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Biotechnological Assessment 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 species *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 genus *Suaeda* obtained 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 analyzing of microplants for the content of soluble in water B vitamins was carried out. As a result of the research, it was found that intact *Suaeda arcuata* plants in their natural habitats produce a large amount of soluble in water vitamins: riboflavin—0.062% and thiamine up to 0.006%. And in regenerated plants obtained on media without hormones, the content of vitamins was: B2 0.053%, B1 0%, respectively, and with a combination of 1/2 MS + 1 mg/l 6-BAP + 0.3 mg/l IAA + 2, 4-D, the content of vitamins varied as follows: riboflavin—0.059%, folic acid—0.030%, and thiamine was not detected. 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 and accumulation of B vitamins.

Keywords

Aralkum, Hyperhalophyte, *In Vitro*, Explant, Sterilization, Seeds, Micropropagation

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 phytome-

liorants, 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.

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. 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.

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 [1]. 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 [2] [3]. As a result, plants of these species can be a source of raw materials for preparations of hypertensive, antibacterial, antioxidant, antitumor, immunostimulating, anti-inflammatory, antiseptic, antimicrobial action [4] [5]. 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]. Also, this plant is among the potentially medicinal plants, as it contains alkaloids, flavonoids, saponins, coumarins, and vitamins [6]. The study of vitamin biosynthesis during plant ontogenesis made it possible to identify high-vitamin plants and rationally use them in medicine, animal breeding, and in the industrial production of vitamin preparations [7].

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 microclonal 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 propa-

gation; sterilization of the green part of the plant, obtaining sterile explants, also for further propagation [8].

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. 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 (Figure 1).

The process of introducing a plant object into *in vitro* culture was carried out in two stages [9]:

1) 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;

2) 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.

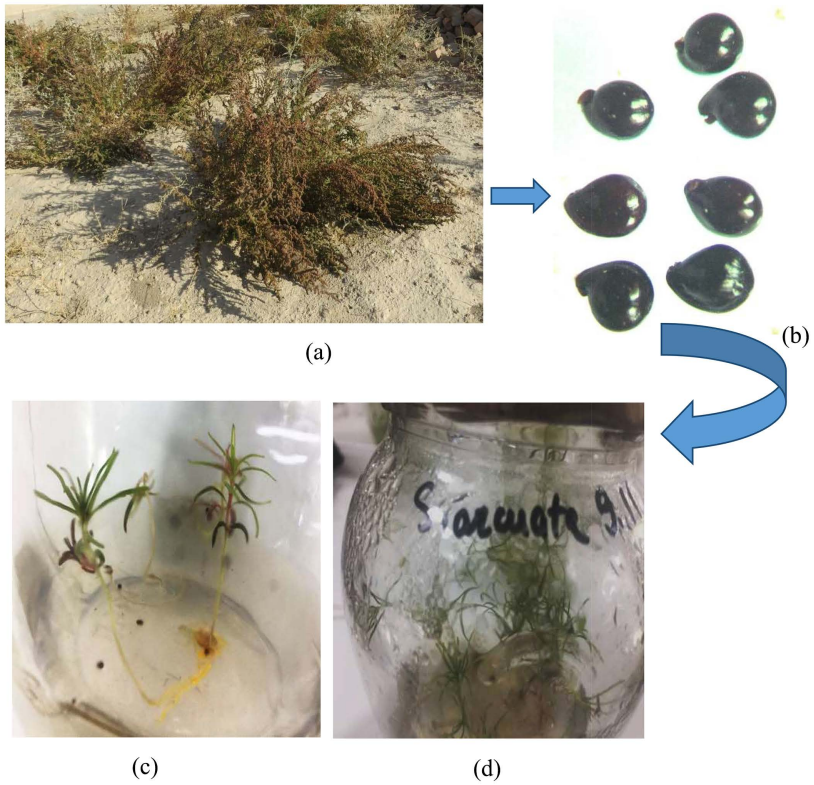

Figure 1. *In vitro* culture introductions of *Suaeda arcuate*. (a) Plants from natural habitats; (b) Seeds introduced into culture *in vitro*; (c) Regenerated on MS nutrient environment without hormones; (d) Regenerated on MS nutrient environment with combinations of various cytokinins and auxins.

To activate the growth, hormones of the cytokinin and auxin series, or their combination, were added to the nutrient medium.

For quantitative determination [7] of group B vitamins, they were extracted from crushed aboveground plant organs with distilled water, then centrifuged at a speed of 6000 rpm, and the supernatant was separated. An equal amount of 10% TFA was added to the supernatant to precipitate proteins and peptides. The formed precipitate was separated by centrifugation at 6000 rpm.

