Contribuţii Botanice, *XXXVIII, (1)*, **2003**
Grădina Botanică "Alexandru Borza"
Cluj-Napoca

INFLUENCE OF GROWTH REGULATORS ON THE *IN VITRO* REGENERATION AND MULTIPLICATION OF APRICOT

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Abstract: Induction of in vitro culture was succesfuly obtained because of high percentage of seed germination deppending on cultivar and composition of culture medium. In the case of „Mamaia" cultivar, the germination was 70% on MS ½ medium and 80% on MS medium. „CMBU" seeds germinated in 50-60% on MS ½ and MS media. In case of „Favorit" cv. the percentage of seed germination was lower than the percentage of seed germination of „CMBU" and „Mamaia" cvs., 40% on MS ½ medium and 60% on MS medium. The growth of plantlets obtained from seeds germination is better on MS medium, than MS1/2 medium. On this medium, plantlets of „CMBU" cv. were 1.6 cm length and plantlets of „Mamaia" cv. were 1.5 cm length. Plant multiplication on media supplemented with growth regulators and adenine sulphate has been obtained with good results especially in case of „Favorit" explants. Plant multiplication of „CMBU" cv. explants was low, the best results have been obtained on medium IV where 2 neoplantlets/explant have been obtained having in average 0.96 cm length. In case of „Favorit" cv. the multiplication was very good, 2.6 neoplantlets/explant have been induced having 1.32-1.4 cm length. Plant multiplication of „Mamaia" cv. was lower, 1.8 neoplantlets/explat have been obtained having 1.3-1.42 cm length. Root induction of „CMBU" plantlets is very low, in average 1.2-1.6 roots/explant have been obtained having 0.36-0.86 cm length. In case of „Favorit" plantlets, root induction is also very low, maximum 1.6 roots/explant have been induced but root growth is good on media III and IV (1.06-1.08 cm length). The maximum number of roots/explant of „Mamaia" plantlets (1.6) was obtained on medium IV, while the roots length on media studied was in average 0.62-1.02 cm.

Introduction

The major key-points of the apricot industry are as follows: (1) Breeding. There is a wealth of diversity in apricot germplasm, but cultivar improvement is slowed by the high degree of heterozygosity within the species. Most of the production in many countries still comes from chance seedlings and/or local cultivars. (2) Cultivar. Adaptation to the environment is one of the major problems. Combining traits such as chilling and heat requirements, blooming time and frost tolerance in a cultivar with good quality fruits is not easily achievable. (3) Fruit quality. It is by far the most pursued goal in improving scion cultivars. Quantitative traits such as size, firmness, aroma, flavor and sugar content could be improved through recurrent selection because large variability exists within the known germplasm. (4) Diseases. Plum pox virus (PPV) is probably the most serious one in Europe. PPV resistance can be found in several genotypes and is rather easily inherited from the progeny. Nevertheless genetic control of PPV susceptibility is not yet completely understood and tolerance can mask tree infection. Other diseases caused by fungi (e.g. brown rot by *Monilinia* spp.) and bacteria (e.g. cankers by *Pseudomonas* sp.) may result in serious damages depending on climatic conditions. Tree decline is still a problem where infection from fungi, bacteria and viruses may worsen the effect of grafting incompatibility. (5) Propagation. A fully compatible rootstock is yet to be found for apricot. Although several good rootstocks have been introduced so far, none of them is widely distributed, scion compatibility and soil adaptation being the major restrains. (6) Processing. Fruits are increasingly processed to make juice, canned and dried fruits, commodities which claim high prices in most markets [3].

There are several studies regarding in vitro propagation of some *Rosaceae* species as *Prunus avium x pseudocerasus* [15], *Prunus cerasus* [16]. Plant regeneration from apricot

protoplasts was also obtained [11]. The influence of phloroglucinol, L-arginine, L-proline have been studied on the in vitro multiplication and root induction of apple tree [17], pear tree, quince tree [6]. Recent researches show that sorbitol ensure the best conditions for plant multiplication of peach than sucrose [12], and fotoperiode and sucrose concentration could also influence plant multiplication of peach [13]. The carbon source has also influence on callus induction from leaf of *Prunus persica* [7].

