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IN VITRO MULTIPLICATION AND CALLUS INDUCTION OF *SYRINGA JOSIKAEA JACQ*. ENDEMIC TAXA FROM ROMANIAN FLORA

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Abstract: Multiplication of *Syringa josikaea Jacq.* has been induced from microcutings prelevated from in vitro regenerated plantlets. After 4 weeks of culture plant regeneration has been obtained on media II, III, VI. Callus wad induced previously on the media I, IV and V, and then after plant regeneration. Shoot induction from microcutings of *Syringa josikaea* has been very low (1.66 plantlets/explant) but the plant growth is good on all media studied. Root induction was not obtained except the media supplemented with 2.0 mg/l 2iP and 0.1 mg/l IBA, where 1-2 roots have been induced. Callus induction was successful induced on all media studied.

Introduction

Transylvanian lilac is a shrub of four meters high having a lilac inflorescence with many flowers slightly pubescent. In wild form merely exist in mountain valleys. Sometimes it is cultivated as ornamental shrub in parks, botanical gardens or privat domains. It could be find in most of the old privat gardens from Bistriţa-Năsăud county. *Syringa josikaea* has been considered as basis for ameliorating process, most of the horticultural forms having flowers with many colors and suave smell have been obtained from the wilt type.

This species is considered as endemic taxa from Romanian Flora [7]. In Romania merely exist in Cluj county (Valea Drăganului, Ciucea, Negreni, on the Someş valley to an altitute of 955 m) and in Bihor county. According to other authors *Syringa josikaea* is considered as endangered species [1,4].

Callus induction and in vitro cell cultures of different species of *Syringa* has been done for phenolic metabolites and hydroxyphenylethanol glycosides production [2]. As we know there is just a preliminary study about in vitro multiplication of Sy*ringa josikaea* [6]. A study of the effect of growth regulators on the multiplication of microcutings explants and callus induction has been done in this paper.

Material and methods

Plant material

The culture was initiated from shoots microcutings (10 mm long) of in vitro cultured plants obtained by in vitro cultivation of young shoots prelevated en 1994-

1995 from Valea Drăganului. After regeneration nodal explants were inoculated into MS medium supplemented with different growth regulators.

Stem explants have been inocultaed on special media for callus induction.

Culture media and conditions

The basal medium consist of Murashige and Skoog (MS) [3] and Shenk and Hildebrandt (SH) [5] mineral salts and vitamins plus 3% sucrose; solidified with 0.8% agar. The medium pH was adjusted to 5.7 with NaOH before autoclaving (120°C for 20 min). The growth regulators were added to the media before autoclaving, in different combinations:

I. MS + 0.1 mg/l BA and 0.1 mg/l NAA;
II. MS + 1.0 mg/l BA and 0.1 mg/l NAA;
III. MS + 0.1 mg/l 2iP and 0.1 mg/l IBA;
IV. MS + 1.0 mg/l 2iP and 1.0 mg/l IBA;
V. MS + 2.0 mg/l 2iP and 0.1 mg/l IBA;
VI. MS + 2.0 mg/l 2iP and 1.0 mg/l IBA;
VII. SH + 1.0 mg/l BA and 0.1 mg/l NAA.

For callus induction the following media have been tested:

I. MS + 0.1 mg/l BA and 0.1 mg/l NAA;
II. MS + 1.0 mg/l 2iP and 1.0 mg/l IBA;
III. MS + 2.0 mg/l 2iP and 0.1 mg/l IBA;
IV. MS + 2.0 mg/l 2.4-D.

Cultures were maintained permanently in a growth chamber at 25-27°C with a total irradiance of 87 μmol/m^2/s provided by fluorescent tubes, under a 16 h daylight regime for plant regeneration and multiplication and for callus induction the explants were kept 2 weeks under dark and then after transferred under a 16 h daylight regime.

The culture was evaluated 8 weeks after inoculation, the number and the length of regenerated plantlets/explant being followed, the number and length of roots/explant as well as the callus induction.

