

**Selection and optimizing of nucleic acids' extraction methods from  
transgenic sugar beet (*Beta vulgaris* L.) plants**

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The stability of the genetic structures in the genome of transgenic sugar beet plants and their expression is not well understood, so finding out the effectiveness of transgenes displaying in sugar beet plants is relevant.

Work objective is selection of the total DNA extraction techniques and total RNA extraction method from transgenic sugar beet plants and optimization of incubation temperature parameters in RNA extraction using phenol.

In this paper we used sugar beet plants that contain anonymous genetic structure of the resistance gene to broad-spectrum herbicide Roundup, which active ingredient is glyphosate.

DNA isolation was performed with usage of cationic detergent CTAB (cetyltrimethylammonium bromide) by two methods. The main steps of DNA isolation from plant samples was lysis of the cell membranes in the presence of saline buffer, protein purification of DNA from the chloroform-isoamyl alcohol mixture and precipitation of the nucleic acid with alcohol solution. Used in work methods differ in composition of the lysis buffer, number of extraction stages and duration of incubation during DNA precipitation.

Evaluation of the DNA preparations and the optimal methods of DNA extraction from plant samples of transgenic sugar beet plants were performed by electrophoretic separation of DNA in agarose gels with ethidium bromide under ultraviolet light.

As a result of DNA fragments' electrophoretic separation, it was found that elected techniques are effective for the DNA extraction from transgenic sugar beet plants. At the same time, by comparing the quantity and quality of DNA preparations in samples obtained by these methods, it is clear that the usage of optimized methods

in the laboratory of advanced agricultural biotechnology ISB UAAN makes it possible to obtain more high-purified DNA from each sample in sufficient quantity for molecular analyzes, including PCR in GMOs identification. However, based on the electrophoregram results, J. Draper's and other methods allow DNA obtaining in more quantity less high-graded. As shown in Fig. 1, DNA is more degraded, contains a lot of low-pieces, there are also considerable differences in the concentration of DNA of individual samples. An important advantage of this method is also much less time for the entire procedure, while the method provides overnight DNA precipitation, which significantly slows down the process and makes this method less appropriate for DNA isolation from sugar beet.

For quantitative and qualitative assessment of RNA preparations that were obtained by phenol extraction as part of *Trizol* reagent and RNA extraction method in the presence of silica sorbent salt buffer concentration and purity of total RNA was measured.

Based on the data presented in the table, the highest number of total RNA was obtained using phenol extraction (500-900 mg/ml), the value of purity was within a 1.0 to 1.6. Concentration of the obtained RNA using sorbent was 40-70 mg/ml, and the rate of purity was 1,8-2,0. Also, the results indicate that the temperature of incubation during the RNA extraction from sugar beet plants with phenolic method affects the yield and purity of nucleic acids. Thus, the concentration of total RNA obtained by incubation at 60°C and 24°C doesn't have significant difference and is in the range of 500-1200 mg/ml. A high concentration observed for the variant with a temperature of 4°C - 450-800 mg/ml. However, if we compare the purity of RNA preparations, it can be noted that the smallest impurities contained in the version of the incubation temperature 60°C (purity value is in the range 1.2-1.6), whereas in the other two cases it ranges from 1.0 to 1.2, indicating a high content of RNA in these preparations of substances that can inhibit the reverse transcription reaction and affect the yield of cDNA. It's worth mentioning also that the purity of RNA during the extraction using a sorbent is much higher than in phenol extraction (1.5-1.9), but its

low concentration shows that this method is less suitable for the RNA extraction from sugar beet plants in the transgenes expression's estimation.

As a result of the research it was found that the method of DNA extraction is optimized in the laboratory of advanced agricultural biotechnology ISB UAAN is more effective to highlight the total DNA from plant material of transgenic sugar beets, as it allows to obtain a highly purified DNA from aligned concentration that can be used for amplification reactions order to identify GMOs. These data suggest that the most appropriate method of RNA extraction from transgenic sugar beet plants is a method of RNA extraction through the usage of phenol and incubation temperature 60°C for 5 min. Thus, the concentration and purity of the obtained RNA preparations allow their usage in reverse transcription reaction and transgenes expression's estimation in of sugar beet plants.