In vitro culture of apricot through meristem tips culture has been obtained. Different growth regulators at different concentrations were tried to promote the development of the meristems to shoots that can be micropropagated. Optimum season to introduce in vitro the meristems was investigated in relation to bud dormancy. Different basal salt media were used during the proliferation stage as well as different growth regulators to optimize the proliferation rate [10]. There are also good results on plant multiplication of apricot by meristem culture on media supplemented with different concentrations of growth regulators and [18].

In Romania the in vitro plant multiplication of apricot has been realized to obtain many plants free of pathogens and root induction of plantlets [1,4,8,9,19]. Induction of resistance against *Monilinia laxa* of two cultivars of apricot "Băneasa" and "Dacia" has been obtained [2].

A study of the effect of growth regulators on the in vitro regeneration and multiplication of three cultivars of apricot (*Prunus armeniaca*) has been done in this paper. Plant material proceed from Research and Production Station Oradea. „Cea mai bună de Ungaria", „Favorit" şi „Mamaia" are considered valuable cultivars, with middle or good vigour, good productivity, low resistance against patogens to „Mamaia" and „Cea mai bună de Ungaria", and very good to „Favorit". The frost resistence is good to „Favorit" and „Mamaia" and lower (up to -20-24 °C), to „Cea mai bună de Ungaria". All of these three cultivars are autocompatible with abundant blooming. Fruits have 60-70 cm diameter to „Mamaia" and „Favorit" and a little smaller (67-68cm) to „Cea mai bună de Ungaria". Fruits are sligthly pubescent to „Mamaia" and „Cea mai bună de Ungaria", and unpubescent to „Favorit". Savory is good, good taste, sligthly acid to „Cea mai bună de Ungaria", strong flavor (Cociu, 1993).

Material and Methods

In vitro culture has been induced from seeds that have been sterilized with Domestos 15 min and then after rinsed several times with sterile water. 10 seeds from three cultivars: "Cea mai bună de Ungaria" (CMBU), "Favorit" and "Mamaia" have been inoculated on liquid MS [14] and MS ½ media with paper support to avoid the sinking of seeds on culture media and hypoxia. The medium pH was adjusted to 5.7 with NaOH before autoclaving (120°C for 20 min). Cultures were maintained permanently in a growth chamber at 25-27°C with a total irradiance of 100 $\mu mol/m^2/s$ provided by fluorescent tubes, under a 16 h daylight regime.

After 4-5 days seeds were germinated and the culture was evaluated four weeks after the inoculation, the percentage of germination, the number and the length of regenerated plantlets/explant being followed as well as the number and length of roots/explant. Subcultures were performed every six weeks, to ensure the plants material for other experiments. Regeneration efficiency was calculated considering the mean number of shoots or roots per explant. Shoots explants have been transferred on multiplication media supplemented with different growth regulators and other compounds as following:

