New aspects of in vitro propagation of German SBT varieties

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Introduction

In the last decades, separate attempts have been made to study the possibilities of clonal reproduction in vitro of sea buckthorn varieties of various geographical origins. Biotechnological methods are used for accelerated reproduction and preservation of valuable agricultural crops.

However, the clonal micropropagation of sea buckthorn (*Hipopphae rhamnoides L*.) is quite difficult due to a number of factors in the form of polyphenol isolation, hidden pathogens in explants, vitrification, and difficulties in rooting.

Introduction

The present investigation describes the successful in vitro propagation of *Hippophae rhamnoides* seabuckthorn through nodal segments.

In this regard, the aim of our research was to optimize the cultivation conditions of *H. rhamnoides L. ssp. fluviatilis v. Soest in vitro* and adaptation of micro-plants to ex vitro conditions.

The research was carried out on the basis of the UBF GmbH laboratory on plant varieties ***Hergo*** and ***Leikora***.

Materials and Methods

- For cultivation, media were used according to the prescriptions of Murasige and Skoog (MS), Gamborg (B5), McCowns Woody Plant (WPM) with the addition of mesoinosite, sucrose, agar and growth regulators, polyvinylpyrrolidone (PVP) 100 mg/l, a mixture of ascorbic and citric acids in a ratio of 1:1.5 – 25 mg/l.
- Cultivation was carried out in the mode of a 16-hour day with an illumination of 2.5-3.5 kcd and a temperature of 22 ± 2 ° C.
- Sea sand was used to root the propagated shoots with subsequent adaptation to soil conditions. Planting in the open ground was carried out in the third year after the adaptation of plants to ex vitro conditions.

Sterilization methods

Rinse the explants with sterile distilled water under laminar flow conditions. Methods:

I. Immerse into Meliseptol (*Etanol 50% Butanon 1%*) for 1 minute, followed by three washings with sterile distilled water.

II. Immerse for 2 minutes into 5% sodium hypochlorite solution with two drops of Tween 20. Then rinse with sterile distilled water 3 times and pour in 0.1% Sulema (*Mercury dichloride*) for 3 minutes. Rinse three times with sterile distilled water.

III. Immerse into Meliseptol for 1 minute, followed by three washings with sterile distilled water. Pour the explants in a 0.1% Sulema for 3 minutes and rinse three times with sterile distilled water.

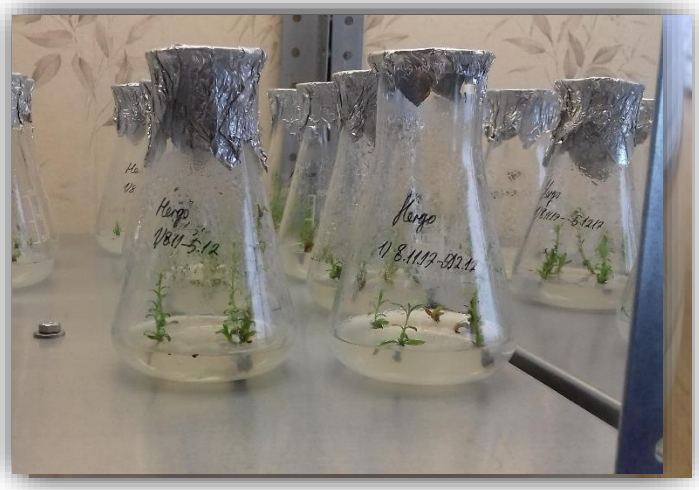
Sterilization methods

At the stage of sterilization of plant tissues, it is necessary to obtain a sterile culture. An important role is played by the quality and technique of sterilization of plant material.

