A PRELIMINARY ANALYSIS OF THE ASPARTATE-AMINOTRANSFERASE ISOZYMES IN *GLEDITSIA TRIACANTHOS* AND *ROBINIA PSEUDACACIA* GERMINATING SEEDS

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Abstract: The extracts made with sodium/potassium phosphate buffer from the probes collected during germination of the seeds from *Gleditsia triacanthos* and *Robinia pseudacacia* was subjected to polyacrylamide gel electrophoresis under native conditions. Then the solution for specific coloring of the bands in which are found the izoenzymes of aspartate-aminotransferase was poured over the gel. The coloring reaction was stopped by rinsing the gel with distilled water when the intensity of the coloration was considered optimal. The results were interesting showing the existence of more than one spot corresponding to aspartate-aminotransferase in almost all analyzed probes. At least one of the spots from each of the probes presents the same Rf as the standard we have used (SigmaAldrich Glutamic-Oxalacetic Transaminase from porcine heart, Type II-A, lyophilized powder, 100-400 units/mg protein).

The main purpose of the paper was to find if there are any different isozymes of the aspartate-aminotransferase in the extracts made with sodium/potassium phosphate buffer from the probes collected during germination of the seeds from *Gleditsia triacanthos* and *Robinia pseudacacia*.

INTRODUCTION

The problem of the existence and the identification of different particular forms of the enzymes is a problem directly related with the genetic variability of each individual of a species (Brown et al., 1981; Huo et al., 2009). Many of the enzymes were analyzed for the existence of their different forms and aspartate-aminotransferase is one of them (Lee et al., 1975; Leuchtmann et al., 1990; Taniguchi et al., 1995; Ferreyra et al., 1996).

Black locust (*Robinia pseudacacia* L.) is a nitrogen fixing leguminous tree species native to the Appalachian Mountains in the United States. It has been largely used for the stabilization of degraded terrains and the production of fence posts. But because of its rapid growth and adaptability to drought it is now widely planted around the entire temperate region of the world (Keresztesi, 1983; Ashby et al., 1985; Tolbert et al., 1985;).

Honey-locust (*Gleditsia triacanthos* L.) is another member of the *Leguminosae* family and is also native from North America. Today it is widely planted as a hardy and fast-growing ornamental being used in extreme urban stress areas such as parking lot islands and sidewalk tree squares. It was also planted for erosion control, for windbreaks and shelterbelts, and as vegetation pioneer for rehabilitation of strip-mine spoil banks. For these qualities it is widely planted around the temperate regions of the globe (Robertson et al., 1976; Potts et al., 1982; Smith et al., 1984).

Both species are well known for their variety of the isozymes that could be found by analyzing different populations and different enzymes (Brown et al., 1981; Surles et al., 1989, 1990; Schnabel et al., 1990).

In this study we try to find aspartate-transaminase isozyme variation in sodium/potassium phosphate buffer extract from probes collected during germination of the seeds from *Gleditsia triacanthos* and *Robinia pseudacacia*.

MATERIALS AND METHODS

The seeds of *Gleditsia triacanthos* and *Robinia pseudacacia* we have used in this experiment came from trees growing naturally in the surroundings of Iasi and in the forests around the village Tudora in the Botosani County. The seeds were well mixed before analysis and all the seeds which are not good (empty, incomplete, with fungi, with holes) were eliminated. All the seeds have been subjected to a two and a half hours treatment with concentrated sulphuric acid and then very well washed, many times, with sterile distilled water (Singh et al., 1991; Deno, 1993). Then they have been spread in sterile Petri dishes, under the sterile hood, onto sterile fabric (the one used to cover the wounds). The seeds received the necessary amount of sterile distilled water and they have been left to germinate under the laboratory temperature and luminosity (that means normal cycles of light and dark). The germination process was considered over when the seedlings have their own chlorophyll (the cotyledons were green and the leaves of the seedling have been formed).

To identify the isozymes of aspartate-aminotransferase we have used the technique of separation of the proteins under native conditions in polyacrylamide gels followed by the identification of specific spots by specific color reaction (Stejskal, 1994).

We have used a continuous native gel of 8% (Sambrook et al., 2001).

Identification of the aspartate-aminotransferase specific spots was done using the method developed by Decker et al., 1963 and MacDonald et al., 1972. The gel was washed with distilled water twice before the coloring solution has been pour over the gel. The color reaction was stopped by rinsing the gel with distilled water.

All the solutions were prepared in distilled water and the reagents came from Sigma and Roth.

Because of the great fragility showed by the colored gel good care is recommended in manipulation of the gel to a dedicated scanner or a transilluminator to be scanned or photographed.

To analyze the gel snapshots have been taken with a dedicated scanner (Amersham ImageScanner) and it was used ImageQuant™ LT / 1D Gel Analysis software from General Electric Healthcare.

RESULTS AND DISCUSSIONS

Analyzing the gels it was observed that both species presents more than one isozyme for aspartate-aminotransferase (fig. 1 and 2).