I. MS + 1.0 mg/l BA + 0.1 mg/l IBA + 0.1 mg/l GA_3;

II. MS + 1.0 mg/l 2iP + 0.1 mg/l IBA + 0.1 mg/l GA_3;

III. MS + 1.0 mg/l 2iP + 0.1 mg/l AIB + 0.1 mg/l GA_3 + 10 mg/l vitamina C;

IV. MS + 1.0 mg/l 2iP + 0.1 mg/l AIB + 0.1 mg/l GA_3 + 10 mg/l vitamina C + 10 mg/l adenine sulphate.

Results and Discussions

The percentage of germination after one week of seed culture is shown in Fig.1. As it could be seen in the case of „Mamaia" cultivar, the germination was 70% on MS ½ medium and 80% on MS medium. „CMBU" seeds germinated in 50-60% on MS ½ and MS media. In case of „Favorit" cv. the percentage of seed germination was lower than the percentage of seed germination of „CMBU" and „Mamaia" cvs., 40% on MS ½ medium and 60% on MS medium. The higher concentration of minerals in MS medium stimulated the length of plantlets obtained from seed germination. The results regarding plantlets length on MS ½ and MS media are shown in Fig. 2. The best results on MS ½ medium have been obtained on „Mamaia" cv. plantlets that reached 1.3 cm length. On MS media, plantlets of „CMBU" cv. were 1.6 cm length and plantlets of „Mamaia" cv. were 1.5 cm length.

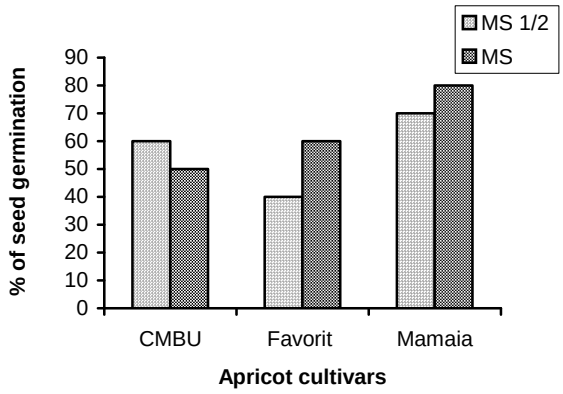

Fig. 1: In vitro seed germination of "CMBU", "Favorit" şi "Mamaia" cultivars.

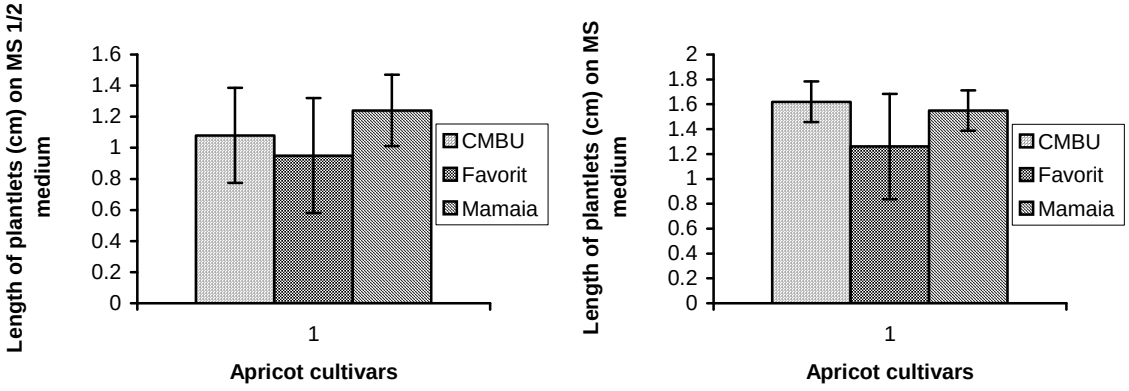

Fig. 2: Length of plantlets obtained by seed germination of "CMBU", "Favorit" and "Mamaia" cultivars (a-on MS medium, b-on MS1/2 medium).

After 4 weeks of culture, shoot explants were detached and transferred on special media for plant regeneration and multiplication. The results regarding plant multiplication and root induction are shown in Fig. 3, 4, 5 a-d. Plant multiplication of „CMBU" cv. explants was low, the best results have been obtained on medium IV where 2 neoplantlets/explant have been obtained (Fig. 3a) having in average 0.96 cm length. As it could be seen in Fig. 3b. the length of plantlets was similar an all media tested. Root induction is very low, in average 1.2-1.6 roots/explant have been obtained having 0.36-0.86 cm length (Fig. 3c-d) because of low concentration of auxin in culture media.

