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Induction of the alternative metabolic route of tyrosine in potato through introduction of tyrosine hydroxylase gene from laboratory rat (Rattus norvegicus)

Abstract

Catecholamines are biogenic aromatic amines common among both animals and plants. This group of compounds was thoroughly studied in animals because of the functions that they perform in these organisms – they are neurotransmitters in the nervous system and hormones regulating many physiological processes, including sugar metabolism. Their function in plants is poorly understood. They can serve there as precursors for other biochemical compounds, including alkaloids (morphine, papaverine), pigments (betalain, melanin) and components of cell wall (amides with hydroxycinnamic acids). Catecholamines are characterized by high antioxidative potential and their derivatives may participate in plant's response to pathogen infection. Because of low concentrations of catecholamines in plants and analogous function to that in animals, like influence on sugar metabolism or modulation of other hormone functions, hormonal activity was proposed for this group of compounds. Changes in their content in stress responses was observed. However, no receptor specific to catecholamines, was found in plants.

All catecholamines are produced from tyrosine, which is formed through hydroxylation of phenylalanine. Both of these aminoacids are the starting pointfor the biosynthesis of a large group of secondary metabolites – phenolic derivatives, which include phenolic acids, benzoic derivatives, lignins or flavonoids. It is accepted that the primary route of catecholamine biosynthesis in plants is based on the transformation of tyrosine to tyramine, which is catalyzed by tyrosine decarboxylase, wnd further hydroxylation of tyramine to dopamine. An alternative route of biosynthesis of these compounds assumes first the hydroxylation of tyrosine to L-DOPA and then its decarboxylation do dopamine. This is the primary route of catecholamine biosynthesis in animals. A gene coding for tyrosine hydroxylase was remains unidentified in plants so far. The aim of this study was generation of this alternative route of catecholamine production in potato by introduction of the sequence coding for tyrosine hydroxylase from laboratory rat (*Rattus norvegicus*) and verification of its functionality and influence on the plant physiology. The selection of potato as the subject of investigations resulted from the studies on the role catecholamines in this organism based on transgenic plants with tyrosine decarboxylase overexpression performed before in the Laboratory of Genetic Biochemistry of the University of Wroclaw. Higher levels of tyramine and catecholamines were measured in those plants and their influence on sugar metabolism was noted. Starch content rise with simultaneous soluble sugar increase was observed in tubers. In addition, presumably because of induced changes in phenylpropanoid content, the plants were more resistant to pathogen infections. In the transgenic plants generated within this study a quite different response was observed. Introduction of rat tyrosine hydroxylase led to increased content of the product of the reaction

catalyzed by this enzyme, namely L-DOPA, though the level of catecholamines (and the products of their catabolism) was decreased compared to the control, which suggests that the main route of catecholamine biosynthesis in potato is that through decarboxylation of tyrosine to tyramine. L-DOPA can be secreted by some plants as an allelochemical and its toxicity lays in its oxidation to chinons with simultaneous production of free radicals. Studies on the potatos expressing the rat tyrosine hydroxylase suggest that the L-DOPA produced in these plants does not serve as a substrate for catecholamine production, but constitutes a stressogenic factor. Higher content of H_2O_2 confirms this hypothesis. The plant's response to this condition is a reaction typical for the plants submitted to oxidative stress – increased production of antioxidants – mainly flavonoids, reinforcement of cell wall (production of amides of tyramine and hydroxycinnamic acids) and activation of genes involved in free radical neutralization. Such a condition of prolonged stress is a reason for plant's increased resistance to pathogen infection. Possibly because of decreased catecholamine level no effect characteristic for the potatoes with tyrosine decarboxylase overexpression, that is starch content decrease, was observed. The productivity of the transgenic potatoes was similar to the control, however, the plants produced higher number of smaller tubers. On the other hand though a somewhat lowered level of glycoalkaloids, which are characteristic for potatoes – α-solanine and α-chaconine and higher protein content in tubers, together with elevated levels of phenolic antioxidants may all be positive characteristics from the consumer's point of view.

The studies on the effect of induction of the alternative route of tyrosine metabolism towards catecholamines resulted in the acquirement of the new knowledge on the possibility of manipulating the metabolic routes in plants by introduction of systems typical for animals. Some new questions arose, as for example how do catecholamines participate in plants' resistance, is it simply an induction of oxidative stress or is it a more sophisticated form of action, like through their influence on transcription factors. The answers will come with further studies on catecholamines in plants.