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BIOCHEMISTRY, BIOPHYSICS, AND MOLECULAR BIOLOGY

Effect of Glutamine Synthetase Gene Overexpression in Birch (*Betula pubescens*) Plants on Auxin Content and Rooting in vitro

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Abstract—The effects of transformation of downy birch (*Betula pubescens* Ehrh.) with the *GS1* gene encoding the cytosolic form of glutamine synthetase on the rooting of plants in vitro was studied. The transgenic plants had an elevated content of glutamine as well as glutamic and aspartic acids and rooted more rapidly than the control plants. Rooting on a medium containing the glutamine synthetase inhibitor phosphinothricin prevented the accumulation of auxin in birch plants carrying the *GS1* gene, indicating the involvement of this enzyme in raising the level of auxins in the transgenic plants. The correlation between the increase in the auxin levels in the transgenic plants carrying the glutamine synthetase gene and the increase in the rooting rate is shown for the first time.

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The regulation of growth and development of roots plays an important role in the adaptation of plants to environmental conditions. However, the root biology has traditionally received less attention as compared to the above-ground parts, in accordance with the adage "out of sight—out of mind." Nevertheless, in the last decade, the interest in the growth and development of roots has markedly increased, due to the possibility of increasing the yield of plants (sort of "underground revolution," similarly to the "green revolution" of the mid-20th century) [1]. It is well known that auxins play an important role in the regulation of root growth and development [2]. These processes greatly depend on the availability of macroelements, in particular, nitrogen [3]. Meanwhile, one of the directions of increasing the productivity of plants by means of genetic engineering is the transfer of the genes encoding nitrogen metabolism enzymes (primarily glutamine synthetase (GS)). Although studies in this direction have been performed since the beginning of the 1990s, the effect of the expression of additional copies of GS genes on the rooting of transgenic plants has not been assessed until recently. In the only available study [4], it was shown that phosphinothricin, a GS inhibitor, had a stimulatory effect on the root system formation in vitro in several transgenic lines of aspen plants carrying the GS gene, unlike the nontransgenic control. A significant increase in the level of 3-indoleacetic acid (IAA) was detected in the leaves of one of the two transgenic lines of hybrid poplar plants carrying the GS gene; however, rooting was not studied in this case [5]. It is known that a low rooting capacity is a problem in vegetative propagation of woody species.

The purpose of this study was to investigate the effect of birch transformation with the glutamine synthetase gene on the auxin content and rooting of transgenic plants in vitro.

This study was performed with the downy birch (*Betula pubescens* Ehrh., genotype Bp3f1) plants and the transgenic line F14GS8b carrying the *GS1* gene, which encodes the cytosolic form of GS from the Scots pine (*Pinus sylvestris* L.). The transgenic plants were obtained by us previously [6] by *Agrobacterium*-mediated transformation of leaf explants with the pGS vector. The plants were propagated on WPM medium supplemented with 0.3 mg/L growth regulator 6-benzylaminopurine, which was excluded from the culture medium at the last passage before rooting. After proliferation, the tips of shoots approximately 2 cm long were planted in rooting medium (WPM medium without hormones) containing or not containing 0.1 mg/L phosphinothricin (the minimum concentration that causes inhibition of root formation in nontransgenic birch plants). All media contained 30 g/L sucrose. The plants were cultured at 22–24°C at a 16 h light/16 h dark photoperiod. The rooting frequency was determined on days 5, 7, and 10 after the

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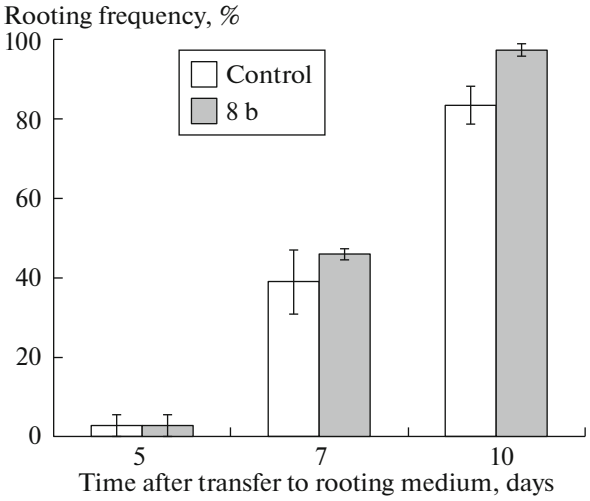

Fig. 1. Rooting frequency of the original (control) and transgenic (8b) birch plants. Data are represented as $M \pm m$, $n = 4$.

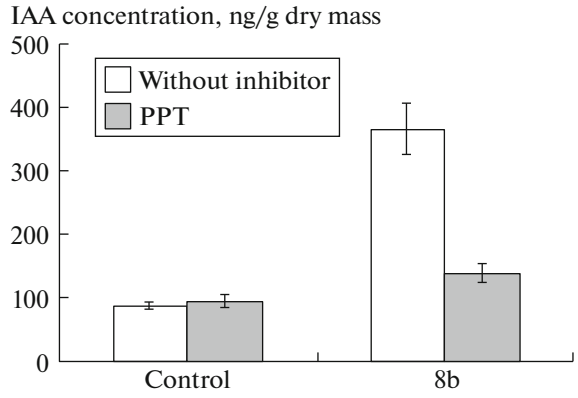

Fig. 2. Concentration of IAA in the original (control) and transgenic (8b) birch plants depending on the treatment of plants with the inhibitor phosphinothricin (PPT, 0.1 mg/L). Data are represented as $M \pm m$, $n = 3$.

beginning of culturing. Auxins were extracted from shoot explants with 80% ethanol on day 18 after transplantation. 3-Indoleacetic acid (IAA) was purified and concentrated by ether extraction using the modified scheme described in our earlier paper [7]. Briefly, IAA was extracted twice with diethyl ether in a ratio of 1 : 3 from an aqueous-residue aliquot acidified to pH 2–3. After extraction, IAA was returned to the aqueous phase with 1% sodium hydrogen carbonate (the ratio of the aqueous and organic phases was 1 : 2), acidified once again to pH 2–3, re-extracted twice with diethyl ether, and then methylated with diazomethane. The samples were dried and dissolved in a small amount of 80% ethanol. Then, immunoassay of auxins was performed using specific antibodies to IAA as described in [8].

