Acta Bot. Croat. 66 (2), 135–142, 2007

CODEN: ABCRA 25
ISSN 0365–0588

Mobilization of albumins and globulins during germination of Indian bean (*Dolichos lablab* L. var. *lignosus*) seeds

VADDE RAMAKRISHNA*

Department of Biotechnology, Sri Krishnadevaraya University, Anantapur 515 003, India.

Albumin and globulin fractions of Indian bean seeds exhibit different mobilization patterns during the 8-day germination period. The albumin degradation was lesser in the early stage and reached a maximum in the later stages of germination. However, the globulins were degraded steadily from the start of imbibition. The influence of the embryonic axis on albumin and globulin degradation was also evaluated by using axis-attached and axis-detached cotyledons. The overall loss of globulins and albumins in axis-detached cotyledons was only 49 % and 60 % of the basal level, while in axis-attached cotyledons it was 81 % and 94 %. These results were confirmed by polyacrylamide gel electrophoresis (PAGE), and reveal the faster degradation of high molecular weight (HMW) polypeptides of globulins in the early stages of germination with increased utilization of albumins in the later stages. The results obtained showed the role of the embryonic axis in seed storage protein mobilization.

Keywords: *Dolichos lablab*, seed, proteins, albumins, globulins, embryonic axis, seed, germination

Introduction

Seed storage proteins were initially classified by OSBORNE (1924), according to their solubility, into albumins (water soluble), globulins (saline soluble), prolamins (alcohol soluble) and glutelins (acid or alkali soluble). During the germination period storage proteins are degraded by a variety of proteases, which convert the insoluble storage proteins into soluble peptides, which are thenfurther hydrolyzed to free amino acids. These free amino acids are mobilized to the embryonic axis to support its growth and also to provide energy (SHUTOV and VAINTRAUB 1987, BEWLEY and BLACK 1994, MUNTZ et al. 2001). Food reserve mobilization and its regulation during germination in dicotyledonous seeds have recevied attention and two hypotheses (*source-sink* and *plant growth substance stimulus*) have been suggested to explain the role of the axis in the process (DAVIS and SLACK 1981, BEWLEY and BLACK 1994, NANDI *et al.* 1995, MUNTZ et al. 2001). Focusing on metabolic changes, we reported in our previous studies (RAMAKRISHNA and RAMAKRISHNA RAO 2005a, b, 2006) on

* Corresponding address, e-mail: vrkrishna70@yahoo.co.in

the pattern of mobilization of seed storage proteins, the development of proteolytic (acidic, neutral and alkaline) enzymes and the role of embryonic axis in protein mobilization regulating the development of proteases by providing phytohormone signals and regulation of their activity *in vivo* by a feedback mechanism during germination of Indian bean seeds. To strengthen the further understanding of protein mobilization, this study aimed at investigating the qualitative and quantitative changes in the major protein turnover and the role of the axis in the mobilisation of albumins and globulins during germination of Indian bean seeds.

Materials and methods

Indian bean (*Dolichos lablab* L. *var lignosus*) seeds were procured from the agricultural farm of Andhra Pradesh Agricultural University, Rekulakunta, Anantapur, Andhra Pradesh. Healthy dry seeds of uniform size and weight were sorted, surface sterilized with 0.1% $HgCl_2$ and rinsed thoroughly with sterile distilled water. The 12 h water-imbibed seeds were germinated at room temperature 29 ± 2 °C for 8 days in sterile Petri dishes lined with moist filter paper. Seedlings were taken at designated time intervals for protein extraction and analyzed as described in our previous study (RAMAKRISHNA and RAMAKRISHNA 2006). To evaluate the influence of the embryonic axis, the seed coat was removed after imbibition and the cotyledons were separated from each other so that the axis remained attached to one of them. The cotyledons with the axis (attached) or without the axis (detached) were incubated with water under the same conditions.

One-gram amounts of dry or germinating cotyledons of Indian bean seeds were extracted with Boulter medium by continuous mild stirring at room temperature for 1h according to the method described by RAJESWARI and RAMAKRISHNA RAO (2002). The pooled extract was centrifuged at 14000 x *g* for 15 min at 4 °C. The albumins and globulins were separated by extensive dialysis of the supernatant against 0.04 M sodium acetate buffer pH 4.8 at 4 °C. The precipitated proteins (globulins) were collected by centrifugation at 18800 x G for 20 min at 4 °C and solubilized in the extraction buffer. The supernatant contained albumins. The extract and fractions obtained from germinated seed cotyledons harvested at different days from germinated seeds were used for quantitative estimations of albumins and globulins by the Lowry method (LOWRY et al. 1951).

