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Changing protein profiles in developing and germinating barley seeds

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ABSTRACT

Though useful information on barley proteins exists from earlier studies, present attempt represents a concerted study with added information on changing protein levels during seed development and seed germination. Albumins represented the major proteins at 4 daf and were overtaken by glutelins on further development. Globulins increased gradually to decline after 30 daf while prolamins which were low initially became second highest from 25 daf onward. New polypeptides were observed in globulin fraction even at 40 daf, no changes occurred in prolamin and glutelin fractions after 20 daf. The intensity of globulin polypeptides decreased after 30 daf and that of glutelin and prolamin polypeptides increased even till 45 daf. During germination, globulins degraded by 5th day but their degradation products stayed even on 10th day. Polypeptides in glutelin and prolamin fractions and their degradation products were not seen after 5th day thus suggesting a relatively more fulfilling storage role for these fractions. Degradation of Mr 62 kDa albumin polypeptide by 4th day pointed towards its storage function. Similar trends followed by prolamins and glutelins during accumulation and degradation vis-a-vis by globulins warrant further work on their molecular mechanisms and on relevance of such varying trends in barley seed biology.

Keyword: Barley, Seed storage protein, Seed development, Seed germination, SDS-PAGE.

INTRODUCTION

Barley (*Hordeum vulgare* L.) represents the 4th major cereal crop standing next to maize, rice and wheat in global production (FAO 2008). It is mainly grown for feed and malting with a very small proportion being used for human consumption. Nutritionally, barley is rich in fibres, β-glucan, phenolics, antioxidants, minerals and vitamins. Protein content in barley seed is known to vary from 10 to 12% [1]. On the basis of their amino acid composition and amino acid sequences, the dominating alcohol soluble prolamins named as hordeins in barley have been classified as sulphur-rich (S-rich), sulphur-poor (S-poor) and high molecular weight (HMW) prolamins [2]. The S-rich prolamins have very high glutamine-proline content and relatively higher cysteine content. These include B-hordeins and account for about 80% of total barley prolamins. The S-poor prolamins lack cysteine and have very low methionine and lysine; these

include monomeric C-hordeins. The HMW prolamins are rich in lysine, glutamine and proline; these are constituted by polypeptide of Mr 105 kDa and have been named as D hordeins by Miflin et al. [3]. Studies by a number of workers [4, 5 and 6] have shown that as compared to other protein fractions, hordeins are accumulated late during seed development. The relative percentage of C hordeins was seen as decreasing and that of B (B1) hordeins increasing during the period of hordein synthesis. On the other hand, synthesis of glutelin polypeptides is reported to begin at 10 days after flowering with their accumulation continuing till maturity [6]. Brandt [7] reported that glutelin bands of Mr 45 kDa and 20 kDa were present at earlier stage while those of Mr 68 kDa and 60 kDa appeared after 20 daf. The other two fractions i.e. albumins and globulins are known to be accumulated at relatively early stages. The albumins are known to dominate with more than 50% proportion at 8 daf and their synthesis approached saturation during 13-20 daf period [7]. It was found that globulins were represented by more of low molecular weight bands at 13 daf while the higher molecular weight components were increasingly synthesized during 20 to 28 daf. Other workers had extracted albumin and globulin fractions collectively as salt-soluble fraction and their electrophoretic analysis showed that the high molecular weight polypeptides were visible by 10^{th} day of seed formation [8] and low molecular weight polypeptides were synthesized at 22 daf stage [6].

In view of the importance of barley in malting and brewing, major studies on its germinating seeds have involved characterization and hormonal regulation of different proteolytic enzymes [9, 10 and 11]. The proteolytic activity of enzymes which has been noticed to be absent until the 2^{nd} day, increases to its maximum on 5^{th} day of germination [10]. Studies on protein degradation during malting have shown that albumins and globulins undergo little degradation; of the various hordein polypeptides, D-hordeins disappeared rapidly followed by B-hordeins and then C-hordeins [12]. On the other hand, Celus et al. [13] showed that with rapid degradation of D hodeins, more of higher molecular weight C hordeins and lower molecular weight B hordeins were degraded in the process. However, little is known about the mobilization pattern of polypeptides present in different fractions over the longer period of germination and seedling growth. In view of this and in view of the varying reports on protein accumulation, present investigations were undertaken for obtaining a clearer picture about the changes occurring in four protein fractions during development and germination of barley seeds.

MATERIALS AND METHODS

Developmental and germination stages

Plants of barley line 'BHS 352' were grown in the experimental plots at Botanical garden of the University at Kurukshetra. Seeds harvested at different developmental stages such as 4 days after flowering (daf), 8 daf, 12 daf, 15 daf, 20 daf, 25 daf, 30 daf, 35 daf, 40 daf and 45 daf (mature). To study mobilization pattern of protein fractions during seed germination, the surface sterilized seeds were germinated at 20 ± 2^{o}C for 10 days on sterilized sand and were collected at different stages of day 1 to day 10 of germination.

