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Salt Stress - Induced Changes in Amino Acid Biosynthesis in Atriplex Aucheri Microshoots

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Abstract: Experiments were conducted by culturing Atriplex aucheri under in vitro conditions on nutrient media supplemented with different concentrations of NaCl (50, 100, 200, 300, 400, and 500 mM). Apical meristem tissues excised from primary seedlings germinated from seeds grown on Murashige and Skoog (MS) medium were used as explants and then transferred to media containing respective NaCl concentrations. The results showed that the tolerance threshold of A. aucheri microshoots to NaCl reached 500 mM, while the optimal NaCl concentration for growth was 200 mM. In addition, the content of free amino acids in salt-adapted microshoots was analyzed using HPLC. The obtained results demonstrated that the free proline content in control plants grown on MS medium was 0.49 mg/g, increasing to 0.53 mg/g at 50 mM NaCl and reaching 0.78 mg/g at 300 mM NaCl. At 300 mM NaCl the levels of tryptophan and phenylalanine were 1.68 mg/g and 0.62 mg/g, respectively. Moreover, at 500 mM NaCl, a significant increase in phenylalanine, isoleucine, and leucine levels was observed compared to the control.

Keywords: Atriplex L., Southern Aral Sea region, in vitro, NaCl, stress, amino acids, proline.

Introduction: In recent years, increasing attention has been devoted to the study of plant species distributed in saline and arid regions with particular emphasis on improving seed productivity and investigating their biological characteristics. Representatives of the genus Atriplex L. have become important research subjects as promising heterocarpic plants with significant forage and phytomeliorative value.

In heterocarpic species, morphological and physiological diversity of seeds plays a crucial role in understanding adaptive strategies to unfavorable environmental conditions (Amanova et al., 2026). The genus Atriplex comprises approximately 300 species of annuals and shrubs, widely distributed in subtropical regions of the world. Most species grow in saline soils and are therefore commonly referred to as “saltbush” (Eman Zaghloul et al., 2023).

In particular, Atriplex aucheri is considered a promising model species for studying drought tolerance mechanisms in desert plants. This species exhibits heterocarpy, producing three morphologically and physiologically distinct seed types (A, B, and C) within a single plant. The functional differentiation of these seed types enhances the ecological adaptability of the species.

A portion of the seeds is capable of rapid and synchronous germination under favorable environmental conditions, enabling efficient utilization of available resources. Another portion remains in a state of deep or non-deep dormancy for extended periods and germinates only after exposure to unfavorable factors such as drought, high temperature, and salinity, thereby ensuring population stability and species persistence.

This strategy has significant adaptive value in desert ecosystems and is considered an important evolutionary mechanism that determines tolerance to extreme abiotic conditions. Seeds of type A are small, black, and possess a five-parted perianth composed of five separate or partially fused segments. Seeds of type B are of medium size and morphologically similar to type A; however, both B and C types are enclosed by enlarged bracteoles, which contribute to seed protection and enhance germination under various environmental conditions.

Type C seeds are large, brown-colored, and also surrounded by prominent bracteoles. In A. aucheri, type C seeds are non-dormant and exhibit high germination rates, whereas type A and B seeds are characterized by shallow physiological dormancy and lower germination rates (Li Jiang et al., 2021).

Studies on A. aucheri have shown that brown seeds (type C) exhibit the highest germination rates and largest size, while black seeds have relatively lower germination rates and are less dependent on maternal environmental conditions. Additionally, variability in seed productivity and seed size is more pronounced under conditions of sufficient water supply but limited nutrient availability (Wang L. et al., 2015).

Several species of the genus Atriplex, including A. halimus, A. lindleyi, A. laciniata, A. confertifolia, and A. leucoclada, have been studied for their antidiabetic, antioxidant, cytotoxic, and antifungal properties (Eman Zaghloul et al., 2023; Chikhi I. et al., 2014; Capua C.J. et al., 2023). In addition, species such as Atriplex canescens (Pursh.) Nutt., Atriplex gmelinii, Atriplex halimus, Atriplex littoralis, Atriplex nummularia, Atriplex sagittata, and Atriplex patula have been extensively investigated by various researchers.

