IN VITRO CULTIVATION OF *RUBUS SANCTUS SCHREB.*

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Abstract: The plants are a major source of natural products used as pharmaceuticals, agrichemicals. Most likely plants will continue to provide novel natural products as well as chemical models for new drugs. In vitro biology and technology have been proposed as contributing toward production of plant natural products, via micropropagation, cell culture. The paper studies “in vitro”behaviour of *Rubus sanctus Schreb.,* a native of Central Asia, used in traditional medicine. It were tested, not only the dedifferentiation capacity, but also the regenerative potential for rapid micropropagation.The initiation of in vitro cultures at *Rubus sanctus Schreb* was achieved from axillary buds, cultivated on different variants of Murashige-Skoog medium. The MS medium supplemented with benzylaminopurine stimulated the direct caulogenesis at *Rubus sanctus Schreb.,* whereas the MS medium supplemented with kinetine generated an intensive proliferative reaction and callus development.The regeneration of whole plants was obtained in two steps: the shoots were excised and transferred to fresh medium and then rooting of these shoots was achieved on the same medium without growth regulators.

INTRODUCTION

Rubus sanctus Schreb. is known to provide extracts which have been used in traditional medicine as antimicrobial (Tosun et al, 2004). Aerial parts of *Rubus sanctus Schreb.* were characterized by their capability of synthesizing and accumulating natural tannin (Hussein et al., 2003). Ethanolic and aqueous extracts from *Rubus sanctus Schreb.* contain a wide variety of interesting antiviral and antibacterial substances: antituberculosis, hepatoprotective and antitumoral activities (Ayoub et al., 2004; Conolly et Hill, 2005; Erdemoglu et al., 2003; Serteser et al., 2008; El-Tawil et al., 2006).

The paper studies “in vitro”behaviour of *Rubus sanctus Schreb.,* which in contrast to several other Rubus species, has not been investigated. From this reason it were tested, not only the dedifferentiation capacity, but also the regenerative potential for rapid micropropagation. The aim of studies is rapid micropropagation of this species and also callus induction, both as an unconventional alternative to exploit the source of raw material for natural compounds.

MATERIAL AND METHODS

Rubus sanctus Schreb., a native of Central Asia Mountains, is a 1-2 m shrub with a prostrate or orching stem, palmate leaves with five leaflets, numerous flowers in lax panicles , fruits with black, glabrous and juicy dropelets (Monasterio-Huelin et Weber, 1996). The explants used to initiate „in vitro” cultures of *Rubus sanctus Schreb.* were harvested in spring from individuals, cultivated in Bucovina, a region of North Romania. Axillary buds of *Rubus sanctus Schreb*. were used not only for callus cultures development, but also as explants for direct micropropagation.The procedures for callus and suspension cultures were established on MS medium. Axillary buds were used as explants for callus production on MS medium at 24 ^{0}C in complete darkness. The explants were sterilized with ethanol 70%, than for 10-15 minutes with sodium hypochlorite solution 3 % and washed with sterilized distilled water.

After 30 days the callus produced was weighted and used to establish cell suspensions, by transferring three sections of calli 1 cm^3 each, from the exponential growth phase into 250 ml Erlenmeyer flasks containing 100 ml of liquid medium. Flasks were rotated 100 rpm in the dark. The cell suspensions were weekly subcultured by filterring and transferring into fresh medium. The submerse cultures were shaken on an orbital shaker at 100 rpm. For cell proliferation, the cultures were maintained in the dark. The regenerative potential of *Rubus sanctus Schreb*. was evaluated, using axillary buds for rapid micropropagation. From this reason, it were tested 7 variants of MS basal medium (Table 1). The regenerated shoots were tested for roots development.

Table 1- Morphogenetical reactions of *Rubus sanctus Schreb*., cultivated „in vitro”

Variants	Growth regulators mg/l				Morphogenetical reaction
	BAP	NAA	IA A	K	
I	1,00	0,1	-	-	caulogenesis
II	1,00	-	0,1	-	caulogenesis
III	1,00	-	-	-	caulogenesis

Variants	Growth regulators mg/l				Morphogenetical reaction
	BAP	NAA	IA A	K	
IV	-	-	0,1	1,00	callus induction
V	-	0,1	-	1,00	callus induction
VI				1,00	calus induction
VII	-	-	-	-	rhysogenesis

BAP- benzylaminopurine: K- kinetine, NAA- naphtalenlacetic acid: IAA- indolylacetic acid

RESULTS AND DISCUSSIONS

After chemical sterilization, the explants were placed on MS medium for callus cultures initiation (Photo 1, 2). Callus cultures were induced with different concentrations of auxin and cytokinin (Table 1). It were used two types of cytokinins: benzylaminupurine and kinetine, in combination with two types of auxins: naphtalenacetic acid and indolilacetic acid. The studies are based on the effect of the above mentioned cytokinins , used alone or in combination with auxins, on cell dediferrentiation or regeneration.