Protein fractionation

Separation of four seed protein fractions was carried out following the methods given by Shewry et al. [14] and Blethen et al. [15] with slight modifications. Sequential extraction involved the use of distilled water for albumins followed by 1 M NaCl for globulins. Prolamins were extracted in 55% propanol having 2% 2-mercaptoethanol at 30^{o}C and glutelins were separated in borate buffer (pH 10) containing 1% SDS and 0.6% 2-mercaptoethanol.

Protein estimation

Semi-micro Kjeldhal method was followed for estimation of protein content in the seeds harvested at various developmental and germination stages; nitrogen determined by the method was multiplied by the standard factor of 6.25 as recommended by AACC. For this purpose, 100 mg of seed meal was completely digested with concentrated sulphuric acid in the presence of a catalytic mixture of copper sulphate, selenium dioxide and potassium dichromate. The digest was heated with 40% NaOH in Markham's distillation assembly and the ammonia so evolved was volumetrically titrated with N/40 HCl. For proportion of different fractions, protein concentration was determined following the method given by Bradford [16].

SDS-polyacrylamide gel electrophoresis

Electrophoretic analysis of four protein fractions was carried out on 14% polyacrylamide gels under reducing conditions [17] using 1.5 mm thick perspex spacers and glass plates of size 24x21 cm. A current of 28 mA was used for electrophoresis of proteins in stacking gel (pH 6.8) and was increased to 32 mA in the separation gel (pH 8.8).

Densitometric scanning

Relative concentration of polypeptides in four protein fractions was determined by densitometric scanning of the gels. Analysis for the purpose was carried out by employing 'TotalLab' software 'TL-100' from Nonlinear Dynamics Ltd. (Downloaded from www.nonlinear.com).

RESULTS

Proteins in developing seeds

Quantitative studies

Barley seeds, harvested at different developmental stages viz. 4 daf, 8 daf, 12 daf, 15 daf, 20 daf, 25 daf, 30 daf, 35 daf, 40 daf and 45 daf, were analysed for various protein characteristics such as protein content, proportion of protein fractions and densitometric scanning of gels. It was observed that protein accumulation occurred at a faster rate during the earlier half of seed development. As can be seen in Table 1, seed protein content increased steadily reaching 6.9% at 25 daf; thereafter it slowed down and showed little increase after 35 daf.

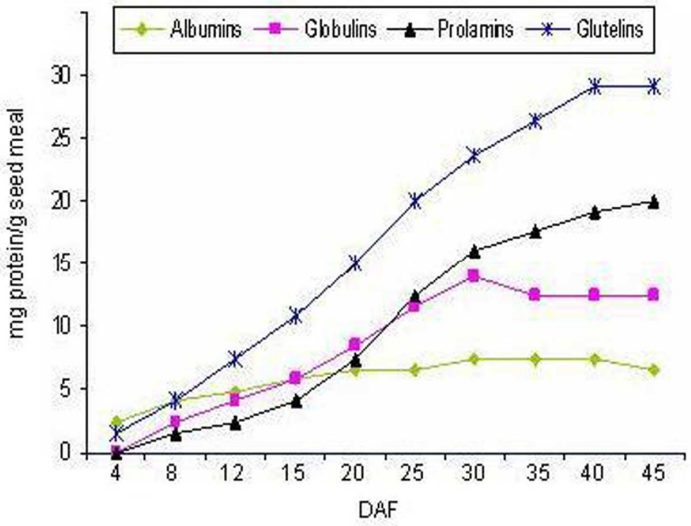
Table 1. Protein content and proportion of four protein fractions at different stages of seed development

Days after flowering	Protein content (%)	Proportion of seed protein fractions (%)			
		Albumins	Globulins	Prolamins	Glutelins
4	1.1	42.9	14.3	14.2	28.6
8	3.1	33.4	20.0	13.3	33.3
12	4.2	26.1	21.5	13.2	39.2
15	5.0	21.9	21.7	15.8	40.6
20	6.0	17.8	22.2	20.0	40.0
25	6.9	13.1	23.0	24.6	39.3
30	7.3	12.3	23.3	26.0	38.4
35	7.5	11.7	19.5	27.3	41.5
40	7.6	11.0	18.3	27.9	42.8
45	7.6	09.8	18.3	28.0	43.9

The concentration of protein in different fractions i.e. albumins, globulins, prolamins and glutelins was very low at the earliest stage of 4 daf. As is vivid from Fig.1, protein concentration in four fractions exhibited varying trends during seed development. The accumulation of glutelins was the fastest with continuous increase from the very initial stages while prolamin accumulation picked up only after 20 daf. On the other hand, globulins showed a gradual but

steady increase up to 30 daf whereafter it was decreased to some extent; the concentration of proteins in albumin fraction remained without much changes at different stages.

Fig. 1 Protein concentration levels of four fractions during seed development.



