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IN VITRO PRESERVATION OF *SYRINGA JOSIKAEA* J.JACK. ex RCHB.

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Abstract: Regeneration and multiplication of *Syringa josikaea* Jack was obtained from different types of explants prelevated from old and youg plants. The explants prelevated from old plants showed a lower percent of regeneration and multiplication than the explants prelevated from young plants. Induction of in vitro culture by seed germination was not possible. From meristem explants plant regeneration was 80% on the media supplemented with 1.0 g/l IBA and 0.1 g/l BA, that is the best media for plant regeneration from shoot tip explants and nodal explants as well. Plant regeneration from flower bud explants have been also very low. On the media supplemented with 1.0 or 2.0 g/l 2iP, callus was induced from meristem explants and low multiplication from shoot tip or nodal explants have been observed. There is also no rooting on these media. Plant aclimatization was succesfull (80%) on mixture of Perlite and peat (1:1) after 14 days of protection and 4 days of watering.

Introduction

Syringa josikaea is considered as endemic taxa from Romanian Flora (Flora RPR, 1961). 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 Olteanu et al., 1994, *Syringa josikaea* is considered as endangered species. This species has many horticultural forms having flowers with many colors and suave smell, being considered as ornamental plant.

The in vitro multiplication of this species is generally difficult. Plant regeneration was allready obtained on MS (Murashige and Skoog, 1962) but multiplication is low. Other experiments carried out on SH medium (Schenk and Hildebrandt, 1972) showed that SH medium is not good for regeneration and multiplication of this plant species (Zăpârţan şi Butiuc-Keul, 2002). Callus induction and in vitro cell cultures of different species of *Syringa* has been done for phenolic metabolites and hydroxyphenylethanol glycosides production (Ellis, 1983). We also obtained callus induction from stem explants of *Syringa josikaea* that presents organogenesis processes on surface (Zăpârţan şi Butiuc-Keul, 2002).

A complet study of the effect of growth regulators and the importance of explant type has been done in this paper.

Material and Methods

Plant material

The culture was initiated in 1994-1995 from different types of explants as following:

1. seedlings obtained by in vitro germination of seeds prelevated in 1994-1994 from plants from Ciucea and Negreni.
2. young shoots prelevated before flowering in 1994-1994 from plants from Ciucea and Valea Drăganului. At the end of april, meristems, nodes and shoot tips were prelevated and inoculated on specific media for plant regeneration and multiplication.
3. young shoots and inflorescence prelevated from 5 years old plants obtained in vitro and transferred to field in the spring of 1995.
4. shoots prelevated from old plants from Botanical Garden from Cluj—Napoca.

Culture media and conditions

The basal medium consist of Murashige and Skoog (MS) mineral salts and vitamins (Murashige and Skoog., 1962) 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 1/2 without growth regulators;
II. MS 1/2 without growth regulators + 3.0 g/l vegetal charcoal;
III. MS + 1.0 g/l IBA and 0.1 g/l BA;
IV. MS + 1.0 g/l IBA and 1.0 g/l BA;
V. MS + 0.5 g/l IBA and 1.0 g/l Kinetin;
VI. MS + 0.5 mg/l NAA and 0.1 g/l Kinetin;
VII. MS + 0.5 mg/l NAA and 2.0 g/l 2iP;
VIII. MS + 1.0 mg/l NAA and 1.0 g/l 2iP;
IX. MS + 1.0 g/l IBA and 1.0 g/l Zeatin;
X. MS + 1.0 mg/l NAA and 2.0 g/l Zeatin.

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 (NARVA LTD 36 W/01D, Germany), under a 16 h daylight regime.

The culture was evaluated 60 days after inoculation, the percent of regeneration, the number and the length of regenerated plantlets/explant being followed as well as the number and length of roots/explant.

Results and Discussions

Plant regeneration

After 30 days of culture plant regeneration has been obtained on all media except media supplemnted with 2iP, where callus induction was observed.

In vitro plant regeneration from shoots of *Syringa josikaea* old plants, after 60 days of culture is shown in Table 1. The percent of plant regeneration from different explants is low on media supplemented with NAA and 2iP and on the media supplemented with IBA 0.5 g/l and Kinetin 0.1 g/l. Higher percent of regeneration from meristem explants has been obtained on MS media supplemented with IBA 1.0 g/l and BA 0.1 g/l (40 %). The highest percent of regeneration (58%) has been obtained from shoot tip explants on MS media supplemented with 1.0 g/l IBA and 0.1 g/l BA. The flower bud explants does not regenerate plants on the most of the culture media tested.

Plant regeneration from shoots of *Syringa josikaea* young plants after 60 days of culture is higher than plant regeneration from shoots of old plants (Table 3). From meristem explants plant regeneration was 80% on the media supplemented with 1.0 g/l IBA and 0.1 g/l BA, that is the best media for plant regeneration from shoot tip explants and nodal explants as well. Plant regeneration from flower bud explants have been also very low.