Results and discussions

Plant regeneration

After 4 weeks of culture plant regeneration has been obtained on media II, III, VI (Fig. 1). Callus wad induced previously on the media I, IV and V, and then after plant regeneration. Plant regeneration is also shown in Fig. 2. On the media SH supplemented with growth regulators, plant regeneration was not induced, the explants survived 3-4 weeks and then after died.

Plant multiplication

After 8 weeks of culture shoot induction from microcutings of *Syringa josikaea* have been very low. In most of the culture media studied 1-1.28 plantlets/explant have been obtained. On the media supplemented with 1.0 mg/l BA + 0.1 mg/l NAA and 0.1 mg/l 2iP + 0.1 mg/l IBA, 1.66 plantlets/explant have been obtained (Fig. 3, a). Plant growth is good on all media as it could be observed

in Fig. 3, b. On the media supplemented with 0.1 mg/l BA +0.1 mg/l NAA or 1.0 mg/l BA + 0.1 mg/l NAA plants were 3.3-4.46 cm heigth. The best media for plant growing are those supplemented with 1.0 mg/l 2iP + 1.0 mg/l IBA where the plants have been 10.22 cm heigth or 2.0 mg/l 2iP and 1.0 mg/l IBA where the plants have been 8.96 cm heigth. On the media supplemented with 2.0 mg/l 2iP + 0.1 mg/l IBA or 0.1 mg/l 2iP + 0.1 mg/l IBA plants were 7.28-7.4 cm heigth.

Fig. 1: In vitro plant regeneration of *Syringa josikaea* on media supplemented with growth regulators (a-MS + 0.1 mg/l 2iP and 0.1 mg/l IBA; b-MS + 0.1 mg/l BA + 0.1 mg/l NAA; c-MS + 0.1 mg/l BA + 0.1 mg/l NAA + 2.0 g/l vegetal charcoal).

Unfortunately, on these media root induction was not obtained except the media supplemented with 2.0 mg/l 2iP + 0.1 mg/l IBA, where after callus induction and plant regeneration, 1-2 roots have been induced having 6.97 cm length. It means that in vitro regenerated plantlets should be transferred on the media supplemented only with auxins for root induction.

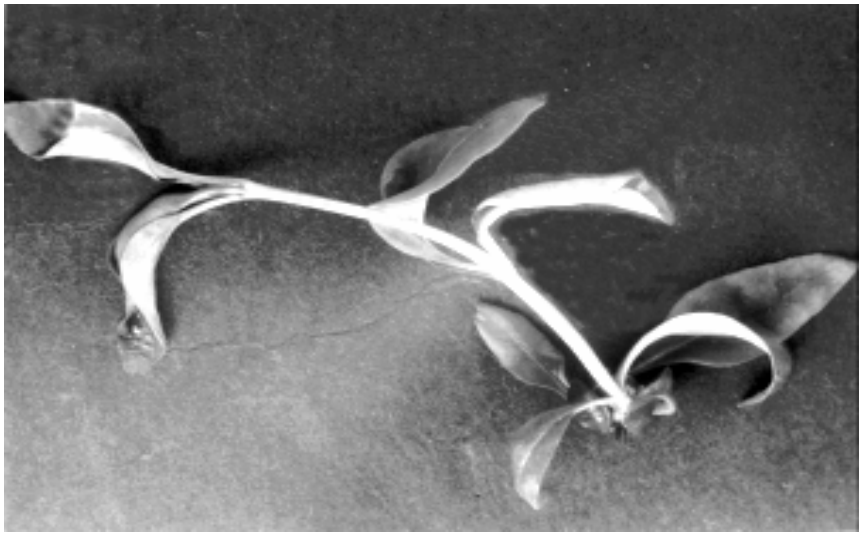

Fig. 2: Plantlet of *Syringa josikaea* obtained by in vitro shoot culture.