The proportion of four fractions based on their protein concentration varied differently with progress in seed development. As can be seen in Table 1, albumins represented the predominant fraction at earliest stages while glutelins represented more than 40% of proportion at maturity. In this way, albumins in the mature seeds were reduced to one fourth of their proportion seen at initial stages of seed development and glutelins exhibited 1.5 times increase in their proportion. On the other hand, globulins exhibited a gradual increase in their proportion from approx. 14% at 4 daf to 23% at 30 daf followed by reduction to 18% at maturity. The proportion of prolamins remained around 13-15% with little changes up to 15 daf and later showed enhanced accumulation reaching 28% proportion in the mature seed. In this way, the rate of albumin accumulation remained more or less the same during seed development and that of prolamins and glutelins exhibited an increase till the end.

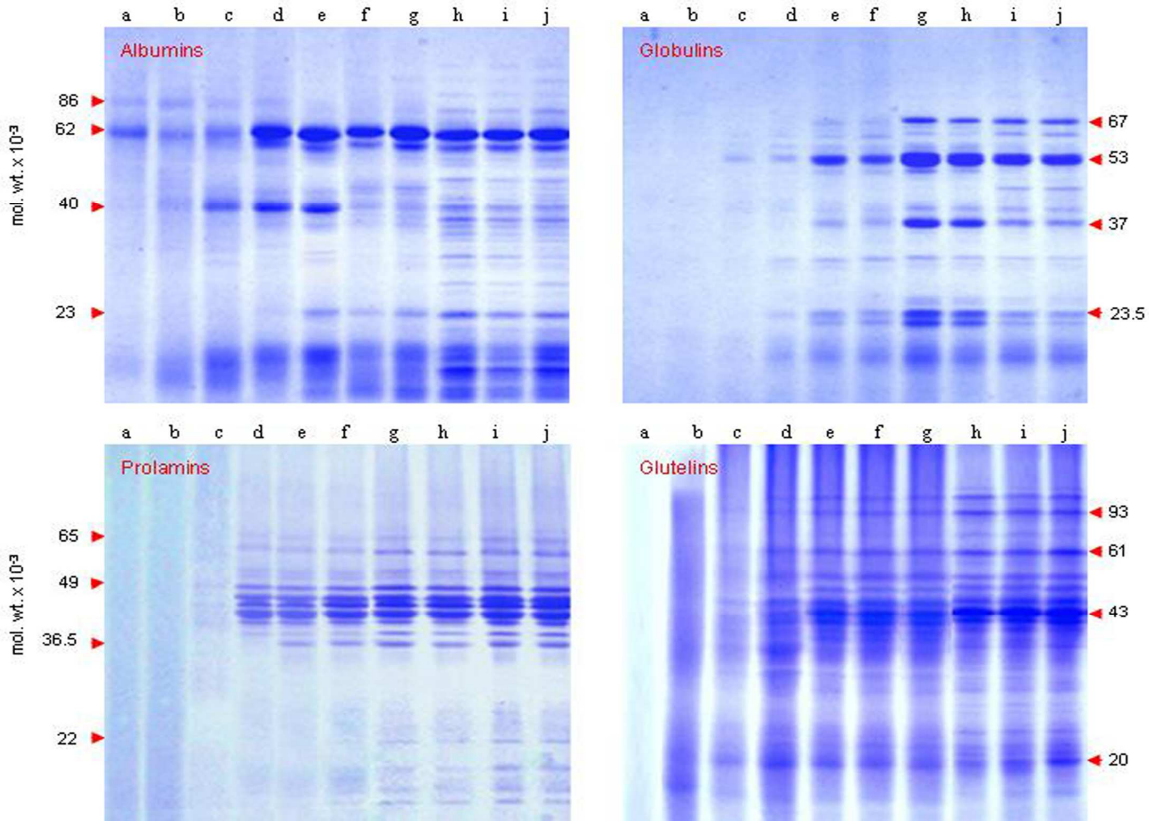
SDS-gel electrophoresis

The four protein fractions separated from seeds of different developmental stages were electrophoretically analysed for appearance and accumulation of their polypeptides (Fig. 2). Whereas albumin polypeptides could be detected at 4 daf stage, globulin and glutelin bands were visible at 8 daf and those of prolamins only at 12 daf stage. Gel electrophoresis followed by their densitometric scanning showed that albumin polypeptides of Mr 86 kDa, 62 kDa, 40 kDa and 13 kDa could be seen at earliest stage followed by those of Mr 57 kDa and 23 kDa at 12 daf. With a number of new polypeptides appearing at 20 daf and 25 daf, further new polypeptides of Mr 47 kDa, 30.5 kDa, 27 kDa, 13 kDa and 12 kDa could be seen at later stage of 35 daf and no qualitative changes were observed after this stage.

