Adaptive and viable characteristics of microclonal seedlings of hyperhylophyte of *Suaeda arcuate* under *in vitro* conditions

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Abstract. This article presents data on the introduction of *in vitro* culture and microclonal propagation of plants identified in the group of hyperhalophytes belonging to the *Suaeda arcuata Bunge*. This study was carried out to optimize the composition of nutrient media for the main stages of reproduction *in vitro*, as well as studies on the rooting and adaptation of regenerants for species of the *Suaeda arcuata* from axillary or apical buds, but more often from stem segments with a node. In this work, hormones of the cytokinin and auxin series, or a combination of them, were added to the nutrient environment for growth activation. The cultivation of regenerates on the environment 1/2 MS + 1 mg/l 6-BAP + 0.3 mg/l IAA + 2,4-D showed the best effect on the growth of regenerants, created the possibility of obtaining the maximum amount of biomass. Our study indicated that *Suaeda arcuata* is a highly salt-tolerant species that survived at 300 mM NaCl most conducive to plant growth, where the quantity of Pro and Met were bigger than the control.

1 Introduction

Over the last years, in our Republic, special attention has been paid to the practical use of plants from understudied territories, in particular, such as phytomeliorants, which can be sources of medicinal raw materials, as well as environmental protection. On the basis of the implemented program activities in this direction, specific results have been achieved, including the determination of the possibilities of the biological resources of the dried bottom of the Aral Sea, their use in medicine, pharmaceuticals and other various branches of chemical production, in part, the use of Aralkum plants as a resource of biologically active compounds.

Formation of the vegetation cover in the arid regions of the Aral Sea is a crucial factor in the development of newly forming lands. Flora of the Aral Sea's dessicated bed and dynamics of its vegetation cover's formation are unique. Despite the fact that the region is the continuation to the Turan Depression, it is quite different from the adjacent desert by the complexity and uniqueness of its natural conditions; due to acceleration of physical-

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geographical and geochemical processes it is a great contrast to this very territory in the past. Soils with various salinities dramatically differ by their physical structures and chemical composition. Groups including various plant species are formed in each type of the soils. In the regions with high concentrations of salts in the soil, the groups of halophytic plant communities are formed.

Despite the complex circumstances in this area, more specifically, high salinity of the soil, dry and sharply continental climate, many plant species are gradually acclimatizing territories freed from water [1].

In recent years, more and more attention has been paid to the search for new medicinal plants and the development of preparations from plant materials used in traditional medicine. Abundant arrays of representatives of the family Chenopodiaceae Vent., taking into account the ecological purity, can be considered from the standpoint of possible use as a cheap source of valuable biologically active compounds

The cultivation of halophyte plants can contribute to the creation of highly productive long-term pasture communities on secondary saline lands, as well as their effective use as medicinal raw materials, since the chemical composition of this family is very diverse and unique. It is represented by various amino acids, carbohydrates, phenolic compounds (flavonoids, isoflavones, xanthones, tannins, etc.), essential oils, etc. In this regard, studies of the component composition of plants growing in the extreme climatic conditions of the chul are promising, for this it is necessary to determine the main classes of biologically active substances, one of which are amino acids, which are the main structural units of the human body and play a huge role in the biosynthesis of biologically active compounds, proteins and peptides [2-4]. The largest number of plant cover species of saline soils in Uzbekistan belongs to the Chenopodiaceae family, many of whose representatives have valuable fodder potential and are medicinal and they are extremely promising for use in the ecological restoration and increase in the productivity of degraded pastoral lands in the arid regions of the world.

Plants of Chenopodiaceae family are succulent, have mostly salt accumulating effect, living on saline and alkaline soils, occupy vast areas of deserts and semi-deserts of Uzbekistan and also play an important role in plant landscapes and are the sources of biologically active substances. Therefore, the study of chemical composition and development of methods of biologically active substances extraction with subsequent biosurfactant screening are of great interest today and are particularly relevant, since they will contribute to creation of highly effective domestic phytopreparations with wide range of pharmacological action.

