SUCROSE ACCUMULATION DURING EARLY SUGAR BEET DEVELOPMENT

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ABSTRACT

This study examined sucrose accumulation in different breeding lines during the first weeks after emergence in order to identify early physiological differences correlated with root sucrose content. At each weekly harvest during the first 10 weeks of growth, roots, leaves, and hypocotyls were weighed and freeze-dried, and hypocotyls diameters were measured. From freeze-dried roots, sucrose was extracted with 80% ethanol and then analyzed with high pressure liquid chromatography (HPLC). Sucrose concentration expressed as fresh weight increased from less than 0.5% at the third week (all germplasm) to over 12% by the tenth week, with measured sucrose levels proportional to those from field-harvested beets. Incremental changes in sucrose levels were not constant during this period, but followed a step-wise trend of rapid sucrose accumulation alternating with low sucrose accumulation. Sucrose concentration expressed as dry weight reached 55% at the 10th week for all lines. During this early developmental stage a time-course differential gene expression analyses (cDNA-AFLP) was performed, and showed that more than 40% of the transcribed genes are differentially expressed in developing roots. Differential gene expression analyses combined with examination of anatomical differences of root tissues during these alternate developmental stages may provide additional insight on the kinetics and molecular mechanisms of sucrose accumulation in sugar beet.

INTRODUCTION

Sucrose content in beet tap roots is inherited as a multigenic trait, in an additive fashion, and with high heritability (Savitsky 1940; Culbertson 1942; Powers 1957; Powers et al. 1963; Zhao et al. 1997). Sucrose distribution within the root is concentrated with the innermost five of the concentric cortical rings, around the point of maximum root girth, and accumulates in vacuoles of parenchyma cells adjacent to vascular tissue (Elliott & Weston 1993). Sucrose biosynthesis, transport, and storage in beets likely occurs by mechanisms similar to other plants, but specific regulatory mechanisms that allow accumulation of sucrose in the roots remain to be identified (Kovtun & Daie 1995, Avigad & Dey 1997, Martin et al. 1997, Bush 1999). Unknown is whether any or all of the enzymes involved in these biochemical processes are important for the accumulation of sucrose in sugar beet roots and which genes, if any, are regulated and would

likely play significant roles during sucrose accumulation. The dynamics of sucrose accumulation during the growing season are of interest since early developmental stages are important for the future storage capacity of the root.

The purpose of this research is to develop a genetic model for heritable differences in root sucrose content in different genotypes of sugar beet, and begin to characterize major genes involved in root sucrose accumulation. The specific objective of this report was to examine early plant development stages and correlate developmental initiation of sucrose accumulation with the changes in gene expression during this developmental phase.

MATERIALS AND METHODS

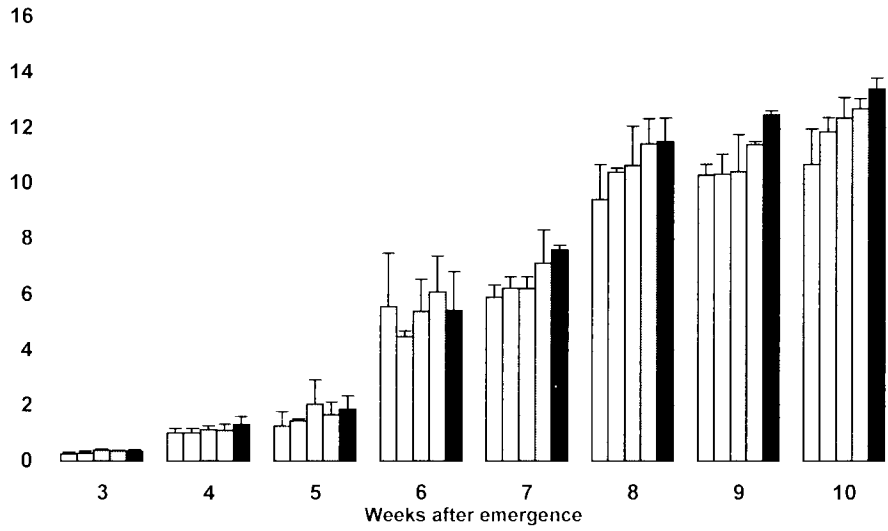
Five germplasm lines (USH20, SR87, SR95, SR96, and SR97, ranging in harvested sucrose contents from 15 to 18%) were planted in the greenhouse (20 to 22.5 C, 16 hr light cycle) with three replications. Plant samples were harvested weekly from the third to the tenth week post-emergence for sucrose analyses, and additionally for mRNA extraction (additionally including the 2nd week). Carbohydrate analyses were performed via HPLC. After freezing, dehydrating, and pulverizing root tissue (<5 week old also included epidermal tissues), sucrose was extracted with 80% ethanol, decanted, vacuum evaporated, and re-suspended in water for HPLC.