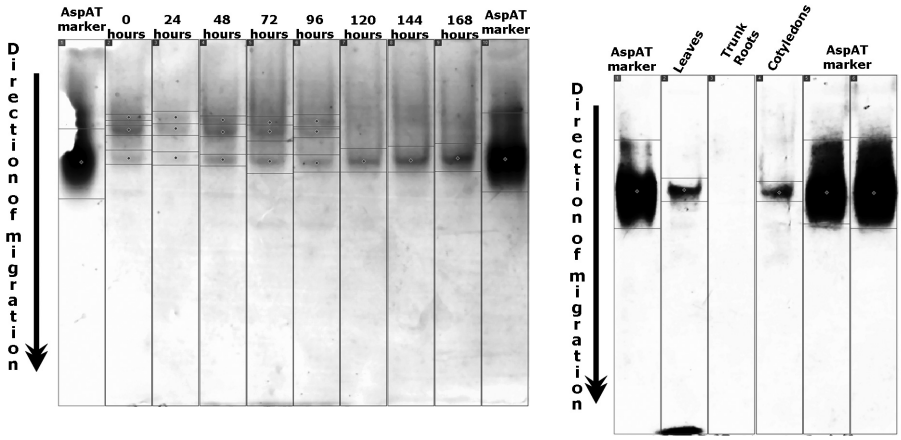

Figure 1. Isozymes identified on the native gel from the sodium/potassium phosphate buffer extracts from *Gleditsia triacanthos* L. seeds collected during germination

Revealing the spots corresponding to the isozymes of aspartate-aminotransferase shows the existence of few isozymes. The profile of these suffers modifications during germination process.

A maximum of three colored spots are present in the beginning of germination, then at 24 hours, 48 hours, 72 hours and 96 hours.

These spots are characterized by Rf's with values around 0.21; 0.25 and 0.3.

The following probes, at 120 hours, 144 hours and 168 hours, exhibits only a single colored spot associated with a single form of aspartate-aminotransferase. The Rf value of this spot is situated around 0.3.

On the probes from the organs of the seedling it is present the same situation like at the end of germination process. Only one spot is identifiable and only in the probes coming from cotyledons and from leaves. In the probe coming of trunk and roots there aren't any spots associated with aspartate-aminotransferase.

The spots identifiable in the probes coming from organs of the seedling have a Rf around the value of 0.3.

The isoenzymic spectrum of aspartate-aminotransferase in the probes made with sodium/potassium phosphate buffer from *Robinia pseudacacia* seeds during germination process exhibit clearly only two spots in all of the probes.

At the beginning of germination, at 24 hours and 48 hours the intensity of the spot with Rf around 0.23 is clearly higher than the intensity of the spot with Rf of 0.33. In the middle of

germination process, 72 hours, 96 hours, 120 hours and 144 hours, both spots shows an almost identical intensity. At the end of germination process, 168, 192 and 216 hours, the spots characterized by the Rf around 0.2 starts to fade.

In the organs taken from seedling of *Robinia pseudacacia* there are two identifiable spots of aspartate-aminotransferase. The Rf's which characterize these spots are similar with those from the seeds during germination process. The intensity of these spots is lower than in the spots from the seeds during germination process.

The obtained results from *Robinia pseudacacia* seeds and seedlings in Romania are in direct correlation with the results found in literature (Surles et al., 1989; Yang et al., 2004).

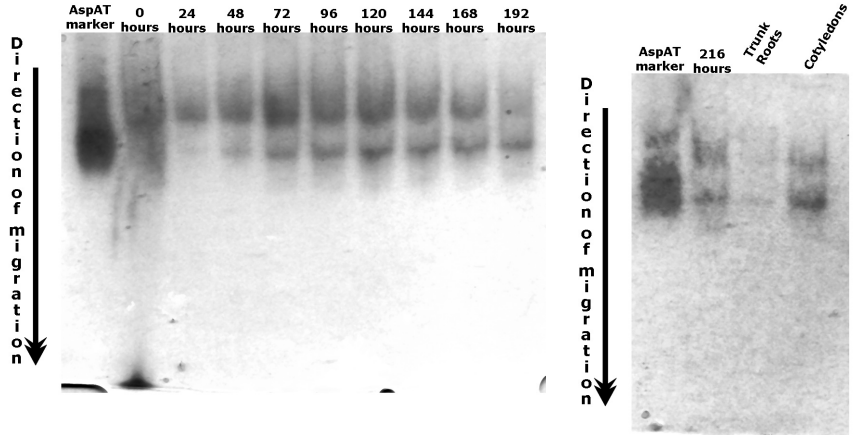

Figure 2. Isozymes identified on the native gel from the sodium/potassium phosphate buffer extracts from *Robinia pseudacacia* L. seeds collected during germination

CONCLUSIONS

It was observed that both species presents more than one isozyme for aspartate-aminotransferase.

In the probes from *Gleditsia triacanthos* it was found that are present a maximum of three spots corresponding to aspartate-aminotransferase isozymes.

Robinia pseudacacia probes exhibit a constant number of isozymes, two, which are present in different proportions over the germination process.

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