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Role of nitrogen metabolites on the regulation of nitrate uptake in maize seedlings

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ABSTRACT The ability of individual amino acids to regulate nitrate uptake was studied in *Zea mays* L. seedlings. The amino acids were applied to the root media or supplied by immersion of the tip-cut leaves before the induction of inducible high-affinity transport system with 0.1 mM KNO_3. NO_3^- uptake was measured by its depletion in amino acid – free medium. Glutamine and glutamate applicated via roots, were found to be the most effective inhibitors of uptake processes. The treatment with Gln via leaves also resulted in a strong inhibition of nitrate uptake rate accompanied with the significant enrichment of root tissue by amino compounds. Exclusion of conversion of glutamine to glutamic acid and to other amino acids by aminooxalacetic acid has showed, that with high probability the glutamine is responsible for the observed nitrate uptake inhibition. Since the lag-phase in inhibitory effect of glutamine was shorter than 2 hours, we suppose the direct inhibition of glutamine on the nitrate carrier itself, rather than on the corresponding gene expression. **Acta Biol Szeged 46(3-4):177-178 (2002)**

KEY WORDS

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Nitrate concentrations in the soil solution vary widely, and plant requirements of N can also diverse at different stages of plant growth. Therefore, nitrate uptake systems as well as the nitrate assimilatory enzymes are subject to regulation by several stimuli, including nitrate, glutamine, asparagine and sucrose. The assimilation of nitrate of most plant species is localised in shoots, therefore the negative feedback control of nitrate uptake is not located in root only, but integrated in whole plant level. Majority of exogenously applied amino acids whether supplied to roots or cotyledons of soybean downregulate the NO_3^- uptake (Muller and Touraine 1992). Glutamate, glutamine, asparagine and aspartate are the representatives of the assimilation of NH_4^+ in GS/GOGAT and AspAT/AS enzyme systems. All four amino acids may have considerable role in these processes, since they are exported from leaves via phloem in considerable amounts (Caputo and Barneix 1997), can be rapidly produced in N – assimilatory processes, and inhibit the nitrate uptake when applied to root medium (Cerezo et al. 2000; Aslam et al. 2001). The aim of the present work was to investigate the role of individual amino-acids on nitrate uptake process in maize roots. Five amino acid (Gln, Glu, Asn, Ala and Ile), involved in NH_4^+ assimilation and/or presented in phloem sap in considerable amounts were exogenously applied on the roots and their short term as well as long term effect on nitrate uptake was measured. We investigated, if the inhibitory effect of Gln on nitrate uptake found in maize roots also occur, when supplied to tip cut second leaves of maize. To exclude the effect of transamination, AOA an inhibitor of amino-transferase were used in one set of experiments. The changes in internal level of all studied amino acids were also determined.

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Materials and Methods

Maize seeds (*Zea mays* L. cv. TO 360) were grown for 10 days under controlled conditions (24ºC, 70% RH, 16/8 h day/night cycle at irradiance 150 µmol m^{-2} s^{-1}). Twenty two hours before the uptake experiments the growth solution was replaced with 1 mM $CaSO_4$ (control), or 1 mM $CaSO_4$ solution containing one of the amino acids (Gln, Glu, Asn, Ala or Ile) and/or aminooxalacetic acid at the required concentration. To screen the effect of Gln on NO_3^- uptake when supplied to the leaves, the 2 mm tip cut second leaves were immersed either in 10-50 mM Gln solution or in 10 mM $CaSO_4$ (control) for 30 hours. In order to trigger the inducible NO_3^- uptake system 0.1 mM KNO_3 was added into solution 6 h before the uptake measurement. In the case of time course effect of Gln (long term effect) HATS for nitrate was induced 24 h before the experiments and the required concentration of glutamine was added at the beginning of the uptake measurement.

Ion uptake experiments were performed with intact plants in the light. Samples of the incubation solution (0.1 mM KNO_3 in 0.5 mM $CaSO_4$) were withdrawn at intervals, and NO_3^- was determined spectrophotometrically (Braun-Systematic, Methodenblatt N60). The NO_3^- uptake rate was calculated on the basis of NO_3^- depletion from the uptake solution and related to the root fresh weight.

