DIFFERENTIAL EXPRESSION OF GLYOXYLATE ENZYMES IN SUGAR BEET RELATED TO SEEDLING VIGOUR

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ABSTRACT

One component of seedling vigour is the efficient utilization of the seed storage reserves to provide energy necessary for growth. This study examined the relationship between the genes of energy metabolism and differences in seedling vigour of sugar beet hybrids under different stress germination regimes. Analyses of 1,718 5' Expressed Sequence Tags (ESTs) from subtracted cDNA libraries, combined with gene expression profiling by northern blots and enzyme activity assays indicated that stress drastically reduces the expression of a α-amylase in a poor-emerging sugarbeet cultivar. In contrast, a good emerging variety exhibited only a moderate reduction in α-amylase gene expression. This pattern of gene expression indicates that mobilization of energy from stored carbohydrates can be limited to various extents by abiotic stresses. As mechanism to cope with reduced carbohydrate catabolism, the good-emerging, but not the poor-emerging, variety appeared to catabolize lipids as supplementary source of energy for respiration and biosynthetic processes. Induction of glyoxylate cycle activity, whose pathway bridges lipid and carbohydrate metabolism in germinating seeds, was indicated by high transcript levels and increased enzyme activity for the key glyoxylate cycle enzymes isocitrate lyase and malate synthase. The differential activity of the glyoxylate cycle is a potential physiological marker to differentiate between high- and low-vigour sugarbeet cultivars.

INTRODUCTION

Our objectives have been to examine germination of sugar beet under sub-optimal environments and to identify physiological and developmental opportunities for intervention by traditional and marker-assisted breeding. As one approach, we developed Expressed Sequence Tags (ESTs) from subtracted cDNA libraries of a high vigour sugar beet hybrid in order to gain some insight into gene expression during germination under stress.

Seedling vigour *a priori* involves the coordinated regulation of many genes in various biochemical pathways, including mobilization of seed storage reserves. Starches are an important energy reserve in beet seed, and lipids and proteins

also are present. Ware (1898) refers to a number of chemical constituents of beet seed, and indicated a starch content of 39.6% primarily located in the perisperm (maternally –derived endosperm-like tissue), a protein content of 27.6% primarily located in the embryo, and 20.5% lipid content distributed throughout the perisperm and embryo. Elamrani et al. (1992) showed virtually no lipid in the perisperm but a lipid content in the embryo around 15%, showing that lipids are likely to be the initial respiratory substrate during germination of sugar beet, with carbohydrates assuming greater importance after radicle protrusion from the seed ball. Similarly, Lawrence et al. (1990) showed differences in organ specific starch, protein, and sugar contents in excised seeds and seedlings, and suggested a specialization of the inner cotyledon (in closest proximity to the perisperm) in carbohydrate uptake.

Involvement of lipid metabolism in beet seedling vigour was suggested by the presence of Expressed Sequence Tags (ESTs) for germination specific, lipid catabolizing enzymes of the glyoxylate cycle in stress germinating beet seed *in vitro*. The abundance of key glyoxylate cycle enzymes isocitrate lyase (E.C. 4.1.3.1) and malate synthase (E.C. 4.1.3.2) in stress- and H_2O_2-induced EST libraries raised the question of the importance of lipids as energy source during germination and seedling emergence under sub-optimal environments. In this study, we present evidence of differential activity of carbohydrate and lipid catabolic pathways in germinating seeds based on gene expression analysis and their physiological importance to seedling emergence and vigour in sugar beet cultivars.

MATERIALS AND METHODS

Seed germination: High quality seedlots (average germination >92%) of hybrids USH20 (strongly emerging) (Coe and Hogaboam, 1971) and ACH185 (weakly emerging) (American Crystal, Moorhead, MN) were used. Germination was performed as described (de los Reyes and McGrath, 2003). Percentage germination (radicle length $\geq$2 mm) was determined daily from four replicate experiments.

Isocitrate lyase activity assay: Soluble protein extracts from control and solution-germinated seedlings were prepared at 2 to 8 days after imbibition. Isocitrate lyase activity was determined by the lactate dehydrogenase (LDH)-coupled continuous assay (Giachetti et al., 1983).

RESULTS

1,718 ESTs (Expressed Sequence Tags) were obtained from three subsets of cDNA. One EST subset was derived from 415 cDNAs that were randomly chosen from an unsubtracted cDNA library of stress-germinated 4-day-old seedlings. Two other EST subsets represent the collections of salt-induced (871 ESTs) and H_2O_2-induced (432 ESTs) genes. This EST collection does not represent the total array of genes that were expressed, but was enriched with genes related to growth and development, stress response and transcription.