The polypeptide of Mr 62 kDa increased in its intensity all through the seed development and represented the highest concentration i.e. 12.1% relative to other albumin polypeptides in the mature grain. On the other hand, intensity of Mr 57 kDa, 23 kDa, 16 kDa and 15 kDa was seen to be high all through the seed development after 20 daf. The polypeptide of Mr 40 kDa which was also observed at all the stages appeared darkest during 12 to 20 daf period of seed development. Polypeptides of Mr 67 kDa, 62 kDa, 40 kDa, 30.5 kDa and 18 kDa belonging to the globulin fraction were seen as very faint bands at the early stage of 8 daf; these along with bands of Mr 53 kDa and 37 kDa were seen as clearly resolved bands at 12 daf. The increasing accumulation of

various polypeptides viz. 67 kDa, 62 kDa, 53 kDa 40 kDa, 37 kDa, 30.5 kDa, 23.3 kDa, 22 kDa and 15 kDa revealed a consistent increase reaching maximum at 30 daf; later these bands looked lighter with progress in seed development. On the other hand, polypeptides of Mr 25 kDa and 47 kDa could be resolved at 30 daf and 40 daf respectively.

Fig. 2 SDS-polyacrylamide gel electrophoresis of protein fractions in developing seeds under reducing conditions. Tracks 'a', 'b', 'c', 'd', 'e', 'f', 'g', 'h', 'i' and 'j' stand for 4 d.a.f., 8 d.a.f., 12 d.a.f., 15 d.a.f., 20 d.a.f., 25 d.a.f., 30 d.a.f., 35 d.a.f., 40 d.a.f. and 45 d.a.f. stages of seed development respectively.



In the mature seed, bands at Mr 67 kDa, 53 kDa, 37 kDa, 22 kDa and 15 kDa positions represented the major globulin polypeptides with their relative concentration of 10.9%, 36.7%, 8.6%, 5.1% and 19.9% respectively, that of Mr 53 kDa had the highest relative concentration during entire course of seed formation. Prolamins from seeds harvested at 4 daf and 8 daf moved as insoluble mass on gels, and could be resolved as very faint bands initially at 12 daf. While polypeptides in the range of Mr 49-41.5 kDa, 35-30 kDa and 22-11.5 kDa could be seen as faint bands at 12 daf, those of Mr 65 kDa, 61 kDa, 55 kDa and 54 kDa were seen at 15 daf followed by Mr 36.5 kDa band at 20 daf, after this stage intensity of polypeptides increased steadily with no qualitative changes occurring any more. The bands of Mr 49 kDa (11.0%), 47 kDa (10.6%), 45 kDa (17.8%), 43 kDa (9.8%), 42.5 kDa (8.8%), 41.5 kDa (8.0%) and 36.5 kDa (5.9%), with their relative concentration shown in parenthesis were seen to accumulate finally as major prolamin polypeptides. The glutelin fraction showed increasing accumulation all through the seed development and included very faint bands in a background of insolubles at very early stages. The first to be resolved were polypeptides of Mr 20 kDa and 13 kDa at the 8 daf stage. On the other hand, polypeptides of Mr 93 kDa, 61 kDa, 53 kDa, 46 kDa and 37.5 kDa were visible as lightly staining bands at 12 daf stage, these got resolved clearly at 15 daf when a band of 105 kDa also could be seen. Certain more polypeptides such as those of Mr 70 kDa and 49

kDa were seen to appear later at 20 daf after which new polypeptides did not appear. A continuous increase in intensity of various polypeptides occurred till the end of seed development.

Proteins in germinating seeds

Quantitative studies

Changing levels of protein content and protein fractions were analysed in germinating barley seeds at different stages i.e. day 1 to day 10. As is vivid from Table 2, 50% of total seed protein was degraded by 5th day of germination. After this period, seed protein content decreased gradually from 3.5% at 5th day to 1.9% at 10th day of seed germination. With progress in seed germination, proportion of globulins and prolamins followed a decreasing trend while that of glutelin fraction registered an increase from 43% at the initial stage to 59% proportion of the proteins left on the 10th day. On the other hand, proportion of albumins increased from 9.8% in the ungerminated seed to 23.4% at 5th day followed by a decrease to 17% on the 10th day of seed germination.

SDS-gel electrophoresis

Changes occurring in the polypeptides of various protein fractions separated from seeds at different germination stages can be seen in Fig. 3. The albumin polypeptides showed little degradation in contrast to those of other protein fractions. The major polypeptide of Mr 62 kDa which was seen without any change up to the 4th day of germination was found to disappear completely after that stage. Polypeptides of Mr 57 kDa, 47 kDa, 30.5 kDa, 23 kDa, 17.5 kDa and 12 kDa were visible over the whole span. The highest intensity and hence highest relative concentration of these polypeptides was found to vary with the germination stage.