Of all hormone combinations used in the medium, three were the most efficient in promoting callus development (variants IV, V, VI). The capacity for callus development was observed to be greater on MS medium with kinetine (1mg/L). Higher levels of cytokinine, such as kinetine tend to favor callus production.The use of benzylaminopurine resulted in a very low growth rate of callus cultures.

The primary callus cultures were readily obtained after 4 weeks. These calli were initially white-green (Photo 3). Cell suspensions of *Rubus sanctus Schreb* were initiated to stimulate cell proliferation, by culturing small pieces of calli in liquid MS medium (Photo 4). The suspensions were maintained in dark, for about 4 subcultures (7 days each). Cell proliferation occurred in either stationary or agitated cultures Changing the level of plant growth regulators and balance in the medium had major effects on „in vitro" reactions.

Shoots differentiation were achieved when the axillary buds were transferred to MS medium, supplemented with benzylaminopurine (variants I, II, II). It is well known that clonal micropropagation utilizes axillary shoot proliferation and the cytokinins , in combination with auxins are added to improve this reaction. The axillary buds of *Rubus sanctus Schreb*. responded differently to exogenous hormones in the medium. Table 1 indicated that axillary buds were more sensitive to benzylaminopurine treatement, followed by shoot development than kinetine treatment. The first plays an important role in shoot growth (Photo 5, 6, 7) and the second, in callus induction. These different responses of the same type of explant can be manipulated by choice of plant growth regulators. Shoot cultures were incubated in a growth chamber at 24 ^{0} C with a 16 h photoperiod. The multiplication capacity of explants cultured on MS medium, supplemented with BAP was maintained upon successive subcultures, to the original medium. Individual shoots were excised and cultured on growth regulators free MS medium (variants VII) (Photo 8, 9). Small rooted plantlets were transferred to MS medium for further development. Plantlets developed on this medium were transferred to soil and gave rise to normal plants. The plants are a source of natural products used as pharmaceuticals. Most likely plants will continue to provide natural products. „In vitro" technologies have been proposed as production of natural products, via micropropagation.

CONCLUSIONS

Procedures for callus and suspension cultures were established from axillary buds of *Rubus sanctus Schreb*. on Murashige – Skoog medium, supplemented with 1 mg/L K or 1mg/L K in combination with 0,1 mg/L IAA or 0,1 mg/L NAA

Direct caulogenesis was achieved on MS medium, supplemented with 1 mg/L BAP or 1 mg/L BAP in combination with 0,1 mg/L IAA or 0,1 mg/L NAA.

Kinetine stimulates the dedifferentiation of explants, whereas, benzylaminopurine stimulates the caulogenesis

Clonal micropropagation of *Rubus sanctus Schreb*. was achieved in two steps: shoots development from axillary buds, followed by roots system development.

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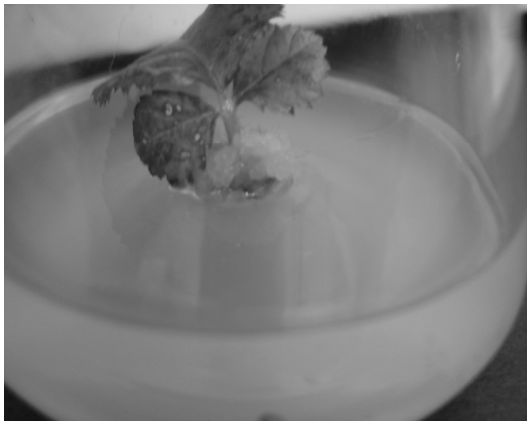