Explants	Sterilization method		
	I	II	III
Hergo	95	100	97
Leikora	100	100	97

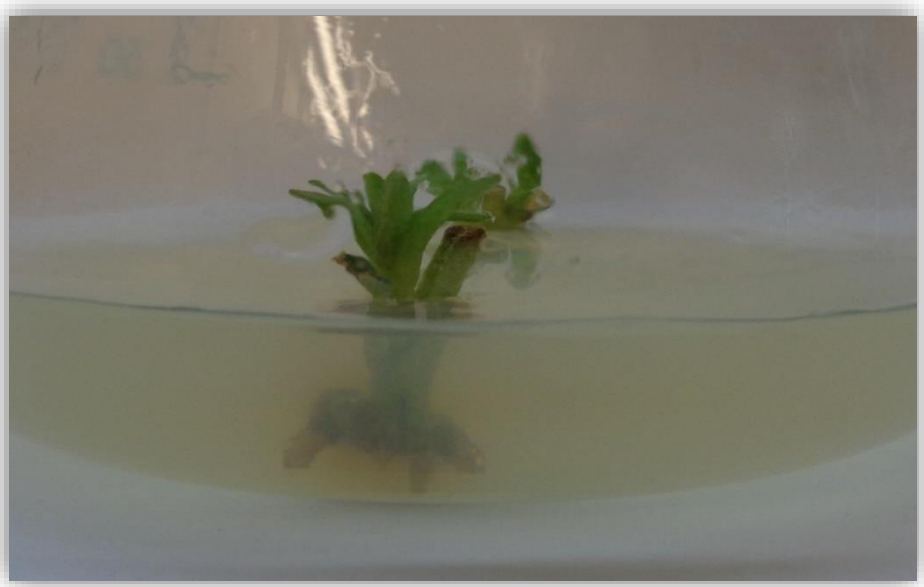
Results and discussion

As a result of the study, it was noted that the introduction of young shoots of container plants from indoor conditions contributed to high sterility and viability, regardless of the variety and methods of sterilization.



Formation of adventitious shoots at the base of the microcutting, variety Leikora, medium B5, day 35 of the passage

Results and discussion

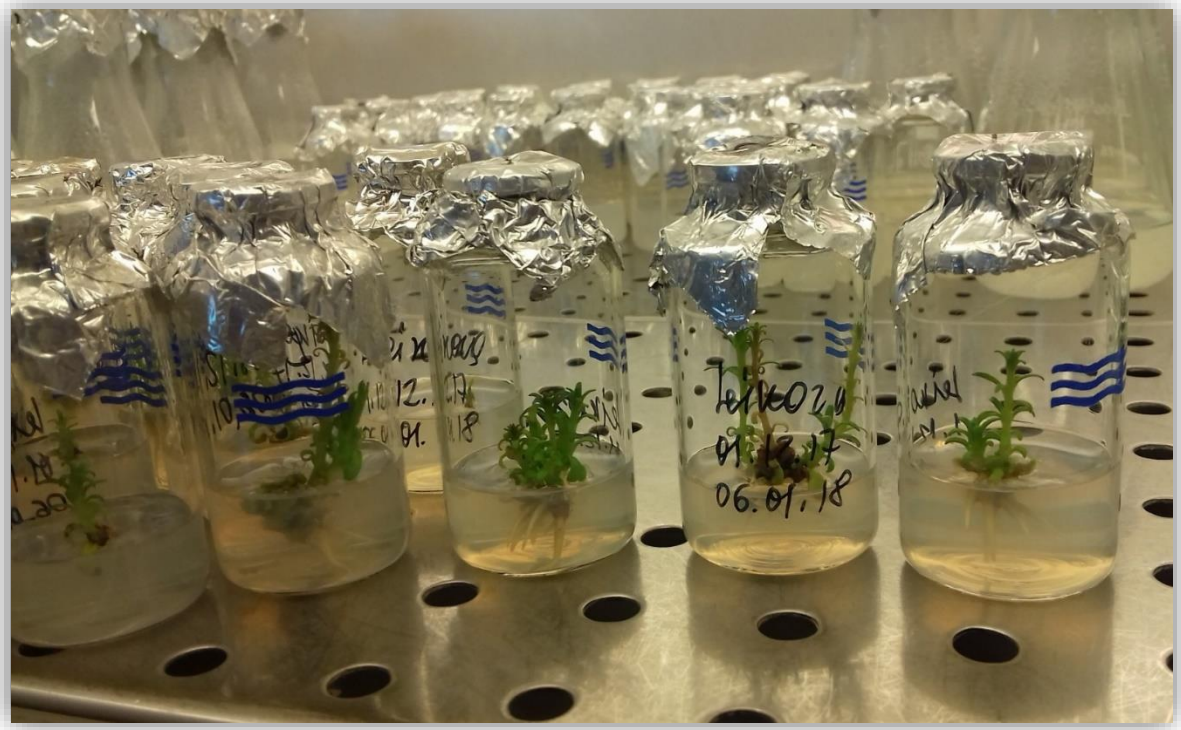


Primary regeneration of shoots from axillary meristems and rhizogenesis on:
B5 nutrient medium + BAP 0.5 µM + Kn 0.5 µM + NAA 0.1 µM
29th day of passage