Amino acids were assayed after precolumn derivatization with diethyl ethoxymethylenemalonate (DEMM). The derivatization reaction time was 50 min, and the derivatives were stable at room temperature. The derivatization was followed by reversed phase high-performance liquid chromatography.

Results and Discussion

Due to the fact that glutamine is the major N-compound being transported to the shoot in most ammonium-grown plants and from leaves to roots by floem transport it has long

time been considered as the main regulatory factor of nitrate uptake (Gojon et al. 1998). Other amino acids have also been considered as feed-back inhibitors of nitrate uptake with aspartate and glutamate being most effective (King et al. 1993). Glutamine, glutamate and asparagine as key intermediates in the pathway of N-assimilation were tested for they ability to regulate nitrate uptake in maize seedlings. Alanine and isoleucine were also included as examples of amino compounds lying outside the main assimilatory pathway, although using Glu as a donor in their synthese processes. Treatment of maize plants with any of the five amino compounds inhibited the NO_3^- uptake. Gln was found to be the most effective inhibitor (about 70%) followed by Glu (65%). The effect of Asn, Ala and Ile was very similar resulting in about 25-35% inhibition of NO_3^- uptake. Downregulation of nitrate uptake rate in roots supplied with the 1 mM Gln was almost the same as 0.1 mM concentration. Gln at 0.075 mM external concentration still inhibited the NO_3^- uptake by 30%, but at 0.05 mM concentration already no inhibition was observed. The strong inhibitory effect of Gln we have found was surprising, because its relatively low effect in comparison to Asp or Glu was described in other plants such as soybean (Muller and Touraine 1992), barley (Aslam et al. 2001), and wheat (Rodgers and Barneix 1993). However, Lee et al. (1992) described almost the same downregulation of nitrate uptake in maize roots provided by Gln, Glu and Asn. Aslam et al. (2001) also found, that DON, which application primarily increased the plant Gln level because of inhibition of GOGAT (Lee et al. 1992), strongly decreased the rate of nitrate uptake. In addition to this, enhanced inhibitory effect was found after externally applied Gln, when added together with DON. Neither DON nor azaserine, the specific inhibitors of GOGAT caused an increase of Gln level in plant tissues alone, but also cause significant increase in the internal tissue level of Asn (Vidmar et al. 2000). Similar results were described after exogenous application of oxalaminoacetic acid (AOA). In good agreement with Lee et al. (1992) AOA also decreased the tissue Asp level, while Glu level remain nearly unchanged. This fact suggests that under AOA treatment asparagine synthetase still produced Asn and Glu from Gln and Asp. Glu can be than used as substrate for glutamine synthetase. Glutamine with or without AOA caused the same efficient downregulation of nitrate uptake, proving its own regulatory role in maize roots.

Glutamine, when applied to the root solution, 1-2 hour lag phase exists until the inhibition is evident. In this period the maize roots probably accumulated glutamine in their tissue, indicated that there was an endogenous effect of Gln on uptake rate. It appears to be direct on the nitrate carrier itself, although the some inhibitory effect on the corresponding genes in some plants was also observed (Vidmar et al. 2000). Nevertheless, in both cases Gln significantly reduced the nitrate uptake and this inhibition increased with the time of exposure of roots to Gln. Gln is able to downregulate nitrate uptake, when externally aplicated via leaves by 50%, when added in 50 mM concentation. This inhibition also occur, when conversion is avoided by aminooxyacetic acid.

The time, until the detectable inhibitory effect was measured was about 14 hours. This lag period is much longer than in experiments with tip-cut soybean cotyledons immersed in to 100 mM amino acid solution (8 h) described by Muller and Touraine (1992). On the other hand the concentration of glutamine was twice as high as in our experiments and absorption surface of cotyledons was also much higher.

The enrichment of the root tissue and basal part of immersed leaves by amino acid supplied to the leaves and some other amino compounds suggest, that Gln was absorbed by leaves from external solution, transported from leaves to roots and also conversed to other amino acids. The This conversion can by at least partially avoided by aminooxyacetic acid.

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