Differential gene expression analysis using cDNA-AFLP was performed as described (Bachem et al. 1996). Amplified fragments originating from amplification with dye-labelled *Eco*RI (5' – GACTGCGTACCAATTCNNN - 3') and *Mse*I (5' – GATGAGTCCTGAGTAANN - 3') primers were separated on 7% poly-acrylamide gels using an LI-COR 4200 Automated DNA Sequencer.

RESULTS

Sucrose accumulation: Sucrose was the main component (>98%) of the extracted sugars, and only traces of glucose and fructose were detected. Sucrose content increased dramatically from less than 2% to more than 10% (fresh weight) between the 5th and 8th weeks (Figure 1). A further smaller increase was observed during the last two weeks of observation when lines reached more than 12% of sucrose in fresh weight. The difference in sucrose content between the lowest sucrose content variety USH20 and the highest sucrose content germplasm SR96 lines was statistically significant after the 6th week post emergence. No significant differences between entries was observed for sucrose content expressed on a dry weight basis, which increased from 5 to more than 55% during the period under investigation (data not shown). Hypocotyl diameters increased exponentially from less than 2.5 to more than 35 mm from the third to the tenth week, without any significant difference between lines.

Figure 1: Sucrose accumulation over eight weeks of early sugar beet growth. Differences between USH20 and SR96 are significant at p=0.10 for weeks 7 & 8, and p=0.05 for weeks 9 & 10).



Transcription profiling: 134 primer combinations were used for cDNA-AFLP analyses. 3,739 amplified fragments, arising from expressed genes, were scored. Most fragments (58%) were invariant, and thus represent constitutively expressed genes. The remaining fragments (42%) varied in their presence or absence in at least one week's sample. The number of fragments detected tended to decrease with as plant age increased, with the exception of the plants at the 2nd week post-emergence (Table 1). Cluster analysis of fragment presence or absence revealed two distinct developmental periods: before and after the 6th week (Figure 2). A developmental shift in growth during the 6th week is postulated to explain this result.

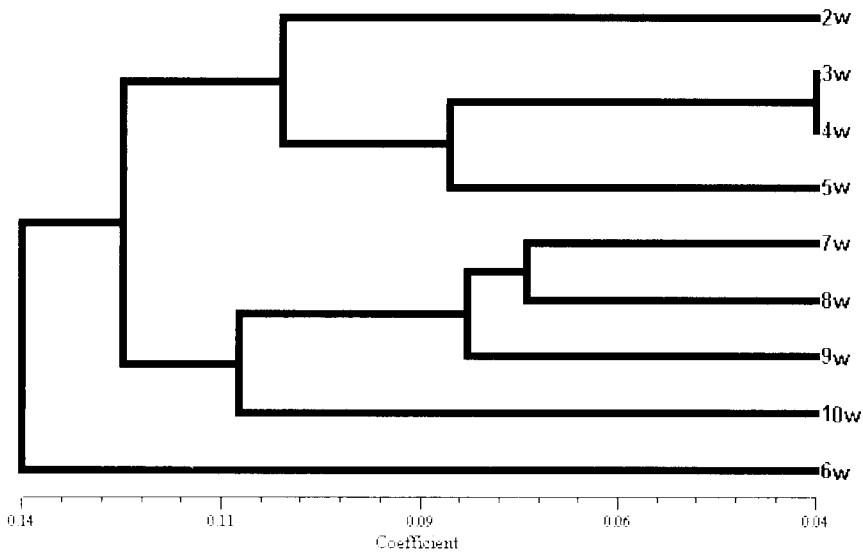
Table 1: Number of fragments representing expressed genes scored over the time course of experiments.

	Number of fragments scored	Percent of total fragments
2	3059	81.8
3	3127	83.6
4	3109	83.2
5	2924	78.2
6	2917	78.0
7	2957	79.1
8	2929	78.3
9	2824	75.5
10	2882	77.1
Total	**3,739**	**100.0**

CONCLUSION

Characterizing genes that play major role in sucrose accumulation will facilitate rapid progress in developing high sucrose germplasm releases after introgression of other favorable traits such as disease resistance and stress tolerance genes from wild and exotic germplasm. Early selection would also be facilitated if sucrose content could be measured early in the season. Each of these enhanced breeding strategies appears feasible on the basis of these preliminary experiments since significant differences in sucrose content (fresh weight) are evident as early as 7 weeks after emergence, and the genes responsible for this increase appear to be expressed as early as 5 weeks post emergence. Interestingly, this period also coincides with the onset of field resistance for many seedling pathogens.

Figure 2: Cluster analysis results from expression patterns (presence vs. absence of 3,739 cDNA-AFLP fragment scored during each week of development from 2 to 10 weeks of age.



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