INTRODUCTION

During the last decade, a notion come into view that nitrate, most available for plants source of nitrogen, is also a signal molecule triggering the mechanisms of various enzyme controlling [1–3]. The evidence for such nitrate action was obtained, in particular, with tobacco mutants deficient in nitrate reduction but nevertheless active in induction of the genes for nitrate transporters, for ammonium assimilation, and also the genes for carbon metabolism enzymes (phosphoenolpyruvate carboxylase, pyruvate kinase, citrate synthase, and NADP-isocitrate dehydrogenase); they also could repress the gene for starch synthesis key enzyme, ADP-glucose pyrophosphorylase [4]. The inhibition of the last enzyme did not influence the total direction of carbohydrate metabolism, because sucrosephosphate synthase, responsible for the synthesis of the pool of transportable sucrose, was not affected. In arabidopsis plants, about 40 genes induced by nitrate were found, including the genes encoding enzymes of carbon metabolism (NAD-malate dehydrogenase, transaldolase, transketolase, glucose-6-phosphate dehydrogenase, and others [5]). On the basis of these data, the notion emerged that nitrate not only induced the pathway of nitrate assimilation in plants but also reprograms carbon metabolism for the creation of more favorable conditions for mineral nitrogen assimilation.

A key point of the initial step of sink organ and tissue providing with carbon is an utilization of sucrose, a universal transport assimilate, with the involvement of sucrose synthase (SS). This enzyme displays a double action: it can synthesize or breakdown sucrose. In the second case, UDP-glucose and other nucleoside diphosphatesugars are produced, which are substrates for various synthesis (starch, cell-wall structural polymers, and glycosides) and monosaccharide mutual conversions. Until now, it is not clear whether this enzyme could be controlled by nitrate.

The objective of this work was to study the in vivo and in vitro effects of nitrate on SS activity in pea plant roots.

MATERIALS AND METHODS

Plant growing and treatments. Experiments were performed with pea (Pisum sativum L.) cultivars Truzhenik and Perfection-80. The seeds were germinated in darkness at 23–25°C in the roll of moistened filter paper. Four-day-old seedlings were transferred in the vessels with 5 l of settled tap water (60 plants per vessel). In experiments performed in vitro, with the isolated SS preparation, the roots of six-day-old seedlings were used. Under low light (climate-controlled chamber, 2.5 klx), nitrate could not activate SS. In in vivo experiments, nitrate activated SS exponentially by a dose-dependent mode with the plateau at 3–5 mM, where its activity was increased by 50%. It is supposed that there is a second constituent in SS activation by nitrate, and it carries information about plant carbohydrate status. Possible mechanisms of nitrate-induced SS activation are discussed.
The light intensity in the greenhouse varied from 3.5–12 klx at cloudy weather to 12–25 klx at clear weather.

In the in vivo experiments, nitrate-containing and nitrogen-free media were used. The first one was the full Knop solution containing KNO$_3$ and Ca(NO$_3$)$_2$ with the total nitrate concentration of 14.2 mM. In some experiments, the medium containing 1.4 mM nitrate was used. The second medium did not contain nitrogen. Calcium and potassium deficit was compensated by addition of K$_2$SO$_4$ and CaSO$_4$·2H$_2$O. Similar concentrations of other Knop macrosalts were present in all media. Plant sampling for SS assay in the roots was performed at the plant age of 13, 17, and 20 days, i.e., in 3, 7, and 10 days after their transfer on different nutrient media. The entire root systems (4–12 g) were analyzed.

**SS isolation and assay.** SS isolation from plant material and its assay (in the reaction of sucrose synthesis) were performed by the methods used by us earlier [6]. Root sample was homogenized at 4°C in the two volumes of the isolation medium comprising 75 mM Tris–HCl (pH 7.5), 10 mM 2-mercaptoethanol, and 5 mM MgCl$_2$. The homogenate was kept at 4°C for 1 h and then centrifuged at 15000 g for 20 min. The supernatant was passed through the column (2 × 6 cm) filled with Sephadex G-25 (coarse) at centrifugation and used for enzyme assay. SS activity was determined on the day after isolation. The preparation was stored in the refrigerator in ice water. Enzyme preparation (15–20 μl) was incubated in the 0.2 ml of the reaction mixture, containing 10 mM D(-)-fructose and 3.12 mM UDP-glucose as substrates and 50 mM Tris–HCl (pH 7.5), 10 mM 2-mercaptoethanol, and 5 mM MgCl$_2$, at 37°C for 15 min. Measurements were performed in five replicates. Control reaction mixture did not contain UDP-glucose. The reaction was stopped by 3-min boiling in the water bath. The amount of sucrose produced was estimated by the method of Pavlinova and Prasolova [7]. Optical density of colored solutions was determined at 520 nm, using a Spekol 21 spectrophotometer (Germany). SS activity was expressed in sucrose micromoles synthesized for 1 min and calculated per 1 g of fresh weight.

When nitrate effects on SS activity were studied in vitro, various KNO$_3$ concentrations were added immediately into the reaction mixture. The range of concentrations (0–50 mM) was chosen on the base of nitrate content in the cytosol (0–5 mM) and vacuoles (40–70 mM) of various plant species [8, 9].

**Sucrose quantification** in the roots, stems, and young expanded leaves of 12-day-old plants (1 g fr wt)