„IN VITRO" MULTIPLICATION AND ACCLIMATISATION IN *Aztekium ritteri* (FAM. CACTACEAE)

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1. Introduction

Aztekium ritteri is a *Cactaceae* species discovered by Friedrich Ritter in the year 1928. Growth rhythm of the plant is very slow. Lodi reported that a seedling of one year old has 1 mm in diameter. The acclimatisation in European conditions is difficult and in most of the collections only if it is grafted on *Peireskiopsis* or *Echinopsis*.

„In vitro" culture at *Aztekium ritteri* was promoted by Sajeva, 1982 when on a MS basal medium supplied with 1 x 10^{-4} M/1 IAA, he obtained a compact callus and buds on it.

Our purpose was to obtain rooted plants with a higher growth rhythm, a higher rate of multiplication than in classical culture, and the acclimatisation of the „in vitro" obtained plants.

2. Materials and method

Biological material was represented by shoots of 5 mm in diameter, harvested from a mother plant of 1o years old, cultivated grafted on Echinopsis sp., or callus and „in vitro" neoformed plants.

Thirteen culture media variants, based on MS basal medium with different hormonal balance and also supplied with biocompatible magnetic fluids were used (Table 1). In this experiment biocompatible magnetic fluids on oil basis of LMO type were used.

For the assepsization of the biological material (shoots of 5 mm diameter) the following protocol was used: strong was in water with tween 20; immersion in ethanol 96.5% (30 sec.); immersion in saturated calcium hypochlorite solution (7%) with agitation (30 min.).

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Table 1.

Review on the experiments with different culture media in *Aztekium ritteri*

Explant type	Culture medium	Phenotypical development
Rib fragments from a „in vivo" plant	MS 0.8 + 0.5 mg/l IAA + 2.5 mg/l KIN	Callus after 40 days and shoots after 90 days
	MS 0.8 + 1.0 mg/l KIN	Friable callus after 30 days
	MS + 0,5 mg/l IAA + 1.0 mg/l BAP	Shoots (4-5 shoots/cm^2)
Rib fragments („in vitro" neoformed plants)	MS + 1.0 mg/l KIN + 20 g/l charcoal	Friable callus with high growth rhythm
Neoformed „in vitro" plants (2 mm ø)	MS + 1.0 mg/l KIN + 45 g/l charcoal	Development of the plants with species characteristic aspects
	MS 0.5 + 1.8 mg/l IAA + 0.011 mg/l KIN (K - medium)	Development of the plants
	MS 0.5 + 1.8 mg/l IAA + 0.011 mg/l KIN + 30 mg/l LMO (M - medium)	Development of the plants and rooting process (83%) after 100 days from inoculation
	Nitsch (macroelements) + MS (microelements) + 1.8 mg/l IAA + 0.011 mg/l KIN + 60 mg/l LMO	Development of the plants and rooting process (100%) at 60 days after inoculation
Neoformed „in vitro" plants (5 mm ø)	MS + 1.0 mg/l KIN	Friable callus after 60 days (dedifferentiation process beginning from inside the plant)
	MS without hormones	Compact callus at the basis of the explant, on the tip shoots
	MS + 1.0 mg/l KIN + 45 g/l charcoal	Compact callus
	MS 0.5 + 1.8 mg/l IAA + 0.011 mg/l KIN + 1380 mg/l LMO (N - medium)	High growth rhythm of the plants: 60% rooting at 100 days after inoculation
	MS 0.5 + 1.8 mg/l IAA + 0.011 mg/l KIN	Development of the plants; callus around the basis of the plant
Callus	MS 0.5 + 1.8 mg/l IAA + 0.011 mg/l KIN supplemented or not with LMO	Roots at the surface of the callus
	MS + 0.5 mg/l IAA + 1.0 mg/l BAP	Compact callus, dark green with many organogenesis centers

On the eleven culture media variants, rib fragments of 4/3 mm size were inoculated in erlenmayer vessels of 100 cu cm. The inoculated explants were maintained in a growth room at a temperature of 24° ± 2°, with a photoperiod of 16 h/day light intensity of 2400 lx, for 100 days. The organogenesis processes

(the diameter and height of the plants, as well as the rhysogenesis process) and stadial development of the plants were recorded.

The „in vitro" obtained plants were acclimatised using a classical method or through their grafting on *Hyllocereus* sp.