Results and discussion

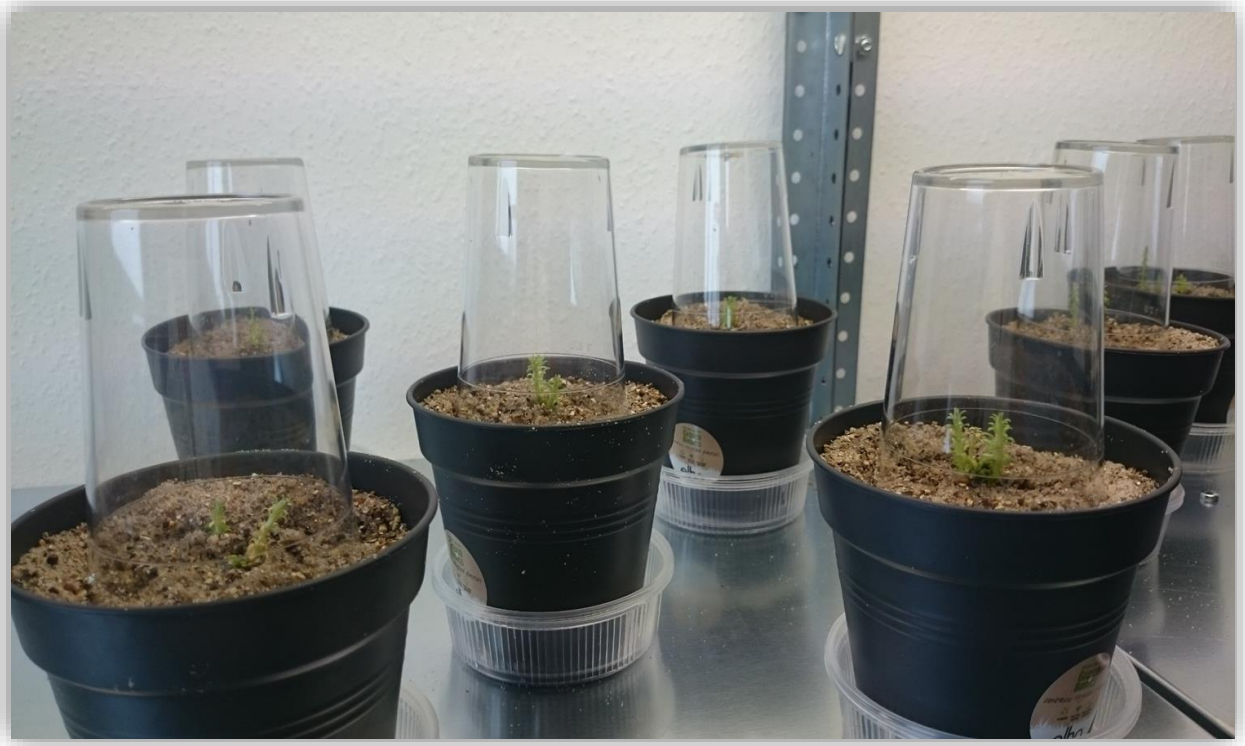
Spontaneous rhizogenesis of sea buckthorn microplants at the reproduction stage

Results and discussion



Seabuckthorn microplants at the **stage of reproduction**

Results and discussion



Adaptation of sea buckthorn microplants to **ex vitro** conditions

Conclusions

- There was no growth of explant and development processes on MS nutrient media.
- On media with a mineral composition according to the WPM recipe, the death of the apex of shoots was observed from the 10th to the 30th day of cultivation.
- Explants planted on **medium B5 were superior** in growth and development of other micro-plants, apical and axillary shoots were correctly formed, without signs of vitrification.

Conclusions

- **The optimal combination** of benzyladenin (BA) 0.5-2.5 μM, kinetine 0.5 μM and 1-naphthyl acetic acid (NAA) 0.1 μM at the breeding stage allowed to obtain up to 6 micro-gears from one cultivar.
- It was found that at the stage of micro-propagation, sea buckthorn shoots actively form the root system. This feature makes it possible to exclude the rooting stage from the technology of clonal micropropagation.

Conclusions

- At the stage of transferring microplants into the ground, significant losses of up to 50% were observed, however, seedlings adapted to greenhouse conditions, well tolerated planting in the field and have already wintered 2 winters.
- The resulting seedlings were well transferred to the open ground, where they successfully grow for 2 years.
- With this micro-propagation method a way to healthy, virus-free plant material was found.
- Productivity compared to green cuttings or wood cuttings is actually less.

Sea buckthorn plants planted in open ground

Summer 2022

Spring 2023

THANK YOU FOR YOUR ATTENTION