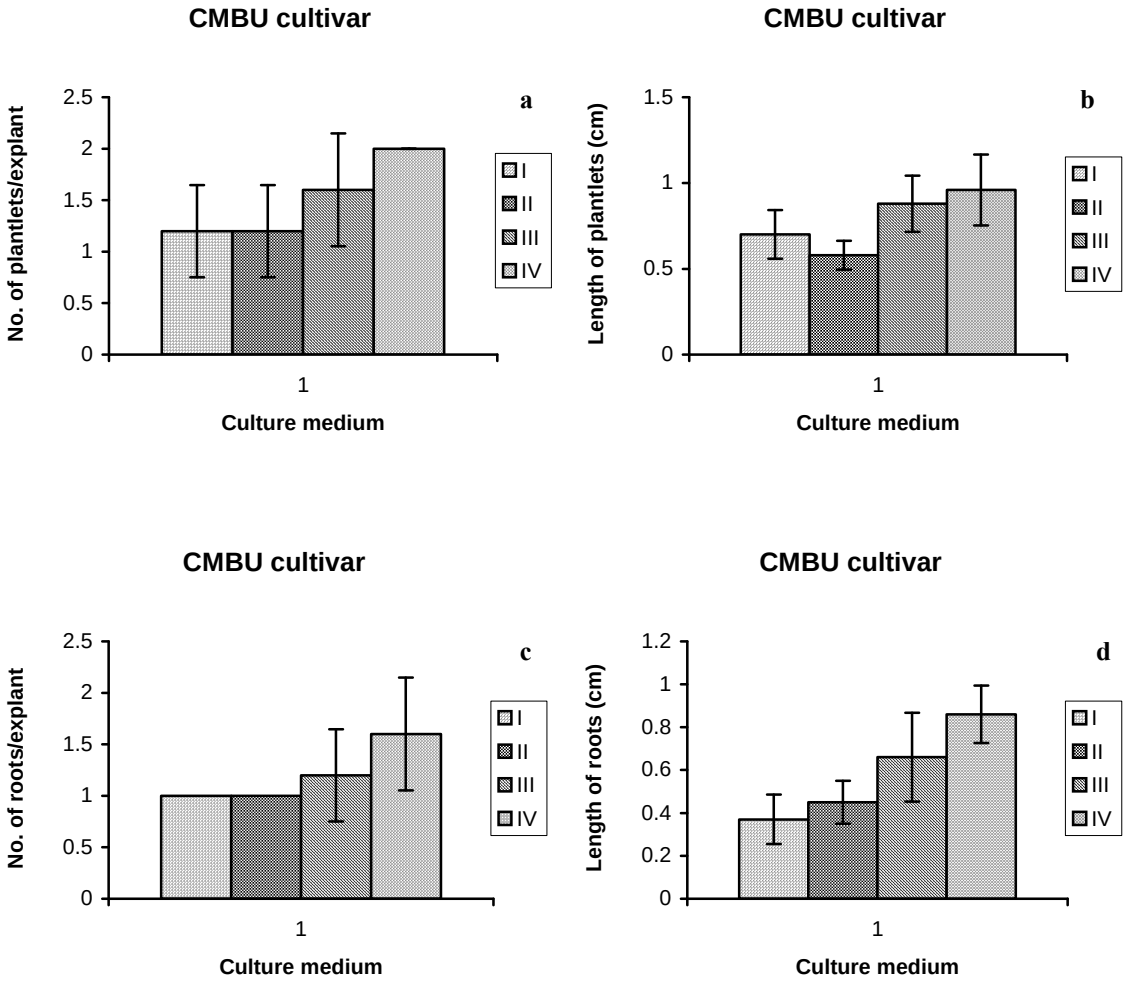

Fig. 3: Regeneration and multiplication of plantlets obtained by seed germination of "CMBU" cv. (a-number of plantlets/explant, b-length of plantlets, c-number of roots/explant, d-length of roots).