The supernatants were neutralized with 0.01 M NaOH solution and then brought to the desired volume. Quantitative determination of vitamins was carried out using HPLC.

Vitamins ("Sigma") were used as a standard: B1 (Cat. No. 59438), B2 (Cat. No. 83885), B3 (Cat. No. 59676), B6 (Cat. No. 58560), B9 (Cat. No. 59303). To build a calibration curve for each standard vitamin and determine the formula of the curve, calibration solutions were prepared: 5 concentrations in the range of 0.01 - 0.200 μg/ml.

Studies were performed on an *Agilent Technologies* 1200 Series chromatograph, column 4.6 × 75 mm Zorbax SB-C18, 3.5 μm, 0.1% H_3PO_4, pH-2.5, and acetonitrile gradient. Vitamins have been detected at wavelengths of 210 and 254 nm. The chromatography process and column calibration gave elution times B1 (3.75 min), B2 (4.56 min), B9 (5.85 min), B3 (7 min), B6 (8.8 min). The trend equations of the calibration curves were: YB1 = 3971.1x − 44.57, YB2 = 4771.1x, YB9 = 3886.3x, YB3 = 2851.1x − 54.75, YB6 = 11811x.

For the quantitative determination [10] of B group vitamins, they were extracted from crushed aerial plant organs with distilled water, then centrifuged at a speed of 6000 r/pm, and the supernatant was separated. An equal amount of 10% TFA was added to the supernatant to precipitate proteins and peptides. The formed precipitate was separated by centrifugation at 6000 r/pm.

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, semi-terete, glabrous, with an expanded base, arcuately curved, alternately arranged, monoclinous and pistillate flowers, almost sessile, on short stalks in multiflorous dense glomerulus. Fruits in October [11].

According to Adylov T. A. [9], 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. The fruit is one-seeded, in a perigonial spathe of 5 tepals fused to 1/2, tuberculate from the surface. The fruits are small, up to 2 mm in diameter. Weight of 1000 pcs. of the fruits 0.8 g.

Yearling long-vegetating halophyte *Suaeda arcuate* was collected in 2022 in the South Aral, Karakalpakstan. Seeds of *Suaeda arcuate* were used for introduc-

tion 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 [12].

Previously, it was shown that callus tissues were initiated in the species *Suaeda nudiflora*, while cultivating some of them, regenerated plants were obtained from a young stem on MS environment supplemented with various concentrations of BAS and 2,4-D. The grown shoots easily rooted on the environment ½ MS with the addition of IBA and NAA, while 80% - 90% of rhizogenesis was achieved. Adapted plants derived from callus did not show any morphological variation and were similar to the parental donor plant [13].

There is evidence in the literature that *Suaeda monoica* and *Suaeda nudiflora* calli were induced from seedling epicotyls on Murashige and Skoog's environment (MS) with the addition of various combinations of auxins and cytokinins. Effective callus regeneration was obtained by exposing the callus tissue in MS environment to 2,4-dichlorophenoxyacetic acid (2,4-D, 1.0 mg/l), benzylaminopurine (BAP, 0.5 mg/l) and 2,4-D (0.5 mg/l), kinetin (Kn, 0.25 mg/l) for S. monoica and S. nudiflora, respectively. A significant increase in proline content was observed and a strong positive correlation was found between total phenol content and antioxidant activity with increasing salt concentration [14].

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 arcuata*

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 [15] 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 stages: 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 *Suaeda 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 *Suaeda arcuata* species into *in vitro* culture. Cultivation conditions: 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 *Suaeda 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.

For the experiment, five variants of nutrient environments were prepared, and the MS nutrient environment without hormones was noted as the best option. In this case, no developmental anomalies were observed in the regenerants, there was no callus formation, the root system developed, therefore, MS environment without hormones was chosen for the control variant.

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, created the possibility of obtaining the maximum amount of biomass and accumulation of B vitamins. 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. Vitamin Composition Analysis

The content of soluble in water B vitamins, playing a large role in cellular metabolism were revealed in the microplants of *Suaeda arcuata*. For this aim, a comparative analysis of the vitamin composition of plants cultivated under *in vitro* conditions and intact models from habitats of the *Suaeda arcuata* species was carried out.

Extracts obtained from the studied plant material were analyzed under specified conditions. The vitamins in each sample were identified by comparing the elution times of the standard vitamins from the column, and their amounts were calculated from the respective vitamin peak areas in the chromatograms and trend equations. The results obtained are presented in **Table 1** and on the chromatogram (**Figure 2**).