3. Results and discussions

Phenotypical development of the explants

The results that were obtained testing different culture media are summarized in Table 1.

Growth rhythm of the „in vitro" neoformed plants

Growth rhythm of the „in vitro" neoformed plantlets was improved by the supplementation of culture medium (MS + 1.8 mg/l IAA + 0.011 mg/l KIN, respectively K - medium) with 1380 mg/l LMO (N - medium). The differences, in comparison with the results obtained on control medium (K - medium), become very significant after 70 days from inoculation. At 100 days after inoculation, the plantlets size that initially had 3.0 mm in diameter and 2.0 mm in height, was of 6.3 ± 0.5 mm in diameter and 5.1 ± 0.2 mm in height, in comparison with the dimensions registered on K - control medium (4.5 ± 0.2 mm in diameter and 3.5 ± 0.3 mm in height; Fig. 1).

Rhysogenesis process

Rhysogenesis process was very difficult to induce. Until arriving at a proper medium formula, we have tested a lot of hormonal combinations without positive results. The supplementation of the culture medium with biocompatible magnetic liquids of LMO type, gave the first satisfactions.

At 55 days after plants on MS basal medium with 1.8 mg/l IAA and 0.011 mg/l KIN, there were obtained 33% rooting plants on M - medium (with 30 mg/l LMO), 34.5% rooted plants on N - medium (with 1380 mg/l LMO) and no rooted plants on control medium (K - medium, without magnetic liquid of LMO type; Fig. 2).

At 100 days after transfer, rooting percent was 83% on M - medium and 100% on N - medium. On the other hand, the number of roots per plant was higher on medium with lower concentration in LMO, respectively 7.5 ± 0.5 roots per plant on M - medium, in comparison with 5.8 ± 0.3 roots per plant on N - medium (with 1380 mg/l LMO).

Acclimatisation

For the acclimatisation in the classical methods, plants with a well developed radicular system were used. The acclimatisation was made in greenhouse, on a characteristic mixture for Cactaceae. After transfer in greenhouse, there is a few weeks period of lower growth rhythm.

For the acclimatisation with success of the „in vitro" neoformed plants, there is needed the graft of the *Aztekium ritteri* plants, on other