The best results regarding plant multiplication have been obtained on „Favorit" cv. explants as it could be seen in Fig. 4 a-d. The best multiplication was induced on MS medium supplemented with 1.0 mg/l 2iP, 0.1 mg/l AIB, 0.1 mg/l GA_3, 10 mg/l vitamina C and 10 mg/l adenine sulphate (medium IV) where 2.6 neoplantlets/explant have been induced. Plantlets growth is good on media III and IV where plantlets reached 1.32-1.4 cm length (Fig. 4b). On media I and II plantlets length is lower, not more than 0.84-0.96 cm. Root induction is also very

low, maximum 1.6 roots/explant have been induced (Fig. 4c) but root growt is good on media III and IV (1.06-1.08 cm length) (Fig. 4d).

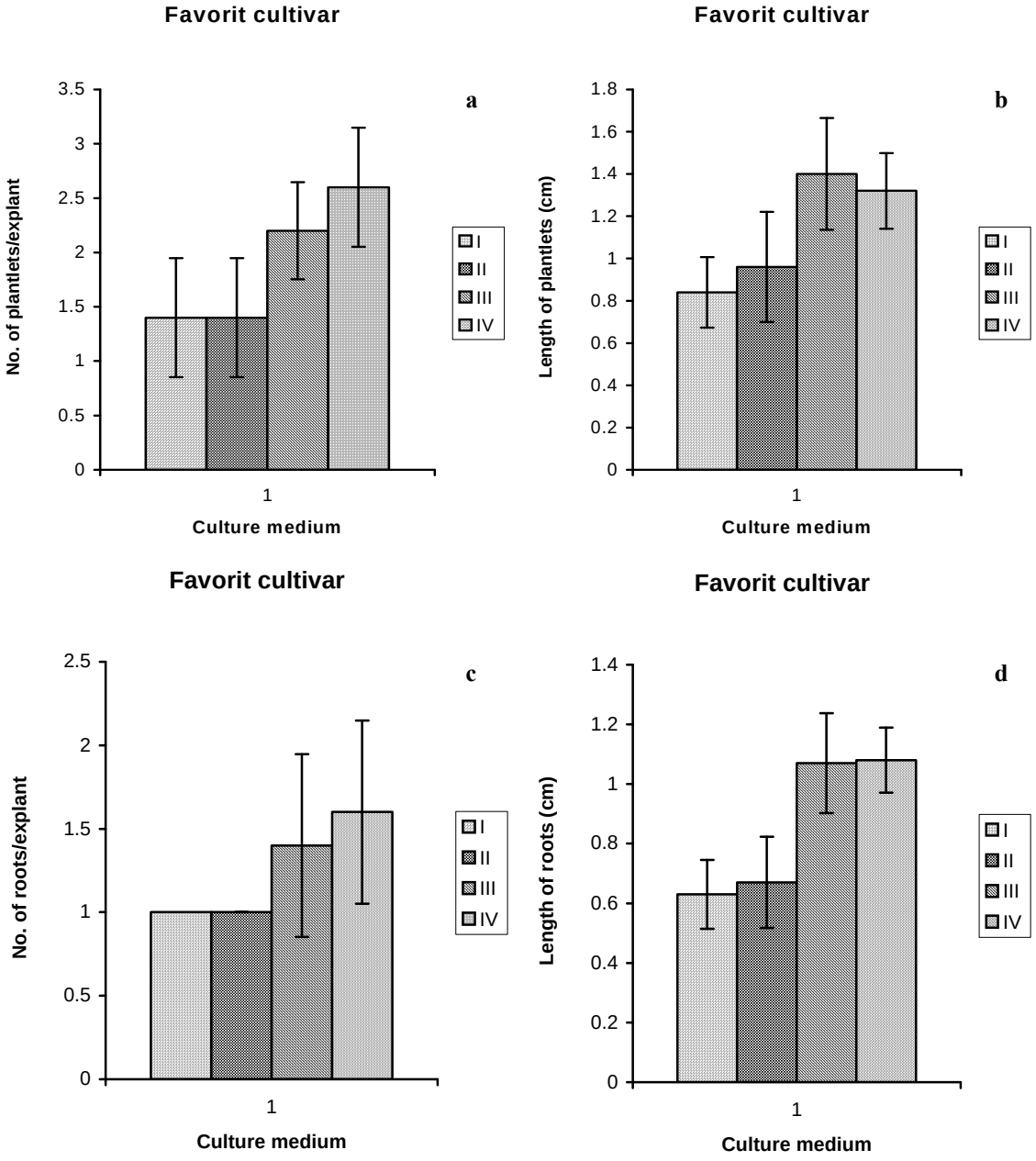

Fig. 4: Regeneration and multiplication of plantlets obtained by seed germination of "Favorit" cv. (a-number of plantlets/explant, b-length of plantlets, c-number of roots/explant, d-length of roots).

The results regarding plant multiplication and root induction of „Mamaia" cv explants are shown in Fig. 5 a-d. In case of plant multiplication the results are lower than plant multiplication of „Favorit" cv. plantlets and similar with plant multiplication of „CMBU" cv. plantlets. The best multiplication (1.8 neoplantlets/explat) was obtained on medium IV (Fig. 5a). The length of these plantlets is good on media III and IV where plantlets reached in average 1.3-1.42 cm length

(Fig. 5b). Root induction is also very low, the maximum numer of roots/explant (1.6) was obtained on medium IV (Fig. 5c) while the roots length on media studied was in average 0.62-1.02 cm (Fig. 5d).

