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EXPERIMENTAL RESULTS REGARDING THE ROOTING AND ACTIMALIZATION CAPACITY OF ALBIZZIA SHOOTS REGENERATED IN VITRO

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Abstract: *The paper proposes a comparative study of two species of the genus Albizzia in terms of the in vitro rooting of the multiplied shoots, but also the behavior of these two species of the genus Albizzia in the acclimatization technique, as a mandatory step for the passage of regenerated seedlings in vitro to life in vivo.*

Two species of this genus were used as biological material, Albizzia julibrissin var. Durazz and Albizzia lebbeck. The culture medium used for rooting was Murashige-skoog (MS) with different concentrations of IBA and the same concentration of GA3. The data recorded in this study referred to the average number of roots/explant but also the average total length of roots/explant.

After the rooting of the shoots obtained in vitro, we moved on to the stage of their acclimatization. Acclimatization is the final but necessary step found in all types of plant micropropagation. During this process, plants must adapt to new environmental conditions such as: lower relative humidity, higher light intensity, temperature fluctuations and stress caused by various diseases (Preece and Sutter, 1991). Two substrates were used for the acclimatization of Albizzia julibrissin and Albizzia lebbeck plants. The first substrate consists of peat, sea buckthorn and perlite in a ratio of 2:1:1 and the second substrate consists of peat pellets (Jiffy).

• Introduction

The culture of plant cells and tissues, an integral part of plant biotechnologies, has allowed the understanding and modulation of the basic mechanisms of the cytodifferentiation and morphogenesis processes in vitro, so that this technique can be adapted to many practices, which can open new perspectives for the exploitation of plants. The knowledge accumulated so far regarding plant regeneration, morphogenesis and cellular development is based on major discoveries, such as totipotency [9] and the ability to regenerate plant cells and tissues [1; 3; 9; 14; 15]. As a result, the association of an appropriate technology of in vitro propagation can become a useful tool, especially for multiplication and respectively for conservative improvement, which can contribute to improving the current situation of many ornamental plants, namely albizia, used especially for landscaping yards, gardens and parks, which became known in our country very recently, but also highly appreciated due to its special decorative aspect [16]. The history of ornamental trees and shrubs is a long one that exceeds 6000 years. In Europe, the taste for ornamental trees and shrubs grows with geographical discoveries. The discoverers and explorers of the world bring with them, in addition to riches, many saplings of ornamental trees and shrubs or seeds, as special as they are interesting [11]. The albizzia or silk tree is also included in the group of these ornamental trees. The scientific name of this ornamental tree is Albizzia. It originates from Southeast Asia and Australia, but due to its special aesthetic aspect, it has been cultivated in all countries with a warm climate in the world, thus becoming a pantropical plant.

• Material and method

In vitro shoots rooting

To carry out the research in this paper, the biological material consists of two species of the genus Albizzia, respectively, *Albizzia julibrissin* var. *Durazz* and *Albizzia lebbeck*. Explant donor plants, from which explants were taken for the initiation of in vitro cultures, together with minishoots obtained after a number of successive subcultures on propagation media, served as biological material for the studies performed in this experiment. For the realization of this scientific paper, the culture of meristems was used in order to obtain plants identical to the mother plants donating explants, since this method is one that does not induce genetic variability except in very rare cases [5]. These two species of Albizzia were selected for the simple fact that they have the largest global distribution, which means that they have been able to acclimatize to very varied climatic conditions. After 1-3 subcultures on the multiplication medium, the differentiated microshoots with a length of 3-4 cm were detached from the culture, individualized and subcultured on a rooting medium represented by a variant of the MS basal medium with the mineral salts reduced by half, the vitamins of the medium LS, indolyl butyric acid (IBA) in different concentrations (0.2; 0.4; 0.6; 0.8 mg/l), 0.1 mg/l gibberellic acid, 30 g/l dextrose as a source of carbon and 7 g/l agar as gelling agent.

