ISOLATION AND ANALYSIS OF NATIVE DNA AND RNA FROM ROOTS OF RUBBER-PRODUCING SPECIES *SCORZONERA TAU-SAGHYZ* LIPSCH. ET G.G. BOSSE

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Scorzonera tau-saghyz Lipsch. et G.G.Bosse, is endemic species, growing in South Kazakhstan and alternative (to *Hevea brasilians*) source of natural rubber, related to the *Asteraceae* Family. Natural rubber (NR) is an indispensable raw material for the production of different products for medicine, automotive and aviation industries. The introduction of tau-saghyz into the culture will make it possible to obtain NC not only in Kazakhstan, but also on the territory of the countries of the Northern latitudes. At the growing phases of ontogenesis of tau-saghyz (April-June) latex is actively synthesized in the leaves and transported to the roots. In our experiments it was necessary to conduct molecular genetic studies of the selected forms of tau-saghyz with high rubber concentration. In this regard, it became necessary to isolate and analyze native DNA and RNA from dry roots. Samples with the highest rubber content were used for DNA extraction. The main problem in the isolation of DNA from the dry roots of tau-saghyz is the significant content of natural rubber. In this research the following commercial kits were used: 1) DNeasy Plant Mini Kit (Qiagene); 2) GenElute Plant Genomic DNA Miniprep Kit (Sigma); 3) NucleoSpin® Plant II / Midi / Maxi kit (Macherey-Nagel). Further DNA isolation was carried out in accordance with the kit manufacturer's protocol. Quantitative DNA analysis was performed using both UV spectrophotometry (Nanodrop) and fluorometric analysis (Qubit) method. DNA extracted by the 2 kit (Sigma) was further purified by precipitation with ethanol and demonstrates satisfactory quality. Functional analysis of isolated DNA samples was performed both by restriction analysis and by DNA amplification. It is worth to mention that for reaction primers that are characteristic of gene AACT (ACETO-ACETYL-COA TRANSFERASE) *Cucumis melo* were used. This enzyme catalyzes the first reaction of biosynthesis in the mevalonate pathway, which the final product is IDP (isopentenyl diphosphate). The cis-prenyltransferase (CPT) enzyme catalyzes the biosynthesis of rubber by the sequential addition of IDP to the terminal group of the initiating allylic pyrophosphate (APP). As a result of PCR analysis a putative 1200 bp AAST gene for tau-saghyz was identified. In this study two methods of RNA isolation were chosen: 1. A method widely used for plants of the *Cucurbitaceae* family; 2. A method approved for plants with a high content of polyphenols.

The RNA isolation was carried out as described in the relevant protocols with minor modifications (Verwoerd TC, Dekker BMM, Hoekema A (1989) A small-scale procedure for the rapid isolation of plant RNAs. Nucleic Acids Res 17:2362). The final precipitates were dissolved in 50 µl of water pretreated with diethylpyrocarbonate. Quantitative analysis of RNA was carried out using UV spectrophotometry (Nanodrop). Functional analysis of extracted RNA samples was conducted by cDNA synthesis with usage of reverse transcriptase Superscript IV with the following amplification in PCR. In this reaction the primers specific for CPT gene of *Taraxacum kok-saghyz* were used. The prospective CPT gene was excised from the gel, purified and cloned into the pJET vector. In the further research it is planned to perform its sequencing and comparison with the CPT gene of *Taraxacum kok-saghyz* and *Hevea brasiliensis*.