Biotechnological Aspects of Micropropagation of Salicaceae Genus Plants

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Abstract – The practical significance of the work is to create a high-performance hybrids of willows, production a large number of healthy, genetically identical plant material in vitro for energy security and environmental management.

It is offered the scheme of biotechnology process of obtaining plants regenerants of Willow family and their adaptation.

Key words – Salicaceae, explant, callus, micropropagation, morphogenes

I. Introduction

The recent energy independence and climate change policies encourage development and utilization of renewable energy such as bioenergy. According to the IEA World Energy Outlook 2012, primary demand for bioenergy will strongly increase up to the year 2035: the demand for biofuels and biomass for electricity is expected to triple.

The growth capacity of plants are the most importance for a tree species which should serve the biofuel sector. The tree species of Willows family are a strong contenders as biomass producers, but also for otherwood assortments.

Creating energy plantations plants of the family Salicaceae Lindl. is one of the pressing problems of our time, requiring scientific justification functioning plant morphogenic mechanisms and establishing incentives to produce quality planting material in vitro. With the active development of biotechnology is possible to accelerate the process of propagation of woody plants and receive a short time the right amount healed and viable plant material.

Among the significant number of members of the genus deserve special attention S. viminalis, S. fragilis, S. babilonica, P. nigra×P. balsamifera, P. tremula green-bark form, S. alba, S. matsudana 'Tortuosa' [2, 3, 5]. As a modern alternative to traditional methods of plant propagation is under conditions in vitro, which allows you to get the required number of genetically uniform planting material healed during the year regardless of the growing season [1]. Although the technology microclonal reproduction of many plants of Salix have been working quite well [1, 3], but the research results that would form the whole process of reproduction are not sufficient or missing. Therefore, the aim of research was the development of biotechnology microclonal plant propagation S. alba, S. fragilis, S. babilonica, S. matsudana 'Tortuosa', P. tremula recovered to produce plant-regenerants with subsequent intended use.

II. Methods and research objects

The object of research were representatives of the genus Salicaceae: S. viminalis, S. fragilis, S. babilonica, P. nigra×P. balsamifera, P. tremula green-bark form, S. alba, S. matsudana 'Tortuosa'

For research were taken parts of annual shoots length of 10-15 cm, which were isolated from 2-10-year-old donor plant in February to June. The process of sterilization was performed with using: 70% ethanol (1 min), 2,5% NaClO (10-20 min.), 1% $AgNO_3$ (10-20 min.) 0,1% $HgCl_2$ (5-10 min.) 25 % H_2O_2 (10 min).

For researching of regeneration capacity of tissues and organs at in vitro condition were used nutrient medium MS [4] with the addition of auxin growth regulators (IBA, NAA, 2,4-D) and cytokinin (BA, TDZ, kinetyn) types of action. Also for medium components added 100 mg · l-1mezoinozytol, 30 $g \cdot l^{-1}$ sucrose, 2 $g \cdot l^{-1}$ activated carbon and 6,7-7,0 $g \cdot l^{-1}$ microbiological agar. Callus tissue have been obtained from sterile leaf plates (0,4-0,5 cm^2) and parts of microshoots (0,5-0,8 cm). Plant material was cultivated in a light room and TS-80 thermostat at 25 ± 10 °C and lighting 2,0-3,0 KLK 16-hour photoperiod and a relative humidity of 70-75%.

III. Results and discussion

Have been established the most effective sterilization which provide for 80% aseptic and active plants of explants: S. alba, S. fragilis, S. babilonica and S. matsudana 'Tortuosa' with using of 1% $AgNO_3$ for 10 min, followed by transfer at 2,5% NaClO. For P. tremula, P. nigra×P. balsamifera, S. viminalis effective sterilization which provide more than 98 % of aseptic plants are consist of the use of 70 % (C_2H_5OH) (30 sec), 1 % ($AgNO_3$) (7 min), washing in sterile water (1 min), 25 % (H_2O_2) (10 min), washing in sterile water (5 min).

For getting explants by artificial activation in vitro meristem in February, it is advisable to use a 0,1 % solution of $MgCl_2$ for 5 min.

Have been selected the optimum compositions of the nutrient mediums for microclonal propagation, rooting and receiving plants regenerants.

Activation of existing meristem explants, microshoots development and formation of the root system of S. viminalis were performed with using 0,1 $mg \cdot l^{-1}$ BA and kinetyn for P. nigra×P. balsamifera (0,25 $mg \cdot l^{-1}$ and kinetyn IBA) for P. tremula (0,5 $mg \cdot l^{-1}$ TDZ)

The Getting of the considerable number of plants microshoots of S. viminalis (by direct morphogenesis) have been fixed in the culture medium with adding of 2,0 $mg \cdot l^{-1}$ BA for hybrid P. nigra×P. balsamifera (mixed type of morphogenesis – direct and indirect) – 0,5 $mg \cdot l^{-1}$ BA and 20 $mg \cdot l^{-1}$ for adenine and P. tremula 0,5 $mg \cdot l^{-1}$ TDZ (mixed type morphogenesis – direct and indirect with leaf, stem and root explants) 0,25 $mg \cdot l^{-1}$ kinetyn – direct morphogenesis, for S. alba (0,25 $mg \cdot l^{-1}$ kinetyn), S. fragilis (without hormones), S. babilonicay S. matsudana 'Tortuosa' (0,25 $mg \cdot l^{-1}$ kinetyn and 2 $mg \cdot l^{-1}$. (Fig. 1).

