In vitro cultures of *B. capillare* and *B. argenteum* were initiated from apical shoots of gametophytes and spores, respectively. Small shoots of *B. capillare* were separated from soil and the rest of substratum, and the material was placed in glasses covered with cheesecloth and rinsed with tap water for 30 min. As most mosses have filoids with one-cell layer and not protection layers, surface sterilization with 70% ethanol was inappropriate, because it damages plants.

Variously dilute solutions of commercial NaOCl bleach (8% active chlorine) were used for sterilization: 0.5; 1; 2; 3; 5; 7; 9; 11; 13%. Rinsed plant material was kept for 5 min in a bleach solution containing a few drops of liquid detergent and rinsed again with sterile distilled water. This method was effective for surface sterilization.

Immature capsules of *B. argenteum* with operculum or both with operculum and calyptra, and undamaged sporophytes were separated from the gametophytes and washed in distilled water. If calyptra was present, it was carefully separated from the capsule, taking care not to separate or damage operculum. Capsules prepared this way were kept in 9, 11, 13, 15% solution of commercial bleach for 5 min and then rinsed 3 times with sterile distilled water.

Capsules were opened by sterilized needle and the spores transferred onto a solid medium. As spores are sterile in undamaged and immature capsules we made advantage of that fact to avoid complex procedures for sterilization of tiny spores invisible to the naked eye. Small shoots of *B. capillare* (about 3 mm long) were isolated aseptically and cultured with tip side up on a solid nutrient medium.

The basal medium (BM) contained MS (Murashige and Skoog 1962) mineral salts and vitamins, 100 mg L⁻¹ myo-inositol, 30 g L⁻¹ sucrose, 0.70% agar (Tolflak purified, Belgrade), and was supplemented with 1.0 mg L⁻¹ 2, 4-dichlorophenoxyacetic acid (2,4-D) and 2.0 mg L⁻¹ kinetin (KIN). The medium pH was adjusted to 5.8 prior to autoclaving, at 115 °C for 20 min. The cultures were grown at 25±2 °C under white fluorescent light (47 μmol m⁻² s⁻¹ irradiance) and a day/night regime of 16/8 h. The plants were subcultured in one month intervals.

Surface moss sterilization with commercial bleach (8% active chlorine) gives the best results. We found that depending on plant morphology of gametophyte (position of filoids, density, etc.) the concentration of bleach solution should be varied from 0.5 to 15% in order to find out the one that provides the best sterilization without tissue damage. The results acquired in this experiment indicate that the best concentration of bleach solution for sterilization of this plant material is 9%
both for gametophytes and sporophyte. It was observed that the addition of a few drops of liquid detergent to the solution of NaOCl bleach solution improves surface sterilization of plant material and enables development in axenic culture. This we may explain by the fact that detergent decreases surface pressure and enables filoids to spread, so the solution of NaOCl bleach can reach most of the shoots.

After 10 to 14 days some changes were observed in the cultures of B. capillare, i.e secondary protonema developed. After a month, the plants gave the caulonema, but even six months after the establishing of in vitro culture, plants still failed to form protonemal and caulonemal buds. Chopra and Rashid (1969) reported that in some pottiateous mosses (gen. Anoectangium, fam. Pottiaceae) bud formation could be induced by substituting Fe-EDTA with a natural iron source (ferric citrate).

This method seems to be simple and much easier than those reported by Basile and Basile (1988) - surface sterilization by the washing machine methods, and by Cano et al. (1996) - ultrasound surface sterilization.

Another simple method that we used to establish B. argenteum culture was to collect plants with immature sporophytes, but not too young (preferably in early spring). In this way, the samples can be kept in dry and cold place for quite a long period of time. Our plants were kept for 10 days before culturing them in vitro.

Considering that capsules have better protection than filoids and young capsules are usually smooth, not plicate as filoids, all concentrations of NaOCl bleach used in this experiment showed good sterilization results.

The germination of spores was obtained 7 days after their transfer to the medium, followed by visible protonema formation within the next 8 days. Development of caulonema and buds, as well as regeneration of the whole gametophytes was obtained 3 days after the beginning of spore germination. Small leafy shoots appeared one month after the germination onset. The MS medium supplemented with with 1.0 mg L⁻¹ 2,4-D and 2.0 mg L⁻¹ KIN was very convenient for spontaneous regeneration of B. argenteum, although bryological literature suggest that Knudson solution (Knudson 1946) is much appropriate for bryophyte growing.

Chopra and Kumra (1988) reported that some mosses could not grow or even germinate without a symbiosis with fungi and bacteria.

According to Doyle (1967), Frahm and Nordhorn-Richter (1984) and Vitt et al. (1985) any plant of the Bryaceae family and certainly not any of the Bryum genus has yet been grown in the axenic culture. Very good knowledge of the biology of bryophyte appears therefore to be a considerable advantage in the process of establishing their in vitro culture.

We have shown in this experiment a very easy, effective and convenient method both for surface sterilization and in vitro culturing of two moss species.