The amino acid content was determined by ion exchange chromatography with a Biochrom 30 amino acid analyzer (Biochrom Ltd., United Kingdom) using a system of lithium citrate buffers as described previously [9].

The transgenic birch plants carrying the GS gene exhibited an increased rooting rate (Fig. 1). Since auxins play an important role in the root system development, it was of interest to compare their level in the transgenic and non-transformed plants. As can be seen in Fig. 2, the auxin content in the F14GS8b plants was more than threefold higher than in the control plants. The inhibition of GS activity by the inhibitor prevented the accumulation of auxins, and the transgenic plants treated with phosphinothricin did not differ in their level from the control plants (Fig. 2), which confirms the involvement of GS in raising the auxin level in the transgenic plants. Thus, the increase in the rooting rate in the transgenic birch plants, which was observed in our experiments, might be due to the higher content of auxins in them.

The assessment of the content of amino acids in plant explants showed that the level of glutamine was 1.5 times higher in the transgenic plants compared to the control (Table 1), which was apparently due to the pine GS gene expression in the transgenic birch plants. In addition, we found a significant increase in the content of aspartic and glutamic acids. However, the total amino acid content remained almost unchanged (increased by 3%).

Hybrid poplar plants carrying the GS gene [5] had higher levels of both individual amino acids (glutamine 2.3 times, glutamate by 51%, and aspartate by 32%) and the total amino acid content (by 20%). However, this may be due to the genotypic characteristics (poplar and birch), culturing conditions (greenhouse and in vitro), as well as to the fact that the poplar was grown under high nitrogen level. A significant increase in the glutamine, glutamate, and aspartate content was also detected in the leaves of tobacco [10] and *Arabidopsis* [11] plants transformed with the chloroplast isoform of GS. It should be noted that the transgenic plants carrying the GS gene had an increased content of those amino acids that play a major role in nitrogen transport and remobilization in plants [11]. However, control and transgenic plants with GS gene did not differ in the amino acid content in the storage organs (sorghum seeds) [12].

Since the initial phase of the tryptophan-dependent and independent pathways of IAA biosynthesis is the transfer of the α-amino group of glutamine to chorismate to form anthranilate [5], the increased IAA concentration in the transgenic plant tissues may be caused by the increase in the content of glutamine. The increased levels of glutamine and glutamic acid in the transgenic plants (the content of the latter was 10% higher than in the control) also indicates that these amino acids may perform a specific function in the

Table 1. Amino acid content in the leaves of the original (bp3f1 control) and transgenic (F14GS8b) birch plants (the amino acids whose content in the transgenic plants was significantly higher than in the control are shown in bold)

Amino acid	bp3f1	F14GS8b
Lysine	13.6 ± 0.2	6.1 ± 0.1
Histidine	10.2 ± 0.4	8.1 ± 0.2
Arginine	70.0 ± 0.6	48.4 ± 0.3
Aspartic acid	**79.1 ± 0.7**	**102.5 ± 0.6**
Threonine	13.0 ± 0.2	14.8 ± 0.3
Serine	64.0 ± 1.3	50.4 ± 0.6
Glutamic acid	**208.0 ± 1.7**	**233.2 ± 0.7**
Glycine	10.2 ± 0.3	8.4 ± 0.3
Alanine	59.6 ± 0.8	48.2 ± 1.0
Valine	8.0 ± 0.3	6.5 ± 0.1
Methionine	3.0 ± 0.1	3.5 ± 0.1
Isoleucine	5.7 ± 0.2	5.5 ± 0.3
Leucine	7.6 ± 0.3	6.6 ± 0.3
Tyrosine	16.0 ± 0.4	18.4 ± 0.5
Phenylalanine	11.5 ± 0.3	14.5 ± 0.3
Asparagine	38.3 ± 0.7	26.0 ± 0.3
Glutamine	**70.0 ± 1.1**	**102.6 ± 4.7**
α-Aminobutyric acid	17.1 ± 0.5	20.8 ± 0.4
Total amino acid content	704.9	724.5

$M \pm m$, $n = 3$.

regulation of auxin synthesis, which is confirmed by the results of assessment of the effect of the glutamine synthetase inhibitor on the IAA accumulation in the transgenic plants. Previously, the effect of glutamate on the root development in *Arabidopsis* plants was shown [13]. The authors of the cited paper explained this fact by the signaling function that involves the glutamate receptor. The discovered coincidence in the localization of glutamate receptor and GS [14] was interpreted as evidence for their interaction and a possible involvement of GS in the regulatory function of the receptor. A reduced glutamate sensitivity, which was detected in an auxin transport mutant, indicates a relationship between glutamate and auxin signaling [15]. In this study, we have shown a correlation between the increase in the level of auxins in transgenic plants, GS gene, and the increase in the rooting rate, which may be a manifestation of the signal transmission mechanism involving the glutamate receptor. Further studies are required to test this correlation in detail.

The phenomenon discovered by us has not only theoretical but also practical significance to improve the clonal micropropagation, when the ex vitro survival of plants depends primarily on the rooting ability of microshoots.

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