THE ROLE OF PHYSICAL AND EPIGENETIC FACTORS ON CALLUS FORMATION *IN VITRO POTENTILLA RECTA L*. SUBSP. *LACINIOSA* (WALDST. ET KIT. EX NESTLER) NYMAN

O. Zaiats, I. Mitrofanova

The possibility of callus formation in vitro has been shown. The strains of Potentilla recta callus which are differ by light – brown color, compact texture and low vitrification have been selected during the experiment. In this case significant discrepancies in the quantitative characteristics of the obtained strains due to the type of explants, its placing on the media and physical factors of culture (darkness or light) have been found.

Potentilla recta, calluogenesis, explant, physical factors, in vitro.

Genus *Potentilla* from Rosaceae family is well known since ancient times due to its healing properties [10]. One of the species from this genus which is used in traditional medicine and homeopathy is *Potentilla recta* L. [1]. This perennial medical herbaceous plant has bactericidal, astringent and hemostatic effect. Rhizomes of *P. recta* are used for treatment and prevention of dysentery, ulcerative colitis, gastritis, cholecystitis and liver cirrhosis. Water and ethanol extracts of *P. recta* are used for wounds` treatment [1, 5, 7, 11].

Nowadays more than one third of medicines are made from stock plant material and chemical structure of substances is so complex that plants will be their only source for long time [6]. Due to the actuality of high quality stock medical material production it`s important to find new alternative and economically valuable sources of biologically active substances of plant origin. One of these sources is plant cell, organs and tissue culture.

Though ornamental and medical effects of species genus *Potentilla* are well known only single publications are devoted to the investigations of their callus formation *in vitro* [2, 8].

The aim of the investigations is to determine special features of *in vitro* callus formation for *Potentilla recta*.

Materials and methods. Objects of our investigations were leafstalks segments and parts of leaves from aseptic culture of *P. recta* L. subsp. *laciniosa* (Waldst. et Kit ex Nestler) Nyman. In our work we used common biotechnological methods [4] and methods developed in Nikitsky Botanical Gardens – National Scientific Centre (NBG-NSC) [3]. Manipulations with aseptic material were made in laminar “Fatran” (Czech Republic) and BP-4-004 (Ukraine). Leafstalk segments and leaf parts were cultured on

the modified Pierik medium [9] submitted with 6-BAP, 2,4-D or kinetin and NAA (Sigma, USA) in different concentrations and proportions. They were cultured in thermostat in dark under the temperature 26±1 °C or in the growth chamber under the temperature 24±1 °C, 16 h photoperiod and light intensity 2000-3000 Lux. Frequency of callus formation was estimated as per cent of explants that formed callus from their common number. For callus morphology analyses stereomicroscope MBS-10 (Russia) was used. Experiment was made in three variants. Statistical analyses were made using criteria Kruskal-Wallis, Mann-Whitney and chi-squared test. For calculation programmer *StatSoft Statistica* 10.0 was used.

Conclusions

It has been determined that culture conditions significantly influence on frequency of callus formation ($p = 0.042$) and frequency of rhizogenesis *in vitro* ($p = 0.001$) in different explants of *P. recta*. It has been found out that light had significantly negative influence on frequency of callus formation from leaf parts in abaxial ($p = 0.04$) and adaxial ($p = 0.02$) positions to the medium. For all types of explants and their positions on the medium significant positive influence of light on the frequency of rhizogenesis ($p \leq 0.05$) has been determined.

It has been noticed that under the light influence accumulation of dry and wet callus mass significantly decreases. Significant effect of culture conditions (light or dark), type of explants and its position on the medium on the accumulation of wet callus mass ($p = 0.029$) has been found out.

It has been demonstrated that leaf parts in abaxial position on the medium under their culture in dark had the highest indexes of accumulation of wet (31.3±18.1 mg per explants) and dry (10.2±8.0 mg per explants) callus biomass.

Список літератури

1. Вторичные метаболиты фенольной природы *Potentilla alba* L. в условиях *in vitro* / М.В. Китаева, А.В. Зубарев, Е.В. Спиридович, В.Н. Решетников // Труды БГУ. – Минск, 2011. – Т. 6, ч. 1. – С. 123–127.
2. Китаева М.В. Введение в культуру *in vitro* редких лекарственных растений *Potentilla* L./ М.В. Китаева // Интродукция, сохранение и использование биологического разнообразия мировой флоры: материалы междунар. конф., посвященной 80-летию Центр. бот. сада Национальной академии наук Беларуси, (Минск, 19–22 июня 2012 г.). – Минск, 2012. – С. 398–401.
3. Калинин Ф.Л. Методы культуры тканей в физиологии и биохимии растений / Калинин Ф.Л., Сарнацкая В.В., Полищук В.Е. – К.: Наук. думка, 1980. – 488 с.
4. Методы биотехнологии в селекции и размножении субтропических и косточковых культур / О.В. Митрофанова, И.В. Митрофанова, А.В. Смыков, Н.П. Лесникова // Сб. тр. Никит. ботан. сада. – 1999. – Т. 118. – С. 189–199.
5. Орлова Л. Лечение корнем лапчатки / Л. Орлова. –Мн. Харвест, 2006. – 64 с.
6. Пасешниченко В.А. Растения–продуценты биологически активных веществ / В.А. Пасешниченко // Соросовский образовательный журнал. – 2001. – Т. 7, № 8. – С. 13–19.
7. Растительные ресурсы СССР: Цветковые растения, их химический состав и использование / [отв. ред. П. Д. Соколов]. – Л.: Наука, 1987.– С. 59-69.

8. Kovalenko P.G. Secondary metabolites synthesis in transformed cells of *Glycyrrhiza glabra* L. and *Potentilla alba* L. as producents of radioprotective compounds / P.G. Kovalenko, V.P. Antonjuk, S.S. Maliuta // Ukrainica Bioorganica Acta. – 2004. – №1-2 – P. 13-22.

9. Pierik R.L. *Anthurium andreanum* plantlets produced from callus tissues cultivated *in vitro* / R.L. Pierik // Physiol. Plant. –1976. – Vol. 37, №1. – P. 80-82.

10. Tomczyka M. *Potentilla* — A review of its phytochemical and pharmacological profile / M. Tomczyka, K.P. Lattéb // Journal of Ethnopharmacology. – 2009. – Vol. 122, Issue 2. – P. 184-204.

11. Tosun A. Antimicrobial activity of some plants used in folk medicine in Turkey / A. Tosun, Ö. Bahadir, N. Altanlar // Turkish Journal of Pharmaceutical Sciences. – 2006. – №3. – P. 167-176.