Grouping ESTs according to putative biochemical function showed that 7.2% of cDNAs from the whole collection represented known genes in carbohydrate or lipid catabolic pathways. For carbohydrate utilization, transcripts encoding starch and polysaccharide hydrolytic and debranching enzymes were numerous (1.5%). Transcripts for α-amylase were the most abundant EST under this functional category (0.3%), and this gene serves as a physiological marker for carbohydrate breakdown. Not all genes in the primary pathways for sugar catabolism, i.e. glycolysis, oxidative pentose phosphate pathway and tricarboxylic acid cycle were represented, but their activities were indicated by the occurrence of transcripts encoding more than half of the enzymes (3.8%).

The importance of lipids during germination was implied by the relatively high frequency of transcripts for lipases and fatty acid hydrolytic enzymes (0.6%). Activity of the fatty acid β-oxidation spiral was indicated by the EST for acetyl-CoA acyl transferase (0.1%). The glyoxylate cycle was also active as shown by high percentage of transcripts (1.2%) for glyoxysomal enzymes isocitrate lyase and malate synthase. Isocitrate lyase is the key glyoxylate cycle enzyme that links fatty acid oxidation and sugar metabolism via the succinate produced from glyoxysomal acetyl-CoA, and is specific to seed germination. These data suggest that glyoxylate cycle activity may be a critical factor for successful emergence under sub-optimal environments.

The effects of H_2O_2, submergence and salt stresses on the expression of stored energy reserve catabolism genes were compared between USH20 and ACH185. In roots and leaves, isocitrate lyase and malate synthase expression was either low or undetectable, acetyl-CoA acyl transferase was expressed at very low levels, while α-amylase was undetectable.

In US H20 seedlings, α-amylase expression was high in control (filter paper germination) and H_2O_2 treatments and was reduced slightly by stress. In contrast, α-amylase expression in ACH185 was high in non-stressed seedlings but severely reduced by solution stress (water and salt).

All solution germinations induced expression of isocitrate lyase, malate synthase, and acetyl-CoA acyl transferase in USH20. In contrast, submergence and salt stress caused severe reduction in these transcript levels in ACH185. Expression in H_2O_2 remained high and was comparable to the filter paper control. Expression of isocitrate lyase, malate synthase, and acetyl-CoA acyl transferase appeared coordinately regulated by stress and H_2O_2.

CONCLUSION

Germination and early seedling growth rely on the maintenance of energy supply from seed storage reserves. The glyoxylate cycle allows plants to utilize lipids as a carbon source. Under optimal conditions, the glyoxylate cycle was highly active in germinating sugar beets. The data presented here provide evidence that oxidation of fatty acids and the glyoxylate cycle in a strongly emerging sugar beet hybrid were more active under stress than under optimal conditions. Activity of α-amylase was significantly reduced by stress, and reduction of the rate of carbohydrate catabolism was more severe in the weakly emerging than in strongly emerging hybrid. Based on this relationship, the

carbon intermediates derived from lipids via the glyoxylate cycle is an important component of seedling vigour in sugar beet.

The glyoxylate cycle has two important physiological functions: 1) the provision of carbon intermediates from lipid metabolism for sucrose biosynthesis, and 2) replenishment and maintenance of the tricarboxylic acid cycle under conditions when most intermediates are being withdrawn for biosynthetic processes (anapleurotic function). The glyoxylate cycle utilizes acetyl-CoA derived from fatty acid oxidation for the biosynthesis of the four carbon compound succinate, which is then exported and converted to malate in the mitochondria via succinate dehydrogenase (Kornberg and Beevers, 1957). Malate can be utilized for sucrose biosynthesis via gluconeogenesis. Sucrose is then transported to different parts of the seedlings to support post-germinative growth. These data indicate that the coordinate induction of isocitrate lyase, malate synthase, and acetyl-CoA acyl transferase by stress occurred both before and after radicle elongation. Since cellular adaptation to stress conditions require massive changes in gene expression, purine and pyrimidine biosyntheses that utilize tricarboxylic acid cycle intermediates as substrate, the carbon intermediates produced from the early induction of the glyoxylate cycle were probably not utilized for gluconeogenesis but as an anapleurotic pathway for the tricarboxylic acid cycle cycle.

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