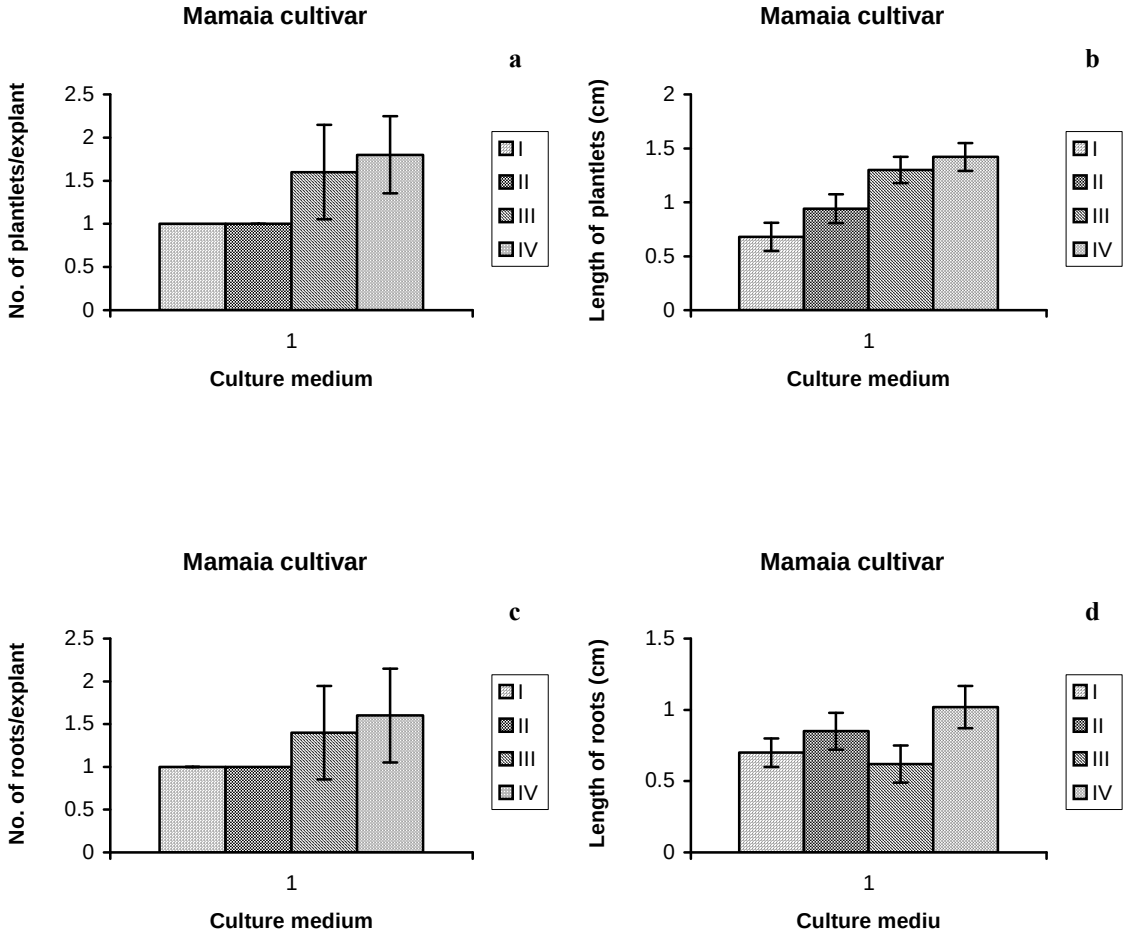

Fig. 5: Regeneration and multiplication of plantlets obtained by seed germination of "Mamaia" cv. (a-number of plantlets/explants, b-length of plantlets, c-number of roots/explants, d-length of roots).

Our results are in concordance with previous results regarding the in vitro propagation of different species of *Rosaceae* [7,11,13,15,16,17,18].

Conclusions

Induction of in vitro culture was succesfuly obtained because of high percentage of seed germination 40-80% deppending on cultivar and culture medium.

The growth of plantlets obtained from seeds germination is better on MS medium.

Plant multiplication on media supplemented with growth regulators and adenine sulphate have been obtained with good results especially in case of „Favorit" explants.

Root induction is low on all media studied.

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INFLUENŢA REGLATORILOR DE CREŞTERE ASUPRA REGENERĂRII ŞI MULTIPLICĂRII IN VITRO A CAISULUI

(Rezumat)

