IN VITRO PROPAGATION OF *MAGNOLIA X SOULANGEANA* SOUL. – BOD. HYBRID – FACTORS AFFECTING AXILLARY BUDS PROLIFERATION

ÎNMULȚIREA IN VITRO A HIBRIDULUI *MAGNOLIA X SOULANGEANA* SOUL.-BOD. FACTORI CARE INFLUENȚEAZĂ PROLIFERAREA MUGURILOR AXILARI

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Abstract *Magnolia genus includes a group of about 80 species with persistent or falling leaves and with bloom before or after coming into leaf. Magnolia x soulangeana Soul. – Bod. is a valuable hybrid for its early bloosom. Continuation of experiences regarding in vitro micropropagation of magnolia aimed to establish culture medium for axillary buds proliferation. In vitro multiplication capacity was influenced by the composition of mineral salts of basic medium, 6-benzilaminopurine (BAP) concentration and the number of subcultures. The best shoot multiplication rate (MR) i.e. 10 plantlets / explant after 5 subcultures, was established with Lepoivre mineral salts, Jaquiot vitamins, 1.0 mg/l naftilacetic acid (NAA) at 5.0 mg/l BAP. At the same time the effect of some antioxidants and absorbant component on browning of plantlets due to the polyphenols emission was studied. None of the tested components eliminated totally the browning of plantlets. Further experiments are necesary for multiplication protocol improving and to establish the conditions of rooting and aclimatization.*

Rezumat. *Genul magnolia cuprinde un grup de aproximativ 80 de specii cu frunze persistente dar şi căzătoare şi cu înflorire atât înainte cât şi după înfrunzire, Magnolia x soulangeana Soul. - Bod fiind un hibrid valoros pentru înflorirea timpurie. Continuarea experienţelor privind multiplicarea magnoliei a avut ca scop stabilirea mediului de cultură pentru inducerea proliferării mugurilor axilari. Capacitatea de multiplicare in vitro a fost influenţată de compoziţia sărurilor minerale, de concentraţia 6- benzilaminopurinei (BAP) şi de numărul de subculturi. Cea mai bună rată de multiplicare (RM) a lăstarilor, 10 plantule /explant după a 5-a subcultură a fost stabilită folosind sărurile minerale Lepoivre (L), vitaminele Jaquiot (JQ), 1,0 mg/l acid naftilacetic (ANA), la o concentraţie de 5,0 mg/l BAP. A fost studiat totodată efectul unor componente antioxidante şi absorbante asupra procesului de brunificare a plantulelor datorat emisiei de polifenoli. Nici unul dintre componentele testate nu a eliminat total brunificarea. Sunt necesare experienţe viitoare pentru îmbunătăţirea protocolului de multiplicare şi pentru a stabili condiţiile de înrădăcinare şi aclimatizare a magnoliei.*

Magnolia x soulangeana group constitutes the largest and best-known category of deciduous flowering magnolias. Cutting propagation is preferred for most magnolias. However, rooting potential of cuttings varies considerably among cultivars as well as among species. In recent years, propagation of wide range of ornamental plants by tissue has become accepted commercial practice. *In vitro* propagation is a desired method for multiplication of valuable plants at faster

rates than conventional procedures. Micropropagation of magnolias has been reported from shoot tip explants and axillary buds (Biederman, 1987; Kamenicka and Lanakova, 2000) and via somatic embryogenesis (Merkle, 1995). The aim of this study was to establish culture media for *in vitro* axillary buds proliferation and at the same time to study the effect of some antioxidants and absorbent component on browning of plantlets due to the polyphenols emission.

MATERIAL AND METHODS

We used magnolia shoots developed during initiation phase. Murashige - Skoog (MS) and Quoirin and Lepoivre (QL) with Jaquiot vitamins was the basic medium. All media contained 30 g/l glucose and 32 mg/l Na Fe EDTA and were solidified with 9 g/l agar. The growth hormones used were NAA in concentration of 1.0 mg/l and BAP in concentrations of 1.0 – 5.0 mg/l (Table 1).

Table 1

Media composition used for shoot multiplication in *Magnolia x soulangeana*

Composition	Medium 1	Medium 2	Medium 3	Medium 4	Medium 5	Medium 6
Macroelements	MS	MS	MS	QL	QL	QL
Microelements	MS	MS	MS	QL	QL	QL
Vitamins	JQ	JQ	JQ	JQ	JQ	JQ
NAA mg/l	1.0	1.0	1.0	1/0	1.0	1.0
BAP mg/l	1.0	3.0	5.0	1.0	3.0	5.0

MS = Murashige and Skoog (1962); QL = Quoirin and Lepoivre (1977); JQ = Jaquiot (1956); BAP = 6- benzylaminopurine; NAA = naftalene acetic acid.