Table 1: In vitro plant regeneration (%) from shoots of *Syringa josikaea* old plants, after 60 days of culture

Explant	Culture media									
	I	II	III	IV	V	VI	VII	VIII	IX	X
Meristem	3	20	40	10	3	10	-	-	20	18
Shoot tip	38	18	58	51	2	8	5	5	50	56
Node	9	25	45	40	2	10	3	3	40	42
Flower bud	-	-	2	3	-	-	-	-	5	8

Table 2: In vitro plant regeneration (%) from shoots of *Syringa josikaea* youg plants, after 60 days of culture

Explant	Culture media									
	I	**II**	**III**	**IV**	**V**	**VI**	**VII**	**VIII**	**IX**	**X**
Meristem	9	40	80	25	30	28	15	18	65	61
Shoot tip	11	28	97	29	20	32	50	50	90	92
Node	8	37	90	20	20	38	33	37	81	84
Flower bud	-	-	4	5	-	-	-	-	11	16

Shoot induction

After 60 days of culture shoot induction from explants of *Syringa josikaea* old plants have been obtained with low percentage. In most of the explants the shoot induction does not occur. In case of shoot induction as it happened from shoot tip explants on media supplemented with 1.0 g/l IBA and 1.0 g/l Zeatin (40%of multiplication) or 1.0 mg/l NAA and 2.0 g/l Zeatin (50% of multiplication) the number of induced shoots was wery low (1-2 small plants) (Table 3).

Table 3: In vitro shoot induction (%) from explants of *Syringa josikaea* old plants, after 60 days of culture

Explant	Culture media									
	I	**II**	**III**	**IV**	**V**	**VI**	**VII**	**VIII**	**IX**	**X**
Meristem	-	2	8	6	-	-	callus	callus	20	20
Shoot tip	3	13	38	20	-	-	-	-	40	50
Node	-	2	15	11	2	2	-	-	35	45
Flower bud	-	-	1	1	-	-	-	-	1	3

The evolution the explants from *Syringa josikaea* young plants, after 60 days of culture is presented in Table 4. The number of plantlets/explant, their length as well as the number of roots/explant being followed. It could be observed that flower bud explants have a low multiplication on the media supplemented with 1.0 g/l IBA and 0.1 g/l BA, where 3 plantlets/explants have been obtained having 2.06 cm length. On the media supplemented with 1.0 g/l IBA and 1.0 g/l BA, 3.8 plantlets/explant have been obtained having 4.18 cm length. Multiplication was improved by addition of 1.0 g/l IBA and 1.0 g/l Zeatin to MS media, when 4.4 plantlets/explant have been obtained but the plant growth was lower (0.66 cm length). On the media supplementd with 1.0 mg/l NAA and 2.0 g/l Zeatin, 7.8 plantlets/explant have been obtained having 0.36. cm length. The number of roots/explant is also reduced on these media. On the media I, II, V, Vi, VII, VIII, there is no multiplication.

On the MS ½ media there are no multiplication and rooting, indeppendent on the type of explant. The multiplication is sligtly increased and rooting was induced by addition of vegetal charcoal to the MS ½ media. Thus, 1.2 plantlets/explant have been induced from shoot tip explants and 2 plantlets/explant from nodal explants. The best media for plant multiplication from meristem, shoot tip or nodal explants are the media supplemented with 1.0 g/l IBA and 0.1 g/l BA. On these media the plant growth and root induction are stimulated as well.

On the media supplemented with 1.0 or 2.0 g/l 2iP, callus was induced from meristem explants and low multiplication from shoot tip or nodal explants have been observed. There is also no rooting on these media.

Media supplemented with 1.0 g/l IBA and 1.0 g/l Zeatin or 1.0 mg/l NAA and 2.0 g/l Zeatin ensure a good plant multiplication (4.4-4.6 plantlets/explant) from shoot tip explants.

Table 4: In vitro shoot induction (%) from explants of *Syringa josikaea* youg plants, after 60 days of culture

Culture media	Meristem			Shoot tip			Node			Flower bud		
	No. of plants	Length of plants (cm)	No. of roots	No. of plants	Length of plants (cm)	No. of roots	No. of plants	Length of plants (cm)	No. of roots	No. of plants	Length of plants (cm)	No. of roots
	1±0	2.26±0.19	-	1± 0	8.04± 0.11	-	1 ± 0	3.3 ± 0.21	-	-	-	-
II	1± 0	7.2± 0.18	1.6 ± 0.54	1.2±0.44	12.24±0.2	1.8 ± 0.44	2 ± 0.7	11.96 ± 0.11	1 ± 0.7	-	-	-
III	3.6 ± 0.54	5.12 ± 0.08	1.16 ±0.54	4.6 ± 0.54	10.28 ± 0.19	1 ± 0	2.8 ± 0.44	7.94 ± 0.26	1 ± 0	3 ±0.7	2.06 ± 0.11	1.4 ± 0.54
IV	2.6 ± 0.56	3.96 ± 0.11	1 ± 0	3.8 ± 0.44	4.16 ± 0.28	1.2 ± 0.44	2 ± 0.7	3.04 ± 0.36	-	3.8 ± 0.44	4.18 ± 0.14	1.6 ± 0.54
V	2.8 ± 0.4	2.38 ± 0.16	2.8 ± 0.44	2 ± 0.7	4.56 ± 1.5	1.8 ± 0.44	2.2 ± 0.83	7.7 ± 0.29	3.2 ± 0.83	-	-	-
VI	1.2 ± 0.58	1.42 ± 0.16	-	1.6 ± 0.54	4.14 ± 0.16	-	2.2 ± 0.44	5.24 ± 0.18	-	-	-	-
VII	Callus	Callus	-	1 ± 0	0.46 ± 0.11	-	1 ± 0	0.48 ± 0.1	-	-	-	-
VIII	Callus	Callus	-	1 ± 0	0.26 ± 0.19	-	1.2 ± 0.44	0.3 ± 0.12	-	-	-	-
IX	1.4 ± 0.54	1.98 ± 0.14	-	4.6 ± 0.54	1.1 ± 0.12	1.2 ± 0.44	2.2 ± 0.44	1.14 ± 0.21	2 ± 0.7	4.4 ± 0.89	0.66 ± 0.11	2.2 ± 0.44
X	1.8 ± 0.44	1.24 ± 0.36	-	4.4 ± 0.54	0.26 ± 0.15	-	1.6 ± 0.54	0.38 ± 0.13	2.6 ± 0.54	7.8 ± 0.83	0.36 ± 0.08	-

