	----	-----	------	------
21.5	6.82	6.60	6.22	6.73	5.23	------	------
20	13.16	13.90	12.98	16.71	16.06	21.77	26.30
18	7.27	9.50	9.96	10.88	11.52	19.12	22.51
16	9.99	10.19	11.22	-----	-----	------	------
13	14.96	14.29	13.12	17.70	23.76	45.05	51.19

and 14.96% to 51.19% when progressed towards later stages of germination i.e from ungermination period of seed to 6th day of germination.

Analysis of germinating seeds showed the pattern as to which proteins were utilized in which order during seed germination. The first to be utilized were the major polypeptides identified as the glutelin polypeptides which had molecular weight 88 kDa, 57 kDa, 53 kDa, 36-40.5 kDa, 32 kDa, 29 kDa and 16 kDa. The polypeptides of molecular weight 32 kDa, 29 kDa and 16 kDa were completely degraded by 3rd day, those of mol.wt. 88 kDa, 60 kDa, 57 kDa, 53 kDa, 36-40.5 kDa disappeared by 4th and 5th day of germination. At later stages of germination i.e. on the 6th day, only the polypeptides of molecular weight 20 kDa, 18 kDa and 13 kDa could be seen. Almost similar kind of degradation pattern for polypeptides had also been reported previously by Horikoshi and Morita (1982) in Japonica rice but with different molecular weight composition of these polypeptides. Their reports clearly showed the deconstruction of Protein Bodies-II

