Contribution of Invertase and Sucrose Synthase Isoenzymes to Sucrose Losses in Sugarbeet

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Introduction

Sugarbeet sucrose metabolism is the very basis of the sugarbeet industry. The unique ability of sugarbeet to accumulate large quantities of sucrose in a large and readily harvested organ is the basis for a domestic industry valued at over 1.2 billion dollars (1). Sugarbeet roots typically accumulate sucrose to 16-18% of their fresh weight. Greater sucrose accumulation is possible, however, and sucrose contents of 20-21% have often been obtained. The final sugar content of a mature root is determined by the balance between sucrose storage and sucrose utilization. Sucrose imported into the root is either sequestered into the vacuole for storage or catabolized to provide for the root's metabolic and material needs. To enhance sucrose accumulation in sugarbeet roots and prevent sucrose losses during development and postharvest storage, an understanding of these processes is essential.

Background

The major enzymes involved in sucrose catabolism are invertase and sucrose synthase. Both of these enzymes occur in plants as a number of different isoenzymes. Invertase catalyzes the hydrolysis of sucrose to glucose and fructose. Invertases are categorized into two groups based on their pH optimum for activity. The acid invertases exhibit a pH optimum in the range of 4.5 to 5.0 (2). These isoenzymes are found in the cell wall and vacuole, and can be insolubilized in the plant cell wall by ionic bonds (3). Generally, acid invertases are associated with young, actively growing regions of the plant. They are thought to be essential for providing carbohydrates to areas of growth by hydrolysis of imported sucrose (4). The neutral or alkaline invertases exhibit a pH optimum at 7.0 to 8.0, and are located in the cytoplasm (5). Unlike the acid invertases, the neutral or alkaline invertases are typically found in mature tissues. Their *in planta* function is generally unknown. It has been speculated, however, that they may regulate hexose levels for the metabolic needs of the mature cell. Sucrose synthase catalyzes the cleavage of sucrose in the presence of uridine diphosphate to form UDP-glucose and fructose in a reversible reaction. This enzyme is also located in the cytoplasm. Sucrose synthase activity is typically found in storage sinks, such as the root or fruit (6). Sucrose synthase activity is usually correlated with increasing sink strength, although its function in sucrose partitioning and metabolism is unclear.

While the enzymes responsible for sucrose breakdown are known, their respective roles in sucrose metabolism during sugarbeet development and storage are unclear. All of these enzymes are found in the sugarbeet root during some stage of development. The acid invertases exhibit greatest activity in young, actively growing roots. With maturity of the root, acid invertase levels decline, and neutral or alkaline invertase and sucrose synthase activities increase. Little is known about the role of these enzymes in sucrose partitioning during growth and development of the

sugarbeet root. Several studies have examined the activity of the invertases and sucrose synthases in relation to postharvest sucrose losses. These studies have yielded conflicting results, suggesting that either an acid invertase or a sucrose synthase is responsible for sucrose losses during storage.

Ongoing and Future Research

It is the objective of ongoing and future research at the USDA Northern Crop Science Laboratory to identify the specific enzymes involved in sucrose breakdown during different developmental stages and postharvest storage of sugarbeet roots. While such studies have been undertaken previously by others, the relationship between the sucrose catabolizing enzymes and the level of extractable sucrose is still unclear. Past studies have often yielded contradictory results, making it difficult to assign function or importance to these enzymes. The discrepancies between studies may arise from the nature of the enzymes involved. In nearly all plants, invertase and sucrose synthase occur not as single enzymes, but as families of isoenzymes. The different isoenzymes of enzyme families usually exhibit different patterns of expression and frequently exhibit different reactivities toward substrates. Different isoenzymes are important at different developmental stages and are thought to perform different functions in the plant. Multiple isoforms for the enzymes of sucrose catabolism occur in sugarbeet. Multiple isoenzymes of acid and alkaline invertase have been found in sugarbeet (4, 5). Sucrose synthase is also likely to occur as more than one isoenzyme, although this has not been definitively proven. Despite evidence for multiple isoenzymes of the sucrose catabolizing enzymes, previous studies have relied on enzyme activity assays. These assays measure total activity for an enzyme family, not the activity of an individual isoenzyme. This approach has probably contributed to the confusion over the function of these enzymes.

In research at the NCSL, the activity of the sucrose catabolizing enzymes will be studied at the isoenzyme level throughout development and postharvest storage. Acid and alkaline invertase and sucrose synthase isoenzymes will be examined for enzyme activity and steady state transcription levels. These will be correlated with the carbohydrate content of sugarbeet roots during development and postharvest storage. Respiration rate will also be examined in stored roots. To measure the contribution of individual isoenzymes, total activity for all isoenzymes will first be determined using colorimetric endpoint assays. The relative contribution of individual isoenzymes to the total activity will be measured by densitometric scanning of activity stained isoelectric focusing or nondenaturing polyacrylamide gel electrophoresis. The expression of individual isoenzymes will also be examined at the transcriptional level. The expression of invertase and sucrose synthase isoenzymes will be determined using RT-PCR and isoenzyme specific primers. These studies should provide clues to the importance of different isoenzymes for sucrose loss at different stages of growth and during storage. Comparison of activity and steady state transcription levels will also provide information on their regulation.

It is hoped that an understanding of the relative contribution of the isoenzymes of invertase and sucrose synthase to sucrose losses will be gained from these studies. This knowledge may potentially aid in maximizing extractable sucrose in sugarbeet roots by providing insights into changes in cultural or storage practices that would enhance sucrose accumulation and

conservation. Alternatively, these studies may identify specific isoenzymes whose expression could be altered by genetic engineering to increase extractable sucrose.

Literature Cited

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