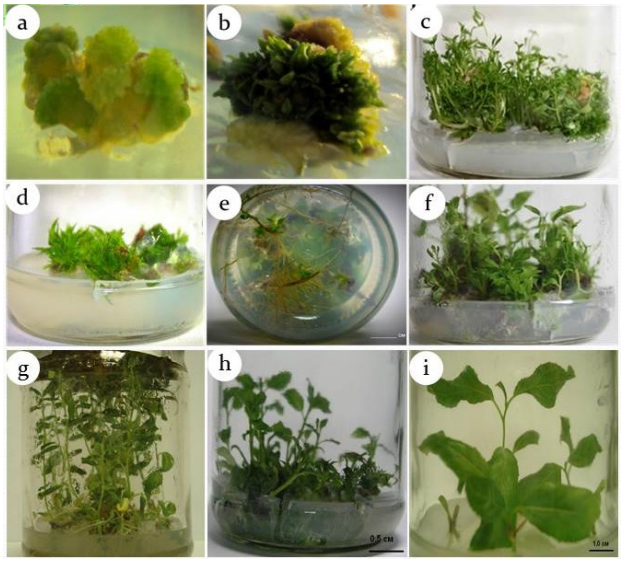

Fig. 1. Stages of direct and indirect morphogenesis and the formation of different representatives Salicaceae plant-regenerants in vitro: a – activation of the synthesis of chlorophyll in the areas of morphogenic callus explants in *P. tremula* (indirect morphogenesis); b - morphogenic callus obtained from leaf explants of *P. tremula*; c – microshoots formation of *P. tremula* by indirect morphogenesis (passage 2); d, e, f - microshoots formation by direct morphogenesis; g – plant regenerants of S. viminalis; h, i – microshoots formation of P. nigra × P. balsamifera (direct morphogenesis).

The use of ½ MS nutrient medium with the addition of sucrose 15 mg·l^{-1} explants S. viminalis, 30 mg·l^{-1},hybrid P. nigra×P. balsamifera and withouth hormones MS, or with the addition of 0,25 mg·l^{-1} for P. tremula made it possible to obtain a significant multiplication factor of plant-regenerants (25,5 and 10,0) for the 28-30-day cycle of cultivation.

For microclonal plant propagation S. viminalis, P. nigra × P. balsamifera and P. tremula studied by indirect morphogenesis processes of induction, intensity and regenerative ability of forming callus explants on different types by using a different growth regulators and duration cultivation. In the nutrition medium with the addition of 1,0 mg·l^{-1} 2,4-D and 2,0 mg·l^{-1} IBA for plants S. viminalis, 2,0 mg·l^{-1} 2,4-D for P. nigra × P. balsamifera and 2,4-D 1,5 mg·l^{-1}, TDZ 0,5 mg·l^{-1} for P. tremula, obtained morphogenic callus tissue and primary callus formation 98,9 ± 0,2%.

It is shown that the most intensive average increase in wet weight callus hybrid P. nigra × P. balsamifera held at the 7th passage in culture medium with 0,5 mg·l^{-1} 2,4-D.

It was investigated and established plant operation mechanisms underlying proliferation, cell differentiation, histogenesis, organogenesis and regeneration of the whole organism cultured from tissues and organs of plants S. viminalis, P. nigra × P. balsamifera and P. tremula in vitro. Investigated regenerative capacity of tissues and organs in vitro plants under the influence of culture media components.

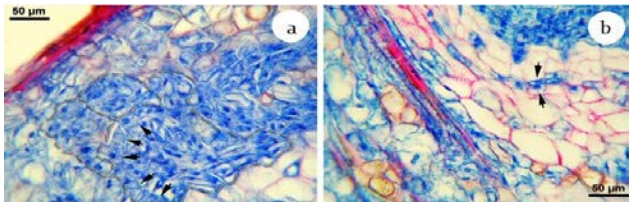

Fig. 2. Initiation of the formation and development of adventitious roots in callus of aspen in vitro: a – the area of the distal leaf petiole, b – in the basal part of the stem (arrows marked formation of protoderm in the bud of root, staining water blue and safranin)

Developed new (direct) way of adapting plants to the conditions regenerants open ground, which eliminates the steps of "climate chamber" and "closed ground" that allows you to get a significant amount of above-ground mass already in the first year of growth for its processing into solid biofuels.

Conclusion

The research of micropropagation of different representatives of Salicaeae were done. It is established that ways of morphogenetic potential tissue explants of plants by in vitro selection, modification of nutrient components and cultivation conditions at different stages of morphogenesis.

The processes of callus induction and indirect morphogenesis for different types of explants of were research. Using cytological and anatamo-histochemical analysis the effect of exogenous hormonal factors on the differentiation of cells and indirect processes of morphogenesis were determined.

The basic conditions for induction the process of root formation of S. viminalis, S. fragilis, S. babilonica, P. nigra×P. balsamifera, P. tremula green-bark form, S. alba, S. matsudana 'Tortuosa were determined. Found most effective adaptation of plants in vitro in a greenhouse, thus ensuring 100% engraftment adapted plants in the environmental conditions.

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