Results and Discussion

Many experiments on micropropagation of different genetic forms of apple show that except the microbial contamination, the browning of cultures due to the exudation of polyphenols are found to be the major bottleneck for the establishment of aseptic cultures. In this experiments, the yellowing of MS medium was observed immediately 2–3 hours after buds inoculation (Fig. 1).

Regarding the effect of nutrient medium supplemented with several antioxidants and some methods of avoiding the phenol releasing from the explants, the type of treatment affected the response percentage during proliferation stage. The apple buds of two cultivars, cultured on MS medium combined with antioxidants (ascorbic acid and citric acid 0.1% each) presented greater survival percentage during the proliferation stage compared to the explants cultured on medium without these acids. The season in which the explants are collected is the major factor that affects the success of cultures establishment. In the other hand, the success of micropropagation is strongly depended from the use of disinfectants, antioxidants and avoiding of polyphenolics methods. Thus, the buds isolated in May, treated with additional agents (citric acid, polyvinyl pyrrolidone PVP and active charcoal) have a higher survival percentage (from 63% to 100%) during the first 2 weeks of proliferation stage (Graphic 1). But, was noticed that the number of survived plantlets was decreased during further periods of culture because of delayed infections symptoms or delayed polyphenol compounds release. In this case was very difficult to establish cultures for further micropropagation.

Figure 1. Development of apple buds in different stages of micropropagation

Figure 2. Plantlets development from apple mature seeds

Figure 3. Comparison of leaves number for both apple cultivars (1-cv. Starking; 2-cv. Gold) in different periods of propagation

Figure 4. Comparison of shoots length intervals for both apple cultivars (1-cv. Starking; 2-cv. Gold) in different periods of propagation

Conclusions

• In order to overcome the problem of media coloring by polyphenols that are released from buds, the most optimal nutrient medium is considered MS medium supplemented with MS vitamins and combined with citokin BAP (1 mg l⁻¹) and auxin IBA (0.1 mg l⁻¹); sucrose 30 g l⁻¹ and also agar 7%; the pH has been checked to be 5.8. Bud explants were treated by some methods in order to avoid the polyphenolic oxidation of the explants.
• Methods: i) MS + ascorbic acid 0.2%, 48 hours in the darkness and twice transfers in fresh medium, ii) MS + ascorbic acid 0.2% and 72 hours in the darkness, iii) MS + polyvinyl pyrrolidone (PVP) 0.1%, ascorbic acid 0.1%, citric acid 0.1%, 48 hours in darkness and some times of transfers in fresh medium, iv) MS + active charcoal 1 g l⁻¹ and 24 hours in darkness, v) MS + ascorbic acid 0.1%, 48 hours 0.05%, 48 hours in darkness and twice transfers in fresh medium, vi) MS medium with ascorbic acid 0.1%, citric acid 0.05% and active charcoal 1 g l⁻¹, 2 days in darkness and onetime transfer to fresh medium.

Conditions of in vitro culture chamber. The test tubes are placed in the culture chamber with controlled physical conditions (temperature: 23°C, photoperiod: 16 hours with light/24 hours).

Figure 4. Comparison of shoots length intervals for both apple cultivars (1-cv. Starking; 2-cv. Gold) in different periods of propagation

References
