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PLANT INDUSTRY

Experimental Morphogenesis in a Curly Birch Tissue Culture

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Abstract—A technology was developed for clonal micropropagation of curly birch (*Betula pendula* Roth var. *carelica Merckl.*) by activation of meristems existing in the plant and induction of the formation of adventitious buds from primary and secondary callus tissue.

Keywords: curly birch, tissue culture, microsprouts, adventive buds, callusgenesis, meristem
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The problem of mass propagation of ornamental trees is becoming urgent in connection with the increasing interest in Kazakhstan in new plants as well as development of indoor and outdoor landscaping of cities with trees and shrubs. This is because of the virtual absence in the country of forest nurseries for their production and decrease in the proportion of imported planting stock, which doesn't always meet the standard.

The most promising at present is the method of in vitro clonal micropropagation of plants, which is used widely in agriculture and more rarely in forestry. For the majority of tree species there are no technologies for their mass propagation under in vitro conditions. Therefore, it is necessary to develop new and improve existing clonal micropropagation technologies.

Of interest to breeders, practical workers, and forestry specialists is the curly birch (*Betula pendula* Roth, var. *carelica* Merckl.), distinguished from other birch species by a highly decorative figured wood widely used in the furniture industry and handicraft trades. The main method of its propagation is by seed, in which case, however, segregation of traits in figured and nonfigured forms occurs in progeny, which leads to loss of economically valuable forms of the given plant species in natural populations. Replacement of the seed propagation method by vegetative eliminates these shortcomings [1–3]. Curly birch belongs to species difficult to propagate by the vegetative method; therefore, investigations to develop new technologies of its propagation with the use of cellular biotechnology methods are needed.

METHOD

One-year-old shoots 15–20 cm long isolated from the crown of a mature curly birch tree were the object of investigation. The shoots contained dormant apical and axillary buds. The cut shoots were placed in a vessel with water and held at room temperature until the stage of appearance of a green growing point and young shoots, which were subsequently used for introduction into an in vitro culture [4].

The shoots were treated with 96% alcohol, after which they were divided into segments (1–1.2 cm) and placed in a saturated sodium hypochlorite solution for 30–45 min or in a 3% chloramine solution. Then the sterilized segments were washed three times with sterile distilled water and placed on sterile Murashige and Skoog (MS) medium [5] containing 0.5 mg/l BAP (6-benzylaminopurine), 0.2–1.5 mg/l 2,4-D (2,4-dichlorophenoxyacetic acid), or 0.5 mg/l BAP and 0.2 mg/l NAA (α-naphthyleneacetic acid), inducing callogenesis or activating the development of existing meristems [6, 7]. Explants were grown in a light room, where a temperature of 26°C, 16-photoperiod, and light intensity of 300 lx were maintained.

Since mainly a balanced complex of phytohormones initiates secondary differentiation of cells, we investigated the effect of auxins IAA, NAA, and 2,4-D in a concentration of 0.5 mg/l and the cytokinins kinetin and BAP in a concentration of 2 mg/l on the formation of morphogenic structures in curly birch callus

Table 1. Callogenesis in curly birch tissue culture depending on 2,4-D concentration

2,4-D, mg/l	Callogenesis, %	Increment of callus tissue, mg
0.2	0	0
0.5	40.1 ± 8.2	39.7 ± 1.4
1.0	66.7 ± 9.8	62.0 ± 2.1
1.5	43.4 ± 7.9	56.0 ± 1.2

Table 2. Morphogenesis in curly birch callus tissue culture under the effect of phytohormones

Index	Kinetin (2 mg/l)			BAP (2 mg/l)		
	IAA*	NAA*	2,4-D*	IAA*	NAA*	2,4-D*
Quantity of morphogenic callus, %	42.3 ± 3.7	33.2 ± 2.6	12.3 ± 2.7	73.6 ± 6.4	45.3 ± 4.7	15.7 ± 4.3
Average number of adventitious buds per callus; (9)	4.6 ± 0.4	3.7 ± 0.3	3.3 ± 0.7	8.1 ± 0.9	4.7 ± 1.3	4.0 ± 1.0

* Concentration, 0.5 mg/l.

tissue. Heterogeneous green globular calli were used as the initial material, and MS medium was the base. Data were recorded 30 days after the moment of culturing the callus tissue on the inducing media.

The shoots that formed were propagated by the method of activating the development of meristems in the plant (micrografting), which is based on removal of apical dominance. In this case, the microshoots are divided into segments containing two axillary buds and cultured on a hormone-free nutrient medium with half macrosalt strength according to the WPM or MS recipe. The propagated microshoots were rooted on WPM nutrient medium containing indolebutyric acid (IBA)in a concentration of 3 mg/l. Adaptation of the microplants was done under conditions of 90–95% humidity at 23–25°C. A mixture of peat and sand in a 1 : 1 ratio was used as the substrate. After 2 weeks adaptation, the plants were transferred to a greenhouse.

RESULTS AND DISCUSSION

During culturing of explants in vitro for 3–4 weeks on MS medium containing 2,4-D and BAP, we observed the formation of disorderly growing callus tissue of the friable type in the basal part of the shoot. It was established experimentally that the process of callogenesis depends directly on the 2,4-D concentration in the nutrient medium. However, at high concentrations of this auxin, a decrease in the value of the index was noted, which affected the growth rate of the callus tissue (Table 1). Thus, the main condition for callogenesis in a curly birch tissue culture is the addition of exogenous auxin 2,4-D in a concentration of 1 mg/l.

After 4 weeks culturing, morphogenic structures, represented by dense dark green sections (meristematic foci) consisting of the growing point and leaf anlagen. Regenerant plants subsequently developed from. Maximum increase of the multiplication ratio was observed with the addition to the culture medium of 0.5 mg/l IAA and 2 mg/l BAP (Table 2). The average number of induced buds de novo in this variant was 8.1 per callus. In other variants the indices were 1.5–2 times lower, and the adventitious buds that formed were characterized by slow growth. Repeated culturing of callus tissue on the investigated nutrient media again led to the formation of adventitious buds at an average rate of 6–8 per callus, from which microshoots subsequently formed.

Under conditions of micrografting and culturing microshoots on hormone-free nutrient medium with half macrosalt strength, we observed active growth of axillary buds and the formation of microshoots characterized by normal morphology. The percent of rooted plants upon propagation of microshoots on WPM medium with 3 mg/l IBA was 85.3.

The height of the completely formed plants after 2 weeks adaptation and transfer of the microplants to the greenhouse reached 24–32 cm after 6 months.

Thus, as a result of multifarious experiments, in vitro curly birch culturing conditions were optimized and a technology of clonal micropropagation of this plant both by callus tissue and by activation of the development of axillary buds was developed.

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