However, according to the available literature, only a limited number of studies have been conducted on Atriplex dimorphostegia and A. aucheri (Karim A. et al., 2011; Asilbekova D.T. et al., 2008; Khalbekova H.U. et al., 2023; Amanova et al., 2026).

To date, there is insufficient information on seed germination under in vitro conditions and on the assessment of salinity tolerance in species of this genus. Therefore, further investigation of Atriplex species represents an important scientific and practical objective.

METHODS

Study object. The study was conducted using Atriplex aucheri Moq., a representative of the genus Atriplex L., collected from the dried bottom of the Aral Sea in October 2025. Under in vitro conditions, seed germination, initial culture establishment, morphobiological responses to different NaCl concentrations, and changes in free amino acid content were investigated.

Taxonomic description: A. aucheri Moq. In Chenop. monogr. enum. (1840) 51. - Опр. раст. Ср. Аз. 3 (1972) 43. DC. Prodr. 13, 2 (1849) 91. Grub. in Pl. As. Centr. 2 (1966) 28. A. amblyo-stegia Turcz. in Bull. Soc. Nat. Mosc. 25 (1852) 416.- Iljin in Fl. URSS. 6 (1936) 85. Botsch. in Fl. Uzbek. 2 (1953) 223. - Golosk. et Poljak. Опр. мар. Казах. (1955) 27.- Golosk. in Фл. Казах. 3 (1960) 207.-Zak. Фл. раст. басс. Зер. 2 (1961) 109.- Prat. ex E. Nikit. in Ф.л. КиргССР. Доп. 1 (1967) 64. A.

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Atriplex aucheri is an annual species growing on takyr soils, gypsum-rich clay slopes, saline habitats, wormwood-saltwort communities, and along roadsides, as well as in ruderal habitats on plains and foothills. It flowers in July and produces fruits in September. In Karakalpakstan, it is distributed in the Lower Amu Darya region, Kyzylkum, Ustyurt, and Aralkum. Its broader distribution includes the Zaysan Basin, Balkhash, Caspian, and Aral deserts, the Kazakh uplands, Betpak-Dala, Muyunkum, Kyzylkum, Tien Shan, Pamir-Alai (southern), and Kopet Dag regions.

Seed germination assessment. Sterilized seeds (Amanova, 2026) were cultured on hormone-free Murashige and Skoog (MS) medium supplemented with sucrose (30 g/L) and agar (7.5 g/L), with pH adjusted to 5.6–5.8. Seeds were sown at 100 per treatment with four replicates. Germination was carried out under thermostatic conditions at 26 ± 2 °C. During the experiment, seeds were incubated in complete darkness; however, daily observations were conducted under natural room light. Germination was monitored regularly over a 21-day period.

Determination of free amino acid composition and content. The composition and content of free amino acids in the samples were determined using the phenylthiocarbamyl (PTC) derivatization method (Steven A. Cohen and Daniel, 1988). Identification of amino acids was carried out based on retention times compared with standard amino acid mixtures, while quantitative analysis was performed by calculating the peak areas of individual amino acids in the chromatograms.

RESULTS AND DISCUSSION

Seed Germination. Seed germination was observed from the first day of the experiment. Mass germination occurred within 5–7 days after the onset of germination. Additionally, analysis on the 7th day showed that a well-developed primary root system had formed in all samples, and the germination rate reached 83.46 ± 0.76%. This result indicates that the seeds of this species are well adapted to in vitro sterile conditions and that the composition of the MS nutrient medium was aduquate for their germination (Amanova, 2026).

Adaptation to Salt Stress. Apical meristematic tissue from the initial seedlings germinated from seeds of A. aucheri grown on MS nutrient medium were used as explants and and transferred to nutrient media containing different concentrations of NaCl (Fig. 1).

