BIOCHEMICAL CHARACTERIZATION OF SALSOLA RICHTERI CALLUS TISSUES UNDER SALINITY STRESS

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Studying desert plants as a source of fodder, food, and medicine not only expands scientific knowledge but also plays a significant role in addressing global challenges related to sustainable development and environmental protection.

Halophytes, although adapted to high salinity, also possess considerable economic potential. They contribute significantly to environmental restoration and regeneration and are widely used as medicinal resources. Most halophytes are perennial salt-tolerant shrubs, while a smaller proportion are annual species [1]. The growing interest in halophyte research highlights the need to recognize their substantial potential as valuable sources of plant-derived products [2].

The objective of this study was to perform microclonal propagation of Salsola richteri (Moq.), a species distributed in the Southern Aralkum region, under in vitro conditions; to establish sterile cultures; to induce callus tissue formation; and to determine the levels of free amino acids and vitamins in callus tissues grown under salinity stress.

S. richteri (Moq.) Kar. ex Litv., Uzbek. Turkest. Otd. Russk. Geogr. Obshch. **4**, 5 (1905) 27. – Il'in, Fl. SSSR **6** (1936) 242, tab. 11, fig. 11. – Bochantsev, Fl. Uzbek. **2** (1953) 282. – Bondarenko, Opr. Vyssh. Rast. Karakalp. (1964) 92. – Pratov, Opr. Rast. Sr. Az. **3** (1972) 95. – Yerejepov, Fl. Karakalp. … (1978) 63. – Korovina et al., Ill. Opr. Vyssh. Rast. Karakalp. i Khorezm. **1** (1982) 122. – Sherbayev, Fl. i Rast. Karakalp. (1988) 156. — **Рихтер шўраги** (Uzbek: черкез; Karakalpak: шеркез).

This shrub flowers in June and bears fruit in September. It grows on sands and saline sandy soils. In Karakalpakstan, it is widespread in the Lower Amu Darya, Kyzylkum, Ustyurt, and Aralkum regions. Its general distribution extends to Iran, Afghanistan, and Central Asia. The areal type is Iran–Turan.

S. richteri dominates the second vegetation layer. It is a psammomesoxerophilous shrub reaching 2–3 m in height. The species forms large associations on sands and saline sandy soils, playing a significant role in stabilizing sands and reducing soil salinity levels [3–5].

One of the important achievements of plant biotechnology is the induction of callus tissue from plant explants. A callus is a mass of unorganized parenchyma cells with several advantages: it is healthy, virus-free, sterile, and allows for the production of numerous regenerants. Callus culture offers an unconventional yet promising approach to vegetative propagation of valuable plant species [6].

Obtaining callus tissues of S. richteri with these advantages can accelerate the production of clean microcuttings in a short time and rapidly increase the number of healthy plants. In this study, seeds of S. richteri were used. A series of experiments were conducted to propagate the selected plant under in vitro conditions. Prior to inoculation, the seeds were stratified at −20 °C for 14 days and subsequently sterilized.

Sterile seeds were sown on hormone-free MS (Murashige & Skoog) nutrient medium [7] containing 30 g/L sucrose, 7.5 g/L agar, and adjusted to pH 5.6–5.8. Several square explants (0.2–0.5 mm) were excised from the main and middle parts of the roots, stems, and leaf blades of microseedlings obtained from the germinated seeds. For callus induction, 10–12 leaf explants were placed in each Petri dish containing MS medium supplemented with 0.5, 1.0, or 2.0 mg/L 2,4-D. Cultures were incubated in the dark at 26 ± 2 °C.

For S. richteri leaf explants, the optimal concentration for callus induction was 0.5 mg/L 2,4-D in the MS medium. To assess propagation under salinity stress, cultures were transferred to MS medium containing 50, 100, 200, or 300 mM NaCl, supplemented with 0.5 mg/L 6-BAP, 0.5 mg/L kinetin, and 0.1 mg/L IBA.

Initial morphological changes in the explants on NaCl-containing media were observed within one week compared with the control. After two weeks, the most vigorous callus growth was recorded in cultures on MS medium supplemented with 0.5 mg/L 6-BAP, 0.5 mg/L kinetin, 0.1 mg/L IBA, and 200 mM NaCl, which was selected as the optimal condition.

In the next stage, the contents of free amino acids and water-soluble vitamins in S. richteri callus tissues grown under salinity stress were determined by HPLC. Ten essential amino acids were detected, with their concentrations increasing compared to the control on medium containing 200 mM NaCl. Threonine content ranged from 3.14–6.53 mg/g, arginine from 0.60–2.26 mg/g, valine from 0.64–0.84 mg/g, histidine from 0.89–1.19 mg/g, tryptophan from 1.78–1.94 mg/g, phenylalanine from 0.36–0.57 mg/g, and lysine from 0.06–0.09 mg/g. Among the non-essential amino acids, glycine and asparagine increased to 2.94–2.96 mg/g, and proline to 1.33 mg/g.

Ascorbic acid, vitamin B_1, B_2, B_3, B_6, and B_9 were identified in S. richteri calluses, with vitamins B_6 and B_9 showing the highest increase (5.83–7.92 µg/g) compared to the control.

These results demonstrate the potential for propagating S. richteri microseedlings under saline in vitro conditions, offering applications in the reclamation of saline soils and the development of salt-tolerant crop varieties.

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