SDS-PAGE ANALYSIS OF PROTEINS FROM THE SEEDS OF HONEYLOCUST (*GLEDITSIA TRIACANTHOS*) DURING GERMINATION

EUGEN UNGUREANU[1]*, VLAD ARTENIE[1], ALICE-MADALINA UNGUREANU[2]

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Abstract: The main objective of the experiment was to observe the variety and evolution of the proteins during germination of the seeds from the honeylocust (*Gleditsia triacanthos*). Using the technique of SDS-PAGE there were discovered a lot of protein fractions. The profile of these fractions varies from the wide distribution along the entire molecular masses spectrum at the beginning of the germination to a poorly represented profile of protein fractions at the end of the germination.

INTRODUCTION

During germination under controlled conditions it was observed the evolution of the protein fractions at a qualitative level by using the technique of SDS-PAGE.

The electrophoretic analysis was performed on proteins soluble in distilled water and on proteins soluble in phosphate buffer 0.2M with pH=7.

MATERIALS AND METHODS

The seeds of honeylocust (*Gleditsia triacanthos*) came from the forests of the Botosani County and the trees from the city of Iasi.

The germination process was conducted in Petri dishes on wet filter paper (the paper and Petri dishes were sterilized before the experiment began and the water was also sterile).

Probes were collected at every 3 days and the collected material was stored in freezer at -20°C until it was analyzed. Extraction of the material was performed first with distilled water for 24 hours under slightly agitation at +4°C and then with phosphate buffer solution with pH=7 and 0.2M.

Electrophoresis was made using the Laemmli system and the resulting gel was colored with Coomasie Brillant Blue R250.

Pictures of the gel were taken and they were analyzed using specialized software, ImageQUANT TL v2005 from Amersham / General Electric Healthcare.

RESULTS AND DISCUSSIONS

At first sight could see that the electrophoretic profile of the proteins separated by SDS-PAGE from the seeds of honeylocust (*Gleditsia triacanthos*) is a very distributed one and that it decrease in quantity and quality from the beginning of the germination until the end of the process.

A rich profile considering the number of discovered bands is observed in the dry seeds and the seeds at the beginning of germination.

The water extract revealed a more equilibrated profile during germination than the phosphate buffer extract.

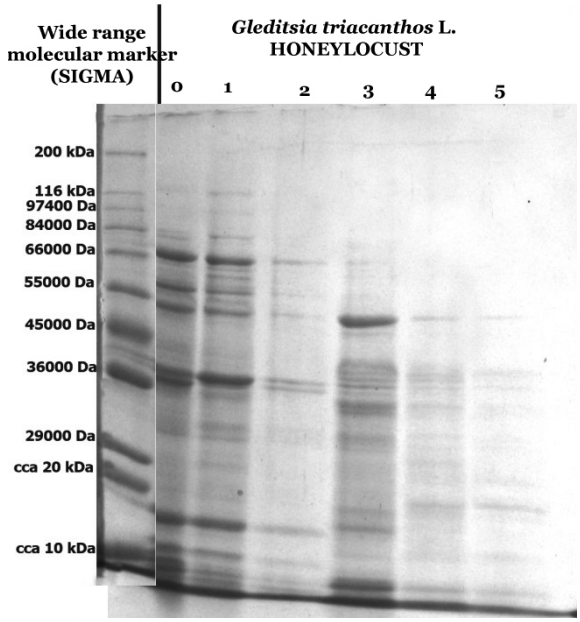

Figure 1 – The electrophoregram of the proteins extracted in distilled water from seeds of the honeylocust (*Gleditsia triacanthos* L.) during germination

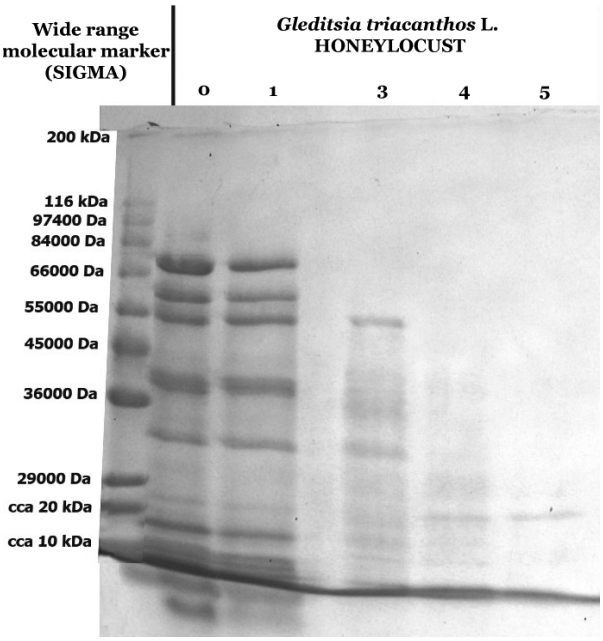

Figure 2 – The electrophoregram of the proteins extracted in phosphate buffer from seeds of the honeylocust (*Gleditsia triacanthos* L.) during germination

