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Nitrogen compounds in organs of two sugar beet genotypes (*Beta vulgaris* L.) during the season

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Abstract

A major aim in sugar beet breeding is to decrease the concentration of soluble N compounds in the beet to avoid negative impacts during beet processing. Due to its importance and analytical limitations the only selection criterion is amino N analyzed in the beet at harvest. It is thus not clear when genotypic differences are established during the season. Furthermore, it is not known whether selection for low amino N affects other N compounds in the beet and the N composition of other plant organs. Therefore, the concentrations of total N and soluble N compounds (protein, amino N, glutamine, betaine, nitrate) were investigated during the season in different organs (leaf blades, petioles, crown, beet) of two sugar beet genotypes differing in the amino N concentration of the beet. Field trials were carried out at three sites in 2002 and in 2003 with harvests at three times from 100 to 170 days after sowing.

Differences between the genotypes in N compounds were already established at 100 days after sowing. Therefore, characterization of genotypic differences seems to be possible earlier than in autumn. In the target organ beet, largest genotypic differences occurred for the target trait amino N and its main constituent glutamine, whereas the concentration of total N, betaine and soluble protein was only slightly lower in the low compared to the high amino N genotype. In the other organs, no consistent changes occurred for the soluble N compounds. The largest genotypic differences were detected in the nitrate concentration of the mature leaf blades (48% lower) and the glutamine concentration of the young leaf petioles (47% higher in the low amino N genotype). Glutamine synthetase activity of the beet was positively correlated to its amino N concentration. It was concluded that differences between genotypes in the amino N concentration of the beet resulted most likely from differences in glutamine synthetase activity in the beet but not in the leaves.

Keywords: Amino N; Glycine betaine; Genotype; Glutamine synthetase; Organ; Quality; Sugar beet

1. Introduction

Soluble N compounds in the beet lower its technical quality because they impair sugar recovery (van der Poel et al., 1998). Soluble N in the beet is composed of about 30% amino N as the sum of amino acids, 30% glycine betaine N and 35% non-defined residual N as well as 5% nitrate N (Hoffmann and Märländer, 2005). Among those, only the concentration of amino N is determined for quality assessment (Buchholz et al., 1995) and, due to the close correlation, taken as representative for the concentration of total soluble N. The reason for focussing on amino N lies in its noxious chemical reactions during processing and the effect on crystallization, as well as in analytical limitations with respect to the other N compounds. Accordingly, in breeding the amino N concentration is the only selection criterion with regard to N components in the beet. It is therefore not known whether breeding for a low amino N concentration in the beet affects the concentration of other N compounds in the beet. Furthermore, there is no information on whether differences in amino N concentration are restricted to the beet or if they occur also in other plant organs such as crown and leaves.

The major amino N compound in the beet is glutamine (Mäck, 1988). Genotypic variability in the glutamine concentration of modern sugar beet varieties ranges from 4 to 6 mmol/kg beet which equals 30–33% of amino N (Hoffmann and Märländer, 2005). In hydroponically grown sugar beet supplied with ammonium as the sole N source glutamine in the beet reached up to 80% of the total free amino acids (Mäck, 2003). This is the highest percentage reported and seems to be the upper physiological limit. Glutamine is synthesized by glutamine synthetase (Miflin and Lea, 1980) of which several

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catalytically active isoenzymes, oligomers and subunits have been separated in sugar beet organs (Mäck and Tischner, 1994; Brechlin et al., 1999, 2000). Genotypic variability in the activity of glutamine synthetase, however, has not been examined for sugar beet so far.

Glycine betaine is the dominant soluble N compound in the beet. It is molassigenic (Smith et al., 1977; van der Poel et al., 1998), but is not analyzed in the quality assessment due to technical limitations. Therefore, data on the genotypic variability in the beet are scarce. A recent survey revealed a range from 10 to 15 mmol/kg beet (Hoffmann and Märländer, 2005). Betaine and glutamine are supposed to be synthesized in the leaves and exported to the beet (Hanson and Wyse, 1982; Burba et al., 1984). Differences between genotypes in the betaine and glutamine concentration of the beet may thus originate from the leaves. Glutamine synthetase activity detected in the beet (Mäck and Tischner, 1990), however, indicates that the beet may contribute to the synthesis of glutamine.