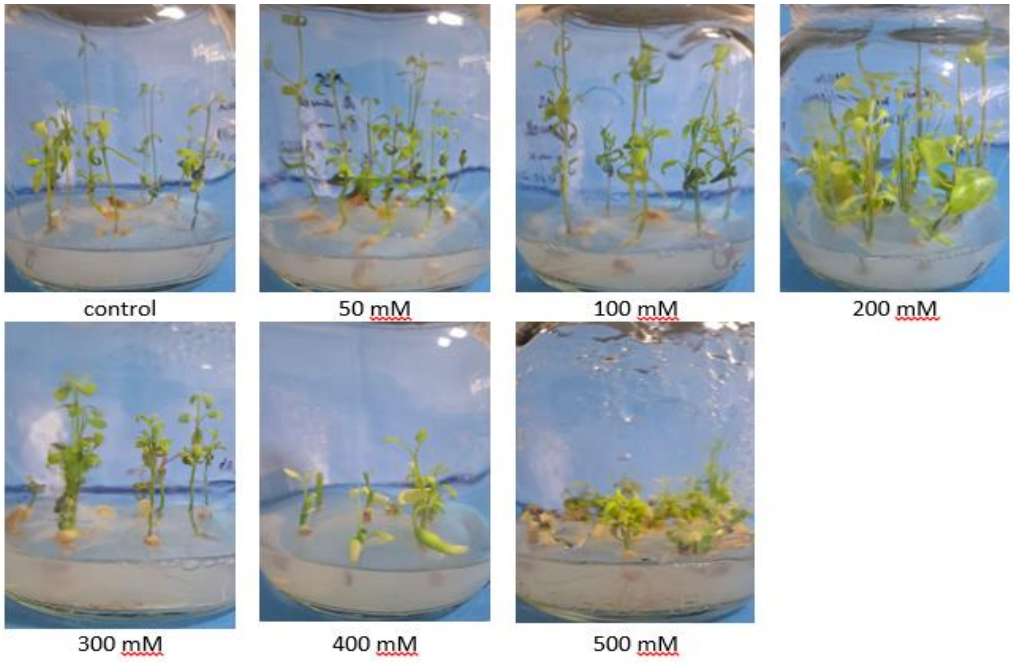

Fig. 1. Microrooted seedlings of A. aucheri cultured on nutrient media supplemented with different NaCl concentrations.

Morphogenetic analysis of A. aucheri micro-seedlings (n = 4)

Growth Parameters (cm)		
Samples	Root	Shoot
Control	0.63± 0.26	3.66± 0.38
50 mM	5.26± 0.28	9.63± 0.18
100 mM	6.50± 0.57	9.13± 0.24
200 mM	10.63± 0.96	13.36± 0.74
300 mM	6.43± 0.66	5.63± 0.50
400 mM	4.21± 0.94	3.23± 0.61
500 mM	2.12± 0.23	2.76± 0.21

Comparative Analysis of Growth in Saline Media. A comparative analysis of a 30-day-old A. aucheri plants grown under saline conditions, relative to the initial explants, revealed significant differences in growth and development. In particular, the length parameters of micro-seedlings at 50 and 100 mM concentrations were close to each other, measuring 9.63 ± 0.18 cm and 9.13 ± 0.24 cm, respectively. At 200 mM NaCl, root length reached 10.63 ± 0.96 cm, while shoot length reached 13.36 ± 0.74 cm, indicating a favourable growth under this condition. At 300 mM NaCl, a decrease in root growth was observed, and shoot length decreased to 5.63 ± 0.50 cm. At 400 and 500 mM concentrations, both root formation and shoot growth were significantly inhibited.

Free Amino Acid Composition and Content in Salt-Adapted Micro-seedlings. Experiments were conducted by cultivating A. aucheri in vitro on nutrient media supplemented with different concentrations of NaCl (50, 100, 200, 300, 400, and 500 mM). The content of free amino acids in 30-day-old salt-adapted microshoots was analyzed using the HPLC.

The results showed that the free proline content in control plants of A. aucheri grown on MS medium was 0.49 mg/g. Under salt stress, a sharp increase in proline accumulation was observed, reaching 0.53 mg/g at 50 mM and 0.78 mg/g at 300 mM. In addition, at this concentration, tryptophan and phenylalanine content were 1.68 mg/g and 0.62 mg/g respectively. Furthermore, at 500 mM, significant increases in phenylalanine, isoleucine, and leucine contents were recorded compared to the control.

CONCLUSION

In this study, it was determined that the tolerance limit of A. aucheri microshoots to NaCl reached 500 mM, while the optimal concentration for growth was 200 mM. These findings expand the potential for the use of Atriplex species in in vitro propagation under saline conditions and provide a basis fo their application in soil reclamation and the development of salt-tolerant crops.

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