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SELECTION AND OPTIMIZING OF NUCLEIC ACIDS' EXTRACTION METHODS FROM TRANSGENIC SUGAR BEET *(Beta vulgaris L.)* PLANTS

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The results of the research of total DNA extraction with CTAB buffer system and extraction of total RNA in two fundamentally different ways: phenol method and RNA extraction with sorbent. The optimal method of DNA extraction, including quantitative and qualitative assessment of preparations of nucleic acids, obtained from transgenic sugar beet plants.

Foreword. The latest agricultural technologies that use genetically modified organisms (GMOs), is the result not only of rapid development of genetic engineering techniques, but also of proper study of the useful traits inheritance problem and characteristics of the transferred genes expression [2]. It is important to search for new approaches to the GMO's monitoring with usage of molecular genetic techniques that many times accelerate the process of creating and controlling the usage of transgenic plants. To evaluate the expression of introduced genes through the usage of PCR with reverse transcription (RT-PCR), because of the existence of certain structures in the plants' DNA is not enough to draw conclusions about the effectiveness of the introduced genes [1].

In particular, 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.

Materials and methods of research. 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 [3, 4]. 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 [5, 8, 9].

The method is optimized in the laboratory of advanced agricultural biotechnology ISB UAAN. Frozen plant material in a fragment of leaf lamina (500 mg) was triturated with 0.6 ml of lysis solution, placed in Eppendorf tubes and incubated for 1 h at 60˚C. To the mixture an equal volume of hloform-isoamyl alcohol (24:1) was added with further centrifugation at 11,000 g, the second material cleaning with a mixture of chloroform-isoamyl alcohol was made. For the DNA precipitation chilled isopropyl alcohol was used in a ratio of 1:0.6 and incubated for 20 min. (18-20˚C). Precipitate was washed with 96% ethanol solution and dried at a temperature 18-20˚C, and then DNA was dissolved in 50 ml of TE buffer [4].

Methods of J. Draper et al. Frozen samples of plant material (500 mg) were triturated and transferred into the Eppendorf test tube, 500 ml of 2˚CTAB (96˚C) and 10 ml of mercaptoethanol were added. Samples were incubated for 20 min. at 56˚C and cooled to 18-20˚C. An equal volume of chloroform-isoamyl alcohol (24:1) was added and further centrifugation at 11,000 g was made. To the resulting supernatant 0.1 volume of 10% CTAB (56˚C) was added, in addition cleaning with chloroform was made. To the collected aqueous phase was added an equal volume of 1% CTAB and solution was incubated for 20-30 min. at 18-20˚C. Precipitate obtained with centrifugation was dissolved in 400 ml of 1M NaCl. To the resulting solution a double volume of chilled 96% ethanol was added and left for the night at -20˚C. Precipitation was made by centrifugation for 2 min. at 9000 g. Precipitate was washed with 65% and 85% solutions of ethanol three times for 1 min. Precipitate was predryed and dissolved in 50 ml of TE buffer [3].

For the quality estimation of obtained DNA from transgenic sugar beet plants electrophoresis was performed in 2.5% agarose gel at a voltage of 150 V for 30 min. with ethidium bromide under ultraviolet light with a wavelength of 321 nm [1].

During the process of the total RNA extraction from transgenic sugar beet plants two approaches were used: RNA extraction method with usage of phenol with multi-temperature incubation and reagent kit for the RNA extraction with sorbent.

RNA extraction using phenol. In this paper, a set of Trizol RNA Prep 100 (LLP "Laboratory Izohen", Russian Federation) reagents was used. The procedure for the RNAextraction by this method consists of the following stages: cell lysis in the presence of guanidinium thiocyanate and phenol, RNA purification from protein and phenol chloroform and precipitation of nucleic acid with isopropyl alcohol. Historical [6, 7, 10, 11] using of Trizol reagent different incubation temperatures are used: 60˚C, room temperature and 4˚C. Plant material with weight 100 mg were triturated in a cooled on ice mortar in the presence of 1 ml Trizol-reagent. The resulting homogenate was transferred into Eppendorf tubes and incubated at indicated temperatures for 5 min., with further addition of 200 ml of chloroform and incubated for 5 min. at 4˚C. The resulting suspension was centrifuged 5 min at 14,000 rev/min. To the supernatant was added to a double volume of isopropyl alcohol. The resulting mixture was kept at -20 ˚ C for 30 min. with further centrifugation for 15 min. at 14,000 rev/min. The precipitate was washed with 75% ethanol solution and predryed for 3 min. at 65˚C. As the solvent EkstraGen E reagent was used, which was part of the set.

