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Monitoring Protein Mobilization During Seed Germination of Broad Bean (*Vicia faba* L.)

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Abstract: Protein mobilization of germinating seeds of *Vicia faba* L. cv. Eresen 87 was studied by SDS-PAGE. The protein mobilization started after the 3rd day in normal seeds and continued more slowly in detached cotyledons. Plant growth regulators were not affected the protein mobilization in detached cotyledons. The dry weight of the embryonic axis increased, whereas it decreased in cotyledons during the germination.

Key words: *Vicia faba*, protein mobilization, SDS-PAGE, seed germination, plant growth regulators

INTRODUCTION

Cereals and Leguminous seeds take a large place of human food consumption. Animal proteins being more expensive, people in developing countries depend on seed protein alone for their entire protein requirement (FAO, 1970; Vitale and Bollini, 1995; Mandal and Mandal, 2000). Grain legumes alone contribute 33% of the dietary protein nitrogen needs of humans (Graham and Vance, 2003).

Reserve proteins can be located in the cotyledons, embryo or endosperm of seeds (Dalling, 1986; Vitale and Bollini, 1995). Seed protein content is over 20% for most situations in *Leguminosae* family plants; even this ratio rises to 40% in soybean (Mandal and Mandal, 2000). Globulins are dominant than albumins in *Leguminosae* seeds. Globulins are storage proteins, whereas albumins are mostly enzymatic and non storage proteins. *Leguminosae* seeds contain two types of globulins; legumin and vicilin (Dalling, 1986; Müntz, 1998; Wang *et al*., 2003; Le Gall *et al*., 2005).

Embryonic axis controls the protein mobilization. Two hypotheses have been proposed concerning the axial control of this process. First, the growing axis may acts as a sink, which draws off the products of reserve mobilization and its excision leads to an accumulation of proteolytic end products (Chin *et al*., 1972; Kern and Chrispeels, 1978; Davies and Chapman, 1979; Mitsuhashi *et al*., 1984). Second, the growing axis may produce plant growth substances, which stimulate the synthesis of proteolytic enzymes for reserve mobilization in the cotyledons. The effect of plant growth regulators on protein mobilization could be specific to species and also to cultural variety. Gibberellins and cytokinins are thought to regulate this process in dicots (Allen *et al*., 1984; Munoz *et al*., 1990; Nandi *et al*., 1995; Yoshida and Hirasawa, 1997). In a similar manner gibberellins, arising from the embryo, influence reserve mobilization especially in the endosperm of cereals (Jacobsen and Varner, 1967; Yomo and Varner, 1973).

SDS-PAGE (Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophphoresis) is widely used for monitoring protein mobilization in early stages of seed germination (Hussain *et al*., 1988; Ahmed *et al*., 1995; Krochko and Bewley, 2000). As well as variations within the species can be determined by electrophoretic method.

Changes in the carboxypeptidase, leucine aminopeptidase and endopeptidase activities of attached (intact seed) and detached cotyledons of *Vicia faba* L. cv. Eresen 87 seeds during 7 day germination have been investigated in our previous study (Kırmızı and Güleryüz, 2006). According to the results of this study, removal of the embryonic axis decreased the endopeptidase activity. As well as, protein mobilization continued more slowly in detached cotyledons and Benzyladenine stimulated the leucine aminopeptidase activity.

The aim of this study was to show additional evidence on protein mobilization with the aid of the electrophoretic method and to show parallelity of these results with fresh and dry weight changes during germination of the seeds of *Vicia faba* L. cv. Eresen 87 during germination.

MATERIALS AND METHODS

Plant material: Seeds of *Vicia faba* L. cv. Eresen 87 seeds were used. Seeds were purchased from Turkish Agricultural ministry Aegean Agricultural Research

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Institute, Menemen, İzmir, Turkey. Seeds were incubated as attached (intact seeds) and detached cotyledons. Some cotyledons were detached from the embryonic axis before incubation. Detached and attached cotyledons were incubated with sterile distilled water or test solutions for 0, 1, 3, 5 and 7 days. To determine if the growth regulators can replace the embryonic axis, detached cotyledons were incubated with 10^{-4} M BA, GA_3, IAA and 10^{-5} M ABA for 3 and 5 days. Seeds were surface sterilized with 2% NaOCI to prevent any contamination. Three seeds or six cotyledons were placed on two sheets of sterilized filter paper in petri dishes. At the end of the incubation, seeds were washed with distilled water, dried and kept at -70°C until use (Kırmızı and Güleryüz, 2006).

Sample preparation: Excised cotyledons (3 cotyledon pairs) or seeds (3 seeds) were homogenized in 11 mL of ice cold buffer (50 mM Tris-HCI pH 7. 4, 10 mM 2ME) at 4°C. The homogenates were centrifuged (10 000g for 20 min, 4°C) (Nandi *et al*., 1995).