The evaluation of multiplication rate (MR) was recorded after each subculture at four weeks. All tests had three replications and the data were analyzed for significance by analysis of variance, with the mean separation by Duncan's Multiple Range test (Duncan, 1995) with S.P.S.S. for Windows Release 14.0.0.

During the course of the present work, the problem of tissue and media browning due to phenolic oxidation was particularly encountered. Thus, to reduce or control phenolic browning we used sterile solution of antioxidants agents such as ascorbic acid, citric acid (0; 25; 50 and 100 mg/l) and soluble PVP (polyvininylpyrrolidone) (100; 500; 1000 mg/l) like absorbent component. Plantlets (60 for each treatment) were soaked for 30 – 60 min. in these sterile solutions and than transferred to the Quoirin and Lepoivre (QL) mineral salts and Jaquiot vitamins with 1.0 mg/l NAA and 5.0 mg/l BAP. Control plantlets were rinsed in water. The viability, color, growth and degree of browning were observed frequently and number of browning plantlets was noticed after 42 days. All cultures were grown at 25 ± 2 ºC under 16 h photoperiod (40 – 50 µE ms ²sec provided by white cool fluorescent lamps.

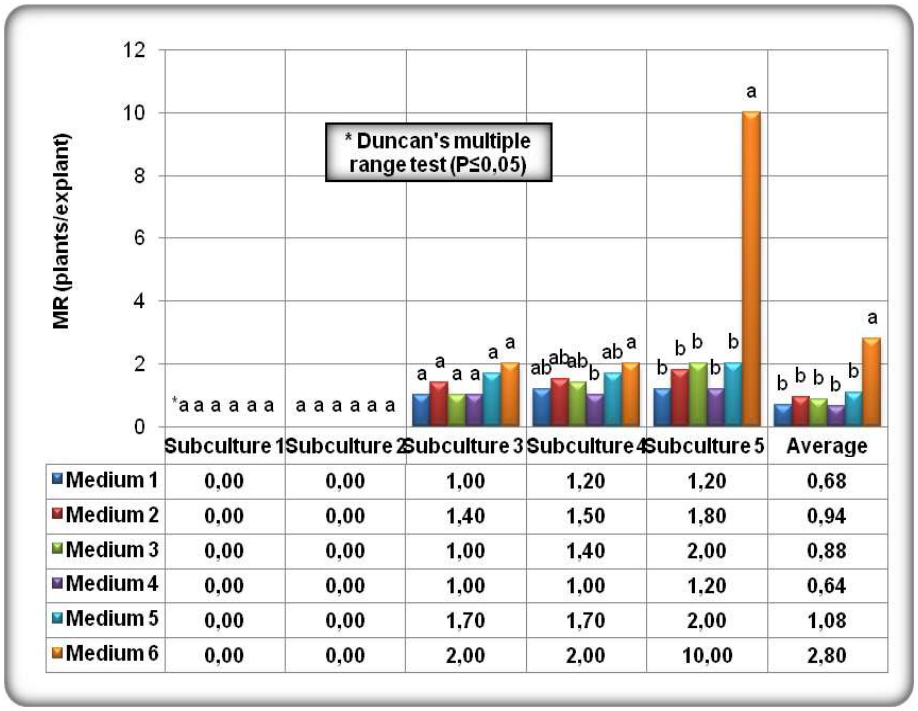
RESULTS AND DISCUSSIONS

Observations regarding micropropagation ability of *Magnolia x soulangeana* showed that shoot proliferation is difficult and required a longer period (12 weeks) of BAP exposure to obtain cytokinin autonomy. The results (Fig. 1) indicated that a good MR was achieved on QL basic media with JQ vitamins comparative with MR obtained when MS basic media with JQ vitamins

were used. Comparative to the MS basic media, which promoted a MR of 2.0 plants/ explant, the QL basic medium proved to be appropriate for shoot multiplication. It allows a MR of 10.0 plants/explant, after 5 subcultures.

Another factor which influenced the shoot proliferation was BAP concentration. Different levels of cytokinin influence shoot quality. Study to optimize factors for development of multiple shoots revealed that BAP had a positive effect in a high concentration. Multiplication factor increased with increasing amounts of BAP. More than two shoots were produced per explant when incubated on media supplemented with 5.0 mg/l BAP and 1.0 mg/l NAA, irrespective of basal medium composition.

Results presented in Fig. 1 shows that also the number of subcultures influenced the MR. No shoot proliferation was observed after the first and the second subculture.