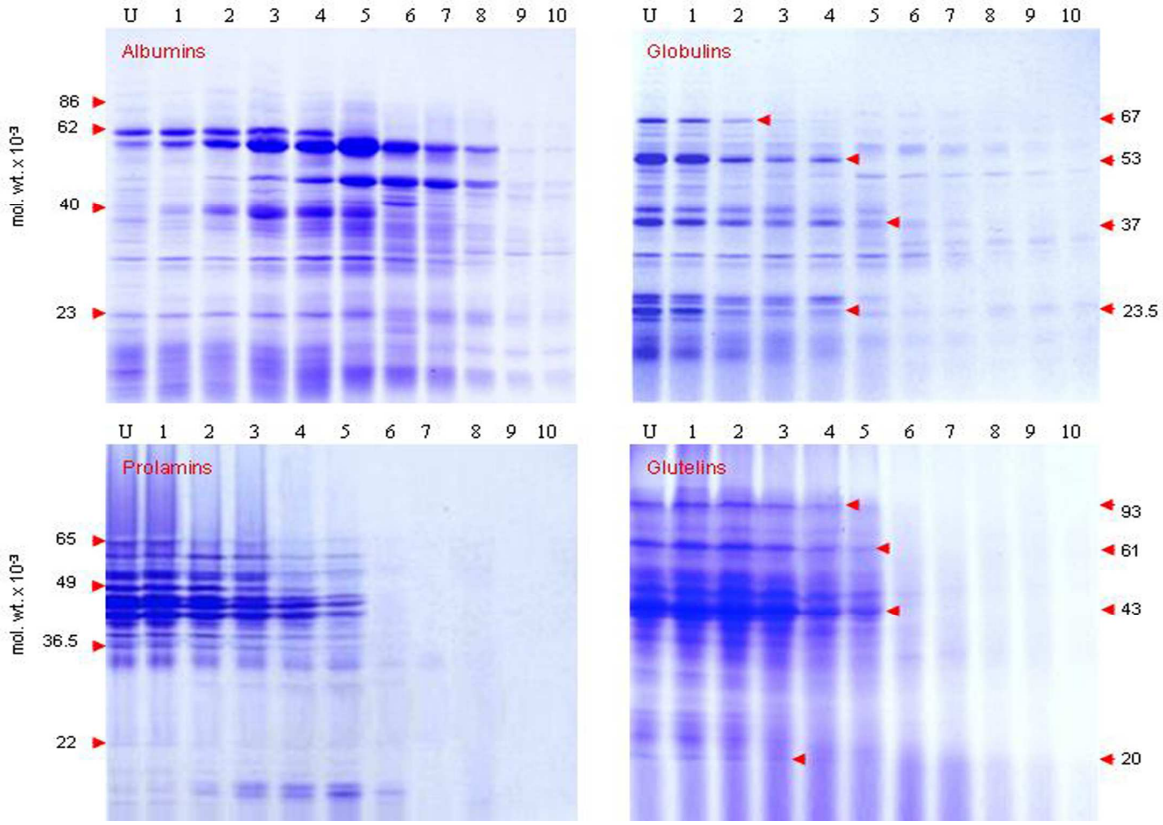
Table 2. Seed protein content and proportion of seed protein fractions at different Germinating stages

Days of germination	Protein content (%)	Proportion of seed protein fractions (%)			
		Albumins	Globulins	Prolamins	Glutelins
Ungerminated	7.6	09.8	18.3	28.0	43.9
1	7.0	10.0	17.5	27.5	45.0
2	5.8	09.3	16.0	26.7	48.0
3	5.4	11.8	15.8	25.0	47.4
4	4.7	16.0	13.3	22.7	48.0
5	3.5	23.4	11.5	20.2	44.9
6	3.1	22.7	10.8	18.9	47.6
7	2.3	20.1	10.4	18.1	51.4
8	2.0	17.9	09.9	17.5	54.7
9	1.9	17.2	08.8	17.4	56.6
10	1.9	17.0	07.6	16.4	59.0

In this way, relative concentration of Mr 57 kDa polypeptide was in the high range of 10.9% to 13.7% during 3rd to 5th day and that of Mr 47 kDa was 10.5% to 15.2% during 5th to 7th day. Similarly, polypeptide of Mr 40 kDa had very high relative concentration i.e. 11.5% to 8.0% between 3rd and 5th day and the intensity of Mr 30.5 kDa was highest at 4th and 5th days. Whereas polypeptides of Mr 45 kDa and 42 kDa were visible only during the middle stages, that of Mr 32.5 kDa could be seen in the later part of germination period. In the globulin fraction, various darkly staining polypeptides such as Mr 67 kDa, 62 kDa, 53 kDa, 50 kDa, 46 kDa, 44 kDa, 40 kDa, 37 kDa, 30.5 kDa, 25 kDa, 24.5 kDa, 23.5 kDa, 22 kDa and15 kDa, and other light bands were seen to decrease in their intensity at different rates. Whereas polypeptides of 67 kDa, 62 kDa, 50 kDa, 46 kDa, 44 kDa and 22 kDa had almost disappeared by 3rd day, that of Mr 53 kDa disappeared slowly i.e. up to the 4th day. These were followed by bands of Mr 40 kDa, 25

kDa and 24.5 kDa which disappeared by 5th day and those of Mr 37 kDa, 30.5 kDa and 23.5 kDa disappearing by 7th day. With disappearance of these polypeptides, new bands of Mr 58 kDa, 49 kDa, 32.5 kDa, 23.5 kDa and 20 kDa appeared on gels at various stages starting from the 3rd day of germination. Out of these, bands of Mr 49 kDa, 32.5 kDa and 23.5 kDa could be seen even on the 10th day while those of Mr 58 kDa and 20 kDa had disappeared by that time.