As a result of the studies, it was found that in intact *Suaeda arcuata* plants from natural habitats at the stage of the juvenile stage, a large amount of soluble

in water vitamins is produced: riboflavin 0.062%, thiamine up to 0.006%, and in regenerated plants cultivated under *in vitro* conditions in combinations of MS without hormones: B2-0.053%, B1-0%, respectively; 1/2 MS + 1 mg/l 6-BAP + 0.3 mg/l IAA + 2,4-D-riboflavin 0.059%, folic acid-0.030%, thiamine was not detected in regenerates, but on both variants of nutrient environments with hormones there were high levels of vitamins B6 and B9—almost two times higher than in intact plants, and vitamins B2 were lower than in intact plants.

4. Conclusions

In this work, for the first time, a comparative analysis of the vitamin composition of plants cultivated under *in vitro* conditions and intact models from the habitats of the *Suaeda arcuate* 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.

Table 1. The content of vitamins in *Suaeda arcuate*, %.

Vitamin	Intact plants		regenerated plants			
			MS		1/2 MS + 1 mg/L 6-BAP + 0.3 mg/L IAA + 2,4-Д.	
	mg/100g	%	mg/100g	%	mg/100g	%
B-2	62	0.062	53	0.053	59	0.059
B-9	29	0.029	18	0.018	30	0.030
B-6	6	0.006	29	0.029	11	0.011
B-1	3	0.003	0	0	0	0

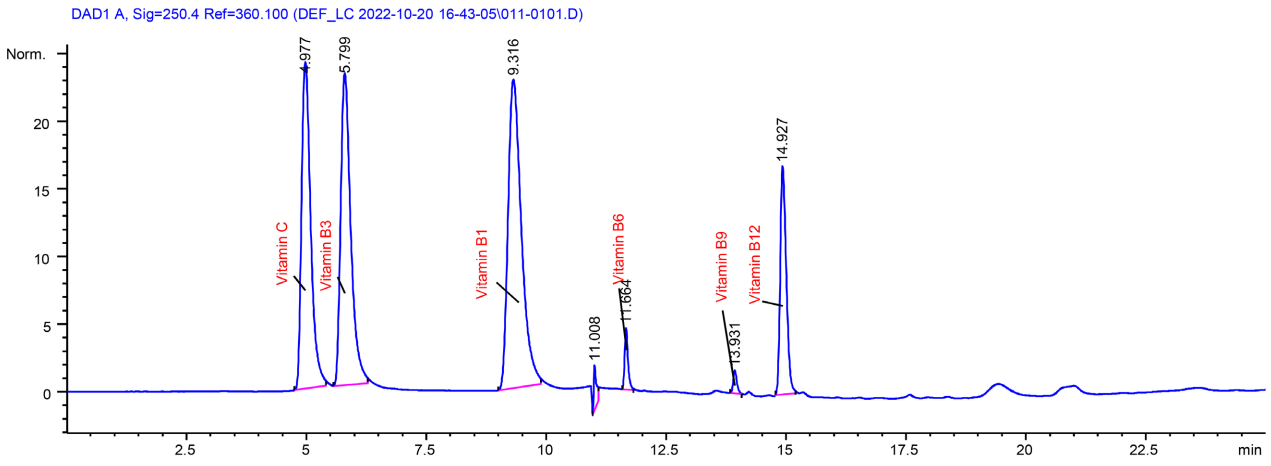

Figure 2. Standard chromatogram of vitamin content *Suaeda arcuate.*

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.

It was shown that, at the juvenile stage, a large amount of vitamins of group B is formed in intact *Suaeda arcuate* plants. It was revealed that the studied cytokinins had a different effect on the morphogenesis of *Suaeda arcuate in vitro*. The tested cytokinins and auxins according to their activity can be designated as follows: 1/2 MC + 1 mg/l 6-BAP + 0.3 mg/l IAA + 2,4-D, cultivated regenerates on this environment created the possibility of obtaining the maximum amount of biomass and accumulation vitamins of group B. It is shown that the composition of the nutrient environment without hormones has a positive effect on obtaining healthy regenerated plants.

In the biotechnological material of this plant, a large amount of mainly pyridoxine and folic acid is formed—almost twice as high as in intact plants. Such a correlation of the amount of vitamins is associated with biochemical biosynthesis in plant tissues. Thus, *Suaeda arcuata* plants obtained as a result of microclonal propagation on the medium 1/2 MS + 1 mg/l 6-BAP + 0.3 mg/l IAA + 2,4-D can be used as a source for obtaining vitamins B6 and B9.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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