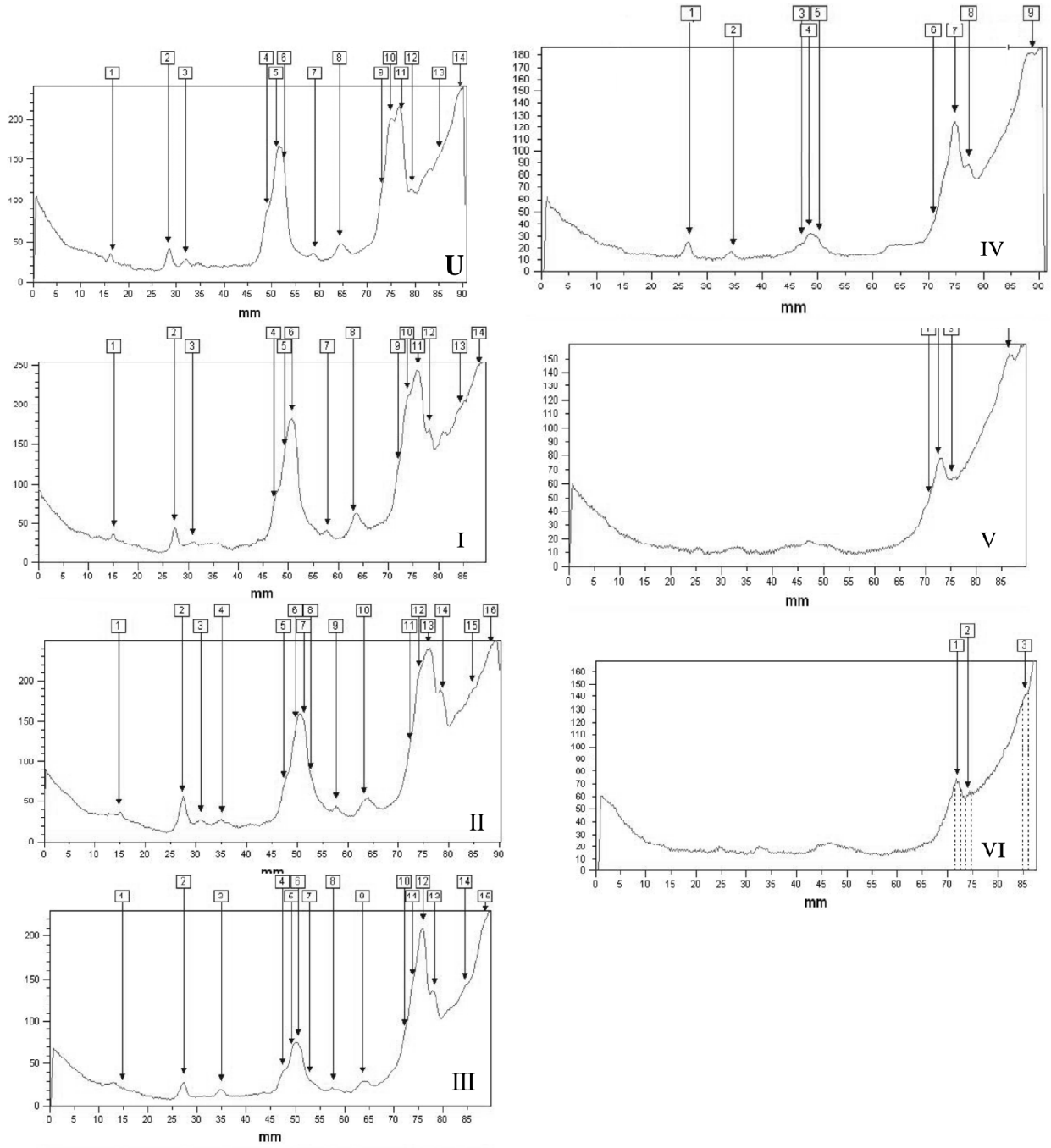

Figure 6: **Densitometric scanning profiles of different polypeptides at different germination stages of rice line Pusa - HH**

(PB-II) at 6 days of germination, starting its degradation from 3 days of germination. The PB-I on other hand reported to be degrading at later stages and could be detected at 9 days of germination. In the present study, the polypeptides which have been utilized from early days of germination belong to major protein fraction-glutelin and the degradation behaviour of PB-II supports our results of early degradation of glutelin polypeptides which are stored in this type of protein bodies. On the other hand, the appearance of new bands at molecular weight of 50 kDa and 35 kDa and the increase in their intensity might be due to the intermediate products accumulated as a result of degradation of polypeptides of higher molecular weights. Thus, in the early stages, polypeptides of different molecular weight are seen to be degraded and in

later stages, their degradation products are also further degraded. Yang *et al.* (2007) have also reported rapid degradation of rice glutelins on imbibition of 48h during seed germination. The process of mobilization of storage proteins is triggered either by activation of proteolytic enzymes already stored in the seed or by newly formed proteinases in protein bodies (Muntz *et al.*, 2001). Seed germination is a complex physiological process which is now known to require a large number of different proteins. Recently, detailed studies have also been initiated in rice for proteins involved in the molecular mechanisms and pathways for mobilization during seed germination (He and Yang, 2013).

Acknowledgements

Supply of seeds from Indian Agricultural Research Institute, New Delhi for the present work is thankfully acknowledged. First author is grateful to Kurukshetra University, Kurukshetra for research support in the form of a University Research Scholarship.

Abbreviation

kDA, kiloDalton; ME, 2-mercaptoethanol; d.a.f., days after flowering; mA, milliamperes

References

Bradford, M.M., (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of dye binding. Anal. Biochem. 72, 248- 254.

Chandna, M., and Matta, N K. (1990). Characterization of pearl millet protein fractions. Phytochem, 29, 3395-3399.

Croy, R.R.D., Gatehouse, J.A., Tayler, M., and Boulter, D. (1980). The purification and characterization of third storage protein (convicilin) from the seeds of pea (*Pisum sativum* L.). Biochem. J. 191, 509-516.

Harris, N., and Chrispeels, M.J. (1975). Histochemical and biochemical observations on storage protein metabolism and protein bodies analysis in cotyledons of germinating mung bean. Plant Physiol. 56, 292-299.

He, D., and Yang, P. (2013). Proteomics of rice seed germination. Plant Sci. 4, 246.

Horikoshi M., and Morita, Y. (1981). Effect of cycloheximide on protein breakdown in germinating rice seeds. Agric. Biol. Chem. 45, 527-529.

Horikoshi, M., and Morita, Y. (1982). Change in ultrastructure and subunit composition of protein body in rice endosperm during germination. Agric. Biol. Chem. 46, 269-274.

Juliano, B. O. (1972). The rice caryopsis and its composition. In DF Huston, ed, Rice: Chemistry and Technology. American Association of Cereal Chemists, St. Paul, MN, 16-74.

Kato, H., and Minamikawa, T. (1996). Identification and characterization of a rice cysteine endopeptidase that digest glutelin. Eur. J. Biochem. 239, 310-316.

Katsube, T., Kurisaka, N., Ogawa, M., Maruyama, N., Ohtsuka, R., Utsumi, S., and Takaiwa, F. (1999). Accumulation of soybean glycinin and its assembly with the glutelins in rice. Plant Physiol. 120, 1063-1073.

Khush, G. S. (1997). Origin, dispersal, cultivation and variation of rice. Plant Mol. Biol. 35, 25-34.