RNA extraction using sorbent. With the implementation of this approach to the total RNA extraction through the usage of the reagents set for RNA ectraction "Ribo-sorb» (LLC «InterLabservis", Russian Federation). According to the manufacturer's instructions, the process of RNA extraction from plant material using sorbent consists of the following steps: lysis of cell membranes,

RNA adsorption on silicate sorbent at a certain pH and in the presence of saline buffer, RNA washing from proteins and phenolic compounds alcohol solutions, sorbent removal and RNA dissolution in buffer. Assessment of the quantity and quality of total RNA was performed using a spectrophotometer [1].

Results. 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 (fig.).

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., 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 [4] is also much less time for the entire procedure, while the method [3] 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 (tabl.).

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

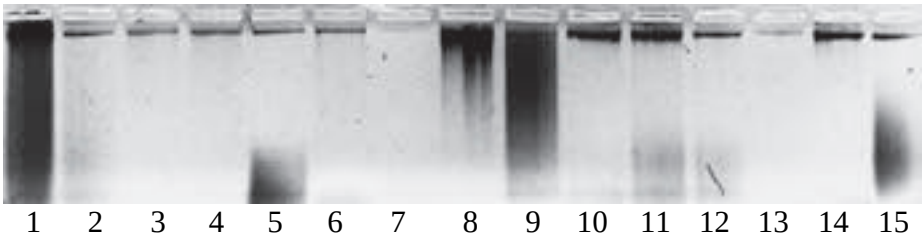

Fig. Electrophoresis of DNA preparations: 2–7 – isolated DNA according to [4]; 10–15 – isolated DNA according to [3]

Table. The concentration and purity of studied RNA

	Phenol extraction method, temperature of incubation						RNA extraction on a sorbent	
	60°C		24°C		4°C		conc. mg/ml	purity 260/280
	conc. mg/ml	purity 260/280	conc. mg/ml	purity 260/280	conc. mg/ml	purity 260/280		
1.	555,5	1,68	510,3	1,02	871,1	1,06	64,5	1,53
2.	831,7	1,24	648,0	1,17	782,7	1,00	45,0	1,96
3.	635,9	1,57	783,7	1,03	655,4	1,10	62,0	1,84
4.	1273,5	1,25	1356,9	1,13	458,2	0,86	61,1	1,64
5.	959,0	1,43	809,9	1,19	689,4	0,96	64,8	1,72

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.

Conclusions

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.

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АНОТАЦІЯ

Присяжнюк Л.М. *Підбір та оптимізація методик екстракції нуклеїнових кислот із трансгенних рослин цукрових буряків (Beta vulgaris L.) // Біоресурси і природокористування. – 2014. – **6**, №5–6. – С.14 –18.*

Наведено результати досліджень екстракції тотальної ДНК ЦТАБ-буферною системою та виділення сумарної РНК двома принципово різними способами – із застосуванням фенолу та за допомогою сорбенту. Підібрано оптимальну методику екстракції ДНК, враховуючи кількісну і якісну оцінку препаратів нуклеїнових кислот, отриманих із трансгенних рослин цукрових буряків.

АННОТАЦИЯ

Присяжнюк Л.М. *Подбор и оптимизация методик экстракции нуклеиновых кислот из трансгенных растений сахарной свеклы (Beta vulgaris L.)// Биоресурсы и природопользование. – 2014. – **6**, №5–6. – С.14 –18.*

Показаны результаты исследований экстракции тотальной ДНК ЦТАБ-буферной системой и выделение суммарной РНК двумя принципиально различными способами – с применением фенола и с помощью сорбента. Подобрана оптимальная методика экстракции ДНК, учитывая качественную и количественную оценку препаратов нуклеиновых кислот, полученных из трансгенных растений сахарной свеклы.