In vitro regenerants acclimatization

Regardless of the in vitro culture method adopted, success in vegetative propagation depends on the ability to transfer plants ex vitro, at an economically acceptable level. This involves the acclimatization or transfer of the plants to conditions of significantly lower atmospheric humidity compared to that inside the bottle, as well as to an increased light intensity. Due to the nutrient substrate rich in carbohydrates, the light conditions and the lack of gas exchange, plants grown in vitro develop a very low capacity for photosynthesis. A reduced activity of photosynthesis is associated with a reduced activity of respiration. During plant acclimation, rapid transition from heterotrophism to autotrophism is required. This transition does not take place instantly, different periods of time being required from one species to another. In most dicotyledons, this transition from one nutritional system to another is achieved following the development of new leaves with photosynthetic competence. The development and growth of these new leaves is carried out on the reserve of carbohydrates provided by the leaves formed in vitro. Taking into account that the transition from one life regime to another is supported with difficulty by the plants, some measures were taken to reduce as much as possible the losses caused by the stress of acclimatization. The seedlings rooted in vitro were carefully removed from the culture vessels, the medium was carefully removed so as not to break the roots and eliminate possible sources for phytopathogenic agents, they were shaped (the roots were shortened and the leaves from the base were removed) and planted in pots. They were placed on "beds" covered with polyethylene film and gauze, to maintain high humidity for 7-10 days. After this period, when the plants began to grow, the space necessary for breathing gradually increased (cultures were progressively discovered), until the plants adapted to the natural living conditions. During the acclimatization period, crop care works were carried out (weeding, phytosanitary protection by spraying with fungicides and insecticides, mobilizing the soil around the plants).

• Results and discussions

Influence of exogenous auxinic concentration on in vitro rooting capacity of Albizzia

Minishoots of the *Albizzia julibrissin* species inoculated on medium variants with a variable auxin content (0-8 mg/l IBA), recorded maximum values of the rooting rate in the interval 26-30 days from initiation, in the case of a concentration of 2 mg/l IBA (Fig. 1).

The observations made during the first 14 days of incubation did not highlight the initiation of the rhizogenesis process. The appearance of the first root primordia was recorded after 18 days of culture, regardless of the medium variant used. Without auxin, the lowest number of minishoots with roots was identified on the control variant, respectively in a proportion of 2 - 21%, at each incubation interval, until the end of the 30 days of the experiment. The use of 2 mg/l IBA led to the best rooting rates (85%) after 30 days of in vitro culture.

To the species *Albizzia lebbeck*, in general, the concentration of IBA (0-8 mg/l) had a similar influence on the percentage of rooted mini-cuttings. Its value was lower compared to that obtained for the *Albizzia julibrissin* species. To the *Albizzia lebbeck* species, the minishoots inoculated on the rooting medium, started the rhizogenesis process after 18 days. On the control variant (without IBA), the lowest number of minishoots with roots was identified, respectively in the proportion of 10-15%, at each incubation interval, until the end of the 30 days of the experiment. The highest values of the rooting rate (79%) were recorded after 30 days of culture at a concentration of 2 mg/l IBA (fig.2).

The qualitative evaluation of the rooted biological material, at the end of the experiment, was assessed according to the two coefficients (C1 and C2), calculated per variant. Thus, maximum values of the C1 coefficient (5.7 roots/explant in the case of the species *Albizzia julibrissin* and 5.1 roots/explant in the case of the species *Albizzia lebbeck*) were recorded on the variant containing 4 mg/l IBA (fig.4). Maximum values of the C2 coefficient were also recorded on the version containing 4 mg/l IBA (fig.5). On the control variant (without IBA), both the average number of roots/explant (C1) and the average total length of roots/explant (C2) were at a low level, for both species studied, indicating that IBA has a qualitatively stimulating effect on rhizogenesis. The overall analysis of the results obtained in the two species highlights a similar reactivity of the minishoots, in the case of supplementing the culture media with IBA.