Electrophoretic separation was performed using native and denatured polyacrylamide gel electrophoresis (PAGE). The qualitative changes of albumins and globulins were analyzed by native PAGE and SDS-PAGE. Electrophoresis was carried out in 10% (w/v) SDS-polyacrylamide slab gels, according to the method of LAEMMLI (1970). Gels were stained with Coomassie Brilliant Blue G (PECK and MORRIS 1986). The molecular masses of the proteins were estimated in relation to the mobilities of the molecular mass standard proteins (Dalton Mark VII-L, Sigma).

Each value presented in the table represents the arithmetic mean ± SE of five independent determinations, unless otherwise stated. The level of significance between germination periods was calculated by DMR (Duncan Multiple Range) test.

Results

The dry seed extract had total protein content of 32 mg per seed including albumins (42 %) and globulins (56 %). The quantitative changes in total proteins, albumin and globulin

contents in intact germinating cotyledons are shown in table 1. The maximal rates of protein depletion were observed during the first (0–2 day) and last stages (6–8 day) of germination. The overall loss of globulins and albumins in intact cotyledons was 70–85 %. The globulin degradation was higher in the early stage (0–2 days) of germination and albumin utilization and its mobilization to the embryonic axis was maximal in the later stages of germination period. Electrophoretic separation provided information on the protein profiles and their mobilization in intact cotyledons during the germination period (Figs. 1, 2). Resolution of dry seed soluble proteins by PAGE under denaturing conditions yielded high molecular weight (HMW) globulins ranging from 10–130 kDa and albumins with polypeptide bands with MW ranging from 16 to 95 kDa. Following imbibition and germination, the high MW polypeptides (132, 98, 78, 70 and 60 kDa) disappeared rapidly but new low molecular weight polypeptides appeared. The new polypeptides – 32, 30, 28, 25, and 22 kDa and low molecular weight peptides started appearing in globulin fraction on day two, and further intensified with the progress of germination. However, in native PAGE the dry and intact cotyledons taken on 0-day showed three prominent high MW globulin proteins and these were degraded and produced low MW proteins during later stages of germination. The albumin fractions of dry seed under native PAGE showed five prominent bands with three high MW proteins, which slowly disappeared in the following 8-day germination period with dramatic reappearance of albumin protein on day 4 in intact cotyledons. However, in SDS-PAGE the partial disappearance of many albumin bands was observed with the appearance of new polypeptides of 70 and 26 kDa on day 0, as well as 60 and 36 kDa on day 2. These polypeptides were later decreased with the progress of the germination period. These results reveal the faster degradation of high MW polypeptides of globulins in the early stages of germination with an increase in the breakdown of albumin in the later stages.

Tab. 1. Changes in the levels of total proteins, globulins and albumins during germination of Indian bean seeds. Each value is the mean ± SE of five independent values. Mean ± SE followed by the same letter do not differ according to DMR test at 5% level of significance ($p<0.01$).

Germination Period (h)	Intact cotyledons			Attached cotyledons			Detached cotyledons		
	Total proteins	Globulins	Albumins	Total proteins	Globulins	Albumins	Total proteins	Globulins	Albumins
Dry seed	30.15±2.50	19.25±2.10	8.96±1.30	30.15±2.50	19.25±2.10	8.95±1.20	30.15±2.50	19.25±2.10	8.96±1.30
0 – day	29.50±2.10	18.10±1.50	8.02±0.62	29.50±2.10	18.10±1.50	8.05±0.75	29.50±2.10	18.10±1.50	8.02±0.62
2 – day	21.96±1.96^{a}	13.40±0.95^{b}	6.38±0.45^{cd}	19.90±2.10^{a}	13.85±0.95	6.15±0.18^{c}	24.12±1.96	17.10±0.98	7.18±0.54^{d}
4 – day	18.48±1.15	10.92±0.76	4.96±0.28	15.65±1.10	10.05±0.89	4.26±0.28	21.86±1.25	14.84±0.95	6.15±0.25
6 – day	16.96±0.95^{e}	8.84±0.52^{f}	2.56±0.20	12.75±1.90	7.85±0.84^{e}	1.96±0.18	18.18±1.25^{f}	11.52±0.68	4.84±0.25
8 – day	13.80±0.84	7.15±0.35	1.58±0.10	9.40±0.45	6.05±0.05	0.89±0.06	15.64±1.4	9.85±0.82	3.98±0.32

