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Amplification of the dreb2a transcription factor gene from salsola richteri (moq.) Kar. Ex litv. Growing in the southern Aralkum

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Abstract: This study investigates halophytic plants of the genus Salsola L., which are widely spread in the southern part of the Aralkum Desert. During the research, a PCR product of approximately 1200 bp associated with the DREB2A gene was amplified in one of the species, Salsola richteri. This gene plays a key role in plant adaptation to drought and salt stress. The obtained data will be used for sequencing the DREB2A gene and analyzing its expression.

Keywords: Salsola richteri, DREB2A, transcription factor, salt tolerant plants, primer.

Introduction: Salsola richteri is a shrub or a small tree ranging from 1 to 3 meters in height. In its young stage, it is covered with finely tuberculate (or papillose) leaves, which later become leafless. The stem is weakly branched, with smooth gray bark, up to 5 cm thick at the base, and its woody branches soon acquire a milky-white color [1]. This species is promising for phytoremediation practices due to its good growth on sandy soils, high seed productivity, ability to propagate by seeds and cuttings, and tolerance to significant salinity. Its powerful root system makes it effective for sand stabilization [4, 5]. The high protein content and the formation of substantial organic mass with economical water use allow S. richteri to be utilized as a valuable forage plant for autumn-winter pastures [2, 3, 5].

Karakalpak scientists studied the growth and development of S. richteri under the ecological conditions of the Karakalpak part of the Kyzylkum Desert from 2014 to 2018. Fruiting of S. richteri began as early as the first year of life, in the third decade of June. Fruits were mainly formed on fourth-order shoots, with the number of fruits per shoot ranging from 16 to 38 and the number of leaves from 14 to 40. Fruiting depended on the age of the shrubs; in the second year of cultivation, a single shrub could produce between 3,000 and 6,000 seeds [5].

S. richteri belongs to the groups of euhalophytes and hemixerophytes, which are adapted to soils with a moderate level of salinity (1.8–2.3%; Cl 0.1–0.23%—

chloride-sulfate and sulfate salinization or 1.3–1.8%; Cl 0.1–0.23%—sulfate-chloride and chloride salinization). This species is capable of successfully growing in such soils, maintaining its dominant position in the phytocenosis even as salinity levels decrease. It forms plant associations and formations over vast areas that have been exposed after water receded. In his research, S. G. Sherimbetov analyzed the chemical composition of plants found in the desiccated regions of the Aral Sea. Among the studied halophytes (Climacoptera aralensis, Kalidium capsicum, Halostachys belangeriana, Salsola richteri, Haloxylon aphyllum, Tamarix hispida), a high content of minerals such as Ca, Cl, K, Mg, and Na was observed.

The plant Salsola drummondii Ulbr., belonging to the genus Salsola L., is capable of successfully growing and completing its life cycle under high salinity conditions (500–800 mM NaCl). This plant adapts to salt stress by reducing the level of photosynthetic pigments, decreasing carotenoid content, and increasing the activity of antioxidant enzymes [6]. Based on the genes of Salsola iberica, which are responsible for resistance to abiotic stress factors, a bank of expressed sequence tags (EST—Expressed Sequence Tag) was created to analyze the molecular mechanisms of adaptation. This bank included 377 ESTs, which were grouped into 227 unique fragments. Similarities were found between S. iberica ESTs and stress-resistance genes, including salt-induced proteins, betaine-aldehyde dehydrogenases, and calcium-binding proteins [7]. Salsola ferganica is a desert herbaceous plant that grows in arid regions of western and northwestern China.

To normalize gene expression in S. ferganica under abiotic stress, nine reference genes (TUA-1726, TUA-1760, TUB, GAPDH, ACT, 50S, HSC70, APT, and U-box) were tested under six stress conditions. The analysis revealed that ACT and U-box exhibited the highest stability among all tested variants [8].

A review of scientific literature has shown that despite S. richteri is tolerance to salinity and drought, there is a lack of data on transcription factors responsible for these adaptation mechanisms. Abiotic stress plays a crucial role in plant growth and development, as plants are exposed to various adverse factors such as drought, low and high temperatures. Under stress conditions, several stress-resistant genes are activated, among which DREB (Dehydration Responsive Element Binding) genes play a particularly important role. These genes encode proteins of the Apetala2/ethylene (AP2/ERF) family, which bind to the dehydration-responsive element (DRE)/C-repeat in the promoter regions of stress-resistance genes. The DRE cis-element, located near the promoter regions of stress-associated genes, serves as the binding site for DREB transcription factors, which regulate osmotic stress in plants. Drought and high salinity levels induce the expression of the DREB2 gene, which plays a key role in regulating abiotic stress-responsive genes.

The expression of OsDREB2A in Oryza sativa is enhanced under salt stress and dehydration, but the gene exhibits low sensitivity to low temperatures and abscisic acid (ABA) [9]. Similarly, in Zea mays, the transcript level of ZmDREB2A increases under high-temperature stress. Arabidopsis thaliana demonstrates DREB2A activation primarily in response to drought and salt stress [10].