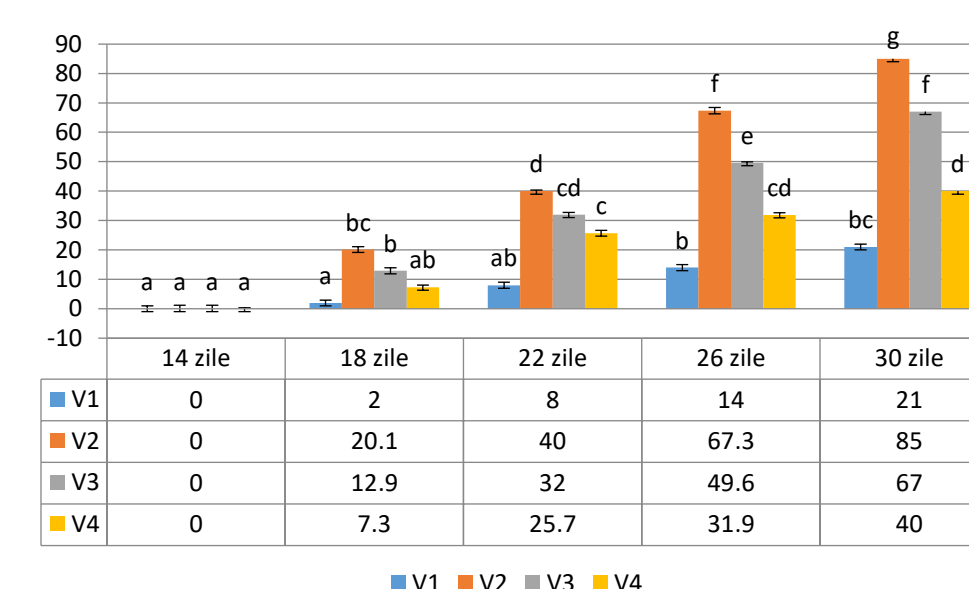


Fig. 1. In vitro rooting ability Albizzia julibrissin according to IBA intake

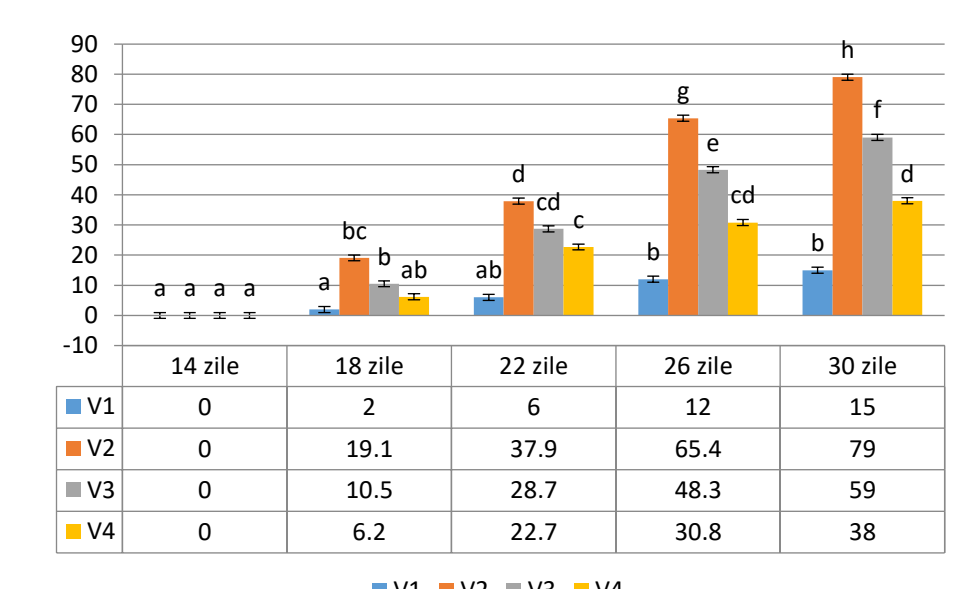


Fig. 2. In vitro rooting ability Albizzia lebbeck according to IBA intake

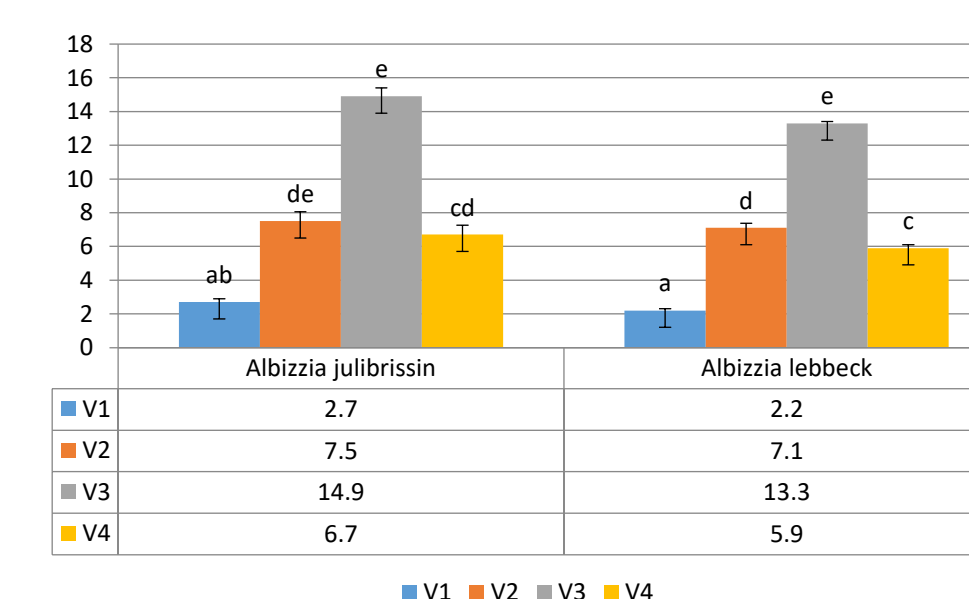


Fig. 3. Variation of the average number of roots / explant (C1), depending on intake IBA after 30 days of starting the experiment