Callus induction

After 4 weeks of culture callus induction was observed but the culture was evaluated after 8 weeks, the diameter of callus, collour, consitence and organogenetic process have been followed. The results are shown in Table 1.

Table 1: Callus induction from stem explants of *Syringa josikaea.*

Culture media	Diameter (cm)	Colour	Consistence	Organogenesis
I	2.34 ± 0.11	Green	Friable	Shoots
II	3.06 ± 0.08	Green	Friable	Shoots
III	3.52 ± 0.14	Green	Friable	Shoots and roots
IV	1.9 ± 0.23	Yellow-green	Friable	-

Callus obtained on media supplemented with auxins in combination with cytokinins is green and friable and organogenetic process took place after 8 weeks of culture. On the media supplementes with 2.0 mg/l 2.4-D, callus was also friable but the colour was yellow-green and organogenesis was not induced.

The culture media supplemented with 2.0 mg/l 2iP and 0.1 mg/l IBA is the best for callus induction because the callus was 3.52 cm size, friable and shoots and roots were induced after 8 weeks of culture, that is very good for plant regeneration and organogenesis. Small peaces of callus have been replaced on special media for organogenesis and 2-3 plantlets were obtained after 6-8 weeks of culture.

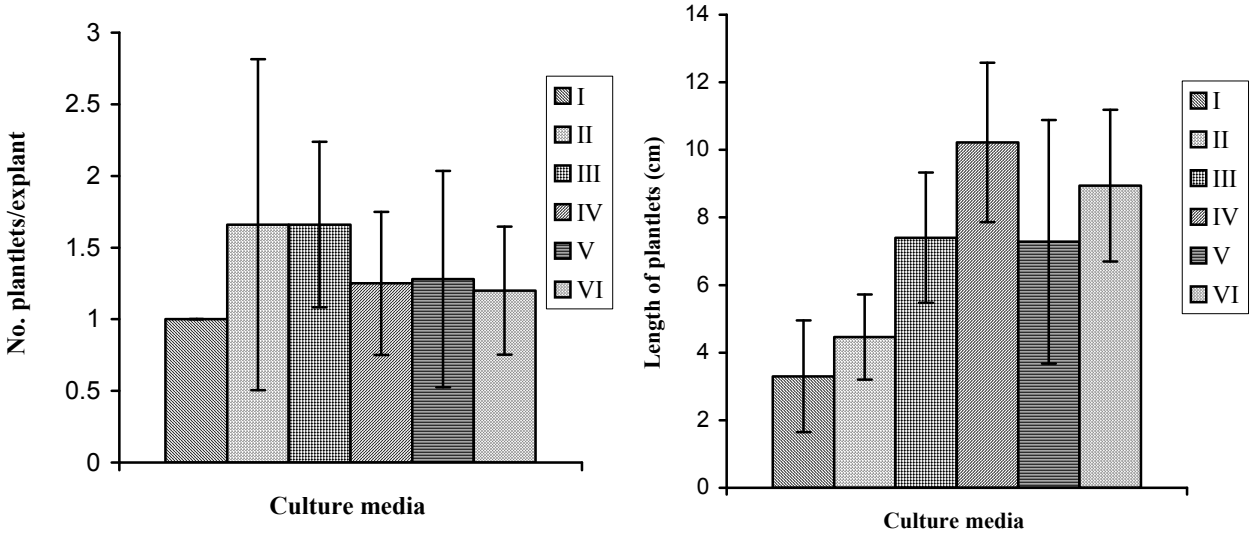

Fig. 3: Plant multiplication of *Syringa josikaea* on MS media supplemented with growth regulators (a - number of plantlets/explant, b - length of plantlets).

Conclusions

After 4 weeks of culture plant regeneration has been obtained on media II, III, VI. Callus wad induced previously on the media I, IV and V, and then after plant regeneration.

On the media SH supplemented with growth regulators, plant regeneration was not induced, the explants survived 3-4 weeks and then after died.