Materialul vegetal provine de la Staţiunea de Cercetare şi Producţie Pomicolă, Oradea. Soiurile luate în studiu: „Cea mai bună de Ungaria", „Favorit" şi „Mamaia"sunt considerate soiuri valoroase, cu vigoarea mijlocie sau mare, productivitate bună, rezistanţă la boli mai redusă la „Mamaia" şi „Cea mai bună de Ungaria", şi foarte bună la „Favorit". Rezistenţa la ger este bună la „Favorit" şi „Mamaia" şi mai scăzută (până la -20-24 °C), la „Cea mai bună de Ungaria". Toate cele trei soiuri sunt autocompatibile, gradul de înflorire abundent, diferenţa dintre ele constă în periada de coacere a fructelor, în iulie la „Cea mai bună de Ungaria" şi „Mamaia" şi în a doua decadă a lunii august la „Favorit". Fructele au textură fermă, sunt mari, 60-70 cm la „Mamaia" şi „Favorit" şi puţin mai mici (67-68cm) la „Cea mai bună de Ungaria". Fructele sunt uşor pubescente la „Mamaia" „Cea mai bună de Ungaria"şi nepubescente la „Favorit". Savoarea este bună, gust bun, echilibrat, uşor acidulat la „Cea mai bună de Ungaria", cu aromă puternică.