Globally, plants of the family (Chenopodiaceae) include about 100 genera and 1400 species, of which 24 genera and 59 species are found in the Southern Aral Sea [3].

Particularly interesting is the list of halophyte species that quickly occupy an areal with high soil salinity. To increase and ensure food security in the republic, studies are also being carried out on halophyte plants intended for use as a component of human nutrition and animal feed. Among the representatives of this family, the genus *Suaeda* can serve as a source of flavanoids, alkaloids, polysaccharides, carotenoids, saponins, coumarins, tannins, other biologically active substances, and vitamins [5-6]. As a result, plants of these species can be a source of raw materials for preparations of hypertensive, antibacterial, antioxidant, antitumor, immunosti- mulating, anti-inflammatory, antiseptic, antimicrobial action [7-8].

A perspective phytomeliorant *Suaeda arcuata* was proposed for use in breeding salt and drought-resistant plants for the rehabilitation of saline desert sandy lands of Uzbekistan [3-4].

The genus of *Suaeda*, is one of the most salt-tolerant representatives; it functions in conditions of high soil salinity and can grow on media with NaCl concentration up to 1

M [9]. It is known that in the membranes of the Golgi complex isolated from the cells of the roots. Cl^{-}/H^{+}- antiporter, which plays an important role in halophytes, where it is involved in the regulation of concentrations Cl^{-} in the cytoplasm under saline conditions. The supposed role of this antiporter is to export ions Cl^{-} from the cytoplasm to the vacuole, which provides plants with salt tolerance [10].

Also, this plant is among the potentially medicinal plants, as it contains alkaloids, flavonoids, saponins, coumarins, and vitamins [11]. However, there are only a few data in the literature concerning the development of individual elements of methods for clonal microclonal propagation of medicinal desert plants.

Currently, the most promising developments include microclonal propagation technologies - *in vitro* production of plants that are genetically identical to the original organism. An important step in the method of *in vitro* plant microclonal propagation is obtaining a sterile, pathogen-free material suitable for propagation, optimizing propagation conditions, including sprout regeneration and development of the root system, and optimizing the conditions for adaptation of the *in vitro* material to the soil, that is, the development of *ex vitro* technologies. Plant material can be introduced into *in vitro* culture in two ways: sterilization of viable seeds, obtaining sterile seedlings intended for further propagation; sterilization of the green part of the plant, obtaining sterile explants, also for further propagation.

2 Materials and methods

The object of study is the one-year long-vegetating halophyte *Suaeda arcuata*, collected in 2022 in the South Aral Sea, Karakalpakstan. It is an annual growing in the solonchaks, blossoming in June and fruiting in September. Distribution in Karakalpakstan: lower reaches of the Amu-Darya, Kyzylkum, Ustyurt, Aralkum.

Seeds of *Suaeda arcuata* were used for introduction into in vitro culture. For micropropagation, it is important to develop an in vitro propagation technique based on the use of mature *Suaeda arcuate* seeds. The process of introducing a plant object into in vitro culture was carried out in two stages [12]:

- Sterilization (decontamination) of plant material with harsh sterilizing agents in order to obtain a material clean from bacterial and fungal infection, treatment with a 0.001% solution of thimerasal, followed by repeated washing in sterile water.
- Cultivation of the experimental material in vitro was carried out using standard methods generally accepted in plant biotechnology, on agar nutrient media. At the end of each passage, the results of the experiments were taken into account. To activate the growth, hormones of the cytokinin and auxin series, or their combination, were added to the nutrient medium.

The salt content in the medium 300 mM NaCl, which corresponds to medium and in high salinity study free amino acids *in vitro* conditions.