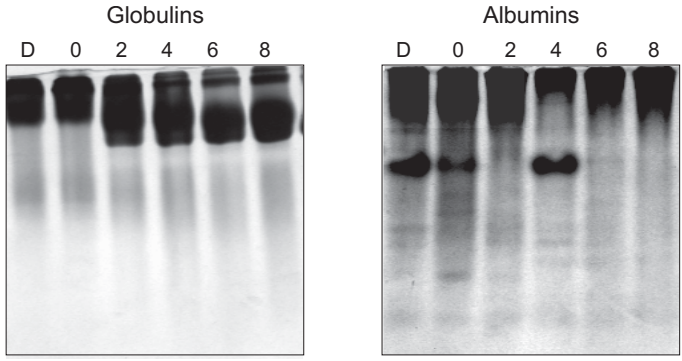

Fig. 1. Changes of globulins and albumins in intact cotyledons under native PAGE during germination of Indian bean.

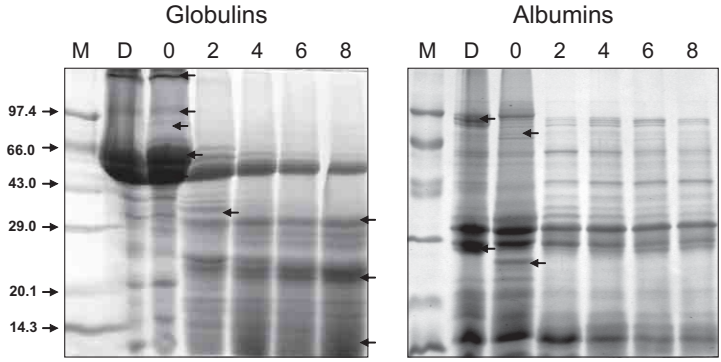

Fig. 2. Changes of globulins and albumins in intact cotyledons under denaturing PAGE during germination of Indian bean.

The influence of the embryonic axis on albumin and globulin degradation was evaluated by using attached and detached cotyledons. Concomitant with the fall in the high MW albumins and globulins, new proteins appeared in attached cotyledons by day 2 and then declined continuously with the progression of the germination period (Tab. 1; Figs. 3, 4). By contrast, a slower rate of degradation of protein (globulins and albumins) was observed in the detached cotyledons. The overall loss of globulins and albumins in the detached cotyledons was only 49 % and 60 %, respectively, of the basal level while in attached cotyledons it was 81 % and 94 %, respectively. It is pertinent to note that in the absence of the embryonic axis the albumin and globulin degradation was retarded.

Discussion

The maximal rates of protein depletion were observed during the first and last stages of the germination period. Albumin and globulin fractions of germinating seeds exhibit different degradation patterns, globulins being continuously degraded after the start of imbibition, whereas albumin degradation was less in the early stages and reached its maximum

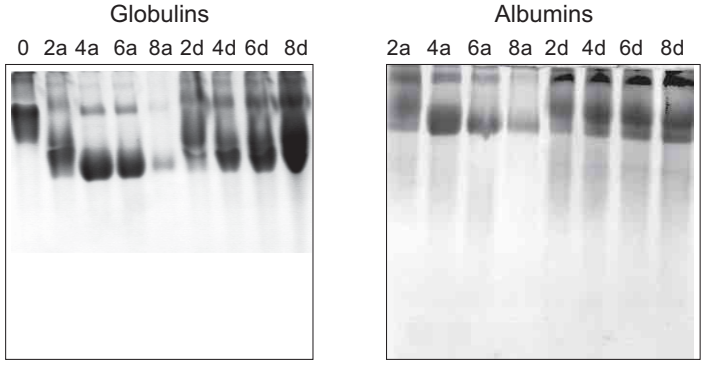

Fig. 3. Changes of globulins and albumins in attached and detached cotyledons under native PAGE during germination of Indian bean seeds. (a – attached cotyledons; d – detached cotyledons)

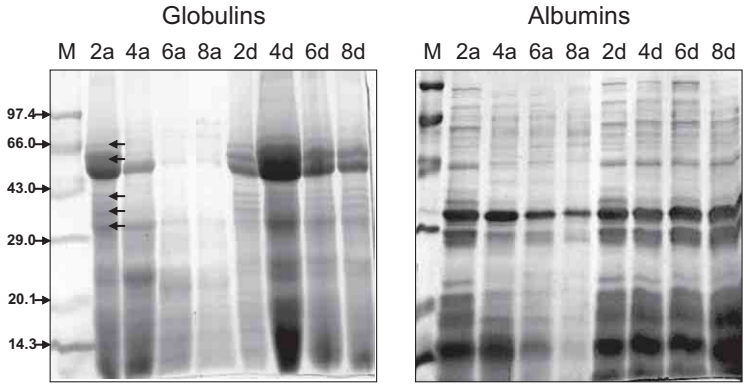

Fig. 4. Changes of globulins and albumins in attached and detached cotyledons under denaturing PAGE during germination of Indian bean. (a – attached cotyledons; d – detached cotyle-