Photo 1- Callus induction at *Rubus sanctus Schreb*

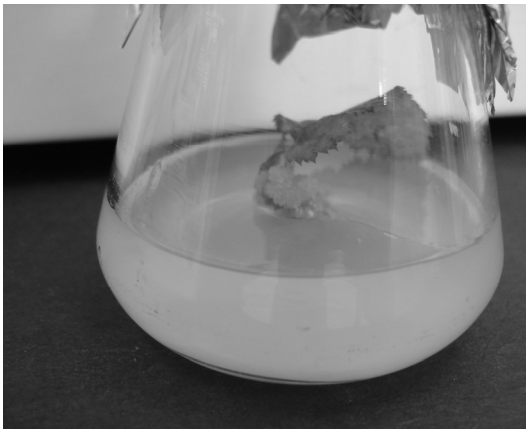

Photo 2- Cell proliferation on the surface of explant

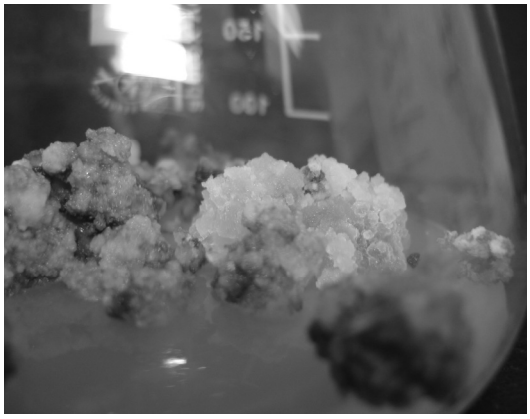

Photo 3- Primary callus cultures

Photo 4- Suspension cultures at *Rubus sanctus Schreb*

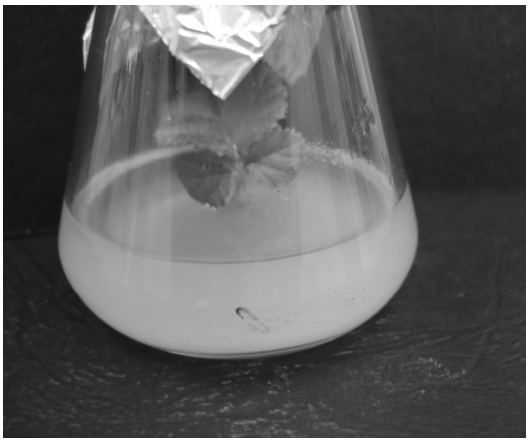

Photo 5- Caulogenesis from axillary bud

Photo 6- Stages of caulogenesis at *Rubus sanctus Schreb*

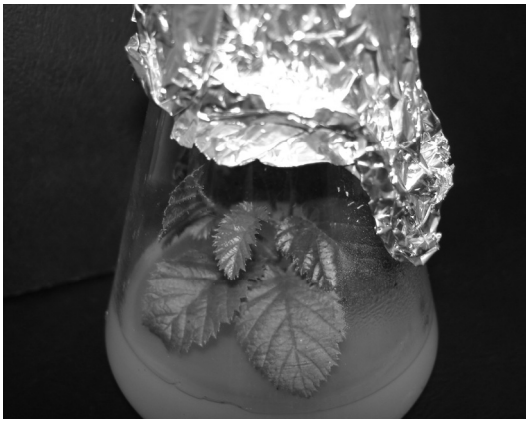

Photo 7-Multiple shoots development at *Rubus sanctus Schreb*..

Photo 8- Initiation of rhysogenesis at *Rubus sanctus Schreb*

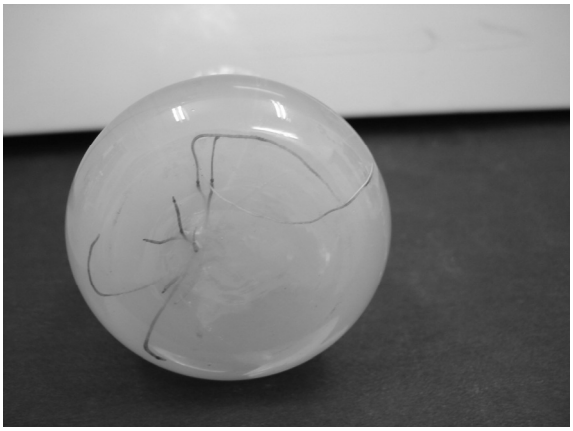

Photo 9- Roots development at *Rubus sanctus Schreb.*