ELECTROPHORETIC ANALYSIS OF PROTEINS DURING SEED GERMINATION IN BLACK LOCUST (*ROBINIA PSEUDACACIA* L.)

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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 black locust (*Robinia pseudacacia* L.). Using the technique of PAA-gel electrophoresis under denaturant conditions there was revealed a lot of fractions which, mainly, decrease form the start time (dry seeds) until the end of the germination.

INTRODUCTION

During germination under controlled conditions it was observed using techniques of PAA-gel electrophoresis the evolution of the protein fractions at a qualitative level.

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 black locust (*Robinia pseudacacia* L.) came from the forests from the Botosani County.

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 they were 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

Under a global view it was observed that in the seeds of black locust (*Robinia pseudacacia* L.) are a lot of proteins and these are widely distributed along the entire spectrum of the molecular masses.

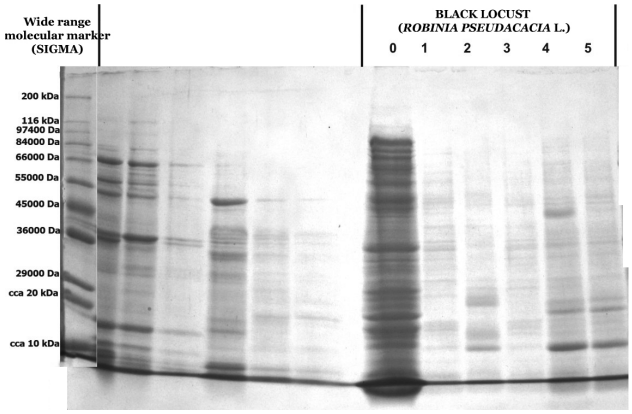

Figure 1 – The electrophoregram of the proteins extracted in distilled water from seeds of the black locust (*Robinia pseudacacia* L.) during germination

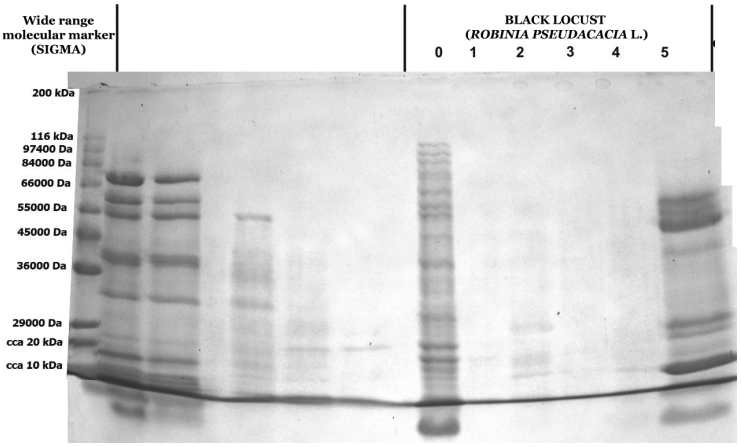

Figure 2 – The electrophoregram of the proteins extracted in phosphate buffer from seeds of the black locust (*Robinia pseudacacia* L.) during germination

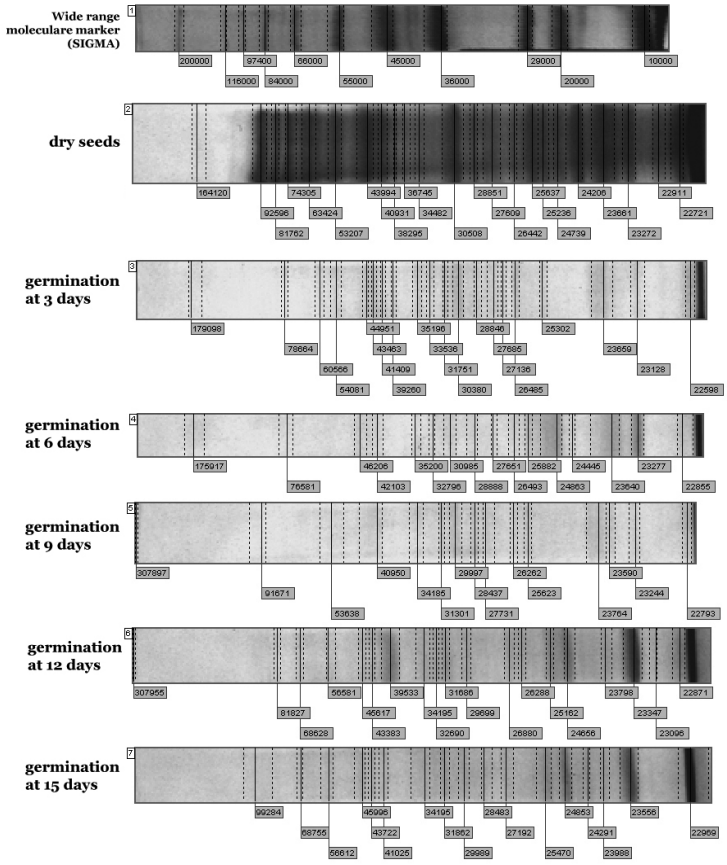

Figure 3 – Distribution of the molecular masses of the proteins separated by SDS-PAGE from the water extracts made from seeds of the black locust (*Robinia pseudacacia* L.) during germination

It was observed on the electrophoregram that in the case of the proteins from the seeds of black locust (*Robinia pseudacacia* L.) in the dry seeds there is lots of protein fractions well represented. As soon as the process of water absorption starts, the content of proteins greatly decreases. It could be the result of the soaking of the seeds and the starting the activity of the hydrolytic enzymes.