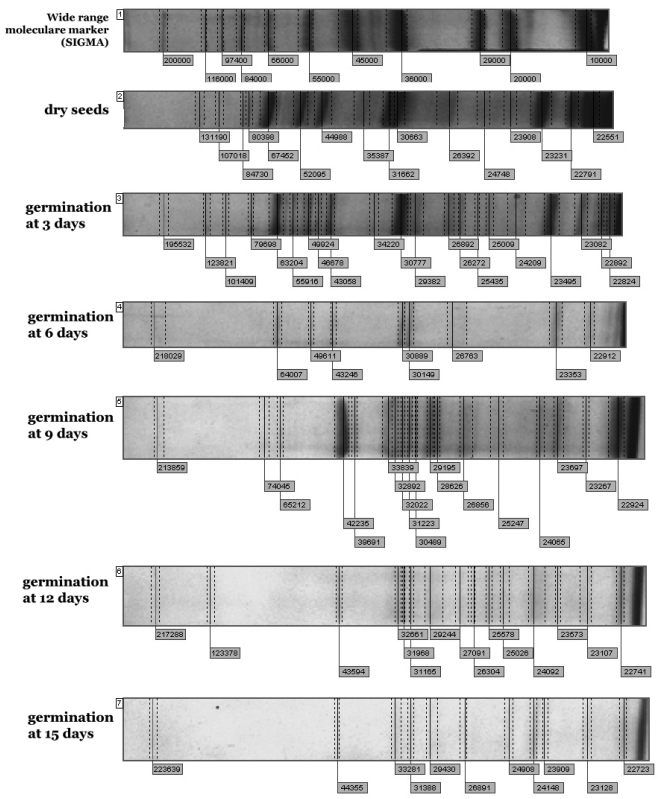

Figure 3 – Distribution of the molecular masses of the proteins separated by SDS-PAGE from the water extracts made from seeds of the honeylocust (*Gleditsia triacanthos* L.) during germination

The analysis shows that there is a decrease of the molecular masses of the proteins on the entire germination process. It could be explained by the hydrolysis of the storage proteins to be utilized as food by the growing embryo. Because of the limitations of the detection method it is possible that many polypeptide fractions to be undetected. More than that because of the small size it is possible that many of these newly formed proteins and polypeptides to pass from the gel long before we stop the electrophoresis.

The number and size of the fractions are bigger in the case of the proteins extracted in distilled water than in the case of the proteins extracted in phosphate buffer.

The fractions that we separated and revealed are well expressed in both extracts.

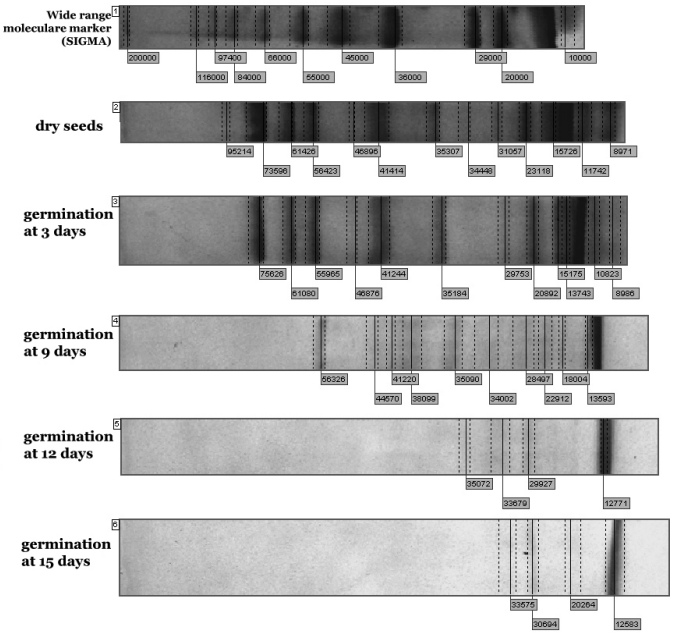

Figure 4 – Distribution of the molecular masses of the proteins separated by SDS-PAGE from the phosphate buffer extracts made from seeds of the honeylocust (*Gleditsia triacanthos* L.) during germination

CONCLUSIONS

There are a lot of well expressed polypeptide fractions in the dry seeds of honeylocust (*Gleditsia triacanthos* L.).

During germination the variety of the proteins tend to decrease, more evidently in the phosphate buffer extracts, which is normal because of the transformations suffered by the proteins.

The number and quantity of proteins with high molecular mass decrease during germination.

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1 – “Alexandru Ioan Cuza” University, 700506, Iasi, ROMANIA
2 – Economic and Administrative College, Iasi, ROMANIA
* - aeu@uaic.ro