Fig. 3 SDS-polyacrylamide gel electrophoresis of protein fractions in germinating seeds under reducing conditions. Track 'U' stands for ungerminated seeds; 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 stand for day 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 of seed germination respectively.



In case of prolamins, various polypeptides were seen to have almost disappeared by 5th day of germination. It was observed that the band of Mr 65 kDa, 61 kDa, 55 kDa, 54 kDa and 49 kDa were degraded at a relatively faster rate; those of Mr 47-36.5 kDa had high intensity even on 5th day. With degradation of these polypeptides, lower molecular weight polypeptides such as those of 31 kDa, 28 kDa, 20 kDa and 15 kDa appeared on the gel; these new polypeptides also disappeared by 8th day of germination. Like prolamins, all glutelin polypeptides had disappeared by 5th day of germination after which these showed very low intensity as compared to that in the ungerminated seeds. The glutelin polypeptides in the lower molecular weight range i.e. 32-13 kDa disappeared in the first three days while those in the higher range were slow with little traces seen even after five days of germination. The degradation of Mr 105 kDa, 93 kDa and 53 kDa was faster as compared to that of Mr 61 kDa, 49 kDa and 43 kDa. As the degradation of glutelin polypeptides occurred, new bands of Mr 34 kDa, 21 kDa and 19 kDa were resolved with increasing intensity initially to disappear later by 7th day of germination.

DISCUSSION

Accumulation of proteins in developing barley seeds has been studied by a number of workers in the past [4, 5, 6, 7 and 18] with a major emphasis on hordeins. Also, the albumins and globulins were extracted collectively as the salt-soluble fraction and were not analyzed as two separate fractions [5, 6 and 8]. Under the electrophoretic conditions used, lesser number of bands could be resolved in earlier studies. Germinating seeds have been analysed mainly for the proteolytic activity in early 2-3 days i.e. limited to the malting period only (12, 13). In this way, information about mobilization of different proteins over the prolonged period of germination and post germination has been lacking. Therefore, with better resolution possible on gels, present studies were planned for a clearer and complete understanding of the changes occurring in protein profiles of barley seeds at various developmental and germination stages.

The albumin polypeptides were seen to undergo qualitative and quantitative changes over a longer period i.e. from the very initial stages until the late developmental stage. These frequent and constant changes in albumin polypeptides may be ascribed to the changing metabolic requirements during seed development. A number of proteins like cysteine proteinases of C4, C5 and C6 type and aspartic proteinases of E group [11], β-amylase and protein Z [8, 19] occur in the developing and ungerminated seeds. The accumulation pattern of albumin bands representing β-amylase (ca Mr 59-54 kDa) and protein Z (ca Mr 40 kDa) is also known to be similar to that of hordeins during seed development [8, 19]. In our study, the bands of Mr 62 kDa, 57 kDa and 23 kDa exhibited increasing accumulation like that in case of prolamin and glutelin polypeptides; the band of Mr 40 kDa which was present in mature seeds, however, was most intense during 12-20 daf period. Most of the bands seen presently in albumin fraction and corresponding to the molecular weight values of above mentioned enzymes in earlier reports are likely to have different hydrolytic functions in germinating seeds.

In the globulin fraction, polypeptides showed maximum intensity and hence increasing rate of their accumulation up to 30 daf stage. Decrease in intensity of these polypeptides after 30 daf may be due to certain globulin-specific proteinases becoming active after this stage. As is known in a number of crops, proteinases also get functional towards degradation of certain polypeptides accumulated during seed development [20]. The Mr 47 kDa globulin polypeptide appearing at 40 daf could be a degradation product of the higher molecular weight polypeptides in the range of Mr 53 kDa. Two dimensional gel electrophoresis followed by MALDI-TOF MS analysis should help confirm any such relationship of Mr 47 kDa polypeptide with these higher molecular weight polypeptides. Disulphide-bonded polypeptide pairs have been extensively characterized in the 11S globulin subfraction of legumes [21] and *Avena sativa* [22]. Two-dimensional gel electrophoresis using non-reducing conditions in the first dimension followed by reducing conditions in the second (our unpublished study), did not reveal the occurrence of disulphide-bonded polypeptide pairs in barley globulins. In view of their polypeptide composition, it may be stated that globulins of barley are similar to the 7S subfraction of leguminous seeds [21]. The globulin polypeptides in rice [23] and wheat [24] are also known to lack disulphide-bonded heterodimers.