Shoot induction from microcutings of *Syringa josikaea* have been very low in most of the culture media, the best media were those supplemented with 1.0 mg/l BA + 0.1 mg/l NAA and 0.1 mg/l 2iP + 0.1 mg/l IBA, where 1.66 plantlets/explant have been obtained. Plant growth is good on all media studied.

Root induction was not obtained except the media supplemented with 2.0 mg/l 2iP and 0.1 mg/l IBA, where 1-2 roots have been induced.

The culture media supplemented with 2.0 mg/l 2iP + 0.1 mg/l IBA is the best for callus induction.

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MULTIPLICAREA IN VITRO ŞI INDUCEREA CALUSULUI LA *SYRINGA JOSIKAEA* JACQ. TAXON ENDEMIC DIN FLORA ROMÂNIEI

(Rezumat)

Syringa josikaea Jack. este considerat unul din cele mai frumoase endemite şi relicte terţiare ale florei României datorită inflorescenţei liliachii frumos mirositoare. Este răspândit în văile Munţilor Apuseni, uneori fiind cultivat ca arbust ornamental în grădini botanice, parcuri sau domenii private. De-a lungul anilor, specia *Syringa josikaea* a fost utilizată ca bază în procesele de ameliorare, multe din soiurile ornamentale de liliac au fost obţinute din aceasta. Datorită frumuseţii sale precum şi datorită importanţei ştiinţifice s-a considerat oportună studierea posibilităţilor de multiplicare vegetativă a acestei specii de plante, prin metode in vitro.

Această specie este puțin studiată din acest punct de vedere existând doar un studiu preliminar referitor la influența unor fitohormoni în regenerarea şi organogeneza in vitro.

Această lucrare prezintă un studiu referitor la rolul reglatorilor de creştere în regenerarea de plante şi multiplicarea in vitro pornindu-se de la minibutaşi prelevați de la plantule regenerate in vitro, precum şi în inducerea calusului. Astfel, s-a constatat că multiplicarea minibutaşilor este foarte redusă pe mediile studiate, rezultate mai bune obținându-se pe mediile suplimentate cu 1,0 mg/l BA şi 0,1 mg/l ANA precum şi pe cele cu 0,1 mg/l 2iP şi 0,1 mg/l AIB, unde s-au obținut în medie 1,66 plantule/explant. Multplicarea plantelor este redusă deoarece prima dată are loc inducerea calusului pe cele mai multe medii apoi are lor organogeneza. Creşterea plantulelor regenerate in vitro este bună pe toate mediile, însă cele mai bune rezultate s-au înregistrat pe mediul cu 1,0 mg/l 2iP şi 1,0 mg/l AIB unde plantule au atins 10,22 cm lungime sau pe mediul suplimentat cu 2,0 mg/l 2iP şi 1,0 mg/l AIB unde plantulele au avut 8,96 cm lungime. Inrădăcinarea acestor plantule nu a avut loc decât pe mediul suplimentat cu 2,0 mg/l 2iP şi 0,1 mg/l AIB, unde după inducerea calusului s-au obținut 1-2 rădăcini având 6,97 cm lungime.

Datorită multiplicării foarte reduse a minibutaşilor s-a încercat inducerea calusului în vederea obținerii organogenezei. Într-o primă etapă s-a testat inducerea calusului pe mediile pe care s-a observat că s-a obținut calus înainte de organogeneză. S-a mai testat şi un mediu specific pentru calus şi anume MS suplimentat cu 2,0 mg/l 2.4-D. Inducerea calusului s-a realizat pe toate mediile însă pe mediile suplimentate atât cu auxine cât şi citochinine, calusul format era verde avea 2,34-3,52 cm diametru, era friabil şi prezenta organogeneză. Prin repicarea unor porțiuni din acest calus pe medii speciale pentru multiplicare s-au obținut 2-3 plantule după 6-8 săptămâni de inoculare. Pe mediul suplimentat doar cu 2,4-D, calusul indus a avut 1,9 cm diametru, era galben-verzui, friabil şi nu a prezentat capacitate organogenetică.