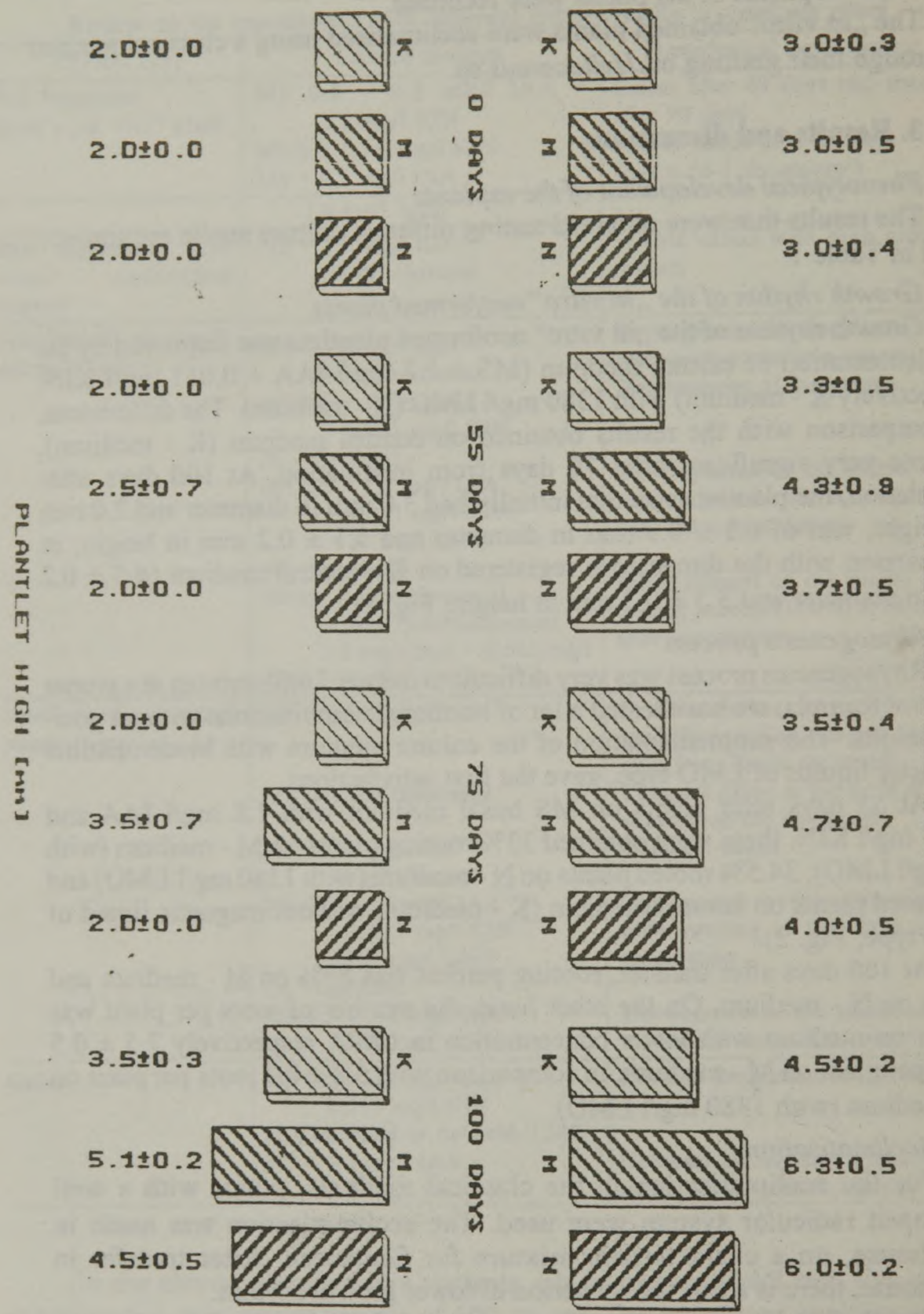

Fig. 1. Aztekium ritteri: the development of „in vitro" neoformed plantlets on media with magnetic liquid.