For the determination of genotypic differences in the N composition of the beet, analyses are usually performed only at harvest in October (Hoffmann and Märländer, 2005), but little is known about the seasonal development of the N compounds. It is thus not clear when differences between genotypes are established.

In the present study two sugar beet genotypes were examined which covered the whole range of differences in the amino N concentration of the beet in October. In order to identify physiological mechanisms responsible for these differences investigations were aimed at (a) whether the differences occurred already during the season, (b) whether the beets differed only in amino N or also in other N compounds and (c) whether the N composition of other organs was affected.

2. Materials and methods

2.1. Material and field trials

Sugar beet genotypes (*Beta vulgaris* L.) were selected according to their amino N concentration in the beet at final harvest. Following extensive analytical procedures carried out only in 2002 on six genotypes two genotypes were selected for this study: a low amino N genotype (KWS OJ_0149) and a high amino N genotype (KWS OJ_0198). Those two genotypes represented the extremes in amino N concentration among the current varieties and differed by a factor of 1.5. They were cultivated at three typical sugar beet growing areas in southern (Seligenstadt, Bayern), northern (Parensen, Niedersachsen), and eastern Germany (Kleinwanzleben, Sachsen-Anhalt) in 2002 and 2003. The trials were carried out in a randomized split-plot design with four replications; the main plot was harvest date, the subplot was genotype. The plots consisted of three rows with a distance of 45 cm and a plot length of 8 m (10.8 m^2). Plants were sown in March/April 6 cm apart within the row and were manually singled to 90–110 thousand plants/ha in May to avoid effects of different population densities (Märländer, 1991). Nitrogen was applied shortly after sowing as calcium ammonium nitrate at a rate of 160 kg N/ha including mineral N at 0–90 cm soil depth in March. Plant protection was carried out according to regional standards.

2.2. Plant harvest and organ separation

Plants were harvested manually at 103, 133 and 168 days after sowing (July, August, September). Mature and young leaves (lamina length <50% of that of mature leaves) were removed from the intact plant at their insertions at the crown and divided into petiole and blade (Fig. 1). In order to avoid artefacts due to rapid water losses, the separated leaf organs were immediately shock-frozen in liquid nitrogen in the field. Therefore, only a limited number of leaves was harvested; 10 young and 10 mature leaves per plot. This material was also used for the determination of glutamine synthetase activity (see below).

For the analysis of the beet, 80–100 plants per plot were harvested manually. The storage root was divided into beet and crown by separating the crown below the oldest leaf insertions. The crown thus consisted of stem tissue whereas the beet consisted of root plus hypocotyl (Artschwager, 1926). The beets were processed in the tare house and the brei was shock-frozen at −80 °C. Due to the laborious work, only the crowns of 50 plants were separated and ground in a large blender (Stephan UM 12, Hameln, Germany) to prepare brei.

For the determination of glutamine synthetase activity special precautions had to be followed during harvest. All steps had to be carried out as quickly as possible to avoid a degradation of the enzyme. Furthermore, organs had to be harvested at a similar time of day at all trial sites to avoid diurnal effects on enzyme activity (Stöhr and Mäck, 2001).

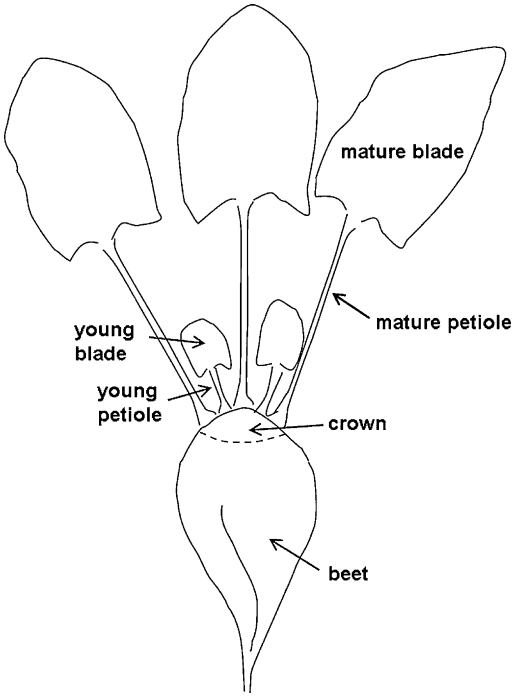

Fig. 1. Separation of the organs of a sugar beet plant.