Plant acclimatization

Plantlets regenerated in vitro have been transferred in four types of substrates as: Perlite, mixture of Perlite and peat (1:1), mixture of sand and peat (1:1) and sand. Plantlets were covered by glass globe 7 or 14 days to protect them against transpiration and watered 2 or 4 days. The evolution of plants transferred ex vitro could be observed in Table 5.

Table 5: Acclimatization of in vitro regenerated plants of *Syringa josikaea* plantlets

Substrate	Days of protection	Days of watering	Plant regeneration (%)
Perlite	7	2	50
	14	4	60
Mixture of Perlite and peat 1:1	7	2	70
	14	4	80
Mixture of sand and peat 1:1	7	2	50
	14	4	50
Sand	7	2	20
	14	4	20

The highest percent of plant regeneration (80%) was obtained on mixture of Perlite and peat 1:1 after 14 days of protection and 4 days of watered. On the same mixture but 7 day of protection and 2 days of watered the plant regeneration was high (70%). A good percent of regeneration (60%) was obtained on Perlite after 14 days of protection and 4 days of watered as well.

In may plants having 4-8 nodes and in average 4 roots of 1 cm length were transferred to hotbed up to september and then after transferred to field (tree-nursery) where they were covered by leaves. Plant survival was deppendent on exposition (north exposition was harmful because of high umidity and frost maintainance) and soil composition.

Conclussions

The explants prelevated from old plants showed a lower percent of regeneration and multiplication than the explants prelevated from young plants.

Plant regeneration and multiplication have been obtained especially from meristem and shoot tip explants. Flower bud explants does not regenerated plants on most of the culture media tested.

Addition of 2iP to the culture media was followed by callus induction.

Induction of in vitro culture by seed germination was not possible.

Plant aclimatization was succesfull (80%) on mixture of Perlite and peat (1:1) after 14 days of protection and 4 days of watering.

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CONSERVAREA IN VITRO LA *SYRINGA JOSIKAEA* J.JACK ex RCHB.

(Rezumat)

Specia *Syringa josikaea* este considerată un endemit pentru flora României, (Flora RPR, 1961), fiind răspândită în județul Cluj (Valea Drăganului, Ciucea, Negreni, pe valea Someşului) şi în județul Bihor.Această specie este răspândită de asemenea în Carpații de Nord şi în Galiția. După Olteanu şi colab., 1994, *Syringa josikaea* este considerată o specie periclitată. De aceea este neceasră conservarea acestei specii. În experimentele noastre s-a realizat regenerare şi multiplicarea in vitro pornindu-se de la diferite tipuri de explante prelevate de la plante tinere şi mature. În urma observațiilor s-a constatat că explantele prelevate de la plantele tinere au prezentat un procent mai mare de regenerare şi multiplicare decât explantele prelevate de la plantele mature. Inducerea culturii in vitro pornindu-se de la semințe germinate aseptic nu a fost posibilă. Regenerarea de plante din explante de meristem s-a obținut în procent de 80% pe mediile suplimentate cu 1,0 g/l AIB şi 0,1 g/l BA. Această combinație de reglatori de creştere a reprezentat varianta optimă pentru regenerarea de plante şi din explantele nodale sau cele prelevate din apexul lăstarilor. Regenerarea de plante din boboci florali este redusă. Pe mediile suplimentate cu 1,0 sau 2,0 g/l 2iP, s-a observat inducerea unui calus din explantele de meristem, iar în ce priveşte multiplicarea din explantele de apex sau nod, aceasta a fost redusă. De asemenea, pe acest mediu, plantele nu au înrădăcinat. Aclimatizarea plantulelor obținute in vitro s-a realizat cu succes (în raport de 80%) pe o mixtură de perlit cu turbă (1:1) după 14 zile de protejare a plantulelor prin acoperire cu globuri de sticlă şi 4 zile de udare.