Protein separation: Protein in different extracts was measured according to the method of Bradford (1976). Separation of protein on SDS-PAGE was conducted on 12% acrylamide containing running gels with a Bio Rad Mini Protean III minigel system. Ten microlitres of denaturized samples were loaded into each lane. The gels were run at constant amperage of 40 mA using Laemli (1970) buffer system. They were stained with Coomasie Brilliant Blue R-250, destained and photographed following electrophoresis. Sigma wide range protein standard was used as standard protein mixture.

Changes in fresh and dry weight: Seeds and detached cotyledons were incubated in darkness at 23°C for 0, 1, 3, 5 and 7 days with distilled water at the end of the incubation. Embryonic axes were dissected from the seeds and fresh weights were measured. Cotyledons and embryonic axes were dried in 70°C oven until their weight became constant and then weighted.

Data analysis: Differences among the days regarding fresh and dry weights of attached and detached cotyledons and embryonic axes were tested by one-way ANOVA. The difference groups among treatment series was performed by Tukey's HSD test. All statistical analyses were based on significance level of 0.05 (Zar, 1984).

RESULTS AND DISCUSSION

Protein profiles of the water incubated attached cotyledons prepared with SDS-PAGE, were shown in Fig. 1. The number of the protein bands has been changed during germination. Some of the protein bands were not present at the initial of incubation, whereas some others were disappeared during the germination period. While the protein band which is equivalent to 29 kDa was disappeared at the initial of incubation, higher molecular weight protein bands than 29 kDa were disappeared from the beginning of the 3rd day.

Protein profiles of the water incubated detached cotyledons were differed from that of attached cotyledons (Fig. 2). For example 29 kDa equivalent proteins and others were not disappeared during the germination period. However, some of the protein bands were disappeared at the day 7. This result suggests that protein

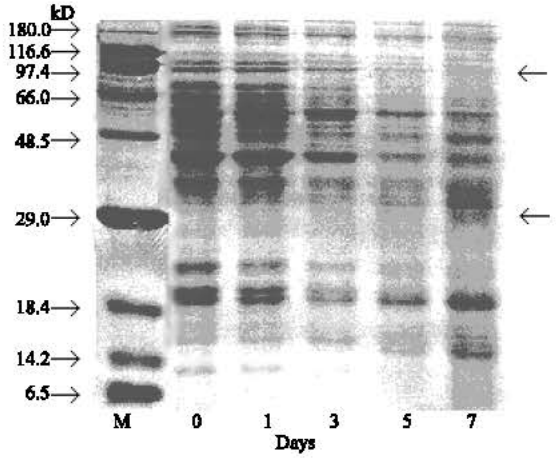

Fig. 1: Electrophoretic profiles of extracts prepared from attached cotyledons of *Vicia faba* L. Eresen 87. M: Marker, lanes 0: imbibed, lanes 1 to 7 germinated for the number of the days indicated

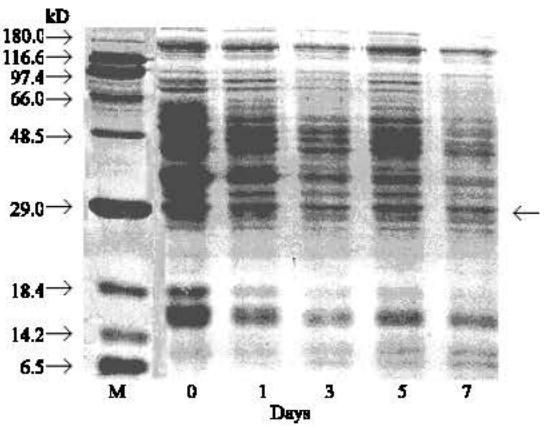

Fig. 2: Electrophoretic profiles of extracts prepared from detached cotyledons of *Vicia faba* L. Eresen 87. M: Marker, lanes 0: imbibed, lanes 1 to 7 incubated for number of the days indicated

mobilization was slow when embryo detached from the cotyledons. Gifford *et al*. (1984) were demonstrated by SDS-PAGE that the mobilization of lectin and albumin proteins continued slowly in excised endosperm of *Ricinus communis*.

In isolated *Gossypium hirsutum* embryos the high molecular weight protein bands decreased within the first 24 h (Vigil and Fang, 1995). In *Zea mays* endosperm while some of the protein bands were disappeared, some other bands were appeared for the first time (Mitsuhashi and Oaks, 1994). Savelkoul *et al*. (1992) have shown clear differences between the days 2 day 3 of the germination in *Vicia faba* L. var. Pistache. They suggested the high molecular weight proteins that disappeared during this period were legumins and vicilins. The disappearance of protein bands was observed after the day 3, even the used cultivar in this study was different from our study. In *Phaseolus vulgaris*, seed proteins showed great changes within the same group or cultivar (Hussain *et al*., 1988).