Varying reports exist on the period for detection and synthesis of hordeins — the most studied protein fraction in barley seed. Whereas bands of Mr 35-46 kDa have been classified as B hordeins, those of Mr 55-70 kDa belong to the C hordeins group [2]. As studied by Rahman et al. [6], more of C hordeins as compared to the B hordeins were accumulated at 22 daf. With seed development, B1 hordein was shown to increase while other polypeptides such as B2, B3 and C1 hordeins registered a decrease in their relative amount. SDS-gel electrophoresis followed by

densitometric scanning in the present study has revealed the presence of B hordein (Mr 36.5-49 kDa), C hordein (Mr 54-65 kDa) and A hordein (Mr 22-11.5 kDa) polypeptides at early stage of 12 daf. Also, there was simultaneous increase in intensity of different polypeptides representing these fractions. Other workers have reported their appearance at 13 daf stage [5, 7] and 10 daf [18] followed by a continuous increase in their intensity during seed development. In glutelins, Brandt [7] had reported only five polypeptides of Mr 68 kDa, 60 kDa, 55 kDa, 45 kDa and 20 kDa; some of these appeared at earlier stages and others after 20 daf. In the present study, a large number of polypeptides e.g. Mr 116 kDa, 105 kDa, 93 kDa, 70 kDa, 61 kDa, 53 kDa, 49 kDa, 46 kDa, 43 kDa, 41 kDa, 39 kDa, 37.5 kDa, 36 kDa, 30 kDa, 29 kDa, 27 kDa, 26 kDa, 24.5 kDa, 24 kDa, 22 kDa, 21 kDa, 20 kDa and 13 kDa have been seen in the glutelin fraction; most of these appeared at 12 daf and thereafter exhibited a simultaneous and consistent increase in their accumulation with progress in seed development.

With qualitative changes ceasing to occur after a relatively shorter period i.e. 20 daf stage, intensity of polypeptides in the two fractions i.e. prolamins and glutelins was seen to increase all through the seed development. This continuous increase in the intensity of prolamin and glutelin polypeptides, unlike that of globulins, points towards the absence of prolamin/glutelin specific proteinases; it is also likely that certain inhibitors of these enzymes are functional in case of prolamin and glutelin polypeptides. It will be of interest to study these enzymes and their inhibitors, if any, for their role in degradation of various proteins during seed development in barley.

The polypeptide patterns and relative intensity of different bands are known to be influenced by a number of factors such as the cultivar used, growing conditions, extraction protocols etc [14, 25 and 26] and these are likely to have played a role for variation in the present results as well. By using higher temperature, Shewry et al. [14] noticed increased extraction of hordeins. However, the solubility of glutelin polypeptides and hence their resolution on gels was affected by elevated temperature used for separation of prolamins [27]. In contrast to earlier studies which involved more emphasis on prolamin fraction, present work aimed at studying changes in all the four fractions. Therefore, prolamin extraction using 2% 2-mercaptoethanol at lower temperature i.e. 30^{o}C was preferred to facilitate enhanced extraction of glutelins without affecting their solubility. In view of the protocol used in present experiments, Mr 105 kDa band observed in glutelin fraction probably represents the D hordein polypeptide; this has been reported by Rahman et al. [6] as well.

The proportion of albumins was the highest at 4 daf and was later equalled by glutelins at 8 daf. However, at 12 daf stage, proportion of glutelins was found to be the highest followed by albumins, globulins and prolamins. This distribution of four protein fractions is consistent with the reports of Ivanko [4] who observed that glutelins constituted the major proportion in 10 days old seeds followed by albumins, globulins and negligible proportion of prolamins. The present study showed that on further progress in development, globulins occupied the second place at 20 daf which were later replaced by prolamins at 25 daf onwards. As reported by Rahman et al. [6], our study also showed that synthesis of globulins is completed by middle stages and that of glutelins and prolamins continues until the end of seed development. As mentioned earlier, use of lower temperature leads to decreased extraction of hordein polypeptides [14]. In this way, lower proportion of prolamins in the present study seems to be due to the extraction protocols followed; in addition, the D hordein component was also shifted in glutelin fraction. In addition to the genetic and environmental factors, activity levels of different proteinases and the type of protein degraded should also contribute towards the final status of a protein fraction and hence its proportion in the seed protein. The expression of genes for various seed proteins in cereals

and legumes is known to be regulated in tissue and time specific manner [28, 29]. In barley, studies have been carried out on changing levels of mRNA populations for different hordein polypeptides [30]. However, such studies on mRNA levels have not been carried out for polypeptides of other barley fractions. In view of the differential and stage-specific accumulation of various polypeptides in present study, work on mRNA levels of globulins and glutelins vis-a-vis those of prolamins in developing barley seeds should provide useful insight into their synthesis.