in the later stages of germination. This result suggests that during albumin depletion there is a rapid transfer of amino acids to the growing embryo. The decrease in protein content in the cotyledons during germination also occurs in horse gram (*Macrotyloma uniflorum)* (KARUNAGARAN and RAMAKRISHNA RAO 1990, RAJESWARI and RAMAKRISHNA RAO 2002), lupine (*Lupinus albus L.)* (NANDI et al. 1995) and *Lathirus sativus* (CHANDNA et al. 1995). Storage globulin mobilization was observed in pea (*Pisum sativum* L.), soyabean (*Glycine max* L.), vetch (*Vicia sativa* L.), and garden pea (*Phaseolus vulgaris* L.) (SCHLERETH et al. 2000, TIEDEMANN et al. 2001).

Many authors reported protein mobilization in terms of measuring the levels of protein in germinating cotyledons, and no reports are available to view the individual protein profile under electrophoretic methods during germination. In this study, electrophoretic separation was performed under native and denatured PAGE to show the protein profiles and their mobilization. The results show the change of protein pattern – the protein bands either disappeared completely or partially with the progress of germination (Figs. 1, 2). These results reveal the faster degradation of high MW polypeptides of globulins in the early stages

of germination with increased breakdown of albumins in the later stages. SUDA and GIORGINI (2000) reported that proteins, including albumins (49%), globulins (51 %) and prolamins (0.3%) in *Euphorbia heterophylla* comprise about a quarter of seed dry mass. These protein fractions exhibit different degradation patterns, globulins being degraded in the early stages after the start of imbibition, and albumins between 60 and 84 hours. Globulin depletion is accompanied by an increase in free amino acids in the endosperm, whereas intense albumin depletion is not, suggesting that during albumin depletion there is a rapid transfer of amino acids to the growing embryo. The pattern of electrophoretic separation of the albumin fraction from mature dry seeds of horse gram yielded five intensely stained bands with MW ranging from 156 to 14.4 kDa and six bands in globulin fraction of MW ranging from 110–21 kDa and also showed the faster degradation of globulins in the early stage with increased mobilization of albumins in the later stages during the 4 day germination period (RAJESWARI and RAMAKRISHNA RAO 2002). Studies with dry seeds of *Lathyrus sativus* revealed various protein fractions ranging in molecular weight from 92–17 kDa with faster degradation of high MW proteins (CHANDNA et al. 1995). The present results revealed faster degradation of high MW polypeptides of globulins in the early stages of germination with increase in breakdown of albumin in the later stages.

The axis-excision affected the total protein mobilization in the Indian bean cotyledons by feedback inhibition by the end products, confirming observations made in our earlier study (RAMAKRISHNA and RAMAKRISHNA RAO 2005a), which showed free amino acid accumulation in detached cotyledons with decreasing protein degradation. Consistent with the same phenomena, in the absence of the embryonic axis, the individual storage albumin and globulin degradation was greatly retarded in detached cotyledons as compared to attached cotyledons, where globulin mobilization is faster than albumin mobilization in the early stages of the 8–day germination period (Tab. 1). The protein profiles under native and denatured PAGE in attached and detached cotyledons revealed the faster disappearance of high MW albumins and globulins in attached cotyledons, as compared to the rate of degradation of proteins (globulins and albumins) in detached cotyledons (Figures 3 and 4) during the 8 day germination period in Indian bean seeds. It is pertinent to note that in the absence of the embryonic axis the albumin and globulin degradation was retarded.

In conclusion, the results obtained from this study indicate that the albumins and globulins are the major storage proteins, exhibit different mobilization patterns, globulins being continuously degraded after the start of imbibition, whereas albumin degradation was less in the early stages and reached a maximum in the later stages of germination. These results were further confirmed by polyacrylamide gel electrophoresis and revealed the faster degradation of high MW polypeptides of globulins in the early stages of germination with increased utilization of albumins in the later stages. The results obtained also showed the definitive role of the embryonic axis in albumin and globulin mobilization during the germination period in Indian bean seeds.

Acknowledgements

The research for this publication was conducted as part of the programme 'Biotechnology for Dry Land Agriculture in Andhra Pradesh' with financial support for the Research and Communication Division, Ministry of Foreign Affairs, the Government of the Netherlands.

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