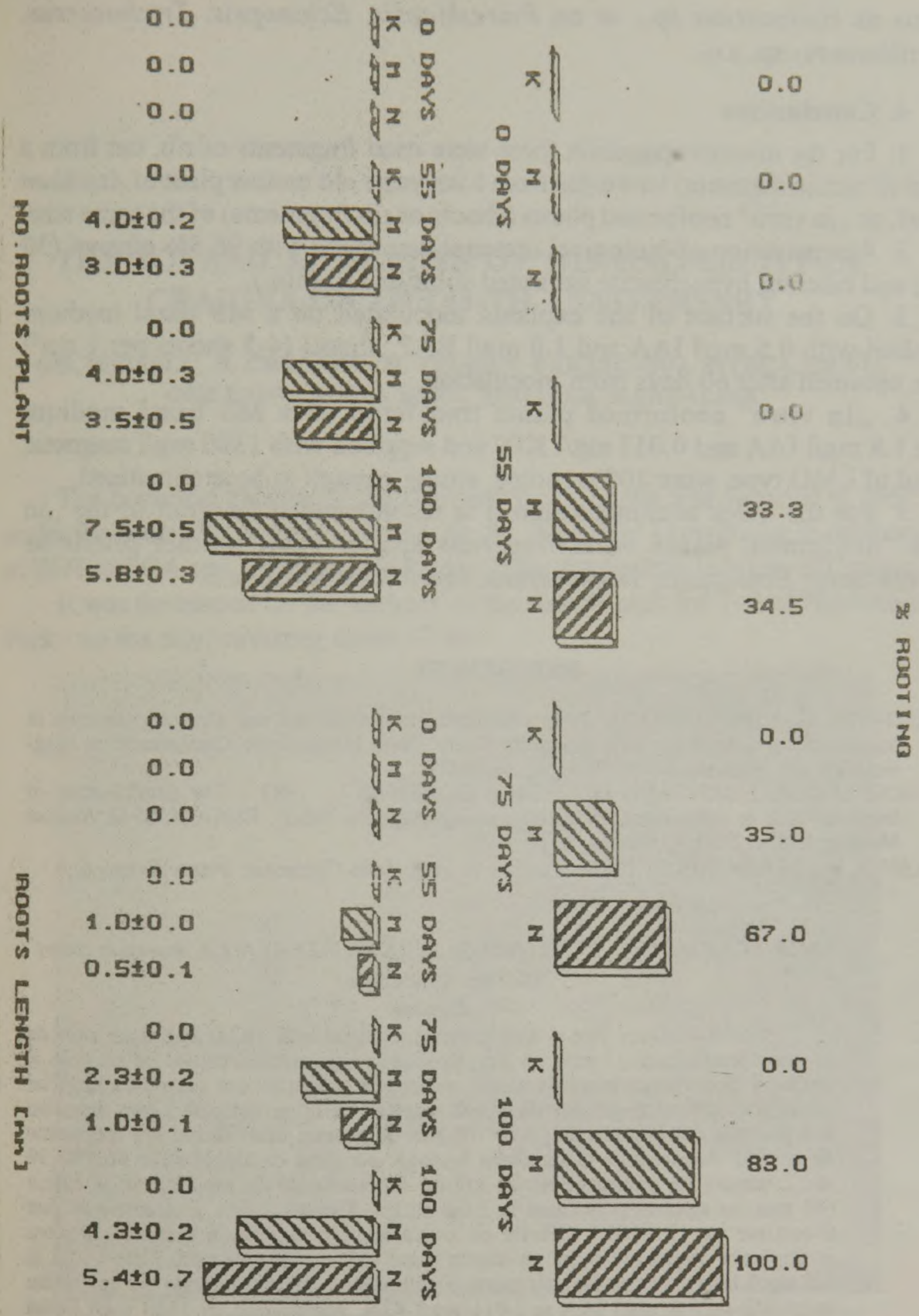

Fig. 2. Aztekium ritteri: rooting process of „in vitro" neoformed plantlets on media with magnetic liquid.

plants as *Hyllocereus* sp., or on *Peireskiopsis, Echinopsis, Trichocereus, Myrtillocactus* sp. a.o.

4. Conclusions

1. For the micropropagation there were used fragments of rib, cut from a shoot (5 mm in diameter) harvested from a ten years old mother plant of *Aztekium ritteri*, or „in vitro" neoformed plants (shoots or rib fragments) of the same size.

2. Assepsization of biological material was made with 96.5% ethanol (30 sec.) and calcium hypochlorite saturated solution (30 min.).

3. On the surface of the explants inoculated on a MS basal medium supplied with 0.5 mg/l IAA and 1.0 mg/l BAP, shoots (4-5 shoots per 1 cm^2) were obtained after 60 days from inoculation.

4. „In vitro" neoformed plants transfered on a MS basal medium with 1.8 mg/l IAA and 0.011 mg/l KIN and supplied with 1380 mg/l magnetic liquid of LMO type, were 100% rooted, strong enough to be acclimatised.

5. For the quick acclimatisation it is recommended the graft of the „in vitro" neoformed plants on *Hyllocereus* sp., or other mother plants as *Peireskiopsis, Echinopsis, Trichocereus, Myrtillocactus* sp. a.o.

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MULTIPLICAREA „IN VITRO" ȘI ACLIMATIZAREA LA *Aztekium ritteri* (Fam. *Cactaceae*)

Rezumat

Aztekium ritteri este o specie descoperită în anul 1928. având un ritm de creștere foarte încet (1 mm pe an). În scopul accelerării ritmului de creștere și obținerii unui număr mare de plante, s-a recurs la cultura „in vitro". Ca material inițial s-au utilizat fragmente de coastă recoltate de la un lăstar de 5 mm diametru sau plantule neoformate „in vitro" (lăstari de aceeași dimensiune sau fragmente de coastă). Asepsizarea materialului biologic s-a făcut cu alcool etilic 96.5% (30 sec.), urmată de imersarea lor în soluție suprasaturată de hipochlorit de calciu (30 min. cu agitare) și spălarea în 3 băi de apă distilată sterilă. Explantele au fost inoculate pe 13 medii diferite de cultură, cele mai bune rezultate pentru multiplicare obținându-se pe un mediu bazal MS suplimentat cu 0,5 mg/l IAA și 1,0 mg/l BAP. Pentru înrădăcinare, plantulele au fost transferate pe un mediu bazal MS cu 1,8 mg/l IAA și 0,011 mg/l KIN, suplimentat cu 1380 mg/l lichid magnetic de tip LMO. Pentru aclimatizare rapidă este recomandată altoirea pe un port-altoi reprezentat de *Hyllocereus* sp., sau o altă specie clasică (*Peireskiopsis, Echinopsis, Trichocereus, Myrtillocactus*).