The SDS-PAGE profiles of the detached cotyledons incubated with plant growth regulators were presented in Fig. 3. A high molecular weight protein band was disappeared in day 3 and day 5 of GA_3 incubated detached cotyledons and amount of the related protein band was decreased in day 5 of BA incubated detached cotyledons. A low molecular weight protein band was found to be disappeared in BA and IAA incubations, but the same protein band was still observed in GA_3 and ABA incubations.

Minor stimulator effects of the plant growth regulators have been detected, but inhibition effect of ABA was not observed in our study. GA_3 promoted the protein mobilization in *Ricinus communis* (Gifford *et al*., 1984). GA_3 promoted and ABA inhibited the protein mobilization in *Anadenanthera peregrina* cotyledons (Barduche *et al*., 1999), disappearing of the protein bands also detected in BA incubated *Helianthus anuus* cotyledons (Allen *et al*., 1984).

Changes of the fresh and dry weights are shown for embryonic axis and cotyledons (Table 1). Significant difference was occurred ($p<0.05$) among the days regarding to fresh weight changes of the cotyledons and embryonic axis. Fresh weight of the embryonic axis was increased during the germination and the highest fresh weight was detected at the day 7. Significant difference was occurred between dry weight of embryo and cotyledons ($p<0.05$). Dry weight of the embryonic axis was not changed until the day 3, but started to increase from day 5 (Table 1). Dry weight of the cotyledons was increased during germination and the highest dry weight was found at the initial day and the lowest dry weight was found in days 5 and 7.

Table 1: The changes in fresh and dry weights of normal seeds and detached cotyledons (per cotyledon pair) during the incubation period (FW: Fresh Weight, DW: Dry weight) ($p<0.05$ significant, $p>0.05$ non significant ±Standard deviation)

	Normal seeds				Detached cotyledons	
	Embrio		Cotyledon		Cotyledon	
	FW	DW	FW	DW	FW	DW
Initial	0.12±0.04^{c}	0.05±0.02^{c}	5.11±0.35^{c}	3.54±0.31^{a}	7.11±0.66^{a}	2.96±0.26^{ab}
1st day	0.20±0.03^{c}	0.05±0.00^{c}	8.00±0.29^{b}	3.32±0.20^{ab}	8.14±0.36^{a}	3.15±0.20^{a}
3rd day	0.26±0.05^{c}	0.05±0.01^{c}	8.00±0.51^{b}	3.11±0.25^{ab}	8.16±1.30^{a}	2.88±0.52^{ab}
5th day	1.20±0.44^{b}	0.11±0.03^{b}	9.59±1.00^{a}	2.98±0.22^{b}	8.20±1.32^{a}	2.69±0.25^{ab}
7th day	1.83±0.34^{a}	0.16±0.02^{a}	9.65±0.57^{a}	2.96±0.14^{b}	8.21±1.38^{a}	2.52±0.82^{b}
α: 0.05	p<0.05	p<0.05	p<0.05	p<0.05	p>0.05	p<0.05

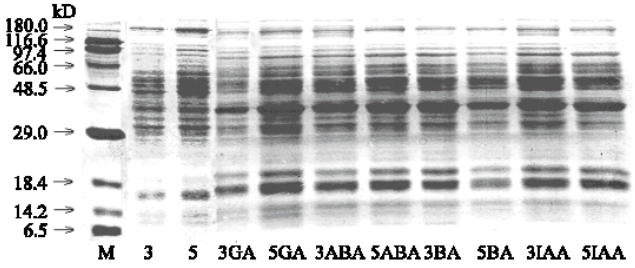

Fig. 3: Electrophoretic profiles of hormone treated and 3 and 5 days incubated detached cotyledons of *Vicia faba* L. Eresen 87. M: Marker, Lanes 3 to 5 : 3 and 5 days of water incubated detached cotyledons, 3GA to 5GA: 3 and 5 days of 10^{-4} M GA3 incubated detached cotyledons, 3ABA to 5ABA: 3 and 5 days of 10^{-5} M ABA incubated detached cotyledons, 3BA to 5BA: 3 and 5 days of 10^{-4} M BA incubated detached cotyledons, 3IAA to 5IAA: 3 and 5 days of 10^{-4} M IAA incubated detached cotyledons

CONCLUSIONS

In this study, the protein profiles of detached and attached cotyledons of *Vicia faba* L. cv. Eresen 87 during germination period has been monitored by SDS-PAGE. The number of the protein bands has been changed during germination. Some of the protein bands were not present at the initial of incubation, whereas some others were disappeared during the germination period. Results from fresh and dry weight measurements and gel electrophoretic patterns, showed parallelity. During the germination, the increases of the fresh and dry weight of the embryo accompanied by the changes on the gel electrophoretic profiles of the attached cotyledons. Whereas, the fresh and dry weights of detached cotyledons more or less remained the same and this accompanied by slow band changes on the gels.

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