GLUTAMINE IN SUGAR BEET: WHERE IS IT SYNTHESIZED?

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ABSTRACT

Glutamine is the predominant amide in the beet. It is assumed that it is produced in the leaves and translocated to the beet (BURBA et al., 1984). The aim of the present study was to evaluate the contribution of the different plant organs in synthesis and accumulation of glutamine when either nitrate (NO_3^-, 5 mM) or ammonium (NH_4^+, 5 mM) is supplied as N source. The plants were grown hydroponically under controlled conditions for 40 days.

The results indicate that glutamine in the beet is imported from various organs, not only from leaf blades. Furthermore, it is snythesized in the beet (crown and root) itself. The contribution in glutamine synthesis of the different organs changes with the N source of the plant. Synthesis of glutamine accumulating in the beet is catalyzed by both the chloroplastic and cytosolic isoenzymes of glutamine synthetase.

ABRÉGÉ - LA GLUTAMINE DANS LA BETTERAVE SUCRIÈRE : OÙ A LIEU SA SYNTHÈSE ?

La glutamine est le principal amide présent dans la betterave sucrière. On suppose qu'elle est fabriquée dans les feuilles et transportée de là vers la betterave proprement dite (BURBA et al., 1984). Cette étude avait pour but d'examiner l'étendue de la synthèse et de l'accumulation de la glutamine dans différents organes. Comme source d'azote, les plantes ont été alimentées soit en nitrate (NO_3^-, 5 mM), soit en ammonium (NH_4^+, 5 mM). Elles ont été cultivées pendant 40 jours de façon hydroponique dans des conditions contrôlées.

Les résultats indiquent que la glutamine est transférée vers la betterave à partir de différents organes, qu'elle ne provient donc pas seulement des feuilles. En outre, la glutamine est fabriquée dans la betterave elle-même (collet et racine de la betterave). La contribution des différents organes à la synthèse de la glutamine dépend de la source d'azote dont dispose la plante. La synthèse de la glutamine issue de la betterave est catalysée par l'isoforme tant chloroplastique que cytosolique de la synthétase de la glutamine.

KURZFASSUNG - GLUTAMIN IN DER ZUCKERRÜBE: WO WIRD ES SYNTHETISIERT?

Glutamin ist das überwiegende Amid in der Rübe. Man nimmt an, daß es in den Blättern gebildet und von dort in die Rübe transportiert wird (BURBA et al.,

1984). Ziel der Studie war es, das Ausmaß von Synthese und Akkumulation von Glutamin in verschiedenen Organen zu untersuchen. Die Pflanzen wurden entweder mit Nitrat (NO_3^-, 5 mM) oder Ammonium (NH_4^+, 5 mM) als N-Quelle versorgt. Sie wurden hydroponisch unter kontrollierten Bedingungen für 40 Tage angezogen.

Die Ergebnisse deuten darauf hin, daß Glutamin aus verschiedenen Organen in die Rübe verlagert wird, also nicht nur aus den Blättern stammt. Darüber hinaus wird es in der Rübe (Kopf und Rübenkörper) selbst gebildet. Der Beitrag der einzelnen Organe zur Glutaminsynthese hängt von der N-Quelle der Pflanze ab. Die Synthese des Glutamins aus der Rübe wird sowohl von der chloroplastidären als auch cytosolischen Isoform der Glutaminsynthetase katalysiert.

INTRODUCTION

Soluble nitrogen lowers the technical quality of the beet because it interferes with sugar crystallisation (HARVEY & DUTTON, 1993). A major aim in breeding is thus to reduce the concentration of this so-called harmful nitrogen. Harmful nitrogen of plants grown in the field is composed from 30 to 40% amino acids, among them predominates glutamine. It is assumed that it is synthesized in the leaves and translocated to the beet (BURBA et al., 1984). The aim of the present study was to evaluate the contribution of different plant organs in synthesis and accumulation of glutamine. Since the N source has a strong effect on the amino-N content of the beet (see other contribution by Mäck in this issue), the plants were supplied with either nitrate or ammonium.

MATERIAL AND METHODS

Two genotypes of sugar beet were selected from field trials; they were characterized by a different amino-N content of the beet (high- and low-N type). Seeds of these genotypes were germinated in vermiculite and thereafter the plants were grown hydroponically for 40 days in a growth chamber under controlled conditions. The plants were supplied with a full nutrient solution containing either 5 mM nitrate or ammonium as sole N source. The pH of the nutrient solution was adjusted to pH 6 and controlled by addition of solid $CaCO_3$ (GOYAL & HUFFAKER, 1986). After 40 days leaves of different stages (old, mature, young) were harvested and separated into blades and stems. Also crown, root and fibrous roots were separated and all organs were immediately frozen in liquid nitrogen prior to analysis of amino acid contents and activity of glutamine synthetase (GS). Amino acids were analyzed with high pressure liquid chromatography and the synthetase reaction of GS was measured as reported (MÄCK & TISCHNER, 1990).

RESULTS AND DISCUSSION

The 40-day-old plants had developed 5 pairs of foliage leaves, a thickened hypocotyl and a thickened root. No difference in plant growth with either nitrate or ammonium was observed at that stage as long as the pH of the nutrient

solution was controlled carefully. The distribution of glutamate, glutamine and glutamine synthetase (GS) activity between the different organs was, however, altered in ammonium-grown plants compared to nitrate-grown plants. Glutamate, substrate of GS, was present predominantly in the blades of young and mature leaves of nitrate-grown plants, but in crown and root of ammonium-grown plants (Fig.1). GS was active predominantly in the blades of mature and young leaves. This activity was most probably due to the chloroplastic isoform of GS which constitutes 80 % of total GS activity in mature blades (MÄCK & TISCHNER, 1994). A significant activity, however, was measured also in crown and root of nitrate- and especially of ammonium-grown plants; in the fibrous roots GS activity was stimulated 6-fold by ammonium compared to nitrate. Besides in crown, root and fibrous roots, GS activity was also stimulated by ammonium in the leaf stems. This points to a response of the cytosolic isoforms (two GS 1 isoforms were separated in sugar beet leaves; MÄCK & TISCHNER, 1994) because cytosolic GS is assumed to be localized primarily in the mesophyll of vascular bundles (EDWARDS et al., 1990) and in roots (STÖHR & MÄCK, 2001).

Most glutamine, product of the GS reaction, was found in the stems of young leaves. In ammonium-grown plants its concentration increased in all organs, most significantly in stems, crown, root and fibrous roots (Fig.1). From the high concentration of glutamine in the root, BURBA et al. (1984) concluded that it is imported from the leaves and that glutamine is the transport form of N in sugar beet. WINZER et al. (1996), however, who analyzed phloem sap of sugar beet leaves found no special N transport form; rather, all amino acids that were present in the leaf blades were translocated via the phloem sap into the roots. The high concentration of glutamine in the stems, as observed here, thus indicates that a large fraction of glutamine was translocated in the xylem.

CONCLUSION

Glutamine is synthesized not only in the leaves but also in the crown and in the root. The absolute highest activity of glutamine synthetase is measured in the fibrous roots when ammonium is supplied as N source. It can be concluded that glutamine in the beet originates not only from the leaves.

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Figure 1: Gutamate, glutamine synthetase (GS) activity and glutamine in the different organs of sugar beet. The plants were grown hydroponically with either 5 mM nitrate (grey) or ammonium (black) as N source for 40 days.