Pentru inducerea culturii in vitro s-au utilizat seminţe de cais provenite de la 3 soiuri şi anume: "Cea mai bună de Ungaria" (CMBU), "Favorit" and "Mamaia" care au fost inoculate pe mediile MS şi MS 1/2 lichide. După

4-5 zile semințele germinează şi s-a notat procentul de germinație. După 4 săptămâni s-a evaluat cultura urmărindu-se lungimea plantulelor rezultate în urma germinării. Procentul de germinare este destul de bun mai ales soiul „Mamaia", unde semințele germinează în procent de 70% pe mediul MS ½ şi în procent de 80% pe mediul MS. De asemenea soiul „Cea mai bună de Ungaria" (CMBU) prezintă procente bune de germinație ale semințelor (50-60%) indiferent de cantitatea mineralelor din mediul de cultură. Procente de germinare uşor mai scăzute (40-60%) prezintă soiul „Favorit". Concentrația de minerale mai mare conținută de mediul MS favorizează în special lungimea plantulelor obținute în urma germinației semințelor. Valorile obținute pe mediul MS sunt uşor crescute față de cele obținute pe mediul MS ½. Cele mai bune rezultate s-au înregistrat şi în cazul acestui parametru la soiul „Mamaia". După 4 săptămâni de cultură, rădăcinițele acestor plantule au fost excizate, iar plantulele au fost repicate pe medii specifice regenerării şi multiplicării plantelor. Mediul de bază a fost MS suplimentat cu reglatori de creştere, vitamina C şi sulfat de adenină.

S-a constatat că, indiferent de compoziția mediului de cultură, multiplicarea este destul de redusă la toate soiurile luate în studiu, chiar dacă procentul de regenerare este 100% pe toate variantele de cultură. Astfel, la soiul „CMBU" se obțin maximum 2 neoplantule/explant pe varianta de mediu IV. Aceste plantule au atins în medie 0,96 cm pe această variantă de mediu, însă lungimea plantulelor este asemănătoare pe toate variantele de mediu testate fiind cuprinsă între 0,58-0,96 cm. Înrădăcinarea plantulelor aparținând soiului „CMBU" este foarte redusă, accidental se formează 2 rădăcini/explant, dar în medie s-au obținut maximum 1,2-1,6 rădăcini/explant. Lungimea acestor rădăcini este foarte redusă fiind cuprinsă între 0,36-0,86 cm. Această înrădăcinare slabă se datorează concentrației foarte reduse a auxinei în mediul de cultură, predominantă fiind citochinina. Cea mai bună multiplicare s-a obținut în cazul soiului „Favorit". Cel mai mare număr de neoplantule/explant s-a obținut tot în cazul variantei de mediu IV (2,6 neoplantule/explant), deoarece aceasta are o compoziție complexă, pe lângă reglatori de creştere conținând şi alte adaosuri care stimulează multiplicarea şi înrădăcinarea plantulelor. Lungimea plantulelor este bună pe mediile III şi IV, plantulele având în medie 1,32-1,4 cm. Pe variantele de mediu I şi II lungimea plantulelor este mai redusă, în medie 0,84-0,96 cm. Înrădăcinarea plantulelor este slabă şi în cazul acestui soi de cais, maximum 1,6 rădăcini/explant, însă lungimea acestor rădăcini este destul de bună. În medie rădăcinile au atins 1,06-1,08 cm pe variantele de mediu III şi IV. În ce priveşte multiplicarea şi înrădăcinarea plantulelor la soiul „Mamaia", rezultatele sunt inferioare celor obținute în cazul soiului „Favorit", însă similare celor obținute la soiul „CMBU". Cea mai bună multiplicare (1,8 neoplantule/explant) s-a obținut de asemenea pe varianta de mediu IV. Lungimea acestor plantule este bună, în special pe mediile III şi IV unde plantulele au atins în medie 1,3-1,42 cm. Înrădăcinarea este redusă şi în acest caz, numărul maxim de rădăcini/explant (1,6) s-a obținut pe mediul IV. Rădăcinile obținute pe cele patru variante de cultură testate au atins în medie 0,62-1,02 cm lungime.