Analysis of free amino acids. The isolation of free amino acids was carried out as follows: the dried plant material was extracted with water (hydraulic ratio 1/10) with constant stirring for 8 hours at room temperature. Proteins and peptides were precipitated from the aqueous extract by adding an equal volume of 10% TCA. After 10 min, the precipitate was separated by centrifugation at 8000 rpm. 0.1 ml of the supernatant was freeze-dried. Determination of free amino acids in the form of their PTC derivatives was carried out by the method of Steven A. and Cohen Daviel J [13]. For the synthesis of PTC (phenylthiocarbomayl) derivatives of amino acids, a solution consisting of TEA + ethanol + water in a ratio of 1:7:1 was prepared and 250 ml were added to the dried samples under study. Kept for 10 min, and evaporated to dryness under vacuum. This step was repeated twice to neutralize the TCAA and to achieve an alkaline reaction medium. Next, a solution was prepared consisting of TEA + ethanol + water + FITC in a ratio of 1:7:1:1 and added to the samples in 250 ml increments. Kept for 30 min, and evaporated to dryness under vacuum. PTC derivatives of standard amino acids were synthesized in a similar way. Identification of PTC-amino acids. The identification of PTC amino acids was carried out on an Agilent Technologies 1200 series chromatograph with a DAD detector. A 75 x 4.6 mm Discovery HS C18, 3.5 µm column was used. Solution A: 0.14M CH_3COONa + 0.05% TEA pH 6.4, B: CH_3CN. Flow rate 1.2 ml/min, absorbance 269 nm. Gradient %B/min: 1-6%/0-5 min; 6-30%/5.1-40 min; 30-60%/40.1-45 min; 60-1%/45.1-50 min. Qualitative analysis and quantitative calculation of the concentration of the studied free amino acids were compared with the peak areas of the standard and studied PTC-amino acids.

3 Results and Discussion

Suaeda arcuata Bunge. is an annual plant, 30 - 50 cm tall, branched from the base. The leaves are meaty, linear-filamentous, semiterete, glabrous, with an expanded base, arcuately curved, alternately arranged, monoclinous and pistil- late flowers, almost sessile, on short stalks in multiflorous dense glomerulus. Fruits in October [8].

According to Adylov T. A. [11], the plant contains in the vegetative organs from 30% to 50% of mineral ions and is adapted to a high salt content in the soil, has a wide areal: Central Asia, Iran. It grows on dry salt marshes and among irrigated crops. It is eaten dry by camels and other animals.

Yearling long-vegetating halophyte *Suaeda arcuate* was collected in 2022 in the South Aral, Karakalpakstan. Seeds of *Suaeda arcuate* were used for introduction into *in vitro* culture. For microclonal propagation, it is important to develop an *in vitro* propagation technique based on the use of mature seeds of *Suaeda arcuate.* Thus, studies were carried out to optimize the composition of nutrient media for the main stages of reproduction *in vitro*, as well as studies on the rooting and adaptation of regenerants for species of the genus *Suaeda* obtained from axillary or apical buds, but more often from stem segments with a node.

There is no information on microclonal propagation of promising medicinal plants growing on the territory of Uzbekistan and on the creation of their biotechnology banks *in vitro*.

3.1 Microclonal Propagation of *Suaeda arcuate*

In this work, hormones of the cytokinin and auxin series, or a combination of them, were added to the nutrient environment for growth activation. To obtain sterile material, after treatment, the plant material was washed twice in distilled water and transferred directly to a nutrient environment. The seeds were transferred to a hormone-free environment with half salts MS, with sucrose with the addition of 7.5 g/l agar as a gel-forming component. Before autoclaving, the pH value of the environment was adjusted to 5.6 - 5.8. Out of 25 seeds planted on a nutrient environment, 22 seeds germinated on average, of which 3 were contaminated with a bacterial infection and were not suitable for further cultivation under *in vitro* conditions.

Seed germination under aseptic conditions was 92%. The total value of the degree of contamination was 8%. The development of plants took place in stag- es: first, a seedling was formed, then the root system developed, after which the shoots lengthened.

Seedlings were transplanted onto fresh MS nutrient environment without hormones and with combinations of various concentrations of cytokinins and aux ins. After 1.5

months, the passivated seedlings looked as follows: there was an active growth and development of *S. arcuata* regenerants under *in vitro* conditions and an active formation of the root system. This indicates the suitability for cultivation of the proposed nutrient environment and the successful introduction of the *S. arcuata* species into *in vitro* culture. Cultivation con- ditions: 25˚C ± 2˚C, illumination 3000 lx, photoperiod 16 hours.