The aim of the study is to design a new primer for obtaining the full DREB2A gene sequence from S. richteri, a plant from the Chenopodiaceae family.

METHODS

As research objects, plant biomaterials of S. richteri collected from the Southern Aralkum in 2021 were used. The plant materials were identified by staff of the Institute of Botany, Academy of Sciences of the Republic of Uzbekistan.

Total DNA was extracted from the leaves of the plant using the PureLink Plant Total DNA Purification Kit (Invitrogen by Thermo Fisher Scientific).

Primer Design

To amplify the DREB2A gene from plants of the Chenopodiaceae family, a search for the nucleotide sequence of the DREB2A gene was conducted in the NCBI database (NCBI - www.ncbi.nlm.nih.gov) using the BLASTN algorithm in the BLAST web application within the GenBank database. Based on the nucleotide sequences of the DREB2A gene from Salicornia brachiata (ID: GU809211.1), Haloxylon ammodendron (ID: KP765243.1), Beta vulgaris (ID: XM_010694618.3), and Chenopodium quinoa (ID: XM_021876866.1) for the reverse primer, as well as Chenopodium album (ID: OX_419225.1) and Salicornia ramosissima (ID: OX_596239.1) for the forward primer from the NCBI database, a design of specific degenerate primers was created for the amplification of approximately 900 and 1500 nucleotide sequences using the CLUSTAL O(1.2.4) Multiple Sequence Alignment program (Table 1).

Table 1.

Oligonucleotide sequence of primers for amplification of the DREB2A gene of some species of the *Chenopodiaceae* family

Primers	5'-3' Oligonucleotide sequence
Pr_D_F	TCGAAGAAAGGDTGTATGAAAGG
Pr_Dr_F1	GGGAWRTTTTAWAWWTTKATTTA
Pr-DOST_R1	AAACCTAYWGAGAATAAGCTT

Fisher Scientific, USA) [7].

Polymerase chain reaction

The amplification of DREB2A gene fragments from the studied halophytic plant Salsola richteri was performed using a MiniAmp™ Plus Thermal Cycler (Applied BioSystems, USA) with specific primers. The polymerase chain reaction (PCR) of the DREB2A gene was carried out according to the protocol of the Phusion™ High-Fidelity DNA Polymerase kit (Thermo

For the quantitative determination of nucleotides in the PCR products, electrophoresis was performed in a 2% agarose gel. The length of the PCR products was determined using a 100 bp DNA Ladder marker (Invitrogen, USA) (Fig. 1).

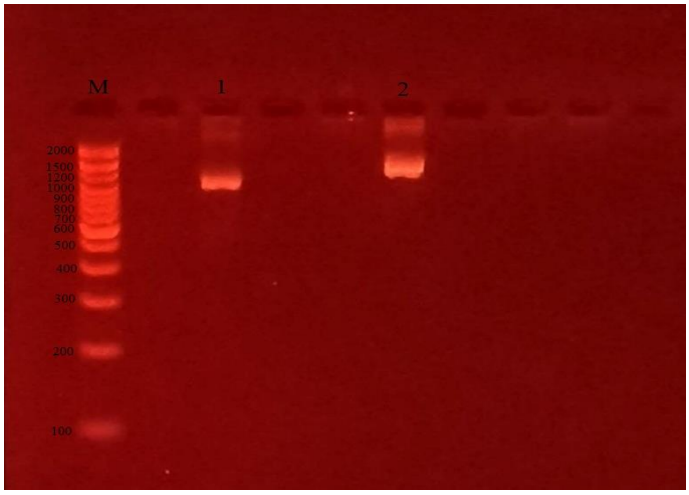

Figure 1. Electropherogram of the PCR product of the DREB2A gene

M - DNA marker; 1. *S. richteri*; 2. *S. richteri*

RESULTS AND DISCUSSION

As a result of the study, a PCR product with an approximate length of 900 and 1200 base pairs was obtained from Salsola richteri. It was established that the PCR product length corresponds to the predicted size during primer design, confirming their specific binding to the complementary sequence. According to NCBI, the length of the DREB2A gene in the registered species Salicornia brachiata (ID: GU809211.1), Haloxylon ammodendron (ID: KP765243.1), Beta vulgaris (ID: XM_010694618.3), and Chenopodium quinoa (ID: XM_021876866.1) ranges from 1100 to 1200 base pairs. The length of the PCR products obtained from S. richteri also falls within this range, confirming their affiliation with the DREB2A gene. The obtained results enable further investigation of the nucleotide sequence of the DREB2A gene in S. richteri and the study of its expression levels under drought and salinity conditions.

CONCLUSION

DNA fragments were isolated from plants of the Chenopodiaceae family distributed in the Southern Aralkum. Using bioinformatics online resources, specific DREB2A primers were designed. The DREB2A gene was successfully amplified from the DNA of Salsola richteri using PCR. It was established that the PCR products obtained from S. richteri were suitable for sequencing nucleotide pair sequences. The results of this study provide a foundation for further analytical research aimed at understanding the functionality of the DREB2A gene in S. richteri.

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