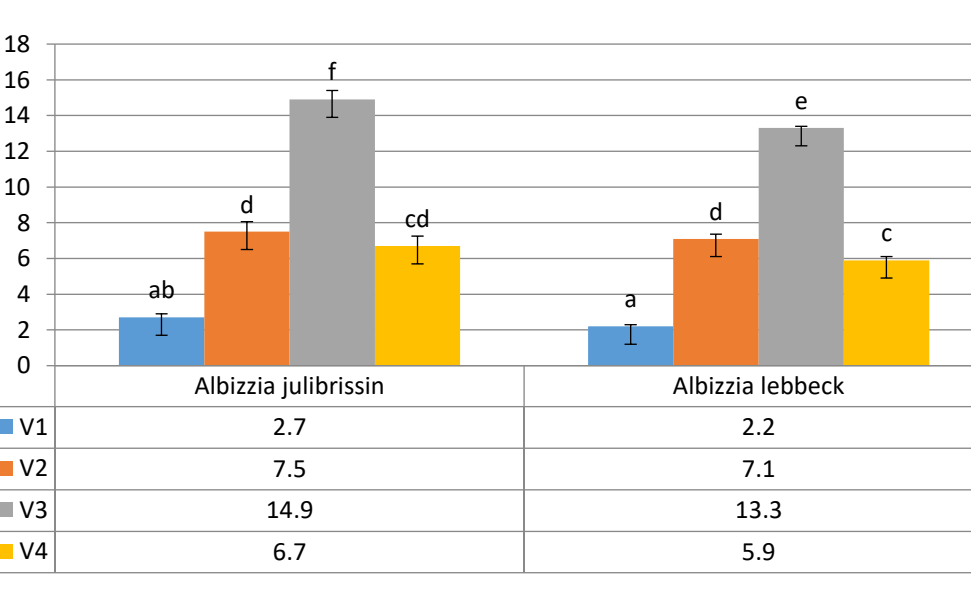


Fig. 4. Influence of IBA concentration on average total length of roots / explant (C2) after 30 days of culture

Regenerants acclimatization

Acclimatization is the final but necessary step found in all types of plant micropropagation. During this process, plants must adapt to new environmental conditions such as: lower relative humidity, higher light intensity, temperature fluctuations and stress caused by various diseases [13]. The intrinsic quality of the plant obtained in vitro is one of the most important factors that depends on its successful survival during the transition process from in vitro to ex vitro conditions. The observations made on the acclimatization of the plants on a substrate consisting of peat pellets (Jiffy)(V.2), led to the conclusion that, in the case of both studied species, the best results were obtained in the case of using peat pellets (81% acclimatization to *Albizzia julibrissin* and 78% to *Albizzia lebbeck*).

Conclusions

The in vitro rooting yield of the studied species was influenced primarily by the genotype and secondly by the culture environment. Only 18 days were needed for the aseptic rooting of Albizzia mini-cuttings. The species *Albizzia julibrissin* had the best behavior. In the case of both species studied, the highest percentage of rooting (85% *Albizzia julibrissin*, respectively 79% *Albizzia lebbeck*) was recorded in the case of adding the medium with IBA in a concentration of 2 mg/l.

The quality of the biological material obtained by in vitro rooting of the minishoots was evaluated by the number and length of the roots. The addition of IBA in different concentrations to the MS1/2 medium caused a proportional increase in the number of roots/explant and the total length of roots/explant. The highest values (9.19 roots/explant and total root lengths of 5.7 cm in *Albizzia julibrissin*; 10.29 roots/explant and 4.3 cm in *Albizzia lebbeck*) were obtained when the culture medium was supplemented with 4 mg/l IBA.

The yield of the acclimatization of the studied species was influenced, along with the genotype, by the nutritional support used. The acclimatization of in vitro regenerated plants was successfully achieved when they had a well-developed root system, capable of further supporting the process of plant growth and development.

The optimal acclimatization conditions proved to be those based on the use of peat pellets as a nutrient substrate that ensured an 81% acclimatization percentage for *Albizzia julibrissin* and 78% for *Albizzia lebbeck*. We recommend peat tablets for the fortification of glass plants in pots.