The explants developed evenly, the height of the shoots cultivated on the studied environment did not vary significantly from 1.5 to 3.2 cm. The cultivation of explants on all nutrient environment for 60 days led to increased growth and development of regenerates on some variants of nutrient environments.

To regulate the process of morphogenesis and assess the content of vitamins in *S. arcuata* regenerants, the following phytohormones were introduced into the nutrient environment: 6-benzylaminopurine (BAP) and auxins, *α*- naphthylacetic acid (NAA), and indoleacetic acid (IAA). Sucrose was added as the main carbohydrate for cultivation at a concentration of 30 g/l.

As a result of the work, it was shown that among all variants with phytohormones, the cultivation of regenerates on the environment 1/2 MS + 1 mg/l 6-BAP + 0.3 mg/l IAA + 2,4-D showed the best effect on the growth of regenerants. As well as, the composition of the nutrient environment without hormones has a positive effect on obtaining parallel developed regenerants. Maintenance of samples in the *in vitro* collection can be carried out on MS environment without hormones and at a low positive temperature of +16˚C, illumination of ~500 lx, and a photoperiod of 8 h.

3.2 Amino acid composition of the *Suaeda arcuata* in the medium 300 mM NaCl

In the studied, 20 free amino acids were identified, of which 10 are essential - threonine, arginine, tyrosine, valine, methionine, isoleucine, histidine, tryptophan, phenylalanine, lysine, leucine. Comparison of the content of amino acids showed that the amount of proline and methionine were the highest. Further, arginine, cysteine, and tryptophan dominated. Proline - improves in all organisms as part of protein molecules and is part of the opioid enkephalin peptide. There is general agreement is a key precursor of the phytohormone ethylene in higher plants. In combination with other biologically active substances (phenolic compounds, polysaccharides, organic acids, macro- and microelements), the therapeutic significance of the studied species is emphasized, which makes it possible to create new drugs of combined action based on these species. Natural and climatic factors affect the content of biologically active substances in plants growing on the dried bottom of the Aral Sea exposed to the strongest salt, dehydration (drought), thermal stress, as well as the parallel effects of many phytopathogenic microorganisms.

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Thus, the collection of plants growing on the bottom of the Aral Sea and their prospect as a source of natural biologically active substances, in our opinion, is quite justified.

4 Conclusion

In this work, for the first time, a comparative analysis of the vitamin composi- tion of plants cultivated under *in vitro* conditions and intact models from the habitats of the *S. arcuata* species was carried out. It was experimentally established that the optimal nutrient environment for the stable development of microshoots without anomalies, without callus formation and initiation of root formation was MS without the addition of hormones. It was revealed that the studied cytokinins had a different effect on the morphogenesis of *Suaeda arcuate in vitro*. The data on the use of halophytes in the generation of plantations for development of forestry in the Republic of Uzbekistan as the basis for the new-stage development of agro-industrial complex.

The studied plants of the genus *Suaeda* are of particular interest both in scientific and ecological terms, which allows us to consider them as promising for introduction into culture in the Aralkum, and the assumption that plants have a selective ability to accumulate chemical elements has been experimentally confirmed. It has been established that the more microelements with a wide range of concentrations are combined in one plant, the greater the ecological amplitude of the growth of this plant and, as a result, the higher its adaptive capacity in conditions of technogenic pollution of new valuable drugs of combined action.

Comparative analysis showed that *in vitro* experiments (300 mM NaCl) in *S. arcuata*, contribute to an increase in amino acid composition, exceeding the threshold level of samples. The high content of 20 free amino acides of *S. arcuata* growing on soil with chloride-sulfate salinity shows that one of the effective mechanisms of physiological adaptation to salinity is the accumulation of proline and water-soluble carbohydrates in cells.

The ability to withstand the type of stress factors is necessary and survival in this environment requires a profound restructuring of plant metabolism, photosynthesis and transpiration, as well as the biosynthesis of adaptogenic substances.

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