During seed germination, a constant decrease was observed in the proportion of globulins and prolamins whereas a reverse trend i.e. an increase in the proportion of albumins and glutelins was observed. It has been seen that glutelin polypeptides are completely degraded by 5th day of germination. However, protein concentration in glutelin fraction remained high and unchanged during seed germination. Briggs and Hough [31] have also reported that glutelins remained unchanged and were present in the spent grain as such during malting. The high protein concentration and apparent increase in proportion of glutelin from 43.9% to 59% may be due to the degradation products of different proteins which are extracted in glutelin fraction due to their changed solubility characteristics. The albumin polypeptides showed varying patterns of appearance and intensity. These can be related to the constantly occurring metabolic changes in the course of seed germination. On the other hand, polypeptide of Mr 62 kDa was visible only up to 4th day of germination. In view of earlier reports on degradation of albumin polypeptides during germination, a storage role has been ascribed to certain albumin polypeptides [8, 32]. It is likely that the Mr 62 kDa polypeptide in barley also serves a similar function of storage. It is also possible that disappearance of this polypeptide after 4th day simply indicates the end of its metabolic role during seed germination. Further characterization of this polypeptide for its amino acid composition may also provide useful information about its nutritional qualities. Like the genes known for albumin polypeptides in brazil nut, sunflower, amaranth etc. (32), genes for barley albumin polypeptides may also prove a good source for genetic manipulations towards improving nutritional quality. It will also be highly relevant to carry out purification studies on individual polypeptides of albumin fraction for further characterization of their specific role, say enzymatic or storage.

During the initial days of germination, a faster rate of degradation was observed for certain polypeptides. These included globulin polypeptides of Mr 67 kDa, 62 kDa, 50 kDa, 46 kDa, 44 kDa and 22 kDa, C hordein polypeptides e.g. Mr 65 kDa, 61 kDa, 55-54 kDa and B hordein polypeptide of Mr 49 kDa in prolamin fraction and those of Mr 105 kDa, a likely equivalent of D hordein, Mr 93 kDa, and 53 kDa in the glutelin fraction. On the other hand, polypeptides such as those of Mr 53 kDa, 40 kDa, 37 kDa, 30.5 kDa, 25kDa, 24.5 kDa and 23.5 kDa (in globulin fraction), Mr 47 kDa, 45 kDa and 43 kDa (in prolamin fraction) and Mr 61 kDa, 49 kDa and 43 kDa (in glutelin fraction) degraded at a slower rate. While some workers have earlier reported rapid degradation of D hordeins followed by B hordeins and then C hordeins [12], others reported more of degradation of higher molecular weight C hordeins and lower molecular weight B hordeins [13]. However, our findings on protein levels during germination showed faster degradation of D hordein equivalent followed by C hordeins and B hordeins. In glutelins, a rapid degradation was observed for the low molecular weight (Mr 32-13 kDa) and Mr 53 kDa polypeptides. It may be stated that degradation of polypeptides during germination, like accumulation of polypeptides during seed development, is also known to vary from cultivar to cultivar [12] and may be the likely factor for present variation also.

In germinating barley seeds, proteolytic activity has been reported to be considerably high on 3rd day reaching maximum on 5th day; thereafter it decreases slightly and then remains stationary up

to the 9th day of germination [10, 33]. More than 40 endoproteinases have been detected in the germinating barley seeds, some being more intimately involved in degradation of storage proteins [11]. Those which are present in the ungerminated seed and remain active through out the germination are called house-keeping enzymes while others which appear on germination and show increased activity during germination have been called as accelerating enzymes [11]. The enhanced intensity of albumin polypeptides of Mr 57 kDa, 40 kDa, 30.5 kDa, 28 kDa and 23 kDa from 3rd to 5th day was concurrent with the disappearance of various polypeptides of the three storage fractions. This indicates the involvement of these albumin bands in degradation of different polypeptides. However, changes in the intensity of Mr 47 kDa albumin polypeptide did not correspond with the degradation period of storage proteins. Studies carried out on barley proteins, as reviewed by Steiner et al. [34], have shown that a large number of proteins such as Mr 10-30 kDa hordeins, 40 kDa protein Z (known to belong to the serpin superfamily of serine protease inhibitors) and LTP 1 (9.7 kDa) are present in the beer and contribute towards haze formation and foam stability.

CONCLUSION

As seen in the present study, all major glutelin and prolamin polypeptides are accumulated and degraded consistently following a similar pattern during seed development and seed germination respectively. This indicates that the two protein fractions have a storage purpose similar to each other but different from that of the globulins. Therefore, it will be of interest to look into the detailed molecular mechanism of their deposition during seed development and utilization at the time of seed germination, and to see whether various players i.e. polypeptides involved in these two events i.e. seed development and seed germination share special relationship with respect to their role in life cycle of plants. Like prolamin and glutelin polypeptides, a large number of globulin polypeptides have been reported in the present study. Genes *Beg*1 and *Beg*1 for the globulin polypeptides present in barley embryo are known to be differently regulated during seed development [35]. Out of a variety of globulin polypeptides in wheat, Loit et al. (24) have shown that some of these might be associated with certain pathological conditions and thus may have implications in human health. Therefore, studies may also be taken up on the regulation of polypeptide level of globulin fraction during seed development and on the role of individual polypeptides in nutrition and health.

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