	Subculture 1	Subculture 2	Subculture 3	Subculture 4	Subculture 5	Average
Medium 1	0,00	0,00	1,00	1,20	1,20	0,68
Medium 2	0,00	0,00	1,40	1,50	1,80	0,94
Medium 3	0,00	0,00	1,00	1,40	2,00	0,88
Medium 4	0,00	0,00	1,00	1,00	1,20	0,64
Medium 5	0,00	0,00	1,70	1,70	2,00	1,08
Medium 6	0,00	0,00	2,00	2,00	10,00	2,80

Fig.1. – Multiplication rate of *Magnolia x soulangeana* related to number of subcultures and culture media

Axillary shoot proliferation in magnolias is difficult because of a high content of phenolic substances and the formation of vitrified shoots (Kamenicka and Valova, 1994.The presence of phenolic compounds and high polyphenol oxidase activity cause explant browning which affects vegetative propagation and limits morphogenic responses. A strategy to control phenolic oxidation becomes a necessity.

This investigation shows that at woody plants with high phenolic acid content, soaking shoots in antioxidants was useful for reducing phenolic oxidation. Plantlets without antioxidants or PVP (control) pretreatment released a brown – red exudates into the medium. Although, none of the tested antioxidant components did not reduced very browning effect, all of the pretreatments showed a positive effect. Among the compounds tested, citric acid (100 mg/l) had significantly less browning of plantlets, than other treatments (Fig. 2). The effect of ascorbic acid was almost as good as of citric acid at the same concentration, but the viability of treated plantlets was low. The pretreatment with PVP who absorb phenols through hydrogen bonding had a good effect. Percentages of shoots showing browning oxidation were fewer than 30 % depending on PVP concentration (Fig. 2). antioxidants protect explants from browning as they act as reducing agents by decreasing the redox potential of phenols in the medium. This is achieved by reverting quinones that are formed by oxidation of phenolic compounds produced in damaged tissue or by competing with free radicals and removing them from the reaction (Debergh and Read, 1991). With PVP, hydrogen bonds absorb polyphenols, reducing their synthesis and thereby preventing browning of explants.

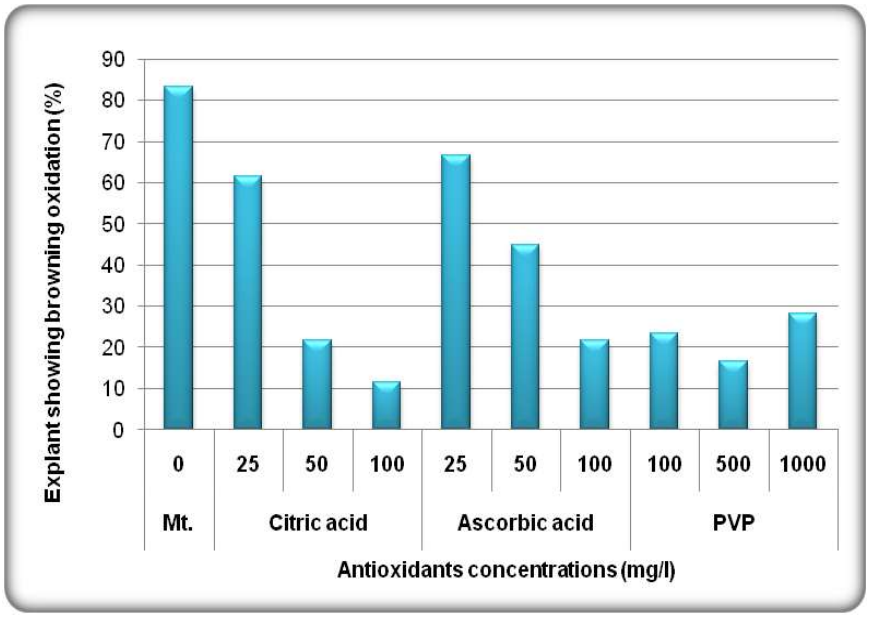


Fig.2 – The effect of antioxidants and absorbent components on browning oxidation of media and *Magnolia x soulangeana* shoots

CONCLUSIONS

Our results demonstrate the critical influence of culture medium, auxins and cytokinins concentration and number of subcultures on axillary shoot formation for *Magnolia x soulangeana* micropropagation.

The best shoot multiplication rate (MR) i.e. 10 plantlets / explant after five subcultures, was established with Lepoivre mineral salts, Jaquiot vitamins, 1.0 mg/l naftilacetic acid (NAA) at 5.0 mg/l BAP.

Although, pretreatements with antioxidants/reducing agents were a good effect on stop the oxidation reactions we consider that is a laborious method and we recommend frequent subculture onto fresh media.

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