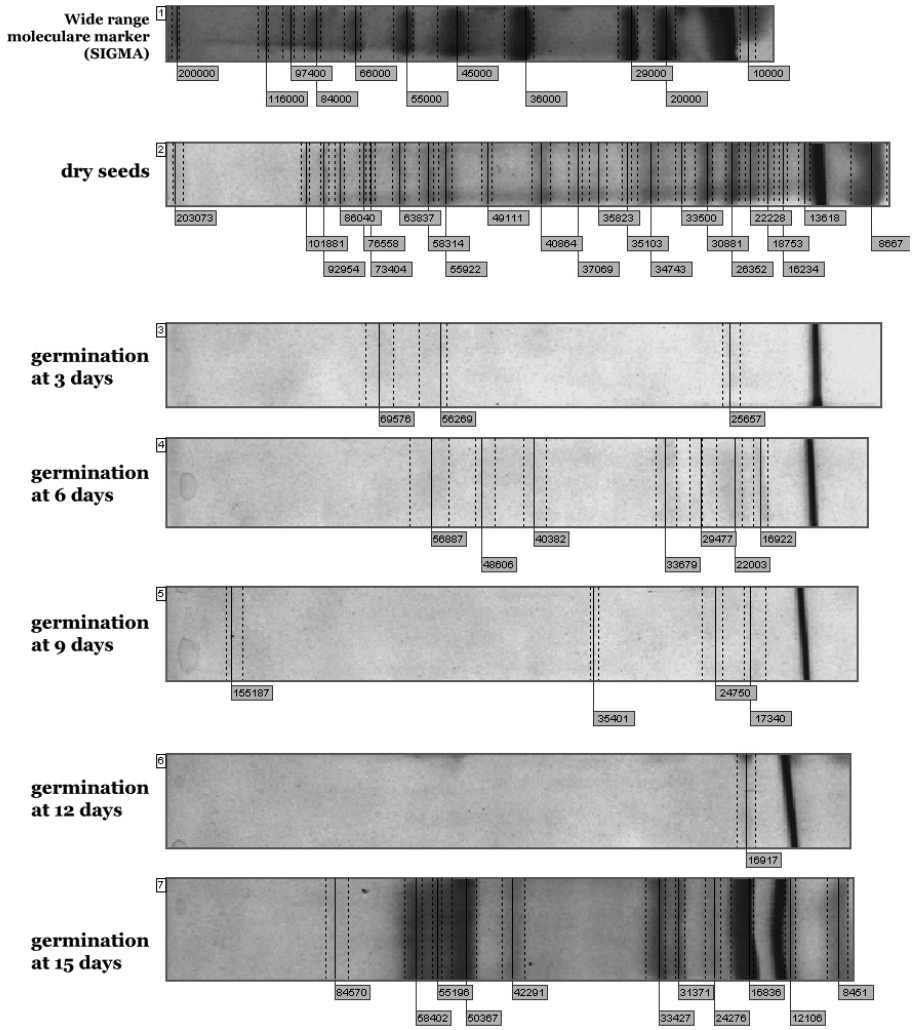

Figure 4 – Distribution of the molecular masses of the proteins separated by SDS-PAGE from the phosphate buffer extracts made from seeds of the black locust (*Robinia pseudacacia* L.) during germination

During the germinations it is observed that the proteins with high molecular mass decrease in quantity. The proteins in the medium and low molecular mass domain are the ones that will be well represented during the germination process.

The proteins with low molecular mass tend to show up and to be well represented at the end of the germination process compared to the proteins with high molecular mass.

An interesting distribution is revealed by the two solvents that were used for the extraction of the proteins. In water there are more solubilized proteins than in the phosphate buffer and, more important, the profile of the proteins is well represented on the entire germination process.

The appearance at the end of the germination of two bands at the 23,5 kDa and other bands suggest that the own mechanism of biosynthesis is active in the plant .

The proteins from the seeds of black locust (*Robinia pseudacacia* L.) are distributed over a wide range of molecular masses in both extracts but the profile is kept only in the water extract during germination process. In the phosphate buffer extracts there are many differences in the proteins profile with many bands represented at the beginning (dry seeds) and at the end (newly formed plant) of the germination process.

CONCLUSIONS

In the seeds of black locust (*Robinia pseudacacia* L.) there are a large variety of proteins.

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.

At the end of the germination process there is a slight tendency of increase in the number of fractions maybe because of the own biosynthetic process of the new plant.

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