Kim, W.T., Li, X., and Okita, T.W. (1993). Expression of storage protein multigene families in developing rice endosperm. Plant Cell Physiol. 34, 595 - 603.

Krishnan, H. B., and White, J. A. (1995). Morphometric analysis of rice seed protein bodies. Plant Physiol, 109, 1491-1495.

Kumar, Y., and Matta, N. K. (2011). Changing protein profiles in developing and germinating barley seeds. Annal. Biol. Res. 2, 318-329.

Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature, 227, 680-688.

Li X., and Okita, T.W. (1993). Accumulation of prolamins and glutelins during rice seed development: A quantitative evaluation. Plant Cell Physiol. 34, 385-390.

Luthe, D. S. (1983). Storage protein accumulation in developing rice (*Oryza sativa* L.) seeds. Plant Sci Lett, 32, 147-158.

Matta, N. K., Gatehouse, J. A., and Boulter, D. (1981). The structure of legumin of *Vicia faba:* A reappraisal. J Exp Bot, 32, 183-197.

Mitsuhashi, W., and Oaks, A. (1994). Development of endopeptidase activities in maize (*Zea mays* L.) endosperms. Plant Physiol. 104, 401-407.

Muntz, K., Belozersky, M.A., Dunaevsky, Y.E., Schlereth, A., Tiedemann, J. (2001). Stored proteinases and the initiation of storage mobilization in seeds during germination and seedling growth. J. Exp. Bot. 52, 1741-1752.

Osborne, T B. (1924). The vegetable proteins. 2nd Edn. Longman Green & Co., London.

Schaeffer, G. W., and Sharpe, F. T. (1990). Modification of amino acid composition of endosperm proteins from *in vitro* selected high lysine mutants in rice. Theor Appl Genet. 80, 841-846.

Shutov, A.D., and Vaintraub, L.A. (1987). Degradation of storage proteins in germinating seeds. Phytochem. 26, 1557-1566.

Singh, A. (2006). Seed storage proteins and grain quality in rice (*Oryza sativa* L.). [PhD. Thesis]. Kurukshetra: Kurukshetra University, India.

Singh, A., and Matta, N.K. (2011). Disulphide linkages occur in many polypeptides of rice protein fractions: a two-dimensional gel electrophoretic study. Rice Sci. 18, 86 - 94.

Takaiwa, F., Kikuchi S., and Oono, K. (1987). A rice glutelin gene family: A major type of glutelin mRNAs can be divided into two classes. Mol Gen Genet, 208, 15-22.

Tanaka, K., Sugimato, T., Ogawa, M., and Kasai, Z. (1980). Isolation and characterization of two types of protein bodies in the rice endosperm. Agric. Biol. Chem. 44, 1633-1639.

Torrent, M., Geli, M.I., and Ludevid, M.D. (1989). Storage protein hydrolysis and protein body breakdown in germinated *Zea mays* L. seeds. Planta. 180, 90-95.

Villareal, R. M., and Juliano, B. O. (1978). Properties of glutelin from mature and developing rice grain. Phytochem. 17, 177-182.

Wen, T.N., and Luthe, D.S. (1985). Biochemical characterization of rice glutelin. Plant Physiol. 78, 172 - 177.

Xu, H., Gao, Y., and Wang, J. (2012). Transcriptomic analysis of rice (Oryza sativa) developing embryos using the RNA-Seq. Technique. PLoS ONE. 7, e-30646.

Yamagata, H., Sugimoto, T., Tanaka, K., and Kasai, Z. (1982). Biosynthesis of storage proteins in developing rice seeds. Plant Physiol, 70, 1094-1100.

Yang, P., Li, X., Wang, X., Chen, H., Chen, F., and Shen, S. (2007). Proteomic analysis of rice (*Oryza sativa*) seeds during germination. Proteomics, 7, 3358 - 3368.

Yano, H., Wang, J.H., Cho, M.J., and Buchanan, B.B. (2001). Redox changes accompanying the degradation of seed storage proteins in germinating rice. Plant Cell Physiol. 42, 879-883.

Zhao, W.M., Gatehouse, A.J., and Boulter, D. (1983). The partial purification and partial amino acid sequence of a polypeptide from the glutelin fraction of rice grain; Homology to a pea legmin. FEBS letter. 162, 96-102.

Zhu, T., Budworth, P., Chen, W., Provart, N., Chang, H., Guimil, S., Su, W., Estes, B., Zou, G., and Wang, X. (2003). Transcriptional control of nutrient partitioning during rice grain filling. Plant Biotech. J.1, 59-70.